HIPPOCAMPAL SYNAPTIC PLASTICITY: EFFECTS OF NEONATAL STRESS IN FREELY MOVING ADULT MALE RATS

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INTRODUCTION

One CNS structure especially vulnerable to the effects of stress is the hippocampal formation (1-5), which has been implicated in many learning and memory processes. Since significant neuro- and synaptogenesis occurs postnatally within specific subfields of the hippocampal formation, the adaptative response to stress which occurs in this structure is of particular interest. Given the predominantly postnatal neurogenesis of the dentate granule cells, it is reasonable to hypothesize that these late-generated cells and their emerging synaptic connections would be particularly vulnerable to the effects of stress during this period of neonatal development. Stress-induced changes in the development of these cells and their connections could thus have a lasting impact on the special neuroplastic capabilities of the hippocampal formation, represented by such phenomenon as long-term potentiation, during both early development and into adults. The phenomenon of LTP, defined as the use-induced enhancement in the efficacy of synaptic transmission within activated brain pathways, has been extensively studied (6-11). A number of reports indicate that stress plays a significant role in altering LTP measures within the hippocampal formation. Most of these studies, performed in adulthood using a wide range of stressors, report an impairment of hippocampal LTP when tested immediately following stress experience (12-15). The majority of these studies, however, were carried out in either "in vitro" slice preparations or anesthetized animals. The present study was undertaken

to determine if there are any impacts on dentate neuroplasticity by repeated isolation stress obtained from freely moving adult (70-90 day old) male rats.

MATERIALS AND METHODS

Sprague-Dawley rats were mated to experienced male breeders and the resulting litters served as subjects for the present study. At birth, litters were culled to 12 pups (6 male and 6 female). The pups were weighed, marked and randomly assigned to one of two treatment categories, isolated or non-isolated with two pups of each sex assigned to each treatment category. In addition, animals from several different litters provided an unhandled group.

Pups were isolated from the nest, dam and siblings for a period of 1 h per day over post-natal (PN) days 2-9. Pups were placed in individual, empty plastic cups (9 cm diameter) in an environmentally controlled chamber mantained at 30°C. On each day of the isolation treatment the dam was separated from the pups and each pup was weighed and marked. Non-isolated siblings were returned to the nest with the mother.

At 70-90 days of age, animals were chronically implanted with stimulating and recording electrodes positioned in the perforant pathway (AP=-7.8; LAT=+4.3; DV=-2.3) and the ipsilateral dentate granule cell layer (AP=-3.8; LAT=+2.5; DV=-3.0), respectively (penthobarbital anesthesia 50 mg/Kg i.p.). Animals were allowed a 48 h recovery prior to recording.

Activation of dentate granule cells by stimulation of the medial perforant path input results in the recording of a triphasic extracellular field potential at the level of the granule cell perikaria. Four component points were identified from each recorded waveform.

Extracellular evoked responses were obtained from the dentate granule cell population in response to single-pulse electrical stimulation of the perforant path. Electrical stimulation was provided by a Grass PSIU-6 photostimulator and consisted of biphasic square-wave pulses (pulse width = 0.125 msec) passed through a pair of Grass PSIU-6 photostimulus isolation units to provide constant current. Responses were amplified

(World Precision Instruments DAM-70) and bandpass filtered from 1 Hz-3 Khz, passed to a digital storage oscilloscope (Nicolet 310) for visual inspection and then to the data acquisition system for digitization (sampling rate = 20 KHz, 12-bit resolution) and subsequent storage and analysis. Animals were attached to the recording apparatus via low-noise cabling and a counter-weighed slip-ring commutator assembly which allowed free movement of the animal about the chamber. A baseline (pre-tetanization) input/output response curve was constructed by recording 10 responses evoked by single-pulse stimulation of the perforant path at each of 9 intensities (200, 300, 400, 500, 600, 800, 1000, 1200, 1500 μA). Stimulations were applied only during quiet waking behaviors at a frequency no greater than 0.1 Hz and intensities were varied in ascending order. Ten responses at each stimulus intensity were averaged to produce a mean response for each intensity. Mean PSA amplitude and EPSP slope measures obtained at each intensity and graphed as a function of stimulus intensity. The stimulus intensity evoking a mean PSA equal to 75% of the maximal response was determined for each animal.

Following determination of the baseline input/output response curve and the 75% maximum PSA level for each animal, tetanizing (conditioning) stimulation was applied to the perforant pathway. This conditioning train consisted of the application of five 500 msec duration bursts of 400 Hz biphasic square-wave stimulation (interburst interval = 5 sec, at the 75% PSA intensity) to the perforant pathway. Change in EPSP slope and PSA measures resulting from tetanization were assessed by recording ten responses to stimulation at the 75% maximum response intensity at 15 and 30 min, 1, 3, 5, 18, 24, 48, 72 and 96 h after tetanization. Animals remained in the recording chamber continuously during the first 5 h post-tetanization, after which they were returned to their home cage. Subsequent to this 5 h period, animals were placed in the recording chamber 30 min prior to recording. Food and water were provided ad libitum between recording periods.

All values of EPSP slope and PSA were first subjected to a repeated measures MANOVA with stress treatment as the between-groups variables

and the time after tetanization as the within-groups variable. Post-hoc analyses were performed on the individual timepoints of baseline (pretetanization), 1 h (establishment of LTP) and 96 h (maintenance of LTP) post-potentiation. Additionally, data for individual animals was transformed to percent change from baseline for each time point. Mean values of percent change for each treatment group were then calculated. For all statistics, F values returning $p<0.05$ were considered significant.

RESULTS

Figure 1 shows the graphic representation of the dentate field response. Two measures of the evoked response resulting at the dentate granule cells after stimulation to the perforant pathway are EPSP and PSA. Figure 2 illustrates the comparison of population spike amplitude values across male treatment categories. The mean percent change in PSA measures indicate the significantly greater degree of enhancement obtained from neonatally isolated males. This figure also indicates that PSA measures for both the non-isolated and unhandled groups returned to baseline levels 48-72 h after tetanization, while PSA measures of the isolated group remained significantly enhanced at the 96 h post-tetanization.

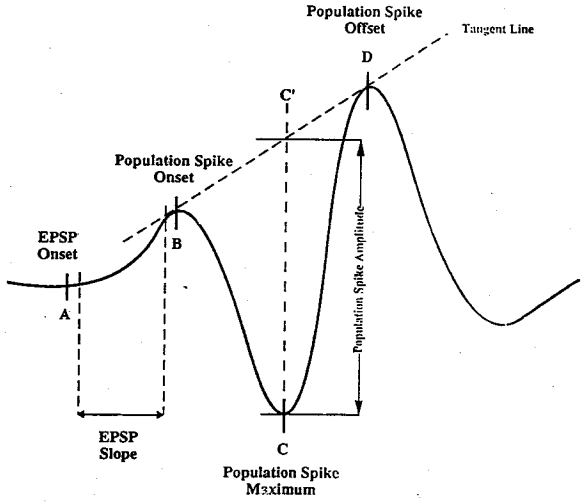

Fig. 1 - Graphic representation of the dentate field response.

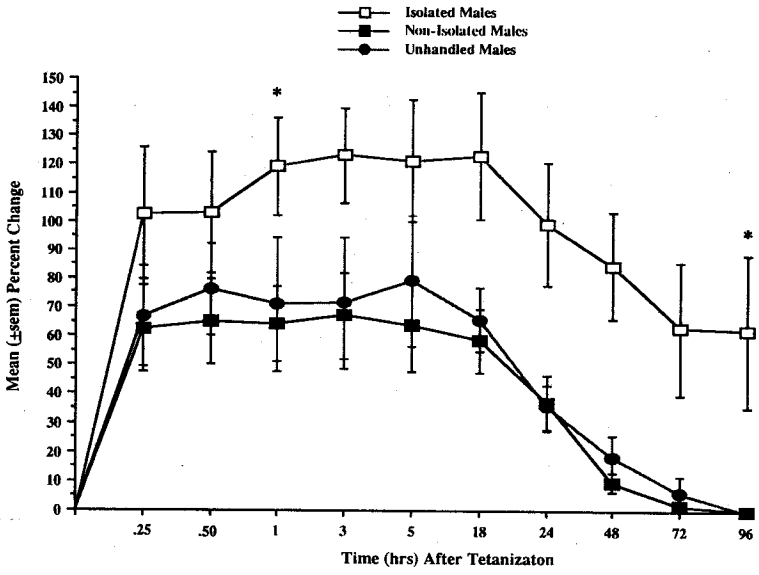

Fig. 2 - Comparison of PSA measures across three treatment categories.

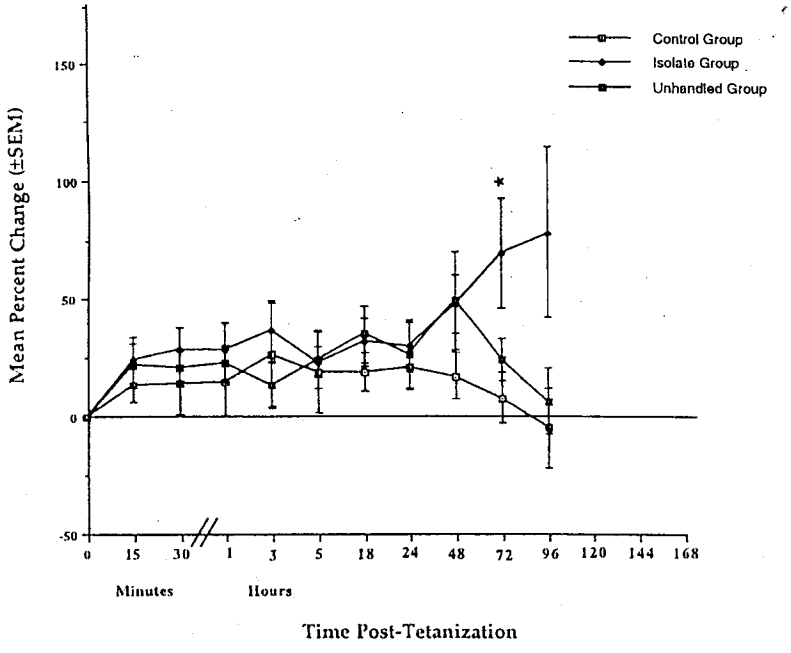

Fig. 3 - Analysis of percent change in EPSP slope measures.

Analysis of percent change in EPSP slope measures for these males revealed that although there was no effect of treatment in the initial degree of enhancement of this measure, enhancement in the neonatally isolated group was significantly greater than that of controls at the 72 h time point (fig. 3).

DISCUSSION

This study examined the impact of repeated neonatal isolation on hippocampal LTP measures obtained from freely moving adult males. Several aspects of this study make it unique from previous work investigating the effects of stress on LTP. First, this study examined the impact of a repeated stress experienced in infancy on measures of LTP obtained from freely moving rats in adult age. Previous studies of stress effects on LTP have utilized either the "in vitro" slice preparation or anesthetized animals. Second, the age of stress experience may be significant when considering its impact on hippocampal neural function. In the rat, the granule cells of the dentate gyrus arise predominantly during the first three weeks of postnatal development (7). Thus, our isolation stress is imposed during the period when significant neuro- and synaptogenesis is occurring within dentate gyrus. During such vulnerable period, stressful experiences may have a profound impact on subsequent development, resulting in lasting alterations to hippocampal circuit performance. A second developmental consideration is that although a dense plexus of GABAergic interneurons has been shown to arise in the dentate gyrus during prenatal development, there are indications that this inhibitory network, responsible for local modulation of granule cell excitability, does not become functionally mature in normal rats until 28-30 days of age (14). This may be particularly significant since neonatal isolation has been shown to alter GABA receptor binding (16).

The timetable of hippocampal development suggests a possible mechanism underlying our findings of both significantly enhanced magnitude and duration of LTP in isolated rats. The greater enhancement of LTP measures obtained from isolated rats in the present study closely parallels result

reported for normal 30 day old rats (17). Thus, the altered response to tetanization seen in our isolated rats may reflect a retarded maturation of functional relationships between the granule cell population and the GABAergic interneurons (basket cells) responsible for modulating granule cell excitability. Such retardation would result in a lower tonic inhibitory influence on the granule cell population and the interneuronal plexus also provides a possible mechanism for the enhancement of LTP obtained from neonatally isolated juveniles.

Isolation has recently been reported to enhance the turnover rate of several monoamine transmitters. Measures of the ratio of parent compound to metabolite level indicate that isolation enhances turnover rates both of norepinephrine and dopamine, suggesting enhanced release of both of these neurotransmitters (18). These changes may also play a role in the greater magnitude and longer duration of LTP seen in our isolated animals. The degree of LTP enhancement has been shown, at least in part, to be dependent on NE level (19,20); thus, results obtained from isolated animals may reflect a stress-induced elevation in norepinephrine and/or dopamine levels and the magnitude and duration of LTP is presently under investigation.

In summary, our findings of enduring alterations to hippocampal LTP obtained from adult male rats following neonatal isolation further substantiate the notion that perinatal stressors may have profound and long-term consequences. Results of the present study indicate that even a mild stressor, which results in a significant impact on neural processes underlies adaptive responsivity in later life.

The present study examines the effects of neonatal isolation on hippocampal LTP in adult male rats. Changes in dentate granule cell population measures, i.e., EPSP slope and population spike amplitude (PSA), evoked by tetanization of the medial perforant pathway were used to assess the effects of neonatal isolation on LTP over a period of 96 h. Following tetanization significant group differences were obtained for input/output (I/O) response measures of EPSP slope and PSA, with isolated males

showing consistently higher values than in the other two groups. Comparisons made at 1 h post-tetanization (establishment of LTP) indicated that isolated males showed significantly greater enhancement than any other group. At 96 h (maintenance of LTP), however, neonatally isolated males showed significantly greater enhancement than either non-isolated siblings or unhandled controls. Additionally, isolation resulted in prolonging the duration of enhancement obtained from males. Thus, males show different enhancement profiles with respect to both the magnitude and duration of LTP and neonatal isolation alters these profiles in profound manner.

1) LOMO T., Exp. Brain Res., 1967, 12, 43-46.
2) MACLENNAN A.J. et al., Science, 1983, 219, 1091-1093.
3) SHORS T.J. et al., Science, 1989, 244, 224-226.
4) SHORS T.J. et al., Brain Res. Bull., 1990, 24, 663-667.
5) SHORS T.J., THOMPSON R.F., Synapse, 1992, 11, 262-265.
6) BAUDRY M., LINCH J., Exp. Neurol., 1980, 68, 202-204.
7) BAYER S.A. et al., Science, 1982, 216, 890-892.
8) CAMP D.M., ROBINSON T.E., Behav. Brain Res., 1988, 30, 69-88.
9) KELLER R.W. et al., Brain Res., 1994, 634, 266-274.
10) SCALZO F.M., HOLSON R.R., Neurotox. Teratol., 1992, 14, 7-14.
11) SEGAL D.S., KUCZENSKY R., Brain Res., 1992, 571, 330-377.
12) COE C.L. et al., p. 127-148 in: Handbook of Squirrel Monkey Research, Plenum Press, New York, 1990.
13) MEANY M.J. et al., Behav. Neurosci., 1985, 99, 765-770.
14) MICHELSON H.B., LOTHMAN E.W., Dev. Brain Res., 1989, 47, 113-122.
15) NISENBAUM L.K. et al., J. Neurosci., 1991, 11, 1478-1484.
16) INSEL T.R., Psychopharmacol., 1989, 97, 142-144.
17) BRONZINO J.D. et al., Brain Res. Bull., 1996, 3, 175-183.
18) HARVEY A.T. et al., Behav. Neurosci., 1994, 108, 171-176.
19) DAHL D., SARVEY J.M., Proc. Nat. Acad. Sci., 1989, 86, 4776-4780.
20) STANTON M.E. et al., Behav. Neurosci., 1988, 102, 692-700.

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