Prenatal stress alters noradrenergic modulation of LTP in hippocampal slices

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Running Head: Prenatal stress affects NE modulation of hippocampal LTP

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ABSTRACT

Long term effects of stress during pregnancy on brain and behavior have been analyzed extensively in recent years. These effects include changes in emotional behavior, a reduction in learning capacity and ability to generate long-term potentiation (LTP) in the offspring. In earlier studies we and others have described a difference in ability to express LTP in dorsal and ventral sectors of the hippocampus (DH and VH, respectively), and its modification by prior stress. We now found that norepinephrine (NE) facilitated conversion of short term to LTP in the normal DH, but not in VH. Prenatal stress (PS) switched the locus of the facilitating action of NE from the DH to the VH. The effects of NE are likely to be mediated by activation of calcium stores. PS also facilitated DHPG-induced LTD in the VH, assumed to be mediated by release of calcium from stores. These observations have important implications for the role of the hippocampus in cognitive and emotional memories.

Key words: prenatal stress, Norepinephrine, ventral hippocampus, LTP,
Evidence for long term effects of prenatal environment on postnatal brain functions has been accumulated in recent years (Laloux et al. 2012; Mychasiuk et al. 2011; Yaka et al. 2007; Yeh et al. 2012). Exposure of pregnant dams to stressful stimulation can cause changes in neurotransmitter metabolism, brain morphology and ability to cope with cognitive and emotional tasks in the juvenile and even in adult life (Maccari et al. 2003,). It was even suggested that prenatal stress (PS) can facilitate emergence of neuropsychiatric conditions such as depression and schizophrenia (Koenig et al. 2005; Markham and Koenig 2011; Seckl and Meaney 2006; Wilson et al. 2012). The molecular mechanisms underlying these long term changes in neuronal functions are beginning to be unraveled, and studies using gene arrays report on gene families that are altered by PS (Afadiel et al. 2010; Biala et al. 2011; Bogoch et al. 2007, Weinstock 2008). Two major chemical families associated with stress are steroid hormones and the neuromodulators, including norepinephrine (NE), serotonin and acetylcholine. There is a growing understanding of the role of steroid hormones in regulation of synaptic plasticity in the adult brain (Maggio and Segal 2007;), and there is evidence that PS causes a rise of corticosterone in the mother (Abdul-Aziz et al. 2012; Yaka et al. 2007) which affects steroid receptors in the pups (Tamura et al. 2011). However, the steroid receptors are developing late in embryonic age, and may not play a major role in PS (Diaz et al. 1998). On the other hand, NE is known to affect plasticity (Bramham et al. 1997; Gray and Johnston, 1987; Hu et al. 2007, Schimanski et al. 2007, Lim et al. 2010 Stanton and Sarvey 1985), and it is assumed to serve an important role in cognitive processes (Sara, 2009). However, there is a paucity of information on the effects of stress on NE modulation of synaptic plasticity. We hypothesized that since NE-containing neurons are formed early in embryonic age, and their development can be derailed by prenatal exposure to drugs (Dey et al. 2007) they may also be affected by PS. In the present study we compared the effects of NE, acting at beta-1 adrenergic receptors, on LTP in acute slices of the
dorsal and ventral hippocampus (DH and VH, respectively), shown to be
differentially affected by stress (Maggio and Segal 2007) in juvenile rats that
underwent PS. Our results indicate that PS caused a shift of the locus of NE
involvement in plasticity from the DH to the VH.

METHODS

Animals: Animal handling was done in accordance with the guidelines of the
Institutional Animal Care and Use Committee of the Weizmann Institute and with
the Israeli national laws on animal care. PS was induced by exposing pregnant
Wistar dams of a local breeding colony (at gestation ages of 14-21) to three
stressful experiences including forced swim in a water bucket (for 20 minutes at
room temperature), placement on an elevated platform (for 30 minutes) and
restraining tube (for 40 minutes), once a day, repeated twice during 6 days of
pregnancy. This protocol was adapted from Weinstock and colleagues who found a
10-fold increase of maternal plasma corticosterone level after forced swim or
restraint (Yaka et al. 2007). Control pregnant dams were left undisturbed.
To find out whether PS affects the offspring via stressed mother during pregnancy
or its effects appear after birth due to changed maternal care, newborn pups were
switched in one experiment between mothers, i.e. prenatally stressed pups were
raised by control foster mother and vice versa. The results of this experiment are
presented separately.

Electrophysiology: Transverse hippocampal slices (400 µm) were prepared from
the hippocampi of 2-3-week-old male rats using a McIlwain tissue chopper. Slices
were incubated for 1.5 h in carbogenated (5% CO₂ and 95% O₂) ACSF at room
temperature. The medium contained (in mM) 124 NaCl, 2 KCl, 26 NaHCO₃, 1.24
KH₂PO₄, 2.5 CaCl₂, 2 MgSO₄ and 10 glucose, at pH=7.4. Recording was made
from slices taken from the dorsal and ventral regions of the hippocampus, as
described elsewhere (Grigoryan et al. 2012). Slices were slightly submerged in a standard chamber at 33.8–34.0 °C with a flow rate of ACSF of 2.5 ml/min. Extracellular recordings of population EPSPs in the stratum radiatum of CA1 region were made through a glass pipette containing 0.75 M NaCl (4 MΩ). Responses were evoked by stimulation of the Schaffer collaterals using bipolar electrodes positioned equidistant on both sides of the recording electrode, in the stratum radiatum of CA1 region such that two independent stimulation pathways were used for each slice (Figure 1A). In previous studies we confirm that sequential tetanic stimulation produces similar LTP in the two pathways. Tetanic stimulation was delivered at a frequency of 100 Hz using a designated number of stimuli. Before applying the tetanic stimulation, evoked EPSPs (50% of maximum amplitude) were recorded for a stable baseline period of at least 10 min. In order to further investigate effects of the PS in DH and VH slices the paired-pulse stimulation protocol was used. Two consecutive stimuli of equal intensity were delivered at varying interpulse intervals (IPIs, ranging from 10 to 100 ms) to stratum radiatum.

The following drugs were used: isoproterenol, a non-selective beta-adrenergic agonist, (Iso, 1 µM; Sigma-Aldrich), atenolol, a selective beta 1 adrenergic receptor antagonist, (2µM; Sigma-Aldrich), ICI-118,551, a selective beta 2 adrenergic receptor antagonist, (0.1 µM; Sigma-Aldrich), forskolin, used to raise levels of cAMP, (0.1 µM, Calbiochem), cyclopiazonic acid, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase reversible antagonist, (CPA, 5 µM; Alomone Labs, Israel), (S)-3,5-Dihydroxyphenylglycine hydrate, a group I metabotropic glutamate receptor agonist, (DHPG, 25 µM, Sigma-Aldrich). Stock solutions were diluted in recording medium immediately before use. Data acquisition and off-line analysis were performed using pCLAMP 9.2 (Axon Instruments). All numerical data are expressed as mean ± SEM. Statistical comparisons were performed by one-way ANOVA test followed by paired
comparisons. P values of <0.05 were considered a significant difference between means.

RESULTS

Isoproterenol effects on short term potentiation in DH and VH
Dependence of EPSP slope on stimulation intensity in the DH and VH was assessed from input/output curves. The DH (n=6) and VH (n=6) slices obtained from control and PS rats exhibited similar input/output relations in stratum radiatum of the CA1 region (Figure 1B). This result suggests that basal synaptic transmission in both DH and VH is not affected by PS. PS did not alter paired-pulse facilitation of population EPSPs in either DH or VH. These results are congruent with previous observations (Yaka et al. 2007; Yeh et al. 2012 (but see Grigoryan and Segal, 2013, where an effect on population spike recorded in stratum pyramidale is found)). Interestingly, Isoproterenol (Iso, 1µM) had no effect on either paired-pulse facilitation or LTP evoked by a full length tetanic stimulation (1 sec, 100Hz) which by itself generated LTP (1.42 ± 0.004 in DH (n=5) compared with 1.41 ± 0.003 in VH (n=5)) (Figure 1C-F).

Short tetanic stimulation, subthreshold for LTP induction (35 pulses, 100 Hz), evoked only a transient enhancement of reactivity to the afferent stimulation (short term potentiation, STP), which was similar in both regions, and returned to baseline within 10 minutes after the tetanic stimulation. Iso did not have any effect on baseline reactivity to the stimulation, but in its presence, the same short tetanic stimulation now converted the STP into a full length LTP in DH slices (1.57 ± 0.01, n=4). Under the same conditions, Iso had no effect in VH slices (n=4) (Figure 2, A-B).

Prenatal stress switches the locus of effect of Iso from DH to VH
In a similar cohort of slices taken from PS rats, there was no difference between DH (n=6) and VH (n=6) in the input/output relations in response to the afferent stimulation (Figure 1B). Surprisingly, in DH slices of PS rats, exposure to Iso did not convert STP to LTP. In sharp contrast, Iso was now able to convert STP to LTP in VH slices (1.41 ± 0.01, Figure 2, C-D).

To test whether PS affects the offspring because of their being handled by a stressed mother after delivery, we repeated some of these experiments with PS rats that were raised by an unstressed foster mother, and vice versa, normal pups raised by stressed mothers. As seen before, Iso did not have any effect on baseline reactivity to the stimulation either in DH or VH slices of both control and PS groups. However, short tetanic stimulation in presence of Iso led to an induction of LTP in DH slices of control (1.58 ± 0.01, n=4) and VH slices of PS rats (1.36 ± 0.01, n=5), but had no effect in either DH of PS (1.04 ± 0.01, n=4) or VH of control rats (1.02 ± 0.02, n=4) slices. These results indicate that PS effects on plasticity are not due to the postnatal handling by a stressed mother.

Pharmacological analysis of Iso action in the hippocampus

To examine the beta adrenergic nature of the action of Iso in the hippocampus in relations to LTP, we used the selective beta 1 adrenergic receptor antagonist atenolol and the beta 2 antagonist ICI-118,551. In either the DH slices (n=5) of control rats, or the VH slices of the PS rats (n=6), atenolol (2 µM; Figure 3, A-B), blocked the effects of Iso, without affecting baseline response properties, indicating that the effects of low concentration of Iso are mediated by activation of a beta-1 adrenergic receptor, as suggested before (Schimanski et al. 2007).

To examine the possibility that a beta 2 adrenergic receptor is involved in the action of Iso, the selective antagonist ICI-118,551(at 0.1 µM), was used. ICI-118,551 did not affect Iso-induced conversion of STP into LTP in control DH (1.49 ± 0.01, n=6) and VH slices of PS rats (1.47 ± 0.004, n=5) (Figure 3, C-D).
Beta adrenergic responses are proposed to be mediated by activation of cAMP cascade, and so we tested the effects of forskolin (at 0.1 µM, a dose that does not affect baseline EPSPs), an activator of the cAMP system, on the ability to convert STP to LTP. Indeed, forskolin at low dose mimicked the effects of Iso, in both the control and PS rats, in the same regions where Iso was effective (1.48 ± 0.01, n=5, in DH slices of control and 1.43 ± 0.004, n=6, in VH slices of PS rats) (Figure 4, A-B).

Finally, we have previously demonstrated that there is a marked difference between DH and VH, in the involvement of calcium stores in the ability to generate LTP (Grigoryan et al. 2012). To test if the effects of Iso are mediated by release of calcium from stores, we exposed the slices to cyclopiazonic acid (CPA), a blocker of release of calcium from stores. Indeed, CPA (5 µM) blocked the facilitating effects of Iso in normal DH (1.05 ± 0.004, n=7) and in VH slices of PS rats (1.04 ± 0.01, n=6, Figure 4, C-D).

Prenatal stress enhances LTD in VH

In a further set of experiments we examined whether PS affects long-term depression (LTD) in DH and VH slices. To this end, we evoked chemical LTD using DHPG, an agonist of group-1 mGlu receptors, which is likely to act by releasing of calcium from stores. At concentration of 25 µM exposure to DHPG led to depression of recorded EPSPs that persisted after 40 minutes of washout of the drug in DH but not in VH slices of control rats (0.82 ± 0.004 (n=5) and 0.96 ±0.01 (n=4), respectively). Strikingly, VH slices from PS rats expressed significantly enhanced LTD (0.74 ± 0.01 (n=5), p<0.001, F= 56.74, compared with control), while DHPG-dependent LTD in DH slices was smaller than that of controls (0.96 ± 0.01 (n=5), p<0.05, F= 16.5). These results indicate that PS switches the locus of action of DHPG from DH to VH, in line with the proposal that PS makes the VH
more metaplastic than the DH, and this is likely to be mediated by release of calcium from stores.

Discussion

The present experiments demonstrate that selective activation of beta-1 adrenoreceptors, known to play an important role in regulating synaptic plasticity in the hippocampus, facilitated conversion of STP to LTP in CA1 region of the DH, but not the VH of normal young rats. On the other hand, the effects of isoproterenol (Iso) were no longer seen in CA1 region of DH slices in PS rats, but were found in VH. These effects were mediated by activation of a beta 1 adrenergic receptor and cAMP generating system, and involved release of intracellular calcium from stores.

The effects of PS on synaptic properties in the hippocampus have been studied by several groups. It has been shown that PS reduces synaptic currents (Saboory et al, 2011) in the infant rats. On the other hand, Yaka et al (2007) did not report on a decrease in basal EPSP size, but on a reduction in ability to express LTP in the offspring of the stressed mothers. That there might be alterations in synaptic properties is indicated by the study of Afadlal et al (2010). In our own study we did not detect changes in basal synaptic activity, but in a specific region-selective ability of norepinephrine to facilitate formation of LTP. As noted before, different magnitudes of PS may contribute to different magnitudes of defects in synaptic properties of the affected individuals.

The present experiments confirm and extend previous studies on the facilitating role of NE in hippocampal synaptic plasticity (Izumi and Zorumski 1999; Katsuki et al. 1997), with several noted additions; the effect shown before (Izumi and Zorumski 1999) was found only in DH slices, and we found that it was absent in VH ones. On the other hand, following PS, while the facilitating action of NE in DH was suppressed, as could have been predicted from other studies showing suppression
of LTP following PS, NE became effective in the VH, and appeared to use the same molecular pathways as in the normal DH LTP.

The involvement of the different subtypes of the beta adrenergic receptors in synaptic plasticity is still not clear. While beta 2 receptors have been found in the hippocampus, Guo and Li (2007) have shown that beta 1 subtype is predominantly distributed in the neuronal membrane in CA1/3 regions of the hippocampus, indicating the likelihood that they mediate the effects observed herein. With respect to their role in synaptic plasticity, beta 2 receptors have been shown to mediate noradrenergic enhancement of spontaneous activity in CA1 region of rat hippocampal slices (Hillman et al. 2005) and to mediate noradrenergic facilitation of LTP (Qian et al. 2012). In contrast, Zhang et al. have recently (2013) proposed that the beta 1 receptor is activated to reduce slow after-hyperpolarization (AHP), enhance excitability and facilitate long term memory. Our results conform to the second option, although we do not exclude the involvement of beta 2 receptors in plastic processes, perhaps on different time scales.

The mechanism(s) underlying the switch from DH to VH modulation of LTP are not entirely clear. In previous studies (Maggio and Segal 2007; Segal et al. 2011) we hypothesized that the effects of stress are related to differential distribution of the steroid glucocorticosterone (GR) and mineralocorticosterone (MR) receptors, but the development of these receptors are primarily postnatal (Diaz et al. 1998), and thus are not likely to underlie the currently observed effects of PS. On the other hand, Tamura et al. (2011) reported that PS reduces expression of MRs in the hippocampus, resulting in delayed maturation of dendrites and spines in the dentate gyrus. In our earlier work activation of MRs is proposed to lead to enhancement of LTP (Maggio and Segal 2007), and so the possible involvement of MRs and GRs in the effects of PS cannot be ruled out. At any rate, a DH/VH differential distribution has not been proposed for NE terminals. In fact, while there is a long array of molecular changes in the brains of prenatally stressed rats, there
is no indication that NE, which is known to be activated by stress and be affected
by prenatal exposure to drugs (Dey et al. 2007), will have a differential and
opposite roles in the two sectors of the hippocampus.
Among several molecular cascades that may be affected by PS is the brain derived
neurotrophic factor (BDNF). It has been suggested that the conversion from pro-
BDNF to mature BDNF is impaired following PS (Yeh et al. 2012) and that BDNF
signaling is reduced in such animals (Neeley et al. 2011), and this may lead to
several impairments, downstream to BDNF. One of them involves maturation of
GABAergic neurons. It was reported that the expression of GABAergic neurons is
reduced in the hippocampus of PS rats (Vaid et al. 1997), and this reduction could
be found also in dissociated hippocampus cultures taken from PS rats (Grigoryan
and Segal 2013). This reduction in inhibitory tone was expressed as a reduction in
paired pulse depression of population spikes (Grigoryan and Segal 2013), which
could be dissociated from a lack of a similar change in EPSP slope, an indicator of
excitatory synaptic function. Thus, a reduction in inhibitory tone may underlie the
enhanced efficacy of NE in the VH following PS. These and other mechanisms
need further explorations.
Another promising molecular species that may be affected by PS involves the
release of calcium from intracellular stores. We (Grigoryan et al. 2012) have shown
a striking difference between DH and VH in ability of calcium released from stores
to enhance LTP. Both the effects of activation of metabotropic glutamate receptors
and Iso share a common molecular path involving release of calcium from stores.
The functional implications of PS have been studied behaviorally by several
groups. The DH is associated with cognitive functions of the hippocampus, and the
connections between the DH and the cortex underlie these functions. In normal
alerted animal, NE release may facilitate transfer of information between DH and
the entorhinal/ cingulate/prefrontal cortex. In contrast, following stress,
hippocampal control switches to VH, and its connections with the amygdala and
the hypothalamus, and NE can then facilitate the activation of these pathways (Lim et al. 2010). The behavioral consequences of this switch may be exhibited by hyperactivity and hypersensitivity of the animal to otherwise neutral stimulation, which may not have adaptive value in the normal organism. In general, PS is reported to cause impairment in cognitive functions (Son et al. 2006; Wilson et al. 2012; Yaka et al. 2007). On the other hand, Fujioka et al. (2001) suggested that mild PS can in fact facilitate learning performance, which is accompanied by reduced emotionality. A similar observation was made by us recently (Grigoryan and Segal 2013), demonstrating that PS rats are more motile in an open field and acquire faster the water maze task than controls. This indicates that the magnitude of the PS, as well as the type of stimulation used and the behavioral tests employed are important factors in determining the possible long term effects of PS on brain physiology and behavior. Another important factor is the gender of the animals studied, as there are major sex-dependent effects of PS on behavior (Abdul Aziz et al. 2012, Bock et al. 2011; Bowman et al. 2004; Paris and Frye 2011; Yaka et al. 2007). There is a need to study the possible DH/VH switch also in female, which we did not do in the current study, for lack of resources. Further experiments are needed to explore specific behavioral functions of the DH and VH and the consequences of the apparent switch between DH and VH-associated functions, and its sensitivity to pharmaceutical intervention.

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**FIGURE CAPTIONS**

**Figure 1.** A: Schematic presentation of positions of recording and stimulating electrodes. Two bipolar electrodes were placed equidistantly on both sides of the recording electrode. B: Input-output curves of EPSP slopes for DH and VH slices of control and prenatally stressed (PS) rats showing no significant difference between groups. C-D: Effect of 1 µM isoproterenol (Iso) on paired-pulse facilitation (PPF) of the EPSP slopes expressed as response to the second stimulation over the first one at different interpulse intervals (IPIs) (10, 20, 40, 60, 80, and 100 ms) in DH (C) and VH (D) slices. No difference in PPF was found in the two regions with the exposure to 1 µM Iso. E-F: Iso had no effect on LTP in DH and VH slices of control rats. LTP was induced by tetanic stimulation, (100 Hz, 1s) at twice the test intensity. The arrows denote the points at which tetanic stimulation was delivered (first to the pathway 1 in the absence ofIso, second to pathway 2 in the presence of Iso, superfused during the bar shown above the record).

**Figure 2.** Effect of 1 µM Iso on EPSPs recorded in stratum radiatum of DH and VH slices from control (A-B) and PS (C-D) rats. The arrows denote the points at which short tetanic stimulation (35 stimuli@100 Hz) was delivered, twice to one pathway. Short tetanic stimulation, given in the presence of Iso (1 µM), produced a full blown LTP in DH slices of control group (A) and in VH slices of PS rats (D).

**Figure 3.** Effect of atenolol, a selective beta 1 adrenergic receptor antagonist, and ICI-118,551, a selective beta 2 adrenergic receptor antagonist, on Iso-mediated conversion of STP to LTP in DH and VH slices of control and PS rats. The effect of Iso was blocked either in the DH slices of control group (A), or in the VH slices of the PS pups (B), by the beta 1 adrenergic antagonist, atenolol (2 µM), but was not
affected by ICI-118,551 (0.1 µM, C-D), indicating that the effects of low
concentration of Iso are mediated by activation of a beta 1 adrenergic receptors.

Figure 4. A-B: Effect of forskolin (0.1 µM) on short-term potentiation (STP) in DH
and VH slices of PS rats. STP was converted into LTP by application of forskolin in
VH slices only. Thus, the effect of Iso was mimicked by forskolin indicating the
involvement of cAMP generating system. C-D. Cyclopiazonic acid (CPA, 5 µM)
blocked the isoproterenol-mediated conversion of STP into LTP in DH of control
and VH of PS slices, demonstrating the involvement of calcium stores in the Iso-
mediated modulation of LTP generation. The arrows denote the points at which
tetanic stimulation (35 pulses@100 Hz) was delivered (twice to one pathway).

Figure 5. Long-term depression (LTD) in DH (A) and VH (B) slices of control and
PS rats. DHPG, an agonist of mGlu group 1 receptors, at concentration of 25 µM
evoked persistent chemical LTD in DH but not in VH slices of control rats. PS
switches the locus of action of DHPG from DH to VH: VH slices from PS rats
expressed significantly enhanced LTD compared to control, while DHPG-
dependent LTD in DH slices of PS rats was lower than controls.