GABA-Dependent Firing of Glutamate-Evoked Action Potentials at AMPA/Kainate Receptors in Developing Hypothalamic Neurons

XIAO-BING GAO, GONG CHEN, AND ANTHONY N. VAN DEN POL
Department of Neurosurgery, Yale University School of Medicine, New Haven, Connecticut 06520

INTRODUCTION

In the adult mammalian CNS, glutamate mediates excitatory synaptic transmission, whereas \( \gamma \)-aminobutyric acid (GABA) is the predominant inhibitory neurotransmitter. The activation of glutamate receptors depolarizes postsynaptic neurons through the opening of nonselective cation channels. The activation of \( \gamma \)-aminobutyric acid-A (GABA<sub>A</sub>) receptors usually hyperpolarizes postsynaptic neurons by opening anion channels and allowing an influx of chloride ions. Together, these two neurotransmitters provide a balance between excitation and inhibition. On the other hand, activation of ionotropic GABA receptors can induce depolarization in developing neurons (Ben-Ari et al. 1989; Chen et al. 1996; Leinekugel et al. 1995, 1997; LoTurco et al. 1995; Obata 1974; Owens et al. 1996; Reichling et al. 1994; Rohrbough and Spitzer 1996; Serafini et al. 1995; Wu et al. 1992). In immature neurons, a high \( [\text{Cl}^–]_i \) may result from unopposed, inward \( \text{Cl}^– \) transport (LoTurco et al. 1995) or from a reduced outward transport (Luhmann and Prince 1991) resulting in GABA-gated \( \text{Cl}^– \) exit from the cell and depolarization. During development, GABA-mediated depolarization gradually shifts to hyperpolarization because of a negative shift in the GABA-gated chloride channel reversal potential (Chen et al. 1996; Obrietan and van den Pol 1995; Wang et al. 1994).

The GABA-mediated depolarization in adult mammalian CNS could play an inhibitory role by shunting other excitatory inputs because of an increase in membrane conductance resulting from the activation of GABA<sub>A</sub> receptors (Staley and Mody 1992) or it could be excitatory (Ben-Ari et al. 1994; Staley et al. 1995; Wagner et al. 1997). Our previous results indicated that GABA-mediated depolarizations could produce a dual effect, either facilitating firing of action potentials or shunting additional depolarizing inputs in developing neurons (Chen et al. 1996). Recently Leinekugel et al. (1997) reported Ca<sup>2+</sup> oscillations mediated by the synergistic excitation actions of GABA<sub>A</sub> and \( N \)-methyl-\( \text{D} \)-aspartate (NMDA) receptors in the neonatal hippocampus. Shunting of NMDA receptor-mediated synaptic responses by GABA was also observed. The conditions under which GABA-mediated depolarization may be excitatory or inhibitory have not been well-characterized. Previous work has focused on the role of NMDA receptors in synaptic strengthening, particularly related to models of Hebbian synapses in development (Crair and Malenka 1995; Kirkwood et al. 1995). The possible role of depolarizing GABA actions in the facilitation of \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-kainate type of glutamate responses has not been studied.

The present study defines some of the conditions within which a GABA-mediated depolarization may be excitatory or inhibitory. We demonstrate here a novel facilitation of GABA on non-NMDA glutamate-mediated excitation in developing neurons that has not been previously reported. A hypothetical synaptic strengthening model is proposed based on the synergistic interaction between GABA- and non-NMDA glutamate-mediated depolarizing responses.

METHODS

Cell culture

Hypothalamic neurons were cultured from rat embryos as described previously (Chen et al. 1995, 1996). Briefly, E15–E18 embryos were removed from pregnant Sprague-Dawley rats heavily anesthetized with a lethal dose of pentobarbital sodium (Nembutal, 80 mg/kg). Medial hypothalami were dissected out of the embryonic brains and cut into small pieces (<1 mm<sup>3</sup>) and then incubated at 37°C for 30–40 min in an enzyme solution containing 10 units/ml papain, 0.5 mM EDTA, 1.5 mM CaCl<sub>2</sub>, and 0.2 mg/ml L-cysteine. The tissue was washed with culture medium containing 10% fetal calf serum after enzymatic digestion and mechanically triturated in culture medium to obtain dissociated cells. The cells were plated in 35-mm culture dishes in a density of 200,000–
30,000 cells per dish, maintained in an incubator at 37°C and 5% CO₂, and fed twice a week. The culture medium contained minimal essential medium (Gibco), 10% fetal calf serum (HyClone, Logan, UT), serum extender (according to the manufacturer’s instruction: Collaborative, Bedford, MA), 20 units/ml penicillin-streptomycin, and 6 g/ml glucose. Cytosine arabinoside (1 μM) was added to inhibit the proliferation of astrocytes. (WaveMetrics, Lake Oswego, OR). In some experiments, the depolarization induced by GABA (at each time point when current was injected or glutamate was applied) was normalized to the percentage of the largest depolarization induced by GABA. All data are reported as mean ± SE. Student’s t-test was used to compare two groups of data.

**RESULTS**

Previous work in our lab has shown that GABA mediates a depolarization instead of a hyperpolarization in developing hypothalamic neurons (Chen et al. 1996). As illustrated in Fig. 1A, a brief GABA exposure via a puffer pipette caused a long-lasting depolarization (n = 55). The top represents the depolarizing response induced by GABA (250 μM, 35 ms) applied from a micropipette during current clamp and the lower trace is an inward current induced by GABA during voltage clamp of the same neuron. Figure 1B shows that the GABA-mediated depolarization can either facilitate or inhibit another depolarizing input. A subthreshold depolarization ± induced depolarization can also be shunted when near the peak of GABA-mediated depolarization (1st response in Fig. 1B). When glutamate is applied simultaneously with GABA (Fig. 1C, left), no action potential is generated; in contrast, when glutamate is applied a few milliseconds after GABA, an action potential is generated (Fig. 1D). These results raise the question as to what physiological factors are involved in the GABA-mediated depolarization shift from inhibitory shunting to excitatory facilitation.

GABA-mediated depolarization facilitates current injection-induced depolarization over a broad window

To explore the potential facilitating effect of GABA-mediated depolarization on other excitatory transmitter actions, we used current injection to simulate the effects of a second excitatory transmitter. Current was injected through the recording pipette into neurons during the depolarization induced by GABA application (250 μM, 35 ms, 1–4 psi). One of three levels of current (low, medium, and high) was injected at different time points during the GABA-mediated depolarization and induced a depolarization corresponding to 75, 87.5, and 100% of the largest depolarization. One-hundred percent would be the current injection that reaches threshold for evoking an action potential in a particular cell. Figure 2 illustrates the typical responses recorded in our experiments (n = 6). All traces were recorded by using gramicidin-perforated whole cell recording. This type of recording does not disturb the normal intracellular Cl⁻ levels, critical for experiments assessing whether or not GABA is depolarizing or hyperpolarizing. The membrane potential
The current injection-induced response was shunted by the atory transmitter in the mature brain. Trace 2 larization.

Trace 2 shows that neither glutamate and GABA (250 μM) applied simultaneously nor glutamate applied alone elicits an action potential. In contrast, D, in same neuron, when glutamate is applied 100 ms after GABA, an action potential (A. P.) is generated. Again, glutamate alone at end of trace does not evoke an action potential.

The facilitating effect of GABA-mediated depolarization appeared from 90 to 50% of the peak GABA response, whereas the subthreshold responses induced by current injection alone before GABA administration. In Fig. 2, the X-axis represents the percentage of its peak depolarization during the decaying phase of the GABA-induced depolarizing potential. The current-injection–induced depolarizing responses recorded before GABA administration served as the control and was normalized as 100% in Y-axis. Figure 3A (low-amplitude current injection) and Fig. 3B (medium-amplitude current injection) show that the current-injection-induced depolarization was significantly shunted (*) only near the peak GABA response (>80% of the peak). When the GABA depolarizing potential decayed to <80% of its peak, the current injection-induced depolarization was not significantly different from the control. In Fig. 3, C and D, the total depolarizing responses induced by GABA plus current injection were significantly larger (*) than the depolarizing response induced by current injection alone before GABA during most of the decaying phase of the GABA-induced depolarizing potential (<90% of its peak; P < 0.05, n = 6). The facilitating effect of GABA-mediated depolarization appeared from 90 to 50% of the peak GABA response, corresponding to the decaying phase of GABA-mediated depolarization from 100 to 1,000 ms after GABA application in our experiment. These results demonstrate that GABA-mediated depolarization facilitates other excitatory input during most of its decaying phase, except a significant shunting near its peak. There is a broad time window for the facilitating effect of GABA compared with the shorter temporal window for the shunting effect.

**Interaction between GABA-mediated depolarization and glutamate-mediated depolarization in developing neurons**

The positive current injection described above was used to simulate the current response to an excitatory input. The next step was to explore the effect of GABA-mediated depolarization on the actions of glutamate, the principal excitatory transmitter in the mature brain.

Our previous experiments with gramicidin-perforated
FIG. 2. Effect of GABA on membrane depolarization induced by current injection in cultured hypothalamic neurons (2 days in vitro). Three levels of currents (low in A, medium in B, and high in C) were injected during depolarization induced by GABA (250 μM, 30 ms). A–C: 10 traces were recorded consecutively with an interval of 10 s. A–C, 1: injection of positive current (60, 70, and 80 pA, respectively, 15 ms). RMP (resting membrane potential) is −70.9 ± 0.5 mV. Depolarizing responses were so greatly shunted near peak of GABA-mediated depolarization (A–C, trace 2) that a suprathreshold depolarizing response was decreased to a subthreshold one (C, trace 2). B: depolarizing responses induced by medium current injection were facilitated to fire action potentials from traces 3 to 8.

whole cell recordings predicted a value of 29 mM [Cl⁻], at a reversal potential of −45 mV in early developing hypothalamic neurons (Chen et al. 1996). Therefore the current experiments were performed with both conventional whole cell recording (n = 12) (with 29 mM Cl⁻ in pipette solution, Fig. 4, A–H) and gramicidin-perforated whole cell recording (n = 4; Fig. 4, I–L). The protocol used in these experiments was similar to that described above with some modification. The current injection was replaced by application of glutamate (250 μM) from micropipette (2–5 μm diam) driven by a picospritzer. The inset in Fig. 4 illustrates the experimental arrangement of GABA and glutamate application in our experiments. In early developing neurons (2–5 days in vitro) GABA and glutamate both induced depolarizing responses in current clamp. Figure 4 illustrates the depolarizing responses of GABA recorded with conventional (Fig. 4, A–H) and gramicidin-perforated (Fig. 4, I–L) whole cell recordings in our experiments. Figure 4B, F and J illustrate glutamate-mediated depolarizations by two glutamate applications (▲) with an interval of more than 1,500 ms. The response induced by the second glutamate application was used as a control. When glutamate and GABA were applied to the recorded neuron simultaneously, the total membrane depolarization induced by these two
FIG. 3. Combined data showing interaction between current injection-induced depolarization and GABA-mediated depolarization. A and B: depolarization response induced by current injection (low level in A and medium level in B) was significantly (*, P < 0.05) shunted when GABA-mediated depolarization was between 80 and 100% of its peak (n = 6). C and D: total membrane depolarization induced by GABA and current injection was significantly (*, P < 0.05) higher than control depolarization induced by current injection alone from 50 to 90% of peak GABA response under 2 levels of current injection (n = 6).

transmitters (1st response in Fig. 4, C and K) was larger than the response induced by glutamate alone (2nd in Fig. 4, C and K), but not large enough to evoke an action potential. In Fig. 4, D and L, the application of glutamate was delayed ~100 ms after GABA. The depolarization induced by glutamate was imposed on the GABA response (1st response in Fig. 4, D and L). The total depolarizing responses because of GABA and glutamate (1st response in Fig. 4, D and L) were greater than either the responses induced by glutamate alone (2nd response in Fig. 4, D and L) or the responses induced by simultaneous administration of GABA and glutamate (1st response in Fig. 4, C and K).

To demonstrate that GABA could facilitate a glutamate response at different durations of GABA application, we used long (Fig. 4, A–D; I–L) applications of GABA where the return to 50% of peak amplitude was 664 ± 220 ms (n = 12) and shorter applications where return to 50% peak amplitude was about 198 ± 42 ms (n = 5). This was done by changing the speed of the perfusion system. In the long applications of GABA (Fig. 4, A–D; I–L), glutamate was facilitated between 100 and 650 ms and in the short one (Fig. 4, E–H) up to ~225 ms. During the shorter application, we focused on brief intervals between 0 and 100 ms delay between GABA and glutamate and found that GABA could facilitate a glutamate-evoked action potential as soon as 10–15 ms after GABA application. In shorter intervals, we would also expect GABA to be facilitative if it could be washed off faster, but this could not be tested in the perfusion system used.

To determine the temporal conditions during which GABA-mediated depolarization could facilitate glutamate excitation in early developing neurons, the timing of GABA application was fixed and the timing of glutamate application was varied from 90 ms before to 210 ms after the GABA application (Fig. 5, A–E). Top traces throughout Fig. 5, A–E are membrane potentials recorded in current clamp and lower traces are inward currents recorded in voltage clamp. When the glutamate application (▲) was 90 ms before GABA (Fig. 5A) subthreshold depolarizing responses were evoked. If the glutamate application was only 30 ms before GABA (Fig. 5B), the GABA-mediated depolarization was facilitated by the glutamate-mediated depolarization and an action potential was triggered (indicated by A. P., truncated). When the glutamate administration (▲) was simulta-
GABA FACILITATION OF GLUTAMATE EXCITATION

**Fig. 4.** GABA depolarization facilitates glutamate-mediated action potential. A–H: experiments were performed by using conventional whole cell recording with 29 mM Cl− in pipette solution and focusing on intervals of either 100–2000 ms (A–D) or 0–100 ms (E–H) between GABA and glutamate (n = 12). I–L: responses were recorded with gramicidin-perforated whole cell recording (n = 4). ▲, application of GABA (250 μM, 30 ms); ▼, glutamate (250 μM, 20 ms). Second response induced by glutamate served as a control (n = 7). Note that synchronous application of GABA and glutamate did not induce action potentials (C and K), whereas delayed application triggered action potentials (D, H, and L). E–H: an example of a series of experiments where presence of GABA was reduced by increasing perfusion and decreasing time of GABA application (250 μM, 10 ms) and interval between GABA and glutamate was varied between 0 and 100 ms. Whereas simultaneous application of GABA plus glutamate did not evoke an action potential (not shown), delaying glutamate by 20 ms (G) or 50 ms (H) did result in an action potential.

Glutamate-evoked depolarization mediated by non-NMDA receptors

Our previous results suggested that a GABA-mediated increase in [Ca2+]i in developing neurons was the result of the GABA-mediated depolarization of the membrane potential and the subsequent activation of voltage-gated calcium channels (Obrietan and van den Pol 1995). In contrast, Ben-Ari et al. (1996) reported that the GABA A and NMDA receptors mediated most of the excitatory synaptic transmission in the immature hippocampal slices, whereas AMPA receptors were quiescent. This was because of GABA-mediated depolarization reducing the voltage-dependent Mg2+ block of the NMDA receptors in immature interneurons (Ben-Ari et al. 1996; Leinekugel et al. 1997). In the present study we demonstrate that GABA-mediated depolarization can facilitate glutamate-mediated action potentials independent of any effect on NMDA receptors in early developing hypothalamic neurons.

Experiments (n = 5) were performed in a bath solution containing 100 μM D,L-AP5, an antagonist of the NMDA receptor. In Fig. 6, A and E, the administration of GABA (▲) induced a depolarizing response. Two depolarizing re-

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GABA facilitates glutamate-mediated action potential

Conventional whole-cell recording

- long interval
- shorter interval

- ▲: GABA
- ▼: glutamate
- ▲+▼: GABA + glutamate

Gramicidin perforated whole-cell recording

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Experiments (n = 5) were performed in a bath solution containing 100 μM D,L-AP5, an antagonist of the NMDA receptor. In Fig. 6, A and E, the administration of GABA (▲) induced a depolarizing response. Two depolarizing re-
FIG. 5. Mutual facilitation of glutamate and GABA ($n = 5$). In this experiment, application of GABA was fixed (vertical dotted line and †), whereas glutamate administration (▼) was timed from 90 ms (A), 30 ms (B), 0 ms (C) before, 30 ms (D), and 210 ms (E) after GABA. Top traces: membrane potential recorded under current clamp. Bottom traces: inward current recorded under voltage clamp. †, where action potentials (with peaks that are truncated here) were evoked ($n = 5$). B: Glutamate-mediated depolarization facilitated GABA-mediated depolarization to fire an action potential during a narrow time window. D and E: the facilitating effect of GABA-mediated depolarization on glutamate excitation occurred within a broad time window. (- - -), baseline (pretransmitter) currents and voltages.

Responses were induced by two applications of glutamate (▲), with a 1,500-ms interval in the presence of 100 $\mu$M AP5 (Fig. 6, B and F), ensuring that the glutamate response was mediated by non-NMDA receptors. When the first administration of glutamate was simultaneous with GABA (Fig. 6, C and G, †), the total depolarization (1st response in Fig. 6, C and G) induced by GABA and glutamate was a little larger than the response induced by glutamate alone (2nd response in Fig. 6, C and G). When glutamate was administered 30 ms (in Fig. 6D) or 100 ms (in Fig. 6H) after GABA, the response to GABA plus glutamate (1st response in Fig. 6, D and H) was greater than either the response induced by glutamate alone (2nd response in Fig. 6, D and H) or the response induced by GABA and glutamate given simultaneously (1st response in Fig. 6, C and G). Under these conditions the time-delayed administration of glutamate and GABA consistently triggered action potentials (Fig. 6, D and H). Addition of the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 100 $\mu$M) to the AP5 blocked the response to glutamate. These results suggest that the glutamate depolarization mediated by non-NMDA ionotropic receptors can be facilitated by GABA-mediated depolarization to initiate an action potential in early developing neurons.

DISCUSSION

The present study demonstrates that GABA can play an excitatory role in early development by facilitating the ability of other excitatory transmitters, particularly glutamate, to induce action potentials. GABA-mediated shunting occurred only in a narrow window near the peak of GABA-induced depolarization. A broad window during the decaying phase of GABA-mediated depolarization was facilitating. This temporally delayed summation between GABA- and glutamate-mediated excitation through non-NMDA receptors has
FIG. 6. GABA-mediated depolarization modulates glutamate response through non-N-methyl-D-aspartate (non-NMDA) receptors \((n = 5)\). Conventional (with 29 mM Cl\(^-\) in pipette solution, traces \(A\)–\(D\)) and gramicidin-perforated whole cell recording (traces \(E\)–\(H\)) were performed in buffers containing 100 \(\mu M\) 2-amino-5-phosphonopentanoic acid (AP5). \(^{1}\) application of GABA (250 \(\mu M\), 30 ms) and application of glutamate (250 \(\mu M\), 20 ms). Second response induced by glutamate served as a control to demonstrate that glutamate alone did not generate an action potential. GABA-mediated depolarization facilitated glutamate excitation dependent on non-NMDA receptors on delayed application of GABA and glutamate in presence of AP5, an antagonist of NMDA receptors.

Facilitating or shunting effect of GABA-mediated depolarization

Both excitatory and inhibitory roles of GABA-mediated depolarization in early development of the CNS have been suggested. Cherubini et al. (1990) reported spontaneous giant depolarizing potentials in the CA3 area of immature hippocampal slices, which were blocked by the GABA\(_A\) receptor antagonist bicuculline. Bicuculline also induced a hyperpolarization in these slices, suggesting an excitatory role for GABA in immature hippocampal slices. Obrietan and van den Pol (1995) reported that GABA elicited a dramatic increase in intracellular Ca\(^{2+}\) in young hypothalamic neurons. This Ca\(^{2+}\) rise persisted in the presence of TTX and could be completely blocked by bicuculline. GABA opened Cl\(^-\) channels leading to a depolarization of neurons and an activation of voltage-gated Ca\(^{2+}\) channels. Chen et al. (1996) demonstrated that in young cultures from rat embryonic hypothalamus, GABA induced depolarizing potentials that often surpassed the threshold for firing action potentials and resulted in an increase in neuronal activity. Owens et al. (1996) found that GABA depolarized neocortical cells and increased [Ca\(^{2+}\)]\(_i\) in embryonic and neonatal cortical slices.

These robust excitatory functions of GABA in early development are in contrast to the finding that GABA-mediated depolarization in adult hippocampal neurons was primarily inhibitory and this was because of its shunting effect on other excitatory input (Andersen et al. 1980; Staley and Mody 1992). Shunting here refers to an increase in conductance across the plasma membrane that may reduce a gluta-
facilitation was largely based on the potentiation of NMDA receptors, to generate a postsynaptic response. This would played by AMPA-kainate glutamate receptors in the adult response and facilitation of AMPA / kainate responses is the (Obrietan and van den Pol 1995). Ben-Ari et al. (1994) 1996). Additionally, given that GABA may exert depolarization than that of the GABAergic system in the CNS (Ben-Ari et particularly in those areas similar to the hypothalamus where normally replaced by glutamate excitation after the establishmen of development, GABAergic transmission may be dominant but instead to facilitate subthreshold glutamate actions. Under our experimental conditions, it was not critical whether or not GABA was applied rapidly or slowly for its facilitation of glutamate. Instead, a critical factor was the level of depolarization relative to the more rapid reduction of current flow and concomitant increase in input resistance during recovery after GABA application. Therefore, within the whole duration of GABA-mediated depolarization, there is a broad window during which a GABA-mediated depolarization could facilitate another excitatory input to generate action potentials. Our data are consistent with observations that in early developing neurons most activity is excitatory (Cherubini et al. 1991; Kriegstein et al. 1987; Muller et al. 1984; Schwartzkroin and Kunkel 1982) and our interpretation would be that this is because both GABA and glutamate exert depolarizing actions at this stage of development.

GABA facilitation of AMPA/kainate type glutamate receptors

The development of the glutamatergic system occurs later than that of the GABAergic system in the CNS (Ben-Ari et al. 1994; Chen et al. 1995; Reynolds and Brien 1992). GABA-mediated depolarization still exists when glutamatergic synaptic transmission appears in immature CNS (Obrietan and van den Pol 1995). Ben-Ari et al. (1994) reported that giant depolarizing potentials (GDPs) resulted from synchronous discharge of an interneuron network mediated by an interaction of GABA<sub>A</sub> and NMDA receptors. Further evidence suggested that most of the excitation of interneurons was determined by cooperation between GABA<sub>A</sub> and NMDA receptors (Khazipov et al. 1996). In the neonatal hippocampal slices, GABA<sub>A</sub> and NMDA glutamate receptors mediated most excitatory synaptic transmission, whereas AMPA receptors were considered quiescent. The facilitation was largely based on the potentiation of NMDA receptors by GABA-mediated depolarization which attenuated the voltage-dependent Mg<sup>2+</sup> block and permitted the activation of NMDA receptors in neonatal interneurons (Ben-Ari et al. 1996). The GABA<sub>A</sub> receptors were suggested to assume a substitute role in the developing hippocampus played by AMPA-kainate glutamate receptors in the adult hippocampus (Leinekugel et al. 1997).

Leinekugel et al. (1997) found relatively little effect of GABA in facilitating non-NMDA receptors. In contrast to the observations from immature hippocampal slices (Ben-Ari et al. 1996; Leinekugel et al. 1997), we found that the NMDA receptor antagonist AP5 did not block GABA facilitation of action potentials in developing hypothalamic neurons, suggesting that GABA-mediated depolarization can facilitate non-NMDA glutamate receptor-mediated depolarization in hypothalamic neurons. When we included the AMPA/kainate antagonist CNQX with AP5, we found no response to glutamate, suggesting that AMPA/kainate receptors are critical for the GABA facilitation in our experiments.

Functional relevance in the development of neural circuits

The development of GABA responses occurs before that of glutamate responses (Chen et al. 1995). In the early stages of development, GABAergic transmission may be dominant and play a major excitatory role. GABA excitation is gradually replaced by glutamate excitation after the establishment of glutamatergic neurotransmission and a negative shift of the reversal potential of GABA responses. There is thus a period during which both GABA- and glutamate-mediated depolarization coexist and this is during a period of intense synapse formation. A facilitative interaction between excitatory GABA and glutamate transmission is described here and leads to the question of what its relevance to development might be.

Our results show mutual facilitation of GABA<sub>A</sub> and non-NMDA glutamate receptors (probably AMPA/kainate receptors) during early development. Our results are not in disagreement with data showing that GABA in developing neurons can enhance NMDA responses in hippocampal neurons (Ben-Ari et al. 1996; Leinekugel et al. 1997). Rather they demonstrate an additional mechanism of GABA facilitation on non-NMDA receptors. Our experiments were done with hypothalamic neurons but should be applicable to other areas of the mammalian CNS during early development, particularly in those areas similar to the hypothalamus where the relative amplitude of NMDA to AMPA/kainate responses may not be as great as in hippocampal neurons (van den Pol and Trombley 1993; van den Pol et al. 1990, 1994, 1996). Additionally, given that GABA may exert depolarizing actions after neuronal injury (van den Pol et al. 1996), GABA could hypothetically exert the same facilitation on glutamate after trauma as described here in developing neurons.

The simultaneous action of both pre- and postsynaptic sites was postulated to stabilize synapses (Hebb 1949). A substantial amount of energy has focused on the importance of relieving the voltage dependent Mg<sup>2+</sup> block of NMDA actions, usually by glutamate activation of AMPA-kainate receptors, to generate a postsynaptic response. This would occur in the event of a simultaneous release of GABA and glutamate, when GABA’s depolarization is at peak amplitude and at the optimal membrane potential for relieving the Mg<sup>2+</sup> block of the NMDA receptor. The critical functional difference between GABA’s facilitation of the NMDA response and facilitation of AMPA/kainate responses is the relative timing of release. Simultaneous activation of GABA and non-NMDA receptors would lead to shunting glutamate
actions, but if glutamate receptor activation was temporally delayed by a finite amount, then GABA would facilitate glutamate actions at AMPA/kainate receptors.

Figure 7 illustrates a simple hypothetical model related to temporal summation of GABAergic and glutamatergic neurons in a developing neural circuit. In Fig. 7, a developing postsynaptic neuron receives axonal innervation from both a GABAergic and glutamatergic neuron. Neither the GABAergic nor the glutamatergic axon by itself can evoke an action potential in the postsynaptic cell. However release of glutamate at a particular interval after GABA evokes an action potential in the neuron postsynaptic to both inputs. The synergistic actions of the two neurons could result in synaptic stabilization or strengthening for either or both of the two synapses. On the other hand, if either presynaptic cell can by itself evoke an action potential in the postsynaptic neuron, simultaneous release of the other input may block the action potential because of shunting. Thus the relative timing of the glutamate and GABA input can take on critical roles in strengthening or weakening potential synaptic inputs from axons containing the other transmitter, particularly in the context of a Hebbian model of synaptic strengthening. GABA-mediated depolarization may thus play a crucial role in shaping the maturation of glutamatergic transmission in early development of the CNS. In the same context, glutamate may play a similar role in potentially stabilizing the synaptic development of the GABAergic system.

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