

**Gowri K. Pyapali, Dennis A. Turner, Christina L. Williams, Warren H. Meck and H. Scott Swartzwelder**  
*J Neurophysiol* 79:1790-1796, 1998.

**You might find this additional information useful...**

---

This article cites 46 articles, 9 of which you can access free at:

<http://jn.physiology.org/cgi/content/full/79/4/1790#BIBL>

This article has been cited by 17 other HighWire hosted articles, the first 5 are:

**Epigenetic mechanisms for nutrition determinants of later health outcomes**

S. H Zeisel

*Am. J. Clinical Nutrition*, May 1, 2009; 89 (5): 1488S-1493S.

[Abstract] [Full Text] [PDF]

**Gestational Choline Supply Regulates Methylation of Histone H3, Expression of Histone Methyltransferases G9a (Kmt1c) and Suv39h1 (Kmt1a), and DNA Methylation of Their Genes in Rat Fetal Liver and Brain**

J. M. Davison, T. J. Mellott, V. P. Kovacheva and J. K. Blusztajn

*J. Biol. Chem.*, January 23, 2009; 284 (4): 1982-1989.

[Abstract] [Full Text] [PDF]

**Prenatal choline availability alters the context sensitivity of Pavlovian conditioning in adult rats**

J. A. Lamoureux, W. H. Meck and C. L. Williams

*Learn. Mem.*, December 2, 2008; 15 (12): 866-875.

[Abstract] [Full Text] [PDF]

**Prenatal choline supplementation alters the timing, emotion, and memory performance (TEMP) of adult male and female rats as indexed by differential reinforcement of low-rate schedule behavior**

R.-K. Cheng, C. J. MacDonald, C. L. Williams and W. H. Meck

*Learn. Mem.*, March 5, 2008; 15 (3): 153-162.

[Abstract] [Full Text] [PDF]

**Phosphatidylethanolamine N-methyltransferase (PEMT) gene expression is induced by estrogen in human and mouse primary hepatocytes**

M. Resseguie, J. Song, M. D. Niculescu, K.-A. da Costa, T. A. Randall and S. H. Zeisel

*FASEB J*, August 1, 2007; 21 (10): 2622-2632.

[Abstract] [Full Text] [PDF]

Medline items on this article's topics can be found at <http://highwire.stanford.edu/lists/artbytopic.dtl> on the following topics:

Veterinary Science .. Synaptic Plasticity

Psychology .. Spatial Memory

Veterinary Science .. Hippocampus

Physiology .. Long-Term Potentiation

Medicine .. Diet

Physiology .. Rats

Updated information and services including high-resolution figures, can be found at:

<http://jn.physiology.org/cgi/content/full/79/4/1790>

Additional material and information about *Journal of Neurophysiology* can be found at:

<http://www.the-aps.org/publications/jn>

---

This information is current as of December 27, 2009 .

# Prenatal Dietary Choline Supplementation Decreases the Threshold for Induction of Long-Term Potentiation in Young Adult Rats

GOWRI K. PYAPALI,<sup>1,5</sup> DENNIS A. TURNER,<sup>1,2,5</sup> CHRISTINA L. WILLIAMS,<sup>3</sup> WARREN H. MECK,<sup>3</sup> AND H. SCOTT SWARTZWELDER<sup>3-5</sup>

<sup>1</sup>Department of Neurosurgery, <sup>2</sup>Department of Neurobiology, <sup>3</sup>Department of Experimental Psychology, and <sup>4</sup>Department of Psychiatry, Duke University, Durham 27705; and <sup>5</sup>Durham Veterans Affairs Medical Center, Durham, North Carolina 27710

**Pyapali, Gowri K., Dennis A. Turner, Christina L. Williams, Warren H. Meck, and H. Scott Swartzwelder.** Prenatal dietary choline supplementation decreases the threshold for induction of long-term potentiation in young adult rats. *J. Neurophysiol.* 79: 1790–1796, 1998. Choline supplementation during gestation in rats leads to augmentation of spatial memory in adulthood. We hypothesized that prenatal (E12–E17) choline supplementation in the rat would lead to an enhancement of hippocampal synaptic plasticity as assessed by long-term potentiation (LTP) at 3–4 mo of age. LTP was assessed blindly in area CA1 of hippocampal slices with first suprathreshold (above threshold for LTP generation in control slices) theta-burst stimulus trains. The magnitude of potentiation after these stimuli was not different between slices from control and prenatally choline supplemented animals. Next, threshold (reliably leading to LTP generation in control slices) or subthreshold theta-burst stimulus trains were applied to slices from control, prenatally choline-supplemented, and prenatally choline-deprived rats. Threshold level stimulus trains induced LTP in slices from both the control and choline-supplemented rats but not in those from the choline-deficient rats. Subthreshold stimulus trains led to LTP induction in slices from prenatally choline-supplemented rats only. These observations indicate that prenatal dietary manipulation of the amino acid, choline, leads to subsequent significant alterations of LTP induction threshold in adult animals.

## INTRODUCTION

Dietary choline is crucial for normal growth and functioning of all mammalian cells (Blusztajn 1995; Blusztajn and Wurtman 1983; Garner et al. 1995; Zeisel and Blusztajn 1994; Zeisel et al. 1991). Choline and its metabolites are important for the structural integrity of cell membranes, cholinergic transmission, and transmembrane signaling during neurogenesis and synaptogenesis, the effects of which may be expressed dramatically later in life (Chung et al. 1995; Durand et al. 1996; Gorry et al. 1992; Holler et al. 1996; Zeisel and Blusztajn 1994). During development there is active formation of neuronal membrane components as neurons divide, grow axons and dendrites, and form synapses. Thus the developing brain may have a high demand for choline, as it functions both as a precursor for major phospholipid components of cellular membranes and as a precursor of the neurotransmitter acetylcholine (ACh).

Availability of exogenous choline during development stimulates ACh synthesis and promotes memory function in the adult (Auerbach and Segal 1994; Barry and Gelperin 1982; Blitzer et al. 1990; Blusztajn et al. 1987; Chung et al.

1995; Drachman and Leavitt 1974; Jackson et al. 1992; Jones et al. 1995; Meck and Church 1987; Pyapali et al. 1997; Sahley et al. 1986; Ulus et al. 1989). More recently Meck et al. (1988, 1989) and Loy et al. (1991) have shown that perinatal choline supplementation has long-term effects on working and reference memory components of performance in the radial-arm maze task in adulthood. Recent studies also indicate that this treatment elevates muscarinic receptor density and choline acetyltransferase levels in the hippocampus, and leads to an increase in the soma size of mainly cholinergic neurons in the diagonal band and medial septum that are also immunoreactive to nerve growth factor (NGF). In addition, perinatal choline supplementation increases hippocampal phospholipase D activity (PLD) activity in the offspring (Holler et al. 1996).

The physiological mechanisms whereby choline administration during gestation improves memory in the offspring is not known. However, a modulation of hippocampal function is one likely mechanism because memory performance is strongly linked to hippocampal function, and perinatal choline supplementation augments cholinergic and PLD activity in the hippocampus. Hippocampal long-term potentiation (LTP) is a manifestation of synaptic plasticity, which is a possible neural substrate for learning and memory (Artola and Singer 1987; Bliss and Collingridge 1993; Deupree et al. 1991, 1993; Drachman and Leavitt 1974; Eichenbaum 1995). We hypothesized that the induction of LTP in hippocampal slices taken from adult rats that had received prenatal choline supplementation would be enhanced and conversely that LTP induction would be diminished in adult rats that had experienced prenatal choline deficiency.

In previous studies we have shown that stimulus trains that were suprathreshold (above the threshold for induction of LTP in control slices) did not lead to LTP induction in slices from animals treated with a choline-deficient diet during the perinatal period (Jones et al. 1995, 1996). Therefore we designed the present experiments to assess the induction of LTP by threshold and subthreshold stimulus trains in hippocampal slices from animals prenatally exposed to control, choline-supplemented, and choline-deficient diets. The induction of LTP by suprathreshold stimulus trains was assessed in slices from animals prenatally treated with control or choline-supplemented diets only to complement the previous data on suprathreshold stimuli (Jones et al. 1995, 1996). This work has been presented in abstract form (Pyapali et al. 1997).

## METHODS

*Prenatal choline treatment*

Pregnant Sprague-Dawley CD strain albino rats were obtained (Charles River, Kingston, NY) at day 9 of gestation (E9). Animals were housed individually in clear polycarbonate cages ( $27.9 \times 27.9 \times 17.8 \text{ cm}^3$ ); food and water were provided ad libitum with a 12 h light/dark cycle. They were fed purified Dyets formula AIN-76A diet (Dyets, Bethlehem, PA) containing 7.9 mmol/kg choline chloride and water. Prenatal choline treatment was carried out from day E12 to E17. The dams were divided into three groups: control ( $n = 9$ ), choline supplemented ( $n = 9$ ), and choline deficient ( $n = 9$ ). The total numbers of offspring used for the experiments from these dams were: control ( $n = 25$ ), supplemented ( $n = 25$ ), and deficient ( $n = 10$ ). However, not all animals yielded hippocampal slices that met electrophysiological criteria for inclusion, so in some instances, the number of slices used in a given experiment did not correspond directly with the number of rats generated for that experiment. Control rats received an AIN-76A diet containing 7.9 mmol/kg choline chloride and water sweetened with 50 mM saccharine, resulting in an average daily choline intake of  $1.3 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ . Dams in the supplemented group received AIN-76A diet containing 7.9 mmol/kg choline chloride and water containing 25 mM choline chloride and sweetened with 50 mM saccharine, resulting in an average daily choline intake of  $4.6 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  (saccharine was used to neutralize the bitter taste of choline in the diet and to equalize the water intake among dams in the treatment groups). Dams in the deficient group received an AIN-76A diet without added choline ( $0.0 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) and water with 50 mM saccharine. After day 17 of gestation, all animals were fed normal AIN-76A diet containing 7.9 mmol/kg choline chloride and saccharine-free water. At birth the pups were cross-fostered to an untreated foster dam and were weaned at postnatal day 24 (P24). At P30 they were housed two to a cage and provided with the standard diet AIN-76A and water ad libitum. All of the rats used in the present study were male.

*Hippocampal slice preparation*

In all instances, the physiological investigators were blind to the treatment condition of the animals before sacrifice. On each test day, one rat was anesthetized with halothane, and the brain was removed quickly and placed in ice-cold, oxygenated artificial cerebrospinal fluid [ACSF; containing (in mM) 124 NaCl, 3.25 KCl, 1.25  $\text{NaH}_2\text{PO}_4$ , 25  $\text{NaHCO}_3$ , 2.4  $\text{CaCl}_2$ , 2.0  $\text{MgSO}_4$ , and 10 dextrose and continually oxygenated with 95%  $\text{O}_2$ -5%  $\text{CO}_2$  to maintain a pH of 7.4]. The hippocampi were dissected from each hemisphere, and 500- $\mu\text{m}$  transverse slices were cut using a manual chopper. The slices were allowed to equilibrate in ACSF for  $\geq 2$  h and then were transferred to an interface chamber maintained at  $36^\circ\text{C}$  and exposed to a mixture of 95%  $\text{O}_2$ -5%  $\text{CO}_2$  gas (see Pyapali et al. 1994, 1996). For some experiments, the calcium and magnesium were reduced to 2.0 and 0.9 mM, respectively (described later).

*Stimulation and recording protocols*

Recordings were made simultaneously from the somatic and dendritic fields of the CA1 region in the slices from each animal. A bipolar, twisted wire electrode was used to stimulate afferent fibers in the stratum radiatum of the CA1 region. Recording electrodes were glass micropipettes filled with 2 M NaCl (2–10 M $\Omega$ ). One was placed in stratum radiatum to record the dendritic field population excitatory postsynaptic potential (pEPSP), and the other was placed in stratum pyramidale to record the combined somatic field pEPSP and population spike. Test stimuli were 100-

ms, monophasic constant-current pulses. Evoked field potentials were amplified 10 times, filtered at 0–5 kHz, digitized at 10 kHz (16-bit) with a laboratory computer system, and stored for off-line analysis.

To assess the induction of LTP, only midtemporal slices from each rat were used. Acceptable dendritic field potentials, recorded from stratum radiatum, exhibited a negative peak of  $\geq 1.0$  mV. Once an acceptable field potential was obtained, the response was allowed to stabilize for  $\geq 10$  min. An input/output (I/O) curve then was generated. At least six stimulus intensities, from 0.5 to 3 mA were used to generate the I/O curve and to determine the maximum dendritic pEPSP response in each slice. There was no significant difference between slices from the three treatment groups in terms of either the stimulus intensity required to obtain the maximum pEPSP slope response ( $H_{[2]} = 3.74$ ,  $P = 0.154$ ), or the maximal pEPSP slope generated ( $F_{[2,27]} = 0.53$ ,  $P = 0.596$ ). Baseline stimulation for the LTP experiments consisted of test pulses delivered once every 30 s at an intensity sufficient to elicit a pEPSP that was 30% of the maximum response on the I/O curve. Stable baseline responses were recorded for  $\geq 10$  min before a stimulus train was delivered through the stimulating electrode to induce LTP. Five baseline dendritic pEPSP slope responses were averaged together to form the baseline (100%) reference value. The suprathreshold, threshold, and subthreshold stimulus trains were designed based on preliminary studies conducted in our laboratory using hippocampal slices from untreated control rats of ages similar to those in the present experiments. Threshold stimuli reliably resulted in LTP generation in these control slices, whereas subthreshold stimuli did not lead to LTP induction.

In the first set of experiments, a suprathreshold theta-burst stimulus train (40 pulses, delivered as 10 mini trains (100 Hz) of 4 pulses each, with an intertrain interval of 200 ms, at 30% of maximum pEPSP intensity) was used to induce LTP. Hippocampal slices were taken from 15 choline supplemented and 15 control rats. Because some animals did not yield slices that met our electrophysiological criteria, there were instances in which data from more than one slice were used from a given rat. In those instances, the data from those slices were averaged and the average included in subsequent statistical analyses. The calcium and magnesium were reduced to 2.0 and 0.9 mM, respectively, throughout these experiments.

In the second set of experiments, either threshold level stimulus trains (20 pulses, delivered as 5 mini trains (100 Hz) of 4 pulses each, with an intertrain interval of 200 ms, at 20% maximum pEPSP intensity) or subthreshold level stimulus trains (20 pulses, delivered as 5 mini trains (100 Hz) of 4 pulses each, with an intertrain interval of 200 ms, at 10% maximum pEPSP intensity) were applied to the CA1 region. The treatment groups in this set of experiments consisted of 10 control, 10 choline-supplemented, and 10 choline-deficient rats. From each animal that yielded acceptable slices, two slices were used. One slice received the threshold intensity stimulus train and the other received the subthreshold stimulus train. The order in which the stimulus trains were applied on a given day was predetermined randomly.

In all experiments, after the application of the stimulus train, responses were recorded once every 30 s for 30 min. The midpoint (50% of the maximum negativity) of each dendritic response was determined, and the pEPSP slope was calculated by defining the point 0.5 ms before the midpoint and 0.5 ms after the midpoint and subtracting the voltage values at these two points. Thus the pEPSP slope was defined in millivolts per millisecond during that 1.0-ms epoch. Five pEPSPs were recorded and averaged at 15 and 30 min each after the stimulus train. These averages were used as dependent measures in the LTP experiments. The pEPSP slope values were normalized to the average of the baseline slope before potentiation, yielding percent potentiation (see Deupree et al. 1991, 1993).

### Statistical analysis

The treatment effects were analyzed using multifactorial analyses of variance, one-way analyses of variance (ANOVAs), and post hoc Mann-Whitney Rank Sum tests when appropriate. As noted earlier, when more than one slice was used from an animal for a given LTP stimulus condition, the data from those slices were averaged before inclusion in the statistical analyses.

## RESULTS

### Suprathreshold stimulus trains

The suprathreshold stimulus trains (40 pulses, 30% maximal intensity) reliably induced robust LTP in slices from both control and choline supplemented animals ( $F_{[2,54]} = 39.16$ ,  $P < 0.001$ ). However, there was no significant effect of the prenatal choline supplementation on the magnitude of LTP. Figure 1 shows representative pEPSP traces from stratum radiatum of CA1 in slices from control ( $n = 18$  slices) and choline-supplemented ( $n = 17$  slices) animals. The traces show examples of potentials at baseline (*left*), 15 min (*middle*), and 30 min (*right*) after the potentiating stimulus train. The magnitude of LTP at 15 and 30 min after the stimulus train relative to baseline pEPSP slope is shown in Fig. 2, with no difference between the two conditions.

### Threshold level stimulus trains

The threshold intensity stimulus trains (20 pulses, 20% maximal intensity) led to LTP induction in hippocampal slices from both control animals and prenatally choline-supplemented animals. However, stimulus trains of this intensity did not induce significant LTP in slices taken from prenatally choline-deficient rats. Figure 3 shows representative traces of the dendritic field pEPSPs recorded from stratum radiatum of CA1 in slices from control ( $n = 9$  slices), choline-supplemented ( $n = 8$  slices), and choline-deficient ( $n = 8$  slices) rats. One rat from the control group and two rats each from the supplemented and deficient groups did not yield healthy slices with acceptable maximum EPSP response and therefore no data could be obtained from these animals. The traces are examples of pEPSPs at baseline (Fig. 3, *left*) and at 15

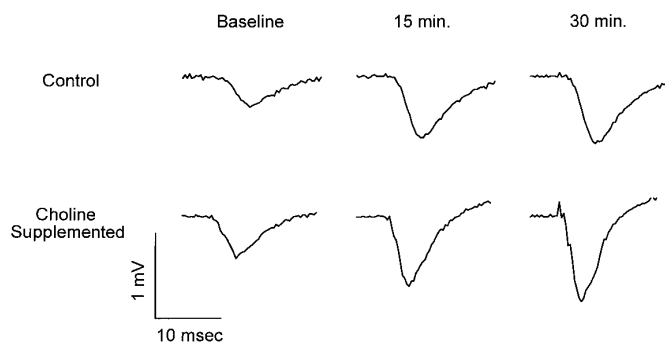


FIG. 1. Representative examples of evoked CA1 dendritic field potentials before (baseline) and at 15 and 30 min after application of a supra-threshold stimulus train (theta-burst, 40 pulses at 100 Hz and 30% maximum baseline I/O, 10 mini trains of 4 pulses each) to the stratum radiatum. *Top*: population excitatory postsynaptic potentials (pEPSPs) from a control slice. *Bottom*: pEPSPs recorded from a slice from the choline-supplemented group.

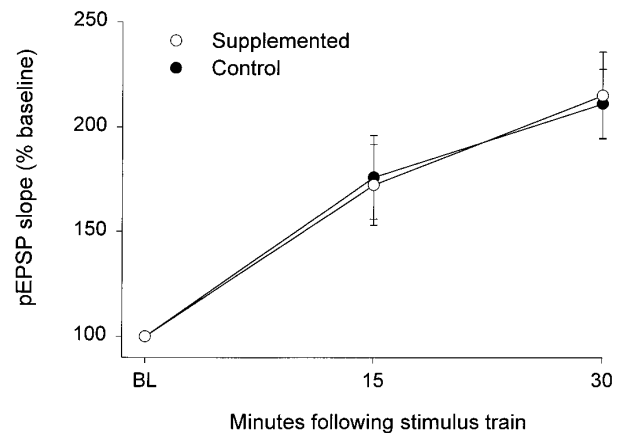


FIG. 2. Summary graph of the effect of the supra-threshold stimulus trains on long-term potentiation (LTP) induction in hippocampal slices from control ( $n = 18$ ) and choline-supplemented ( $n = 17$ ) rats plotted at 15-min intervals. LTP was induced in slices from both groups, but there was no significant difference in the magnitude of LTP between groups. Each point represents mean  $\pm$  SE of the percentage baseline pEPSP slope.

(*middle*) and 30 min (*right*) after application of the stimulus train.

The magnitude of LTP at 15 and 30 min after the stimulus train relative to baseline pEPSP slope is shown in Fig. 4. Slices from both control and choline-supplemented rats showed significant potentiation. One-way ANOVAs indicated that there were significant differences between the treatment groups at both 15 min ( $F_{[2,24]} = 3.89$ ,  $P = 0.034$ ) and 30 min ( $F_{[2,24]} = 3.71$ ,  $P = 0.041$ ) after the stimulus trains were applied. Post hoc Mann-Whitney rank sum tests indicated a significant difference in the magnitude of pEPSP slopes in slices from control compared with choline-deficient rats ( $P = 0.03$ ) but not in those from control compared with choline-supplemented rats ( $P = 0.27$ ) 30 min after application of the stimulus train. It is noteworthy that five of the eight slices from rats in the prenatally choline deficient

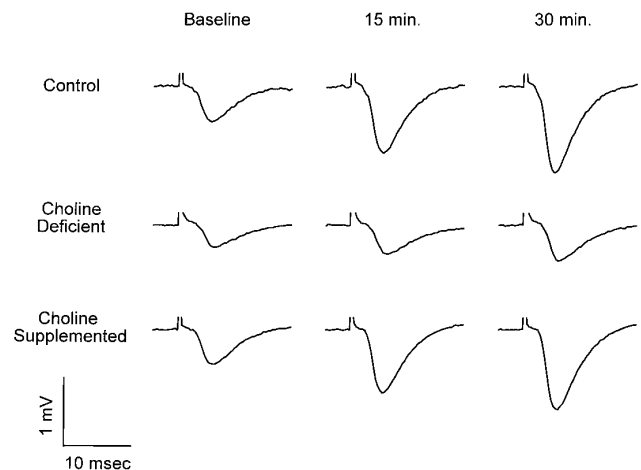


FIG. 3. Representative examples of evoked CA1 dendritic field potentials before (baseline) and at 15 and 30 min after application of a threshold intensity stimulus train (theta-bursts, 20 pulses at 100 Hz and 20% maximum baseline I/O, 5 mini trains of 4 pulses each) to the stratum radiatum. *Top*: pEPSPs from a control slice. *Middle*: pEPSPs from a deficient slice. *Bottom*: pEPSPs recorded from a slice from the choline-supplemented group.

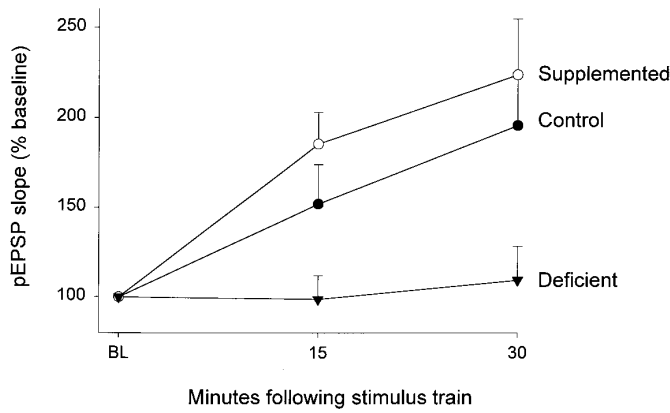


FIG. 4. Summary graph of the effect of threshold level stimulus trains on LTP induction in hippocampal slices from control ( $n = 9$ ), choline-deficient ( $n = 8$ ), and choline-supplemented ( $n = 8$ ) rats plotted at 15-min intervals. LTP was induced reliably in slices from control and choline-supplemented rats but not in those from choline-deficient rats. Each point represents mean  $\pm$  SE of the percentage baseline pEPSP slope.

group showed moderate depression of the pEPSP slope after the stimulus train.

*Subthreshold stimulus trains*

In contrast to the effects of the threshold intensity stimulus trains, subthreshold stimulus trains induced LTP only in slices from the choline-supplemented rats. Because the higher intensity threshold level stimulus trains had not elicited LTP in slices from choline-deficient rats, we included only two slices from choline deficient animals in this experiment. Figure 5 shows representative traces of the dendritic field pEPSPs recorded from stratum radiatum of area CA1 using the subthreshold stimulus trains in slices from control (*top*;  $n = 9$  slices), choline-deficient (*middle*;  $n = 2$  slices), and choline-supplemented (*bottom*;  $n = 8$  slices) rats. The traces show examples of potentials recorded at baseline (*left*) and at 15 (*middle*) and 30 min (*right*) after the stimulus train.

The magnitude of LTP at 15 and 30 min after the stimulus

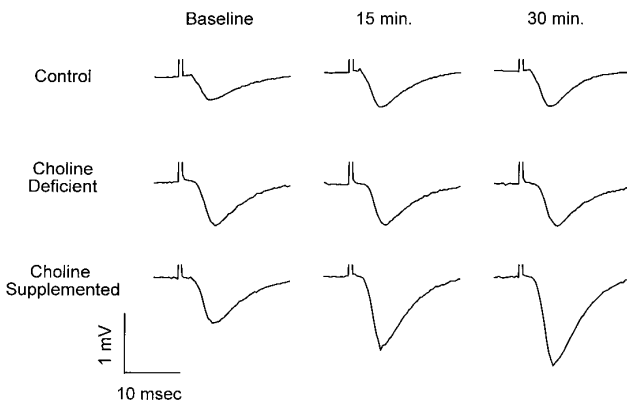


FIG. 5. Representative examples of evoked CA1 dendritic field potentials before (baseline) and at 15 and 30 min after application of a subthreshold intensity stimulus train (theta-bursts, 20 pulses at 100 Hz and 10% maximum baseline I/O, 5 mini trains of 4 pulses each) to the stratum radiatum. *Top*: pEPSPs from a control slice. *Middle*: pEPSPs from a deficient slice. *Bottom*: pEPSPs recorded from a slice from the choline-supplemented group.

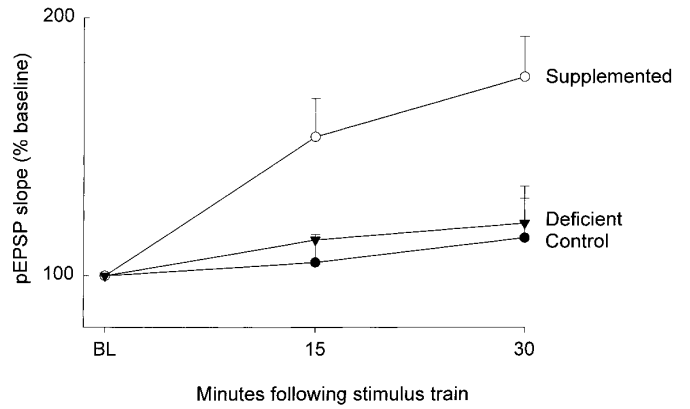


FIG. 6. Summary graph of the effect of subthreshold stimulus trains on LTP induction in hippocampal slices from control ( $n = 9$ ), choline-deficient ( $n = 2$ ), and choline-supplemented ( $n = 8$ ) rats plotted at 15-min intervals. LTP was induced reliably only in slices from choline-supplemented rats. Each point represents mean  $\pm$  SE of the percentage baseline pEPSP slope.

train relative to baseline pEPSP slope is shown in Fig. 6. One-way ANOVAs indicated that there were significant differences between the treatment groups at both 15 min ( $F_{[2,18]} = 3.94, P = 0.035$ ) and 30 min ( $F_{[2,18]} = 3.80, P = 0.045$ ) after the stimulus trains were applied. Post hoc Mann-Whitney rank sum tests indicated a significant difference in the magnitude of pEPSP slopes in slices from control compared with choline-supplemented rats ( $P = 0.004$ ) but not in those from control compared with choline-deficient rats ( $P = 0.36$ ) 30 min after application of the stimulus train.

The overall results of these experiments are summarized in Table 1.

*Baseline control experiments*

The slice selection criteria and baseline monitoring periods were intended to ensure that only slices that produced stable baseline pEPSPs were used. However, we also were concerned about the possibility of drift of the pEPSPs slope across the 30-min period after the application of the stimulus train. Therefore we selected three slices from control rats and treated them exactly as those used in the experiments described above except that no stimulus trains were applied. In these slices, pEPSP slopes were monitored for  $\leq 2$  h after stabilization, establishment of the I/O curve, and restabilization. There was only minimal and nonsignificant drift of the pEPSP slope values. For example, the average pEPSP slope at 15 min after restabilization was  $93 \pm 13\%$  (SE) of baseline and at 30 min the average pEPSP slope was  $103 \pm 11\%$  of baseline.

TABLE 1. Mean percent of baseline pEPSP slope 30 min after theta burst stimulus trains

	Control	Supplemented	Deficient
Suprathreshold	211 $\pm$ 16 (18)	215 $\pm$ 21 (17)	—
Threshold	196 $\pm$ 27 (9)	224 $\pm$ 31 (8)	109 $\pm$ 19 (8)
Subthreshold	115 $\pm$ 15 (9)	177 $\pm$ 16 (8)	120 $\pm$ 15 (2)

Values are means  $\pm$  SE. Number of slices is in parentheses.

## DISCUSSION

The principal findings from this study are that the induction of LTP was enhanced in hippocampal slices from adult rats after prenatal supplementation of dietary choline and diminished in those from adult rats after prenatal choline deficiency. The distinctions between LTP induction in hippocampal slices from treated versus control rats was evident only when relatively mild stimulus trains were used, a finding that is consistent with LTP studies using tissue from aged animals (Deupree et al. 1991, 1993; Moore et al. 1993). The suprathreshold stimulus trains were of little value in discriminating between the capacity for LTP induction in slices from animals in the different treatment groups. In our previous studies, such trains also failed to elicit LTP in slices from prenatally choline-deficient animals (Jones et al. 1995, 1996), so these experiments were not repeated. In the present study, the suprathreshold stimulus trains elicited comparable levels of LTP in slices from both control and prenatally choline-supplemented rats. However, the lower intensity stimulus trains (threshold and subthreshold) were valuable in separating the potential for LTP induction among slices from animals in the three treatment groups. That is, the threshold level stimulus trains reliably elicited LTP in slices from control and prenatally choline-supplemented rats but not in those from prenatally choline-deficient rats, whereas subthreshold stimulus trains elicited LTP only in slices from prenatally supplemented rats. Thus while LTP was induced reliably in control slices by the threshold stimulus train and not the subthreshold stimulus train, neither train elicited LTP reliably in slices from choline-deficient animals and both trains elicited LTP reliably in slices from choline-supplemented animals. These distinctions suggest that the threshold for eliciting LTP in slices from choline-supplemented animals is lower than control. However, even with suprathreshold stimuli, LTP could not be generated in choline-deficient animals (Jones et al. 1995, 1996), suggesting a profound deficiency of LTP induction that could not be overcome with an increased stimulus.

These results indicate that enhanced or decreased availability of choline during prenatal development results in enduring alterations of hippocampal synaptic plasticity that may underlie the changes in spatial memory capacity in rats treated similarly (Meck and Williams 1997a–d). In addition, these findings are consistent with previous findings from our laboratory that indicated that pre- and perinatal choline supplementation resulted in an apparent lowering of LTP threshold. Those studies have been published in abstract form (Jones et al. 1995, 1996).

#### *Direct or indirect cholinergic mechanisms*

The mechanisms underlying these results may relate to direct effects on cholinergic neurotransmission that outlast the prenatal period, alterations in the sensitivity of second-messenger systems that are persistent, indirect effects on the enhancement of glutamatergic neurotransmission, or other effects of choline on cellular metabolism or membranes. One possibility may be direct alterations in the regulation of the synthesis and release of ACh. ACh is one of the major neurotransmitters in the central and peripheral nervous sys-

tems, mediated via both muscarinic and nicotinic receptors. Increasing evidence suggests that ACh may participate in regulation of higher cognitive functions such as memory and learning (Bartus et al. 1985; Ohno et al. 1994; Segal 1982; Vannucchi and Pepeu 1995). Prenatal choline alterations may exert a profound effect on either muscarinic or nicotinic cholinergic neurotransmission, potentially by altering the number of cholinergic receptors or second-messenger systems mediating cholinergic activity (particularly the muscarinic activity). The changes in choline availability may show critical times of sensitivity, where a profound effect may persist past development. Aspects of cholinergic neurotransmission may be upregulated and retain an enhanced effect compared with the control animals or those with decreased choline availability. This effect also may spread to include either second-messenger systems involved in cholinergic responses or even a permanent change in nuclear sensitivity to synthesis, availability, or responsiveness of these second-messenger systems.

Prenatal choline supplementation results in an increase in basal, and glutamate activated, PLD activity in the hippocampus and the developmental time course of this effect correlates with synapse formation and hippocampal maturation ( $\leq 2$  wk after birth). PLD has been implicated in vesicle trafficking and neurotransmitter release. Diacylglycerol, produced from PLD, activates protein kinase C, a mediator thought necessary to maintain LTP (Holler et al. 1996). It is possible that effects such as these in prenatally choline-supplemented animals could result in a secondary enhancement of LTP through changes in shared second-messenger and linkage systems without any direct involvement of cholinergic neurotransmission.

Choline also may affect cognitive function via effects on NGF-related systems and basal forebrain cholinergic neurons (Gibbs 1994). In addition to dopaminergic pathways, choline supplementation also can modify the muscarinic, GABAergic, and noradrenergic systems (molino et al. 1997). However, an agonist for dopamine receptors, apomorphine, the  $\gamma$ -aminobutyric acid receptor agonist muscimol and alpha-adrenergic receptor agonist clonidine do not result in any significant improvement in memory in aged rats, though these have not been tested in the current circumstance of choline alterations.

#### *Mechanisms of action of choline supplementation on excitatory neurotransmission*

Our observations suggest an alteration of glutamatergic neurotransmission because *N*-methyl-D-aspartate (NMDA) receptors in particular are necessary for LTP (as we have induced it) in the CA1 region of the hippocampus (Bliss and Collingridge 1993; Durand et al. 1996). There are several lines of evidence indicating an enhancement of both glutamatergic neurotransmission and LTP after cholinergic activation (Auerbach and Segal 1994; Blitzer et al. 1990; Burgard and Sarvey 1990; Huerta and Lisman 1993; Markram and Segal 1990; Segal 1982). For example, it is possible that presynaptic muscarinic cholinergic receptors may modify glutamatergic excitatory synaptic transmission. In this case, the prenatal choline manipulation could influence hippocampal LTP through a change in presynaptic muscarinic

receptors or their activation (Sheridan and Sutor 1990). Such changes would be different from the primary muscarinic cholinergic effect of mild depolarization due to closure of a potassium channel and decreased M current (Cole and Nicoll 1983), which also could be upregulated in the brain in the prenatally choline supplemented animal.

Although a direct effect of prenatal choline manipulations on hippocampal function is possible, an equally likely possibility is that an effect on cholinergic neurons elsewhere could influence subsequent hippocampal function. For example, immunotoxic lesions after IgG-saporin injections into the medial septal cholinergic neurons showed that the cholinergic projections to the hippocampus facilitate the acquisition of information but are not involved in the retention of information (Shen et al. 1996). Thus an effect of prenatal choline manipulations on cholinergic neurons that project to the hippocampal formation also could be a site of mechanistic significance. Direct tests of cholinergic mechanisms through physiological manipulation and the use of cholinergic agonists and antagonists may be required for assessing these relative possibilities. For example, activation of muscarinic receptors have been shown to promote the induction of LTP (Burgard and Sarvey 1990; Huerta and Lisman 1993; Ulus et al. 1989). Another alternative is that second-messenger systems shared by both cholinergic and glutamatergic neurotransmission may be upregulated by the prenatal choline and that our LTP results would occur in the absence of direct cholinergic stimulation at the receptor level.

*Relationship of LTP to behavioral memory*

LTP, like memory, is identified with hippocampal function (Barnes 1995). Its onset is rapid and incremental, and it is of long duration in response to short bursts of activity at specific synapses, parallel to the activation and time course of memory (Barnes 1995; Eichenbaum 1995; Eichenbaum and Otto 1993). A number of studies involving behavioral and physiological assessments from individual animals have shown very high correlations between spatial memory and LTP. For example, rats that demonstrated efficient behavioral retention of spatial task in vivo also yielded hippocampal slices that produced greater potentiation (Deupree et al. 1991, 1993; Moore et al. 1993). In addition, drug-induced blockade of NMDA-receptor-dependent LTP and blockade of molecular triggers for LTP result in severe memory impairments in animals, supporting a connection between LTP and memory (Frey and Morris 1997). Finally, genetic manipulations, such as specific gene “knockouts” of glutamate receptors and  $\alpha$ -calcium-calmodulin-dependent kinase II genes, eliminate LTP and hippocampal-dependent learning (Mayford et al. 1996; McHugh et al. 1996). The data linking the specific group of processes loosely called LTP to behavioral aspects of memory remain highly correlational, though in some instances this correlation is quite strong.

We thank C. Vipperman and Y. D. Philips for providing technical assistance.

This work was funded in part by National Institute of Aging Program Project Grants AG-09525 (to W. H. Meck, H. S. Swartzwelder, and C. H. Williams) and AG-13165 (to D. A. Turner) and Veterans Affairs Merit Review Awards (to H. S. Swartzwelder and D. A. Turner).

Address for reprint requests: H. S. Swartzwelder, Building. 16, VA Medical Center, Durham, NC 27705.

Received 9 September 1997; accepted in final form 8 December 1997.

REFERENCES

ARTOLA, A. AND SINGER, W. Long term potentiation and NMDA receptors in rat visual cortex. *Nature* 330: 649–652, 1987.

AUERBACH, J. AND SEGAL, M. A novel cholinergic induction of long-term potentiation in rat hippocampus. *J. Neurophysiol.* 72: 2034–2040, 1994.

BARNES, C. A. Involvement of LTP in memory: are we searching under the street lights? *Neuron* 15: 751–754, 1995.

BARRY, S. R. AND GELPERIN, A. Exogenous choline augments transmission at an identified cholinergic synapse in terrestrial mollusk *Limax maximus*. *J. Neurophysiol.* 48: 439–450, 1982.

BARTUS, R. T., DEAN, R. L., PONTECORVO, M. J., AND FLICKER, C. The cholinergic hypothesis: a historical overview, current perspective and the future directions. *Ann. NY Acad. Sci.* 444: 332–358, 1985.

BLISS, T. AND COLLINGRIDGE, G. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.

BLITZER, R., GIL, O., AND LANDAU, E. Cholinergic stimulation enhances LTP in the CA1 region of the rat hippocampus. *Neurosci. Lett.* 119: 207–210, 1990.

BLUSZTAJN, J. K. Choline: the essential nutrient for brain development. *Biosci. Biotech. Biochem.* (Nippon Nogeikagaku kaishi) 69: 578–582, 1995.

BLUSZTAJN, J. K., LISCOVITCH, M., MAURON, C., RICHARDSON, U. I., AND WURTMAN, R. J. Phosphatidylcholine as a precursor of choline for acetylcholine synthesis. *J. Neural Transm.* 24 Suppl.: 247–259, 1987.

BLUSZTAJN, J. K. AND WURTMAN, R. J. Choline and cholinergic neurons. *Science* 221: 614–620, 1983.

BURGARD, E., AND SARVEY, J. Muscarinic receptor activation facilitates the induction of LTP in the rat dentate gyrus. *Neurosci. Lett.* 116: 34–39, 1990.

CHUNG, S. Y., MORIYAMA, T., UEZU, E., UEZU, K., HIRATA, R., YOHENA, N., MASUDA, Y., KOKUBU, T., AND YAMAMOTO, S. Administration of phosphatidylcholine increases brain acetylcholine concentration and improves memory in mice with dementia. *J. Nutr.* 125: 1484–1489, 1995.

COLE, A. AND NICOLL, R. Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. *Science* 221: 1299–1301, 1983.

DEUPREE, D. L., BRADLEY, J., AND TURNER, D. A. Age-related alterations in potentiation in the CA1 region in F344 rats. *Neurobiol. Aging* 14: 249–258, 1993.

DEUPREE, D. L., TURNER, D. A., AND WATTERS, C. L. Spatial performance correlates with in vitro potentiation in young and aged Fischer 344 rats. *Brain Res.* 554: 1–9, 1991.

DRACHMAN, D. A. AND LEAVITT, J. L. Human memory and the cholinergic system. A relationship to aging? *Arch. Neurol.* 30: 113–121, 1974.

DURAND, G. M., KOVALCHUK, Y., AND KONNERTH, A. Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* 381: 71–75, 1996.

EICHENBAUM, H. The LTP-memory connection. *Nature* 378: 131–132, 1995.

EICHENBAUM, H. AND OTTO, T. LTP and memory: can we enhance the connection? *Trends Neurosci.* 16: 163–164, 1993.

FREY, U. AND MORRIS, R.G.M. Synaptic tagging and long-term potentiation. *Nature* 385: 533–536, 1997.

GARNER, S. C., MAR, M. H., ZEISEL, S. H. Choline distribution and metabolism in pregnant rats and fetuses are influenced by the choline content of the maternal diet. *J. Nutr.* 125: 2851–2858, 1995.

GIBBS, R. B. Estrogen and nerve growth factor-related systems in brain. Effects on basal forebrain cholinergic neurons and implications for learning and memory processes and aging. *Ann. NY Acad. Sci.* 743: 165–196, 1994.

GORRY, E., LOY, R., BLUSZTAJN, J., MECK, W., AND WILLIAMS, C. L. Gonadal steroids and choline interact during development to improve radial arm maze performance and increase hippocampal NGF in adult rats. *Soc. Neurosci. Abstr.* 22: 339, 1992.

HOLLER, T., CERMAK, J. M., AND BLUSZTAJN, J. K. Dietary choline supplementation in pregnant rats increases hippocampal phospholipase D activity of the offspring. *FASEB J.* 10: 1653–1659, 1996.

HUERTA, P., AND LISMAN, J. Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature* 364: 723–725, 1993.

Downloaded from jn.physiology.org on December 27, 2009

- JACKSON, D., MECK, W., WILLIAMS, C. L., AND BLUSZTAJN, J. Supplementation with choline in-utero reduces brain choline acetyltransferase (CAT) activity during postnatal development in the rat. *Soc. Neurosci. Abstr.* 22: 340, 1992.
- JONES, J., MECK, W. H., WILLIAMS, C. L., AND SWARTZWELDER, H. S. Choline availability to the developing rat fetus produces organizational changes in adult hippocampal long-term potentiation. *Soc. Neurosci. Abstr.* 21: 161, 1995.
- JONES, J., MECK, W. H., WILLIAMS, C. L., AND SWARTZWELDER, H. S. Prenatal and pre+postnatal choline supplementation reduces the threshold to induce long-term potentiation in hippocampal slices from adult rats. *Soc. Neurosci. Abstr.* 22: 138, 1996.
- LOY, R., HEYER, D., WILLIAMS, C. L., AND MECK, W. H. Choline-induced spatial memory facilitation correlates with altered distribution and morphology of septal neurons. *Adv. Exp. Med. Biol.* 295: 373–382, 1991.
- MAYFORD, M., BACH, M. E., HUANG, Y. Y., WANG, L., HAWKINS, R. D., AND KANDEL, E. R. Control of memory formation through regulated expression of a CaMKII transgene. *Science* 274: 1678–1683, 1996.
- MARKRAM, H. AND SEGAL, M. Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. *J. Physiol. (Lond.)* 427: 381–393, 1990.
- MC HUGH, T. J., BLUM, K. I., TSIEN, J. Z., TONEGAWA, S., AND WILSON, M. A. Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. *Cell* 87: 1147–1148, 1996.
- MECK, W. H. AND CHURCH, R. M. Cholinergic modulation of the content of temporal memory. *Behav. Neurosci.* 101: 457–464, 1987.
- MECK, W. H., SMITH, R. A., AND WILLIAMS, C. L. Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Dev. Psychobiol.* 21: 339–353, 1988.
- MECK, W. H., SMITH, R. A., AND WILLIAMS, C. L. Organizational changes in cholinergic activity and enhanced visuospatial memory as a function of choline administered prenatally or postnatally or both. *Behav. Neurosci.* 103: 1234–1241, 1989.
- MECK, W. H. AND WILLIAMS, C. L. Characterization of the facilitative effects of perinatal choline supplementation on timing and temporal memory. *Neuroreport* 8: 2831–2835, 1997.
- MECK, W. H. AND WILLIAMS, C. L. Choline supplementation during pre- and postnatal development reduces proactive interference in spatial memory. *Dev. Brain Res.* 1997b In press.
- MECK, W. H. AND WILLIAMS, C. L. Perinatal choline supplementation increases the threshold for chunking in spatial memory. *Neuroreport* 8: 3053–3059, 1997.
- MECK, W. H. AND WILLIAMS, C. L. Simultaneous temporal processing is sensitive to prenatal choline availability in mature and aged rats. *Neuroreport* 8: 3045–3051, 1997d.
- MOLINENGO, L., ORSETTI, M., AND GHI, P. Behavioral and neurochemical effects of a chronic choline-deficient diet in the rat. *Behav. Brain Res.* 84: 145–150, 1997.
- MOORE, C. I., BROWNING, M. D., AND ROSE, G. M. Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged F344 rats. *Hippocampus* 4: 11–18, 1993.
- OHNO, M., YAMAMOTO, T., AND WATANBE, S. Blockade of hippocampal M<sub>1</sub> muscarinic receptors impairs working memory performance of rats. *Brain Res.* 650: 260–266, 1994.
- PYAPALI, G. K. AND TURNER, D. A. Denervation-induced alterations in CA1 pyramidal neurons following kainic acid lesions in rats. *Brain Res.* 652: 279–290, 1994.
- PYAPALI, G. K. AND TURNER, D. A. Increased dendritic extent in aged CA1 neurons from aged Fisher 344 rats. *Neurobiol. Aging* 17: 601–611, 1996.
- PYAPALI, G. K., WILLIAMS, C. L., MECK, W. H., TURNER, D. A., AND SWARTZWELDER, H. S. Enhancement of long-term potentiation following prenatal choline enrichment. *J. Neurosci. Abstr.* 23: 2129, 1997.
- SAHLEY, C. L., BARRY, S. R., AND GELPERIN, A. Dietary choline augments associative memory function in *Limax maximus*. *J. Neurobiol.* 17: 113–120, 1986.
- SEGAL, M. Multiple actions of acetylcholine at a muscarinic receptor studied in the rat hippocampal slice. *Brain Res.* 246: 77–87, 1982.
- SHEN, J., BARNES, C. A., WENK, G. L., AND MCNAUGHTON, B. L. Differential effects of selective immunotoxic lesions of medial septal cholinergic cells on spatial working and reference memory. *Behav. Neurosci.* 110: 1181–1186, 1996.
- SHERIDAN, R. AND SUTOR, B. Presynaptic M<sub>1</sub> muscarinic cholinergic receptors mediate inhibition of excitatory synaptic transmission in the hippocampus in vitro. *Neurosci. Lett.* 108: 273–278, 1990.
- ULUS, I. H., WURTMAN, R. J., MAURON, C., AND BLUSZTAJN, J. K. Choline increases acetylcholine release and protects against the stimulation-induced decrease in phosphatide levels within membranes of rat corpus striatum. *Brain Res.* 484: 217–227, 1989.
- VANNUCCHI, M. G. AND PEPEU, G. Muscarinic receptor modulation of acetylcholine release from rat cerebral cortex and hippocampus. *Neurosci. Lett.* 190: 53–56, 1995.
- ZEISEL, S. H. AND BLUSZTAJN, J. K. Choline and human nutrition. *Annu. Rev. Nutr.* 14: 269–296, 1994.
- ZEISEL, S. H., DA COSTA, K.-A., FRANKLIN, P. D., ALEXANDER, E. A., LAMONT, J., SHEARD, N. F., AND BEISER, A. Choline, an essential nutrient for humans. *FASEB J.* 5: 2093–2098, 1991.