

(Section Head)

# Development and Modulation of GABA<sub>A</sub> Receptor-mediated Neurotransmission in the CA1 Region of Prenatally Protein Malnourished Rats

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Prenatal protein malnutrition has been demonstrated to result in alterations in the serotonergic and GABAergic neurotransmitter systems in the rat hippocampus. In the present study, whole-cell patch clamp recordings of CA1 pyramidal cells were employed in an effort to gain insight into the specific cellular locus and functional consequences of the previously reported changes. Hippocampal slices were prepared from Sprague-Dawley rats whose dams were fed either a normal (25% casein) or low (6% casein) protein diet during pregnancy. The development of GABA<sub>A</sub> receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) and their modulation by the benzodiazepine agonist zolpidem were compared in cells from the two nutritional groups at postnatal days 7, 14, 21 and >90. The modulation of mIPSCs by serotonin was also examined in cells from 21 day old rats. No significant differences were observed in the characteristics of mIPSCs in cells from control vs. prenatally protein malnourished rats at any of the ages studied, although there was a trend for a higher frequency of mIPSCs in adult (> p90) prenatally protein malnourished rats. At all ages, zolpidem produced a significant increase in the

mean decay time of mIPSCs that was not significantly different in cells from the two nutritional groups. Serotonin application resulted in a significant increase in the frequency of mIPSCs in CA1 pyramidal cells but there was no significant difference between cells from the two nutritional groups in the characteristics of this effect. These data demonstrate that the previously observed alterations in the serotonergic and GABAergic systems that result from prenatal protein malnutrition do not have significant functional consequences at a single cell level in the CA1 region of the rat hippocampus as measured *in vitro*.

*Keywords:* Hippocampus, *in vitro* slice, mIPSC, Serotonin, Whole cell patch clamp

## INTRODUCTION

Prenatal protein malnutrition has been shown to alter the development and, to a degree, the

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function of the hippocampal system of the rat (for review: Galler *et al.*, 1997; Morgane *et al.*, 1992; 1993). The hippocampus is a useful model system for the investigation of the effects of malnutrition on the central nervous system because of its well-defined system of inputs and outputs, its sensitivity to pre- and peri-natal insults, and its key role in learning and memory processes. Inhibitory synaptic inputs from GABAergic interneurons play an important role in the regulation of the activity and final output of the principal glutamatergic pyramidal cells in the hippocampus (for review: Freund and Buzsáki, 1996). These morphologically and neurochemically diverse populations of interneurons receive significant afferent input from subcortical monoaminergic nuclei including a major input from serotonergic neurons of the brainstem raphe nuclei (for review: Gulyás *et al.*, 1999). Monoaminergic afferents play a key role in the regulation of the flow of information through the hippocampal formation by altering the excitability of the GABAergic interneurons, which in turn modulate the activity of the principal cells. There is growing evidence indicating that both the baseline GABAergic neurotransmitter system and the important modulation of this system by serotonin in the hippocampus may be altered by prenatal protein malnutrition.

Evidence for prenatal protein malnutrition induced alterations in the GABAergic system of the hippocampal formation *per se* has been obtained from behavioral, receptor-binding and electrophysiological studies. First, prenatal malnutrition alters the behavioral sensitivity of rats to the benzodiazepine receptor agonist chlordiazepoxide when administered systemically and learning assessed by the Morris water maze spatial task (Tonkiss *et al.*, 2000a). In addition, infusion of chlordiazepoxide into the medial septum produces less disruption of performance on the Morris water maze spatial task in prenatally protein malnourished compared to control animals (Tonkiss *et al.*, 2000b) Second, *in vitro* ligand binding assays have revealed mean increases of

10–15% in GABA<sub>A</sub> (<sup>3</sup>H]-muscimol) and benzodiazepine (<sup>3</sup>H]-flunitrazepam) receptors in all areas of the hippocampal formation in both p15 and p90 prenatally malnourished compared to control rats (Fiacco *et al.*, 1998). Third, neurophysiological examination of paired-pulse responses in the dentate gyrus *in vivo* have demonstrated marked increases in GABAergic inhibitory responses in granule cells of prenatally protein malnourished compared to control animals (Bronzino *et al.*, 1991a,b; Austin *et al.*, 1992). This effect is limited to the early, GABA<sub>A</sub> receptor-mediated, inhibitory phase of the paired-pulse response, as demonstrated by the increased amount of inhibition of the second response at paired-pulse intervals of 20–50 ms in the prenatally malnourished animals. Finally, it has recently been reported that GABA<sub>A</sub> receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) in CA1 pyramidal cells are increased in frequency in adult rats exposed prenatally to protein malnutrition, although their modulation by chlordiazepoxide is unaltered (Luebke *et al.*, 2000).

In addition to the apparent impact of prenatal protein malnutrition on GABAergic neurotransmission and hence presumably on GABAergic interneurons in the hippocampus, there is growing evidence for an alteration in the important serotonergic inputs to the hippocampus which play a key role in the regulation of interneuronal activity. Diaz-Cintra *et al.*, (1981) have reported significant decreases in the numbers and dendritic arborizations of serotonergic cells of the dorsal raphe nucleus in animals exposed to prenatal protein malnutrition. These changes are accompanied by a reported 33% decrease in serotonergic fibers present in the hippocampal formation of prenatally protein malnourished compared to control rats as revealed by immunocytochemistry (Blatt *et al.*, 1994). In addition, significantly increased levels and release of 5-HT as measured by HPLC coulometric detection in *in vitro* slices (Chen *et al.*, 1995; 1997) and by *in vivo* microdialysis (Mokler *et al.*, 1999) have been reported in the hippocampal formation of

prenatally protein malnourished compared to control rats. Taken together these studies indicate that the raphé-hippocampal system is significantly impacted by prenatal protein malnutrition.

Given the reported alterations in the serotonergic and GABA<sub>A</sub> systems of the hippocampus in prenatally protein malnourished rats, the present study was undertaken to elucidate the locus and functional consequences of these alterations in the principal cells of the CA1 region. In particular, three key questions were addressed: (1) Does prenatal protein malnutrition alter fundamental GABA<sub>A</sub> receptor-mediated neurotransmission (as measured by mIPSCs)? (2) Does prenatal protein malnutrition alter the modulatory effect of zolpidem on the GABA<sub>A</sub> receptor? and (3) Does prenatal protein malnutrition alter the modulatory effect of 5-HT on GABA<sub>A</sub> receptor-mediated mIPSCs?

## MATERIALS AND METHODS

### Housing and Nutritional Treatment of Experimental Subjects

Rats were derived from a colony maintained at the Center for Behavioral Development and housed in strict accordance with animal care guidelines as outlined in the NIH Guide for the Care and Use of Laboratory Animals. Detailed protocols for the nutritional, mating and fostering procedures employed have been described previously (Tonkiss and Galler, 1990; Rushmore *et al.*, 1998). Briefly, nulliparous female rats, obtained five weeks prior to mating (Sprague-Dawley VAF plus; Charles River Laboratories, Kingston, MA) were allowed *ad lib* access to a diet of either adequate (25% casein) or low (6% casein) protein content. For one week prior to mating males obtained from the same source were acclimated to the same diet as the females. One male was mated to two females receiving the same diet and vaginal smears were obtained to determine whether mating had occurred. Fol-

lowing parturition, litters were culled to 8 pups (2 female, 6 male) and fostered to lactating, well-nourished mothers which had given birth within the previous 24 hours. Pups born to dams fed the low protein (6%) diet and cross-fostered to dams given the adequate protein (25%) diet are assigned the abbreviation 6/25 (prenatally protein malnourished). Pups born to dams fed the adequate protein (25%) diet and cross-fostered to other dams given the adequate protein (25%) diet are assigned the abbreviation 25/25 (well-nourished controls). After weaning at 21 days, all offspring were given *ad lib* access to Purina rat chow (formula 5001). One rat per litter was used for all experiments described in this report.

### Preparation of Slices

Slices were prepared as described previously (St. John *et al.*, 1997; Luebke *et al.*, 1993; 2000). Briefly, rats at post-natal day 7 (p7), p14, p21 and >p90 (adult) were decapitated and the brain rapidly removed into ice-cold oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Ringers solution. The composition of the Ringers solution was: (in mM): 26 NaHCO<sub>3</sub>, 124 NaCl, 2 KCl, 10 Glucose, 2.5 CaCl<sub>2</sub>, 1.3 MgCl<sub>2</sub>; pH=7.4, (chemicals from Fluka, NY). The hippocampus was dissected out and 500-micron thick transverse sections were cut on a vibrating microtome and placed in a holding chamber containing room temperature, oxygenated Ringers solution for a minimum of one hour. Following equilibration, a single slice was positioned in a submersion type slice recording chamber (Medical Systems Corp., Greenvale, NY). During experiments, slices were constantly superfused with oxygenated Ringers at room temperature (26 ± 1 °C) at a flow rate of approximately 2 ml per minute.

### Whole Cell Patch Clamp Recordings

Standard tight-seal, whole cell recordings (Edwards *et al.*, 1990; Luebke *et al.*, 1992; 2000; St. John *et al.*, 1997) were made with patch pipettes

fabricated on a Flaming and Brown horizontal micropipette puller (Model P-87, Sutter Instrument Co., Novato, CA). Pipettes were filled with an internal solution of the following composition (in mM): 140 cesium chloride, 2 MgCl<sub>2</sub>, 0.5 EGTA, and 10 Na-HEPES (pH adjusted to 7.2) and had a resistance of 6–10 MΩ. Patch pipettes were lowered into the CA1 pyramidal cell layer, pressure on the pipette was released and a several-hundred MΩ seal was usually obtained. Application of gentle suction resulted in the formation of a GΩ seal, and when suction was increased, whole-cell access was usually achieved. The membrane potential of the cells dropped to 0 mV (the chloride equilibrium potential) within approximately 1 minute of obtaining whole cell access. Access resistance was measured from responses to small hyperpolarizing current pulses and was checked at least every 5 minutes during the course of all experiments. Only those cells that maintained stable conditions such as failure rate and access resistance were used for the analysis of mIPSCs. Voltage clamp recordings were performed with a List EPC-7 patch clamp amplifier (70–90% series resistance compensation) and “Pulse” acquisition software from HEKA elektronik (Lambrecht, GDR) with a Power Mac 120 computer.

Once stable recording of a cell was obtained, spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the voltage clamp mode at a holding potential of –80 mV. Spontaneous events were measured for a period of at least 3 minutes in each cell. Following collection of sIPSC data, the perfusate was switched to control Ringers with the following drugs added: 600 nM tetrodotoxin (TTx), 10 μM 6,7-dinitro quinoxaline-2,3-dione (DNQX, to block glutamatergic AMPA responses) and 40 μM (±)-2-amino-5-phosphonopentanoic acid (APV, to block glutamatergic NMDA responses). Under these recording conditions, GABA<sub>A</sub> receptor-mediated mIPSCs were examined at a holding potential of –80 mV in isolation from action potential dependent sIPSCs and from glutamatergic mEPSCs. In

some experiments the GABA<sub>A</sub> receptor antagonist bicuculline methiodide (10 μM) was applied. Following acquisition of baseline mIPSC data, either the benzodiazepine agonist zolpidem (1 μM), 5-HT (10–100 μM) or 5-HT (10–100 μM) plus the 5-HT<sub>3</sub> antagonist ICS-205,930 (2 nM) were bath-applied to the slice. Recording of mIPSCs commenced approximately 1 minute after drug application and continued for another 5 minutes. All pharmacological agents were purchased from Sigma/RBI (St. Louis, MO).

### Data Analysis and Interpretation

Electrophysiological data were acquired with the “Pulse” acquisition program (HEKA elektronik), stored on hard disk and subsequently analyzed using the “MiniAnalysis” program from Jaejin Software (Leonia, N.J.). Events were required to exceed a detection threshold set at the maximum of the noise level (5 pA) and an area under the curve (fc) threshold of 30 fc. The following characteristics of mIPSCs were determined: frequency, amplitude, rise time constant and decay time constant (e.g. Connors *et al.*, 1988; Edwards *et al.*, 1990; DeKoninck and Mody, 1994). Rise times were measured from 10–90% of the peak amplitude and events that exceeded 4 ms rise time were rejected. mIPSCs occurring during the entire 5 minute recording time period were analyzed such that each sample contained several hundred synaptic events. Electrophysiological data were incorporated in a data base (“Excel”, Microsoft, Corp.) and analyzed for statistical significance using the Student’s *t*-test (two-tailed), with significance defined at  $p < .05$ . Data are given as the mean ± standard error of the mean.

### RESULTS

These studies were performed on hippocampal slices from control and prenatally protein malnourished male Sprague-Dawley rats. At birth, the mean weights of rats in the two experimental

groups were significantly different ( $p < .01$  Students *t*-test, two tailed) with the control pups weighing  $6.29 \pm 0.47$  (SD) grams and the prenatally protein malnourished pups weighing  $5.56 \pm 0.42$  (SD) grams.

The results presented are based on stable, long term recordings from 112 CA1 pyramidal cells in

hippocampal slices. Of these, all 112 experiments were designed to examine the baseline characteristics of mIPSCs across development, 42 specifically to examine the effects of zolpidem on mIPSCs across development, and 30 to examine the effects of 5-HT on mIPSCs in p21 rats. For exact numbers of cells at each developmental stage and nutritional group see the figure legends.

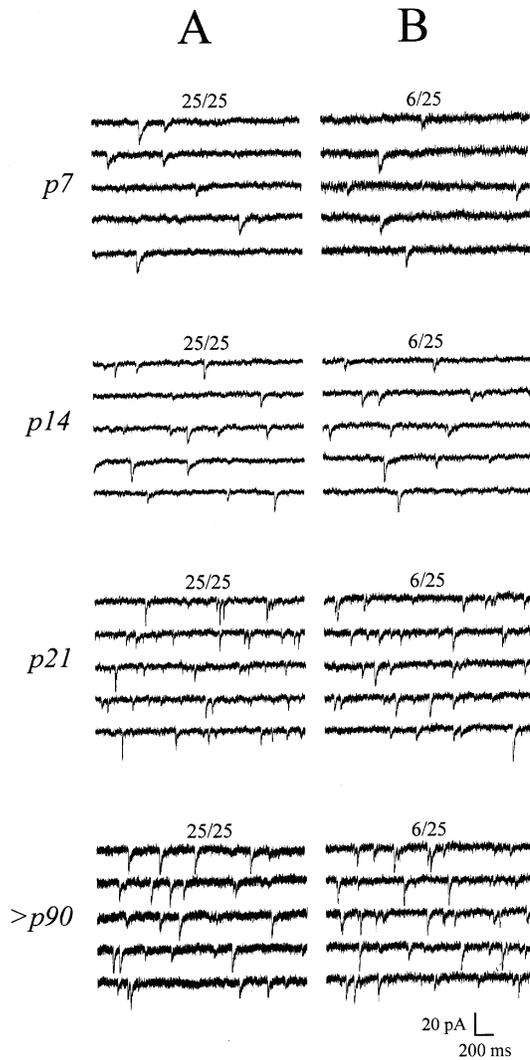


FIGURE 1 Electrophysiological traces of GABA<sub>A</sub> receptor-mediated mIPSCs across development. (A) Representative electrophysiological traces of GABA<sub>A</sub> receptor-mediated mIPSCs at p7, p14, p21 and > p90 in CA1 pyramidal cells from well-nourished rats. (B) Representative electrophysiological traces of GABA<sub>A</sub> receptor-mediated mIPSCs at p7, p14, p21 and > p90 in CA1 pyramidal cells from prenatally protein malnourished rats.

### Development of GABA<sub>A</sub> Receptor-mediated mIPSCs

mIPSCs measured in the presence of TTx, DNQX and APV exhibited a reversal potential of 0 mV (the chloride equilibrium potential) and were completely blocked by the application of bicuculline methiodide ( $10 \mu\text{M}$ ) confirming that the currents were mediated by GABA<sub>A</sub> receptors (data not shown). Representative traces of GABA<sub>A</sub> receptor-mediated mIPSCs in CA1 pyramidal cells from control and prenatally protein malnourished rats at p7, p14, p21 and p90 are shown in Figure 1.

The characteristics of mIPSCs in cells from control vs. prenatally protein malnourished rats at each of the four ages studied are graphically represented in Figure 2. The frequency of events increased significantly with development with mean values of  $0.11 \pm 0.03$  Hz (control) and  $0.07 \pm 0.004$  Hz (prenatal protein malnourished) at p7 to  $1.68 \pm 0.13$  Hz (control) and  $1.9 \pm 0.19$  Hz (prenatal protein malnourished) at p21 (control  $t = 10.8$ ,  $df = 31$ ,  $p < .001$ ; prenatal protein malnourished  $t = 16.6$ ,  $df = 31$ ,  $p < .001$  Students *t*-test, two tailed). However, there was no significant difference in the mean frequency of mIPSCs between cells from the two nutritional groups at any age (Figure 2A). In the adult, cells from prenatally protein malnourished rats exhibited a greater frequency of mIPSCs (mean =  $2.4 \pm 0.73$  Hz) than those from control rats (mean =  $1.5 \pm 0.38$  Hz), but this difference was not statistically significant ( $t = 1.69$ ,  $df = 14$ ,  $p < .1$ ). The mean amplitude of the mIPSCs did not change significantly with development and were not different

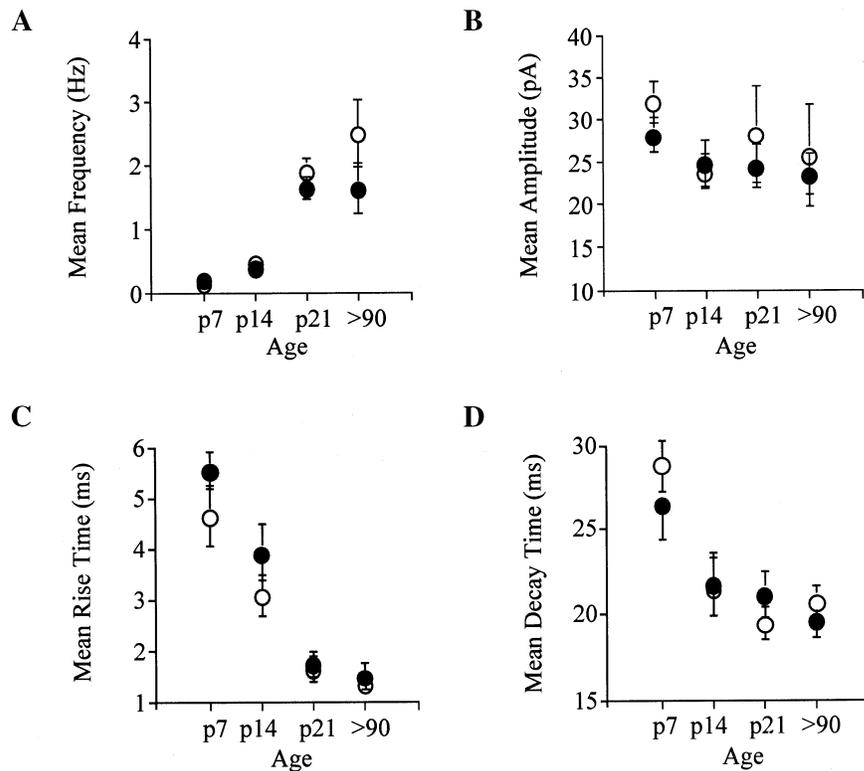


FIGURE 2 Characteristics of GABA<sub>A</sub> receptor-mediated mIPSCs across development in 25/25 vs. 6/25 CA1 pyramidal cells. (A) Mean frequency of mIPSCs across the four developmental ages, showing an increasing frequency of events with age but no effect of prenatal protein malnutrition on the increase. (B) Mean amplitude of mIPSCs across the four developmental ages, showing no change in the mean amplitude of events with age and no effect of prenatal protein malnutrition on amplitude at any age. (C) Mean rise time of mIPSCs across the four developmental ages, showing a faster rise time with age but no effect of prenatal protein malnutrition on rise time. (D) Mean decay time of mIPSCs across the four developmental ages, showing a faster decay time with age but no effect of prenatal protein malnutrition on decay time. Filled circles = 25/25; open circles = 6/25. p7  $n = 13$  control, 13 prenatally protein malnourished; p14  $n = 14$  control, 16 prenatally protein malnourished; p21  $n = 20$  control, 20 prenatally protein malnourished; > p90  $n = 8$  control, 8 prenatally protein malnourished.

between cells from the two nutritional groups (Figure 2B). The mean rise time (Figure 2C) and mean decay time (Figure 2D) of mIPSCs decreased with development but there were no effects of diet at any of the ages examined. By p21 mean mIPSC characteristics were not different from those seen in the adult in cells from both nutritional groups.

#### Effect of Zolpidem on GABA<sub>A</sub> Receptor-mediated mIPSCs

Bath application of 1  $\mu$ M zolpidem produced a significant increase (mean =  $51.2 \pm 4.4\%$ ) in the decay time constant of GABA<sub>A</sub> receptor-mediated mIPSCs in CA1 pyramidal cells at each

developmental age (Figure 3), but had no effect on the frequency, amplitude or rise time constant of the mIPSCs (data not shown). The effects of zolpidem on mIPSCs did not change significantly with development and were not significantly different in CA1 pyramidal cells from rats exposed to prenatal protein malnutrition compared to controls at any age (Figure 3B).

#### Effect of 5-HT on GABA<sub>A</sub> Receptor-mediated mIPSCs

Bath application of 5-HT (10–100  $\mu$ M) produced an inward current in all CA1 pyramidal cells examined and had a mean value of  $-30 \pm 4$  pA

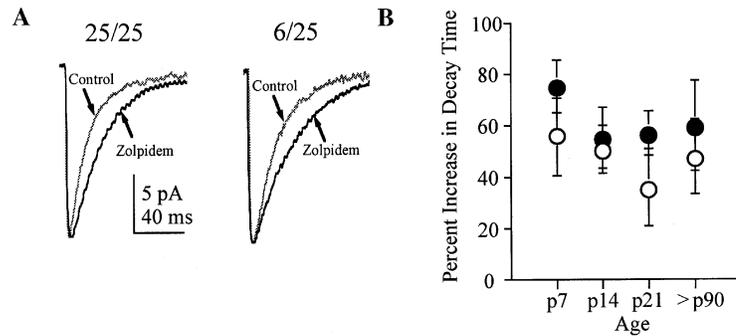


FIGURE 3 Effects of zolpidem on the decay time of GABA<sub>A</sub> receptor-mediated mIPSCs in 25/25 vs. 6/25 CA1 pyramidal cells. (A) Representative, averaged traces showing the effect of zolpidem (1  $\mu$ M) on the decay kinetics of representative 25/25 (left) and 6/25 (right) pyramidal cells. (B) Graphical representation of the effect of zolpidem on the decay time constant of mIPSCs across the four age groups. Filled circles = 25/25; open circles = 6/25. p7  $n=8$  control, 6 prenatally protein malnourished; p14  $n=4$  control, 6 prenatally protein malnourished; p21  $n=6$  control, 4 prenatally protein malnourished; > p90  $n=4$  control, 4 prenatally protein malnourished.

in cells from control animals compared to  $-31 \pm 0.4$  pA in cells from prenatal protein malnourished animals (data not shown). There was no significant difference in the magnitude of the inward current in cells from prenatally protein malnourished animals compared to controls. 5-HT also produced a rapid and significant increase in the frequency of mIPSCs (Figures 4 and 5). Electrophysiological traces showing the dramatic effect of 5-HT on the frequency of mIPSCs in representative CA1 pyramidal cells

from control and prenatally protein malnourished rats are shown in Figure 4.

In cells from control rats the mean frequency of mIPSCs increased from baseline levels of  $2.06 \pm 0.46$  Hz to  $14.7 \pm 2.9$  Hz in the presence of 5-HT ( $t=6.34$ ,  $df=28$ ,  $p<.001$ ) while in cells from prenatally protein malnourished rats the frequency increased from baseline values of  $2.38 \pm 0.5$  Hz to  $11.9 \pm 2.3$  Hz in 5-HT ( $t=6.03$ ,  $df=28$ ,  $p<.001$ ). This increase in mIPSC frequency had a duration of  $17.8 \pm 3.2$  seconds

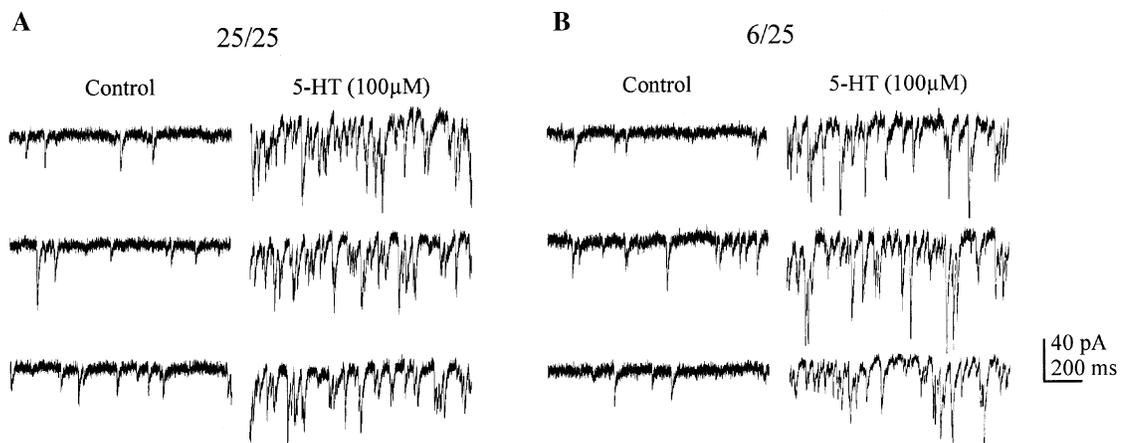


FIGURE 4 Electrophysiological traces demonstrating the effect of 5-HT on GABA<sub>A</sub> receptor-mediated mIPSCs. (A) Electrophysiological traces demonstrating the effect of 5-HT (100  $\mu$ M) on mIPSCs in a representative CA1 pyramidal cells from a control rat. (B) Electrophysiological traces demonstrating the effect of 5-HT (100  $\mu$ M) on mIPSCs in a representative CA1 pyramidal cell from a prenatally protein malnourished rat.

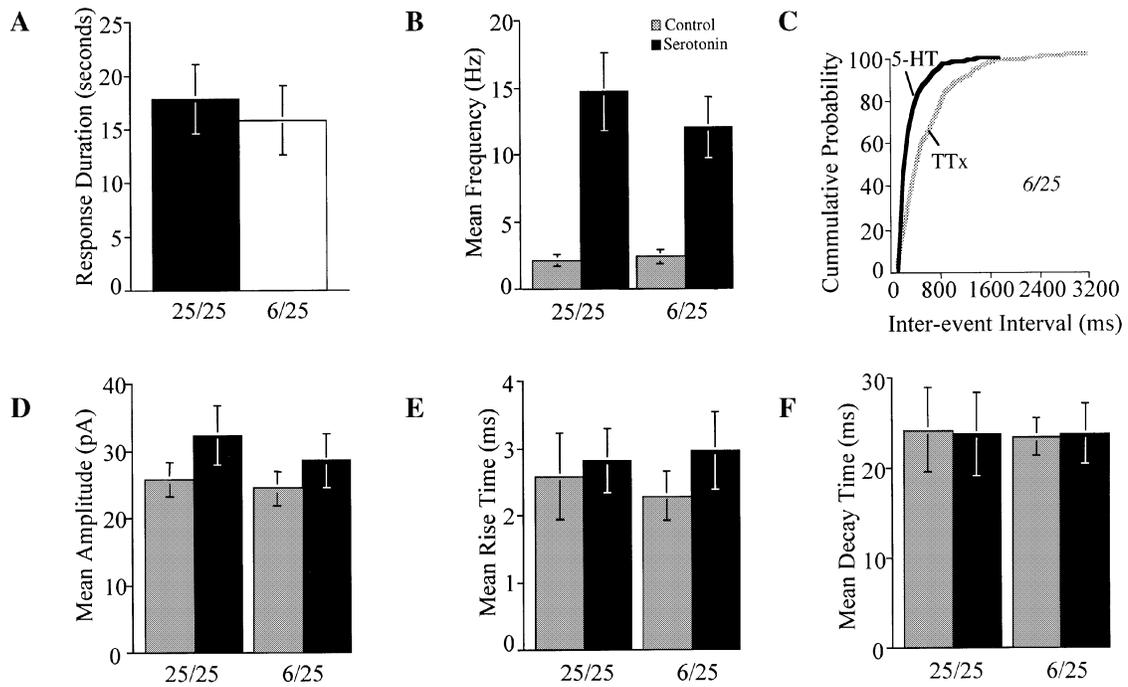


FIGURE 5 Effect of 5-HT on GABA<sub>A</sub> receptor-mediated mIPSCs in 25/25 vs. 6/25 CA1 pyramidal cells. (A) Bar graph demonstrating no difference in the duration of the 5-HT response in 25/25 vs. 6/25 cells. (B) Bar graph demonstrating no difference in the effect of 5-HT on the frequency of mIPSCs in 25/25 vs. 6/25 cells. (C) Cumulative probability histogram of inter-event intervals under control (APV/DNQX/TTx) conditions and in the presence of 5-HT in a representative 6/25 CA1 pyramidal cell. (D) Bar graph demonstrating no difference in the effect of 5-HT on the amplitude of mIPSCs in 25/25 vs. 6/25 cells. (E) Bar graph demonstrating no effect of 5-HT on the rise time of mIPSCs in either 25/25 or 6/25 cells. (F) Bar graph demonstrating no effect of 5-HT on the decay time of mIPSCs in either 25/25 or 6/25 cells.  $n = 15$  control, 15 prenatally protein malnourished.

(control) and  $15.8 \pm 3.2$  seconds (prenatal protein malnourished), and was not significantly different between the two nutritional groups. 5-HT also increased the mean amplitude of mIPSCs in cells from both nutritional groups, but this increase did not achieve statistical significance. In cells from control rats the mean amplitude of mIPSCs increased from baseline levels of  $25.7 \pm 2.5$  pA to  $32.3 \pm 4.5$  pA in the presence of 5-HT ( $t = 1.9$ ,  $df = 28$ ,  $p < .1$ ) while in cells from prenatally protein malnourished rats the amplitude increased from baseline values of  $24.5 \pm 2.3$  pA to  $28.4 \pm 4$  pA in 5-HT ( $t = 1.7$ ,  $df = 28$ ,  $p < .1$ ). The rise and decay times of mIPSCs were unaltered by 5-HT in cells from both nutritional groups. In all cells examined, the application of the 5-HT<sub>3</sub> antagonist ICS-205, 930 (2 nM) completely blocked the 5-HT induced

changes in mIPSC frequency and amplitude ( $n = 15/15$ , data not shown), indicating that the 5-HT response was mediated by activation of 5-HT<sub>3</sub> receptors. In summary, no significant differences in the modulatory effect of serotonin on mIPSC parameters were observed between cells from control vs. prenatally protein malnourished rats (Figure 5).

## DISCUSSION

The present study was motivated by previous findings indicating a significant effect of prenatal protein malnutrition on the serotonergic and GABAergic neurotransmitter systems of the hippocampus (Austin *et al.*, 1992; Bronzino *et al.*, 1991a,b; Fiacco *et al.*, 1998; Luebke *et al.*, 2000;

Mokler *et al.*, 1999; Tonkiss *et al.*, 2000a,b). The question of the possible locus and functional consequences of these alterations was directly addressed in this study by the examination of the developmental characteristics and modulation by zolpidem and serotonin of GABA<sub>A</sub> receptor-mediated mIPSCs in hippocampal CA1 pyramidal cells. Interestingly, no significant alterations in the properties of these neurotransmitter systems were observed in this cell population in response to prenatal protein malnutrition. Specifically, no significant differences between cells from control versus prenatally protein malnourished rats were observed in: (1) the frequency, amplitude or kinetics of mIPSCs across four developmental ages; (2) the modulation of mIPSC decay time by the benzodiazepine zolpidem; and (3) the modulation of mIPSC parameters by serotonin.

### Development of mIPSCs

Over the period of post-natal development from p7 to p21, a significant increase in the mean frequency of mIPSCs, a significant decrease in the mean rise and decay times and no change in the mean amplitude of mIPSCs was observed. By 21 days of age all mIPSC parameters had achieved adult (> p90 values). These data are in agreement with other reports on the development of mIPSCs in dentate granule cells (Holrigel and Soltesz, 1997) and in CA3 pyramidal cells (Takeo and Yoshioka, 2000). Previously, it was reported that CA1 pyramidal cells from adult animals exposed to prenatal malnutrition show a moderate but significant increase in the mean frequency of mIPSCs (Luebke *et al.*, 2000). The present study revealed a trend toward increased frequency of mIPSCs in cells from adult prenatally protein malnourished subjects that did not achieve statistical significance. The reasons for this discrepancy are unclear but perhaps may be explained by the fact that fewer adult cells were examined in the present study than in the previous report (Luebke *et al.*, 2000).

These data provide conclusive evidence that the fundamental characteristics of GABA<sub>A</sub> receptor mediated transmission is virtually unaffected by prenatal protein malnutrition in CA1 pyramidal cells when measured in *in vitro* slices. Thus, it can be postulated that earlier findings of altered levels of GABA<sub>A</sub> receptors and benzodiazepine receptors in this hippocampal subfield (Fiacco *et al.*, 1998) are not of a sufficient magnitude to alter the functional properties of GABAergic neurotransmission in CA1 pyramidal cells. Alternatively, some form of currently unidentified compensatory mechanism may account for the unaltered electrophysiological measures of baseline GABAergic transmission (mIPSCs) in CA1 pyramidal cells. An additional possibility is that cellular characteristics measured in the *in vitro* slice preparation do not exactly mirror events occurring *in vivo* due to deafferentation of the slice or other unavoidable perturbations inherent to the slice technique. These findings do indicate that since basic GABAergic signaling appears intact in CA1 pyramidal cells, the previously reported behavioral (Tonkiss *et al.*, 2000a,b) and neurophysiological (Bronzino *et al.*, 1991a,b; Austin *et al.*, 1992) findings of altered GABAergic systems are likely to be accounted for by fundamental alterations elsewhere in the hippocampus, such as in the dentate gyrus or the CA3 subfield. Further studies are required to determine whether GABAergic neurotransmission is altered at a single cell level in these areas as a consequence of prenatal protein malnutrition.

### Modulation of mIPSCs by Zolpidem

It has been demonstrated that prenatally protein malnourished rats have an altered sensitivity to the benzodiazepine agonist chlordiazepoxide in the Morris water maze, a spatially oriented learning task that is associated with hippocampal function (Tonkiss *et al.*, 2000a). In addition, preliminary findings have indicated altered benzodiazepine binding in the CA1 region of the hippocampus of prenatally protein malnourished

rats (Fiacco *et al.*, 1998). To test the hypothesis that these effects are associated with alterations in the modulation of GABA<sub>A</sub> receptors in CA1 pyramidal cells, the effect of zolpidem on GABA<sub>A</sub> receptor-mediated mIPSCs was examined and found to be unaltered in cells from prenatally protein malnourished rats. Again, these data indicate that the altered sensitivity to benzodiazepines in prenatally protein malnourished rats observed in behavioral studies is likely due to altered modulation of GABA<sub>A</sub> receptors in brain areas other than the CA1 subfield.

### Modulation of mIPSCs by 5-HT

As discussed above, prenatal protein malnutrition has a significant impact on the serotonergic neurons of the raphe nuclei (Diaz-Cintra *et al.*, 1981), in the related innervation of the hippocampus by serotonergic fibers (Blatt *et al.*, 1994) and in the release of serotonin in the hippocampus (Chen *et al.*, 1995; 1997; Mokler *et al.*, 1999). The application of serotonin resulted in an inward current in all cells examined that was likely due to the activation of 5-HT<sub>1A</sub> receptors as described in previous studies (Andrade and Nicoll, 1987). There was no difference in the magnitude of this effect in cells from control and prenatally protein malnourished rats. In addition to producing an inward current, 5-HT application resulted in a significant, burst-like increase in the frequency of mIPSCs measured in CA1 pyramidal cells that was not statistically different in the two nutritional groups. The 5-HT response was fully blocked by the specific 5-HT<sub>3</sub> receptor antagonist ICS-205,930 and is therefore likely to be mediated by 5-HT<sub>3</sub> receptors as has been previously reported in hippocampal principal cells (Piguet and Galvan, 1994; Ropert and Guy, 1991).

Taken together, these data indicate a normal sensitivity of CA1 pyramidal cells to 5-HT in prenatally protein malnourished rats despite the reported increase in the release of 5-HT in the hippocampus both *in vitro* (Chen *et al.*, 1995; 1997) and *in vivo* (Mokler *et al.*, 1999). As a major

function of 5-HT in the hippocampus is to modulate the release of GABA from interneurons (Piguet and Galvan, 1994; Ropert and Guy, 1991), the present study was designed to test the hypothesis that increased levels of 5-HT seen in prenatally protein malnourished rats results in a perturbation of the hippocampal GABAergic system; such as, for example, increased levels of GABA release. However, this is clearly not the case in the CA1 region as assayed by whole cell patch clamp recordings of mIPSCs in the slice, and further studies are required to determine whether a similar scenario of serotonergic hyper-innervation coincident with normal physiological responses to serotonin holds true in other regions of the hippocampus.

The results of this study, together with previous reports of unaltered membrane properties and unaltered glutamatergic synaptic response properties of CA1 pyramidal cells (Rushmore *et al.*, 1998) strongly indicate that the functional properties of principal cells of the CA1 region are not significantly impacted by prenatal protein malnutrition when these properties are measured with whole cell patch clamp techniques in *in vitro* slices. These findings are important when considering the possible cellular mechanisms that underlie observations of altered performance on hippocampally-mediated behavioral tasks and indicate that these alterations are not likely to be accounted for by significant changes in basic signaling within the CA1 subfield.

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