

Nutritional deficit and Long Term Potentiation alterations

M. Petrosino, G. Pizzuti

Dipartimento di Neuroscienze, Sezione di Fisiologia, Università di Napoli Federico II

KEY WORDS: rat, prenatal malnutrition, hippocampus, dentate gyrus, granule cell, freely moving, neuroplasticity

Abstract

In the present work we examined the ability of prenatally malnourished offspring to produce and maintain long-term potentiation (LTP) of the perforant path/dentate granule cell synapse in freely moving rats at 15, 30, and 90 days of age. Population spike amplitude (PSA) was calculated from dentate field potential recordings prior to and at 15, 30, 60 min. and 3, 5, 18 and 24 h following tetanization of the perforant pathway. All animals of both malnourished and well-nourished diet groups at 15 days of age showed potentiation of PSA measures but the measures obtained from 15-day-old prenatally malnourished animals were significantly less than that of age-matched, well-nourished controls. At 30 days of age, remarkable effect of tetanization was likely observed from PSA measures for this age group followed much the same pattern. At 90 days of age, PSA measures obtained from malnourished animals decreased from pretetanization levels immediately following tetanization. At this age, however, at three hours time recordings, this measure growing up to a level which did not differ significantly from that of the control group. These results indicate that the width of tetanization-induced enhancement of dentate granule cell response in preweaning rats (15-day-old animals) is significantly affected from gestational protein malnutrition and this trend is kept in animals tested at 30 and 90 days of age. The fact, however, that considerable limitation in LTP generation was gained from prenatally malnourished animals at 90 days of age, implying that dietary rehabilitation starting at birth is an intervention strategy not capable to improve the effects of the gestational stress.

In previous reports (Bronzino *et al.* 1994, 1997, Hernandez *et al.* 2008, Diaz-Cintra *et al.*, 1991), it was shown that prenatal protein malnutrition, followed by dietary rehabilitation starting at birth, results in abolition of both the establishment and maintenance of long-term potentiation in anesthetized rats. The present study was performed to investigate the impact of prenatal protein malnutrition on these same measures during development.

Materials and Methods

Animals: Virgin female Sprague-Dawley VAF+ rats (Charles River breeding) were provided ad libitum access to one of two diets for a period of 5 weeks prior to mating. The control diet contained 25% casein as the principal protein source, and the test diet contained 6% casein. The diets are isocaloric and supplemented with methionine since this amino acid is absent in casein. Within 24 h from the birth each pups was separated in two different litters. Groups were designated 6/25 or 25/25 to indicate the pre/postnatal dietary regimen. This paradigm provides for dietary protein restriction only during gestation with prenatally malnourished pups beginning dietary rehabilitation within 24 h of birth.

Surgery in Prewaning Animals: At 14 days of age male Sprague-Dawley rats were chronically implanted under pentobarbital anesthesia (37 mg/kg, IP) with stimulating and recording electrodes positioned in the perforant pathway (AP = -6.4; LAT +4.3) and the ipsilateral dentate granule cell layer (AP = -3.0; LAT +2.0) respectively. Stimulating electrodes consisted of a concentric bipolar electrode (barrel = 29 ga. stainless steel hypodermic tubing; tip = 0.005 formvar insulated stainless steel wire; tip separation = 0.5 mm). Recording electrodes were constructed of epoxyite insulated #00 stainless steel insect pins. Ground and indifferent electrodes consisting of 0.013" diameter stainless steel flat point wires were positioned on the surface of the contralateral parietal cortex. All connections were led to gold pins fixed to the skull with dental acrylic. Animals were individually housed and provided ad libitum access to water and a mixture of Purina Lab Chow (5001) softened

with water and were maintained on a 12-h/12-h light/dark cycle. Animals were allowed a 24-h recovery prior to recording.

Surgery in Postweaning Animals: At 27 or 90 days of age male Sprague-Dawley rats were chronically implanted under pentobarbital anesthesia (50 mg/kg, IP) with stimulating and recording electrodes positioned in the perforant pathway (30 day AP = -7.4; LAT +4.1; 90 day AP = -7.8; LAT = +4.3) and the ipsilateral dentate granule cell layer (30 day AP = -3.3; LAT = +2.3; 90 day AP = -3.8; LAT + 2.5), respectively. Final dorso-ventral positioning of both the stimulating and recording electrodes was determined by visual observation of the dentate granule cell field potential evoked by stimulation of the perforant path, and the electrodes were fixed at the positions which provided maximum population response at the lowest stimulus intensity. All connections were led to gold Reliatac pins in a block assembly fixed to the skull with dental acrylic. Animals were individually housed with ad libitum access to food (Purina Lab Chow) and water and maintained on a 12-h/12-h light/dark cycle. Weanlings began recordings at 30 days of age, and adults were given a 7-day recovery period prior to recording.

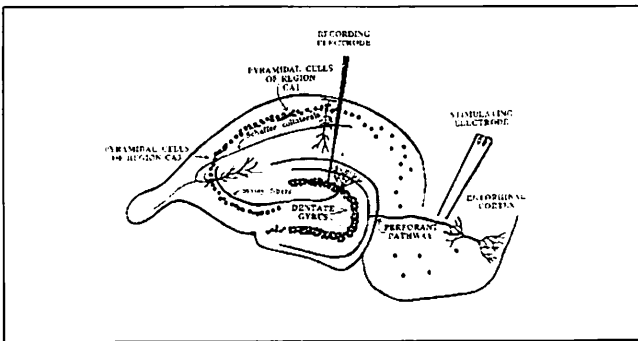


Fig. 1

Stimulation and Recording: In Fig 2. Is shown the field potential of the dentate granule cell and the most significant points in the records (A = EPSP onset, B = population spike onset, C = population spike maximum, D = population spike offset). Extracellular evoked responses were obtained from the dentate granule cell population in response to single-pulse electrical stimulation of the

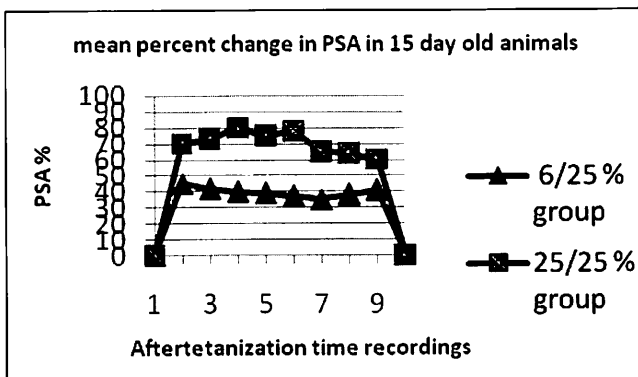


Fig. 2

perforant path. Electrical stimulation was provided by a grass-stimulator and consisted of biphasic square-wave pulses (pulse width = 0.125 ms) passed through a pair of Grass PSIU-6 photo-stimulus isolation units to provide constant current. Evoked responses were amplified (World Precision Instr. DAM-70) and bandpass filtered from 1 Hz to 3 KHz, passed to a digital storage oscilloscope (Nicolet 3 10) for visual inspection and then to the analog/digital conversion system (National Instruments, sampling rate = 20 KHz, 12 bit resolution). The software package imports the digitized waveform to the screen as a series of points, each representing .05 ms of the response. The algorithm then determines the time and DC voltage coordinates associated with the following points: EPSP onset, population spike onset, population spike minimum, and population spike end. The X- and Y-coordinate values of each point are then stored as the features set for the individual waveform. Component analysis of these field potentials provides a means of quantifying changes in cellular discharge (PSA). Measurements of PSA were then determined for individual waveforms, and mean values calculated for these measures were used to assess changes in the evoked activity resulting from tetanization of the perforant pathway.

Determination of Baseline Input/Output (I/O).

Response Measures: Prior to tetanization each animal was placed in a sound-attenuating recording chamber and attached to the recording apparatus via low-noise cabling and a counter-weighted slip-ring commutator assembly which allowed free movement of the animal about the chamber. A baseline (pretetanization) input/output response curve was constructed for each animal by recording ten responses evoked by single-pulse stimulation of the perforant path at each of nine intensities (200, 300, 400, 500, 600, 800, 1000, 1200, and 1500 μ A). These stimulations were applied at a frequency not greater than 0.1 Hz, and the intensities were varied in ascending order. Stimulations were applied during inactive (quiet) waking behaviors (defined by visual inspection as periods when the animal was sitting, immobile, and awake). The ten responses recorded at each stimulus intensity were averaged to provide the mean baseline response at each stimulus intensity prior to tetanization. The PSA measures obtained at each intensity were determined for the individual waveforms, and mean values of PSA measures were calculated for each intensity and used to construct the baseline (pretetanization) I/O response curve for each animal. Group mean (SEM) baseline I/O response curves were then constructed for each diet group at each of the three ages under investigation.

Long-Term Potentiation Paradigm: Following determination of individual baseline I/O response curves and the 75% maximum response level, tetanizing stimulations were applied to the perforant pathway.

Tetanization consisted of the application of five 500-ms-duration bursts of 400-Hz biphasic square-wave stimulation (interburst interval = 5 s) to the perforant pathway electrode. The stimulus intensity used for the tetanizing train was equal to that intensity, determined from the individual animal's I/O response curve, which resulted in evoking a PSA equal to 75% of the maximal response for that animal. Prior to tetanization, potentiation was operationally defined as enhancement of PSA measure at least 15% (i.e., above the pretetanzation baseline levels at the 5% stimulus intensity determined for each animal) occurring during the 1st h of post-tetanization recording. This 15% criterion level was established to ensure that the enhancement of PSA following tetanization was significantly above the level of variance associated with the animal's mean baseline measures, and would be above the increases in these measures attributable to normal development over the period of 15-30 days of age (Bronzino *et al.*, 1994). Animals failing to reach this criterion were designated non-potentiating and, for statistical purposes, were grouped separately for each diet group at each age. Tetanization-evoked changes in PSA measures were assessed at 15, 30, 60 min, and 3, 5, 18, and 24 h after tetanization by recording ten responses evoked by single-pulse stimulation of the perforant pathway at the baseline (pretetanzation) 75% maximal PSA intensity for each individual animal. Evaluation of tetanization-induced changes in response measures consisted of comparing the mean values of PSA obtained for the 75% maximal response prior to tetanization with those values obtained at each of the post-tetanization recordings. For each age, group mean values of PSA obtained prior to and following tetanization from the prenatally malnourished and normally nourished animals were subjected to a multivariate analysis of variance (MANOVA repeated-measures design) with diet as the between-groups factors and time after tetanization as the within-groups factor. Values of F returning $p < 0.05$ were considered significant. Following determination of the group statistics, each animal's post-tetanization data were converted to measures of percent change from that animal's baseline (pretetanzation) 75% maximal levels. This comparison provided the ability for each animal to serve as its own control. Group values of mean percent change were then calculated for each age and diet group to provide descriptive measures of the manner in which age and diet affected the response to tetanization. diet groups prior to tetanization.

Results

PSA Measures: 15-day-old animals: Twenty-three 15 day old animals were examined: $N = 10$ control (25/25), $N = 13$ malnourished (6/25) animals. All animals from both diet groups evidenced potentiation (based on the 15% criterion). Fig. 3 illustrates the mean percent change in PSA measures obtained following tetanization. All animals of the 25/25 diet group showed initial potentiation of the PSA at the 15-min post-tetanization recording period. This level of

enhancement was maintained without significant change through the first 24 h following tetanization. All animals of the 6/25 group also showed initial enhancement of the PSA at the 15-min recording period (Fig. 3). This degree of enhancement was significantly lower than that of controls ($p < 0.001$). The 6/25 animals maintained this initial level of enhancement with no significant change over the first 24 h post-tetanization, reaching levels equivalent to those of controls at 24 h post-tetanization.

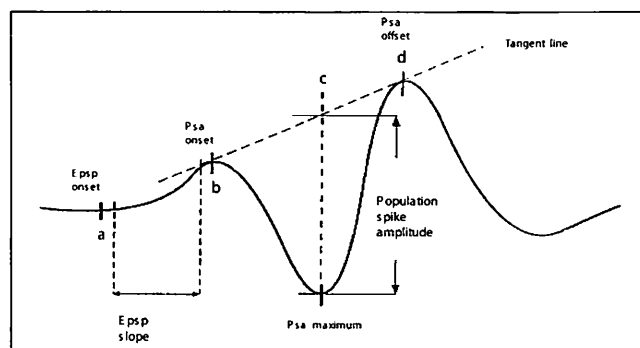


Fig. 3

30-day-old animals: A total of 22 control (25/25) and 16 prenatally malnourished (6/25) animals were examined. Sixteen of 22 animals of the 25/25 group showed initial enhancement of the PSA measure, while only six of 11 animals of the 6/25 group showed enhanced population spike measures at the 15-min post-tetanization recording period (mean = +37%). 30-day old control animals maintained this level of enhancement through the 5-h post-tetanization period, after which the measure slowly decayed to baseline by the 24-h post-tetanization recording (Fig. 4).

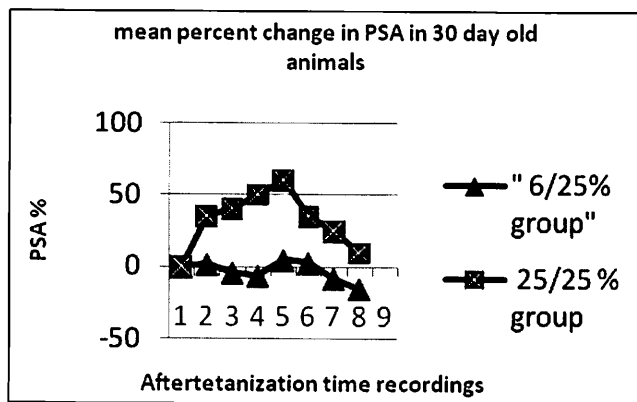


Fig. 4

Animals of the 6/25 group which showed a few initial enhancement of the PSA maintaining on the baseline for all the 24-h recording measures. However, measures of PSA obtained from malnourished 6/25 animals ranged from not differ significantly from baseline levels over the 24-h recording period.

90-day-old animals: A total of 16 control (25/25) and 12 prenatally malnourished (6/25) animals were examined.

25/25 animals showed potentiation of the PSA measure at the 15-min post-tetanzation period. These animals maintained this level of PSA enhancement until 3 h posttetanzation, after which this level began to decay, reaching baseline levels by the 24-h post-tetanzation recording (Fig. 5). No initial enhancement of the PSA was obtained from 6/25 animals at this age. However, 3 h after tetanzation, levels of enhancement obtained from these animals did not differ significantly from either potentiating 6/25 or control diet animals.

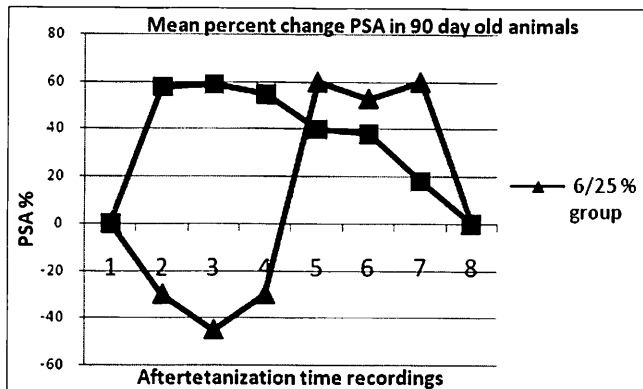


Fig. 5

Discussion and Conclusion

This study investigated the effects of a gestational insult (protein malnutrition) on the subsequent faculty of freely moving offspring to start and maintain LTP of the perforant path dentate granule cell synapse. The results indicate that the prenatal dietary insult has a significant and enduring impact on granule cell responses to tetanzation which resists to improvement by dietary rehabilitation in a significant percentage of affected animals. Of particular interest at 15 days of age is the finding of significant shortening of latency measures in response to tetanzation obtained from animals of both diet groups. Although the methods employed in the present study are incapable of determining the source of this shift, there is a question that it results directly from the tetanzating stimulus. It is, therefore, interesting to suggest that this tetanzation-induced shift in latency, which was not obtained in older animals of either diet group, may represent either the differential development of the various components of the NMDA receptor ionophore complex or tetanzation-induced changes to other membrane properties regulating response sensitivity, which do not occur in older animals. The present findings in control animals also point out that although the dentate is heavily invested with a well-developed plexus of prenatally arising GABAergic interneurons, these inhibitory cells do not appear to be functionally mature until the 3rd week of postnatal development. Thus, the population of granule cells in place in control animals at 15 days of age is under relatively less inhibitory modulation via these interneurons than that seen in adulthood. We suggest that this fact may play a role both in the greater magnitude and longer duration of potentiation obtained in these animals, as tetanzation-induced potentiation of feed-forward inhibitory modulation

of granule cell activity would be relatively non-existent in these younger animals. The fact that prenatal malnutrition has its greatest impact in these preweaning animals on the magnitude of enhancement, rather than its establishment, argues that secondary sources modulating granule cell excitability may play a more significant role in these animals. This notion is furthered by the finding of no significant between-diet group differences in I/O response characteristics, suggesting that granule cell response and nominal levels of synaptic transmission function are relatively unperturbed by the gestational insult. As further evidence of a significant role for systems modulating granule cell activity, the initial lack of PSA enhancement obtained from 30-day 6/25 animals and the significant decline in these measures obtained from 90-day-old malnourished animals suggest that changes in local GABAergic inhibitory systems modulating granule cell excitability may be involved in the diet-related alterations in LTP magnitude and duration obtained in the present study. In this regard, potentiation of GABAergic inhibition has been reported to occur as a result of tetanzation in normal animals. These findings imply that the functional relationship between excitation and inhibition within the dentate gyrus of malnourished animals can be significantly disrupted by regular perforant path granule cell activation. The fact PSA enhancement obtained from a significant proportion of malnourished animals at each of the three ages studied were well below those of controls also suggests that local circuit inhibition of dentate granule cells, via the GABAergic basket cell population, may be enhanced to a greater degree in these animals as a result of tetanzation. A relatively greater enhancement of inhibitory activity can, thus, be hypothesized as contributing to the delayed onset of enhancement seen in 90-day-old malnourished animals. On the last point for consideration is the division of both the 30- and 90-day malnourished animals into groups which show initial enhancement equal to that of controls and a second group which either shows no significant response to tetanzation (as with 30-day-old animals) or shows an initial decrement followed by delayed enhancement of response. The questions these results raise include why the effect of dietary rehabilitation are not equally expressed by all individuals. Our own work in malnutrition and that of others indicate that rehabilitation may achieve only minimal reversal or amelioration of anatomical, neurophysiological, and behavioral deficits dependent upon the timing, degree, and duration of both the insult and the rehabilitation regimen.

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