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Perinatal choline deficiency produces abnormal sensory inhibition in Sprague-Dawley rats

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Abstract

Adequate choline levels in rodents during gestation have been shown to be critical to several functions, including certain learning and memory functions, when tested at adulthood. Choline is a selective agonist for the $\alpha 7$ nicotinic receptor which appears in development before acetylcholine is present. Normal sensory inhibition is dependent, in part, upon sufficient numbers of this receptor in the hippocampus. The present study assessed sensory inhibition in Sprague-Dawley rats gestated on normal (1.1 g/kg), deficient (0 g/kg) or supplemented (5 g/kg) choline in the maternal diet during the critical period for cholinergic cell development (E12-18). Rats gestated on deficient choline showed abnormal sensory inhibition when tested at adulthood, while rats gestated on normal or supplemented choline showed normal sensory inhibition. Assessment of hippocampal α -bungarotoxin to visualize nicotinic $\alpha 7$ receptors revealed no difference between the gestational choline levels. These data suggest that attention to maternal choline levels for human pregnancy may be important to the normal functioning of the offspring.

Introduction

Recent studies have highlighted the effects of manipulating maternal dietary choline levels during gestation on a variety of outcomes in the adult offspring. In rodents, the level of choline in the maternal diet during 2 critical periods (E12-13 and E17-18) of cholinergic cell development, during which the cells are born and migrate to their final position (Semba and Fibiger 1988), has a significant effect on a number of different functions. Specifically, choline supplementation during the E12-18 time frame improves several aspects of learning and memory even into late adulthood (Brandner 2002; McCann et al 2006; Meck and Williams 1997; 1999; 2003; Meck et al 1989; 2008; Mellott et al 2004), increases attention (Brandner 2002), alters cell morphology (Li et al 2004; Williams et al 1998), increases cell proliferation and reduces apoptosis in progenitor cells (Albright et al 1999a,b) and increases the number of hippocampal $\alpha 7$ nicotinic receptors (Stevens et al 2008). Choline deficiency during this developmental time frame causes, among other changes, a reduction in auditory and visuo-spatial memory functions (McCann et al 2006; Meck and Williams 1999), impaired divided

attention and accelerated age-related decline in temporal processing as measured by the timing of simultaneous auditory and visual signaling (Meck and Williams, 1997), a reduction in sensitivity to long-term potentiation (LTP) (Jones et al 1999) and lessened viability of hippocampal slices (Montoya et al 2000).

Sensory inhibition, or filtering of repetitive stimuli, is a normal function of the brain (Adler et al 1998). In normal individuals, the evoked responses to paired identical auditory stimuli presented at a 500 msec interstimulus interval show a decrement to the second stimulus (Adler et al 1998). This inhibition of response to the second stimulus helps to protect the brain from over stimulation which has been postulated to lead to sensory overload (Venebles 1964; 1994). Abnormal sensory inhibition is a hallmark of schizophrenia (Adler et al 1998) and is also observed in other mental diseases such as bipolar disorder and mania (Franks et al 1983). In these patients, the response to the second stimulus is not reduced compared to the response to the first stimulus. Studies in both humans (Leonard et al 2000) and rodents (Stevens et al 1996) have correlated abnormal sensory inhibition with reduced numbers of hippocampal $\alpha 7$ nicotinic receptors. Exogenous stimulation of this receptor subtype with selective or non-selective agonists improves deficient sensory inhibition in both rodents (Stevens and Wear 1997; Stevens et al 1998) and humans (Adler et al 1993; Olincy et al 2006).

Choline, while being an essential nutrient and a component of cell membranes (Blusztajn 1998; Zeisel 2000; Zeisel and Blusztajn 1994), is also a select agonist for the $\alpha 7$ nicotinic receptor (Alkondon et al 1997). This receptor is found early in the developing rat hippocampus (Adams et al 2002), prior to the availability of acetylcholine (Matthews et al 1974). Thus, choline may act as the endogenous ligand for this receptor early in development. Cheng and colleagues (2006) found a possible connection between perinatal choline supplementation and $\alpha 7$ nicotinic receptors. The present study assessed the effect of maternal dietary choline manipulations, both deficiency and supplementation, upon sensory inhibition in Sprague-Dawley rats, assessed at adulthood and maintained on a normal choline diet from weaning.

Results

Animal weights

At the time of surgery to assess sensory inhibition parameters, the rats were between 11 and 12 months of age and weighed between 295 and 625 g. A two-way ANOVA for weight by group (control, deficient or supplemented choline) and sex found no a significant interaction between group and sex ($F_{(1, 17)}=3.33, p=0.086$). However, there was a significant effect group ($F_{(1, 17)}=8.128, p=0.011$) with the deficient rats being the heaviest and the supplemented rats being the lightest (Figure 1). There was also a significant effect of sex ($F_{(1, 17)}=96.574, p<0.001$) with male animals being heavier overall than female (Table 1).

Sensory inhibition

Five sensory inhibition parameters were assessed: conditioning amplitude—amplitude of the response to the first stimulus; conditioning latency—time from stimulus onset to the peak of the conditioning response; test amplitude—the amplitude of the response to the second stimulus; test latency—time from stimulus onset to the peak of the test response; and TC ratio—test amplitude/conditioning amplitude (this is a measure of the amount of inhibition of the test response; a TC ratio <0.05 is thought to be normal sensory inhibition, while ratios >0.05 are thought to be deficient). A 2-way ANOVA for TC ratio showed a significant effect of prenatal diet on sensory inhibition

($F_{(2,28)}=6.327, p=0.005$), but no effect of sex ($F_{(1,28)}=0.119, p=0.773$) or significant group by sex interaction ($F_{(2,28)}=1.296, p=0.290$). Tukey-Kramer *a posteriori* analysis revealed that

animals gestated on the deficient diet had significantly higher TC ratios than either control or supplemented rats ($p < 0.05$) (Figures 2 and 3). A 2-way ANOVA for conditioning amplitude showed no significant effect of group ($F_{(2,28)} = 1.763$, $p = 0.190$), or group by sex interaction ($F_{(2,28)} = 0.111$, $p = 0.896$) but there was a significant effect of sex ($F_{(1,28)} = 5.027$, $p = 0.033$) (Figure 4A) with males having higher conditioning amplitudes than females. A 2-way ANOVA for test amplitude revealed a significant effect of group ($F_{(2,28)} = 5.516$, $p = 0.010$), but no effect of sex ($F_{(1,28)} = 0.953$, $p = 0.337$) or group by sex interaction ($F_{(2,28)} = 0.897$, $p = 0.419$). A *posteriori* analysis revealed that the deficient group had significantly higher test amplitude than the other 2 groups (Figure 4C), accounting for the significant increase in TC ratio in this group. Conditioning latency showed no interaction between group and sex ($F_{(2,28)} = 1.00$, $p = 0.381$) nor were group or sex significant individually ($F_{(2,28)} = 1.061$, $p = 0.360$; $F_{(1,28)} = 2.137$, $p = 0.155$, respectively). Similarly, there was no group by sex interaction for test latency ($F_{(2,28)} = 0.877$, $p = 0.427$), nor were there individual differences for group or sex ($F_{(2,28)} = 0.010$, $p = 0.990$; $F_{(1,28)} = 0.000$, $p = 0.992$, respectively) (Figures 4B & D).

α -Bungarotoxin binding

Assessment of the level of α -bungarotoxin binding in the hippocampus (dentate gyrus, CA3 and CA1) among the 3 diets by 2-way ANOVA showed that there was no difference in binding levels across any region of the hippocampus for any gestational choline level, nor were there differences by sex or for the group by sex interaction ($F_{(2,22)} = 0.752$, $p = 0.483$ for group for the dentate gyrus, $F_{(1,22)} = 1.354$, $p = 0.257$ for sex and $F_{(2,22)} = 0.674$, $p = 0.520$ for the interaction; $F_{(2,22)} = 0.333$, $p = 0.720$ for group in the CA3, $F_{(1,22)} = 3.060$, $p = 0.094$ for sex and $F_{(2,22)} = 0.579$, $p = 0.569$ for the interaction; and $F_{(2,25)} = 0.356$, $p = 0.705$ for group in the CA1, $F_{(1,22)} = 0.594$, $p = 0.449$ for sex and $F_{(2,22)} = 0.326$, $p = 0.900$ for the interaction) (Figure 5A,B,C).

Discussion

The weights of the rats at the time of surgery were found to vary with gender, males being heavier than females, and by group, choline deficient rats being heavier than either the control or supplemented rats. The finding that the supplemented rats were the lightest may be spurious since only a single male choline supplemented rat was studied, thus this overall group weight may be skewed to the light side, inappropriately.

Sensory inhibition in Sprague-Dawley rats generally falls into the range considered to be normal (TC ratio < 0.05), whether the animals are recorded awake (Adler et al 1987; Stevens et al 1991) or anesthetized (Bickford-Wimer et al 1990; Krause et al 2003). In the present study, rats gestated on the control (1.1 g/kg) or supplemented (5 g/kg) choline diets had TC ratios very close to the normal pattern of sensory inhibition with reduced amplitudes in response to the second (test) stimulus relative to the first (conditioning) stimulus. Rats gestated on the deficient (0 g/kg) choline diet did not show this pattern, but rather had amplitudes in response to the test stimulus which were significantly larger than either of the other two groups. In populations of humans which show abnormal sensory inhibition (primarily schizophrenia patients and their first degree relatives) the response to the conditioning stimulus is not different from individuals who have normal sensory inhibition; however, the response to the test stimulus is not diminished as it is in the normal population (Cullum et al 1993), similar to what was observed in the rats gestated on deficient choline.

Choline has been shown to be critical during the developmental period (Zeisel 2006) with both deficiency and supplementation having developmental consequences. Choline is a precursor to acetylcholine, is a selective agonist of $\alpha 7$ receptors (Alkondon et al 1997; Papke et al 1996), and chronic postnatal administration leads to an increase in the expression of $\alpha 7$ receptors in rat brain (Morley et al 1977). In this context, it is worth noting that $\alpha 7$ nicotinic receptors are expressed early in rodent hippocampus during prenatal development (Adams et

al 2002), apparently before acetylcholine is locally available (Nadler et al 1974). Thus, it may be that choline is the endogenous ligand for the $\alpha 7$ receptor during this early developmental time frame.

Sensory inhibition has been shown to depend, in part, on hippocampal $\alpha 7$ nicotinic receptors. These receptors, which are found on hippocampal interneurons (Frazier et al 1998; Freedman et al 1993; Leonard et al 1996), receive input from medial septal cholinergic neurons, and in turn, release GABA onto the pyramidal cells (Adler et al 1998). Studies in both rodents (Stevens et al 1996) and humans (Leonard et al 2000) have shown a correlation between the numbers of hippocampal $\alpha 7$ nicotinic receptors and sensory inhibition, such that lower numbers of receptors are associated with abnormal sensory inhibition. In both rodents and humans, deficient sensory inhibition is improved by administration of either nicotine (Adler et al 1993; Stevens and Wear 1997) or a selective $\alpha 7$ nicotinic agonist (Olincy et al 2006; Stevens et al 1998). A recent study in DBA/2 mice, which have deficient sensory inhibition and reduced numbers of hippocampal $\alpha 7$ nicotinic receptors, found that perinatal choline supplementation increased the numbers of these receptors as well as improving sensory inhibition (Stevens et al 2008). The present study did not demonstrate improved sensory inhibition with perinatal choline supplementation, however, since normal Sprague-Dawley rats already show normal sensory inhibition (Adler et al 1986) and have high levels of hippocampal $\alpha 7$ nicotinic receptors (Adams and Freedman 1997), a change was not necessarily expected. The present study did demonstrate a loss of sensory inhibition with gestation on deficient choline, but no significant change in hippocampal $\alpha 7$ nicotinic receptors. Studies in human subjects have shown positive correlation between P50 sensory inhibition and attention measures, that is, better sensory inhibition is correlated with improved attention (Wan et al 2008). The present data are thus in concert with the previous studies showing a reduction in attention with deficient perinatal choline (Brandner 2002).

A possible explanation for the reduced sensory inhibition without concomitant changes in hippocampal α -bungarotoxin binding in the rats gestated on deficient choline is that there were fewer hippocampal interneurons present though the actual number of $\alpha 7$ receptors was not reduced. Studies have demonstrated that a choline deficiency during gestation in mice increases expression of 2 kinase inhibitors (p15^{INK4b} and Kap) in the fetal hippocampus through decreased DNA methylation (Niculescu et al 2006). Increases in these 2 inhibitors may be responsible for the reduced levels of cell proliferation and increased apoptosis since these kinases effect CDk2 and Cdk4 both of which are cell cycle regulators (Davies et al 2002; Dyson 1998; Hannon et al 1994). Similar results were observed in cell culture studies (Niculescu et al 2004; Yen et al 2002). Stimulation of these receptors by medial septal cholinergic neurons in response to the first stimulus in the sensory inhibition paradigm (Miller and Freedman 1993) releases GABA in the vicinity of pyramidal cells, inhibiting firing of a subpopulation of these cells in response to the second stimulus in the paradigm (Leonard et al 1996). Fewer interneurons, even with increased expression per interneuron, could reduce the level of sensory inhibition in this paradigm. The present study did not address interneuron numbers. Alternately, the α -bungarotoxin binding may not have been sufficiently sensitive to detect changes in $\alpha 7$ numbers. In a recent study, a change in regional hippocampal $\alpha 7$ receptor numbers of as little as 20% was sufficient to produce differences in sensory inhibition in a strain of inbred mice (Stevens et al 2008). Alternate explanations for the reduced sensory inhibition found with gestational choline deficiency could include an overall reduction in auditory-responsive neurons due to insufficient choline for adequate cell membrane production, or possibly changes in the DNA methylation leading to altered expression of some component of the normal sensory inhibition circuit not necessarily related to $\alpha 7$ nicotinic receptors.

In summary, manipulation of perinatal choline levels in Sprague-Dawley rats demonstrated a loss of sensory inhibition in rats gestated on deficient choline levels but failed to show

improvement in rats gestated on supplemented choline. No concomitant reduction in hippocampal $\alpha 7$ nicotinic receptors was observed, though this may be due to reduced numbers of hippocampal interneurons without an overall reduction in $\alpha 7$ nicotinic receptor numbers, insufficient sensitivity of the binding assay, or some other, as yet, undefined change. These data may have implications for prenatal nutritional choline supplementation in humans given that pregnancy significantly depletes choline stores (McMahon and Farrell 1985; Zeisel 2006) and sufficient choline levels during development are critical for a number of other neurological processes (for review see Zeisel 2006) including, possibly, normal sensory inhibition.

Experimental Procedures

Manipulation of Prenatal Choline Levels

Test animals were generated at Boston University. Pregnant female Sprague Dawley rats were fed chow (AIN-76A, Dyets, Inc, Bethlehem, PA) containing either normal (control, 1.1 g/kg choline), deficient (0 g choline) or supplemented (5 g/kg choline) levels of choline-chloride during gestational days E12-E18. At all other times during gestation, the dams consumed the normal choline level diet (1.1 g/kg). At weaning, the offspring were group housed by sex/litter and placed on normal level choline chow, which they consumed thereafter. At adulthood, the offspring were shipped to the University of Colorado Health Sciences Center for sensory inhibition testing. Upon receipt, rats were same-sex paired housed and maintained on the normal level choline chow. Animals were allowed at least 1 week for acclimatization to the higher altitude before recording for sensory inhibition. Each group represented 3 different litters of offspring, but litter notation was lost in transit so evaluation of litter effects was not possible. Rats were shipped at 11 months of age and were tested between 11 and 12 months of age.

Electrophysiological Characterization of Sensory Inhibition

Rats were anesthetized with chloral-hydrate (400 mg/kg IP) and pyrazole (400 mg/kg IP) pyrazole, to retard the metabolism of the chloral hydrate. When surgical plane of anesthesia had been achieved, the rats were mounted in a stereotaxic instrument, the scalp incised and burr holes opened over the dorsal hippocampus and the contralateral anterior cortex. A recording electrode, consisting of a teflon-coated stainless steel wire (127 μ m) was lowered to the CA3 region of the hippocampus (-4.0 mm from bregma, 3.5 mm lateral to midline and 2.5-3.2 mm below dura, Paxinos and Watson, 1998). Final electrode placement was determined by depth from brain surface and the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller et al 1992). A reference electrode, of the same type wire, was placed over the contralateral anterior cortex. Paired tones, 3000 Hz, 10 msec duration, 80 dB, SPL, were presented through hollow earbars. The interval between tones was 0.5 sec and the interval between pairs, 9 sec. Evoked potentials were band-pass filtered from 10-250 Hz. The responses to 16 pairs of tones were averaged for each record. The average of 3 records were analyzed to assess sensory inhibition parameters for each rat. The parameters assessed were the latency of the conditioning response, the amplitude of the conditioning response, the latency of the test response, the amplitude of the test response, and the TC ratio (test amplitude/conditioning amplitude). Waveform amplitudes were measured from N40 trough to preceding positive peak. This complex has greater reproducibility than other methods of measurement (Hashimoto et al 2005).

Autoradiographic Localization of $\alpha 7$ -nicotinic receptors

Tissue processing—Following recording of sensory inhibition responses, the rat was decapitated, the brain removed, hemisected and frozen in dry ice snow. Transverse sections

(12 μ M) through the dorsal hippocampus of the hemisphere, which was not implanted with the recording electrode, were collected for generation of [125I]- α -BTX autoradiograms.

[125I]- α -BTX autoradiograms—The tissue sections were incubated in a solution containing 50 mM Tris-HCl, 120 mM NaCl and 2 mg/ml bovine serum albumin (Tris/BSA buffer, pH 7.4) either with (nonspecific condition) or without (total condition) 5 mM nicotine for 30 minutes at room temperature. The tissue sets were then incubated in Tris/BSA buffer containing [125I]- α -BTX (5 nM, specific activity 2000 Ci/mmol, Amersham Pharmacia Biotech, Piscataway, NJ) at 37°C for 3 hours. At the conclusion of the incubation period, the tissue was rinsed in Tris/BSA buffer for 5 minutes, in Tris buffer without BSA for 15 minutes, and in phosphate buffered saline (pH 7.4) for 5 minutes, all at 37°C. The tissue sections briefly dipped in distilled water, dried in a stream of cool air, and apposed to β -max film (Amersham) with [14C]standards of known radioactivity at room temperature for 3 days. Autoradiograms were then quantified with a computer-based image analysis system (C-Imaging Systems, Compix, Inc., Cranberry Township, PA) using calibrated standards of reference. A calibration curve of optical density against radioligand concentration (fmol/mg tissue) was constructed using the standards. Optical densities in discrete regions of the autoradiographic images were measured and corresponding values of radioactivity were determined by interpolation from the calibration curve.

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA) with Tukey-Kramer *a posteriori* analysis where appropriate

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Figure 1.

Mean ($\pm$ SEM) animal weights at the time of recording for sensory inhibition. Rats were 13-14 months old at the time of recording. Choline deficient rats (DEF) were the heaviest followed by normal choline rats (CON) and the choline supplemented (SUP) were the lightest (** $p < 0.01$; CON $n=14$; DEF $n=13$, SUP $n=7$).

Figure 2.

TC Ratios for rats gestated on control choline levels (CON, 1.1 g/kg of diet), deficient (DEF; 0 choline) or supplemented groups (SUP; 5 g/kg diet). Both the control and supplemented had TC ratios in the generally normal range for Sprague-Dawley rats. However, the rats gestated on the deficient diet showed significantly higher TC ratio's indicating loss of sensory inhibition. Data are mean $\pm$ SEM, CON $n=14$; DEF $n=13$, SUP $n=7$; ** $p<0.01$.

Figure 3. Representative wave forms for a rat gestated on control (A) and a rat gestated on deficient (B) choline diets. The choline supplemented rats did not differ from the control rats. The arrows note stimulus onset, the tick marks note the wave of interest.

Figure 4.

Sensory inhibition parameters for rats gestated on control (CON), deficient (DEF) and supplemented (SUP) choline diets. Conditioning amplitude (A) showed no effect of maternal dietary choline levels, while test amplitude (C) showed a significant increase in amplitude for animals gestated on the choline deficient diet. The conditioning latency did attain statistical significance in the ANOVA, though no 2 specific groups differed significantly (B). Test latency (D) did not differ between the groups. Data are mean $\pm$ SEM; CON $n=14$; DEF $n=13$, SUP $n=7$; ** $p<0.01$.

Figure 5. α -bungarotoxin binding levels in 3 regions of the hippocampus for rats gestated on control (CON), deficient (DEF) and supplemented (SUP) choline diets. None of the regions of the hippocampus (A) Dentate gyrus, (B) CA3 or (C) CA1, showed significant changes in levels of α -bungarotoxin binding as a result of the differences in choline levels in the maternal diet during gestation. Data are mean $\pm$ SEM, CON $n=14$; DEF $n=13$, SUP $n=7$.

Table 1

Weights of rats at time of recording for sensory inhibition

Treatment	Sex	N	Range	Average
Deficient choline	F	8	325-355	349
	M	6	400-590	523
Normal choline	F	7	355-425	391
	M	6	540-610	566
Supplemented choline	F	6	295-440	372
	M	1	625	625