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Review

Metabolic imprinting of choline by its availability during gestation: implications for memory and attentional processing across the lifespan

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Abstract

A growing body of research supports the view that choline is an essential nutrient during early development that has long-lasting effects on memory and attentional processes throughout the lifespan. This review describes the known effects of alterations in dietary choline availability both in adulthood and during early development. Although modest effects of choline on cognitive processes have been reported when choline is administered to adult animals, we have found that the perinatal period is a critical time for cholinergic organization of brain function. Choline supplementation during this period increases memory capacity and precision of the young adult and appears to prevent age-related memory and attentional decline. Deprivation of choline during early development leads to compromised cognitive function and increased decline with age. We propose that this organizational effect of choline availability may be due to relatively permanent alterations in the functioning of the cholinergic synapse, which we have called 'metabolic imprinting'.

Keywords: Choline; Aging; Spatial memory; Simultaneous temporal processing; Attention; Brain development; Cholinergic synapse; Acetylcholine

Contents

1. Introduction

Choline (trimethyl-beta-hydroxy-ethylammonium) is a quaternary ammonium compound that is an important nutrient for humans and other animals [118]. Although choline is classified as one of the B-complex vitamins, unlike other B-vitamins it serves as a structural component of tissues rather than a co-factor in metabolic reactions. In fact, it has been proposed that choline would be more properly classified as related to the amino acids. In addition to the critical role of choline as a precursor of phosphatidylcholine (PC) and sphigomyelin, two phospholipids that serve as components of biological membranes and as precursors for some intracellular messengers (e.g. diacylglycerol or ceramide) choline has several other functions: it is involved in fat metabolism in the liver; it plays a non-specific role as a source of biologically labile methyl groups; it is the precursor of two signaling lipids (i.e. platelet

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activating factor and sphingosylphosphorycholine), and of the neurotransmitter acetylcholine [16]. The work described in this review suggests that choline may, through one of these mechanisms, promote 'tuning' of cellular networks in the basal forebrain, hippocampus, and frontal cortex during early development, thereby leading to the long-term modification of cognitive processes [61,115].

The developing brain may have especially high demands for choline. Recently, the Food and Nutrition Board of the Institute of Medicine—United States National Academy of Sciences issued a report on B vitamins that provided recommendations for the adequate intake of choline [39]. This recommendation, to classify choline as an essential nutrient, was based, in part, on studies showing that the availability of choline during a specific prenatal period in rats [embryonic days (ED) 11 through 17 of the 22-day gestation period] has profound effects on cognitive performance throughout the lifespan [13,79–81]. This review will describe the known effects of alterations in dietary choline availability on attentional and memory processes during development, in adulthood, and in old age. This organizational change in brain function may be due to relatively permanent alterations in the functioning of the cholinergic synapse, which we have called 'metabolic imprinting'.

2. Choline as an essential nutrient in development

The nutritional requirements of the developing nervous system are complex and remain inadequately understood. Protein malnutrition during pregnancy results in multiple changes of brain development in the offspring which manifest as inferior scores on a variety of behavioral and cognitive tests in both human subjects and in experimental animals—for a review see Ref. [87]. Among the needs for individual nutrients, the requirement for essential fatty acids in nervous system development [32] and for folic acid during the periconceptual period in the prevention of neural tube defects [98] are generally acknowledged. The latter research findings led to new public policy aimed at women of childbearing age to ensure that their diets contain adequate amounts of folate. Less is known about the requirements of choline in pre- and early postnatal nutrition.

Choline is an essential nutrient necessary for the growth of mammalian cells [37]. Dietary choline deficiency in adult animals [50] and humans [20,120] causes a variety of systemic abnormalities including: fatty liver, infertility, hypertension, decreased plasma choline and more—for a review see Ref. [118]. In rats consuming a normal diet, pregnancy causes a dramatic depletion of choline pools [122], suggesting that choline requirements during pregnancy are increased and that the need for this nutrient by the mother and the fetuses may exceed the amounts provided by standard laboratory chow (and perhaps by some human diets as well). Increased need for choline continues into the period of lactation. Human milk and rat milk contain approximately 2 and 6 mM of choline-containing compounds, respectively [23,55]. Most of this choline is derived from maternal plasma. Thus, the transport of choline from mother to infant via the mammary gland constitutes an appreciable drain on maternal choline stores and makes her even more vulnerable to the effects of choline deficiency. Hepatic choline stores are lowest in lactating rats, followed by pregnant rats, with non-mated rats having the highest levels [122].

During normal development from fetus to adult, there is a progressive decline in blood choline concentration that begins in utero. In fact, serum choline concentrations are 6–7-fold higher in the fetus and neonate than they are in the adult [121,123]. This decline in serum choline concentration, towards adult levels, occurs during the first weeks of life [69]. High levels of choline circulating in the fetus presumably ensure enhanced availability of choline to tissues. We know that the developing rat brain efficiently extracts choline from blood and that blood and brain choline levels vary with choline availability [17,29]. In fact, supplementing choline to the diet of the mother rat during the perinatal period can further increase blood and brain choline metabolite concentrations of the fetus/pup [44]. When choline availability is modified during ED 11–17, choline deficiency decreases, and choline supplementation increases, the rate of cell division in the neuroepithelial layer of E18 hippocampus and septum. Modifications of choline availability also change the timing of migration, and commitment to differentiation of progenitor neuronal-type cells in fetal hippocampal regions [1–3]. Together, these data demonstrate that alterations in maternal dietary choline can directly affect choline availability to the brain and alter embryonic development.

3. Evidence that prenatal choline supplementation impacts the attainment of developmental milestones

During ontogeny, spatial navigation processes mature at different rates. For example, rats can locate goal objects by means of proximal cues that are co-occurrent with the goal object soon after they open their eyes. However, navigation using relational or distal cues, which requires the calculation of an unmarked location given multiple spatial coordinates, cannot be accomplished until 3–4 days later. In adult rats, performance on these two types of spatial localization tasks is dependent upon different neural substrates [97]. Hippocampal lesions disrupt performance on tasks that require distal/relational cue navigation, whereas performance using proximal cues is left intact [88]. Thus, it is possible that the onset of the ability to navigate using distal cues may be due to some aspect of the maturation of hippocampal function.

We have recently shown that the offspring of Sprague Dawley rat dams supplemented with 4.6 mmol choline chloride/kg/day from days 11 to 17 of pregnancy show a precocious ability to use relational cues to navigate in

a water maze task compared to offspring of dams fed a standard diet (1.3 mmol choline chloride/kg/day) [33,114]. This was particularly striking because all pups were raised in mixed litters by untreated foster mothers and there were no significant differences in pups' body weights or growth curves. Therefore, maternal factors and postnatal experience are not likely to have contributed to this alteration in maturation rate.

Specifically, we have found that prenatal choline supplementation advances, at different developmental periods for the acquisition and retention phases of the test session, rat pups' ability to use relational cues to learn and remember the location of the hidden platform. Naïve 18–19 day old choline-treated rats demonstrated learning during the acquisition trials of their first testing session. In contrast, naïve control rats did not demonstrate learning during the acquisition trials of the first testing session until they were 21–22 days of age. Both groups of rats demonstrated learning during the acquisition trials when they were re-tested three days after the initial training at 21–22 days of age. Therefore, at 21–22 days of age, both naïve and experienced control rats could use relational cues to navigate.

Results for retention trials revealed a slightly different pattern. Experienced 21–22 day old choline-treated rats demonstrated memory during the retention trials, while control rats demonstrated memory during the retention trials only when they reached 24–25 days of age, three days after the choline-treated rats. These results may occur because there appear to be ontogenetic differences in the timing of when water maze spatial learning can be retained. Forgetting occurs after a three-day delay in preweanling rats, but not in adults, and forgetting in juveniles can be alleviated by a single retraining trial [18]. Thus, differences between our acquisition and retention trials are probably due to the development of memory systems more generally.

Together, these results indicate that prenatal choline supplementation accelerates the maturation of relational cue processing. This comparison of water maze performance for prenatal choline-supplemented versus untreated rats at three developmental time-frames between postnatal day (PD) 18 and 28 provides strong evidence that the development of relational, but not proximal spatial cue processing is advanced by as much as three days for choline-treated rats. How and in what way this alteration in brain development is manifested in the adult rat is described in Section 4.

4. Cognitive enhancing effects of choline supplementation administered in adulthood and old age

Under certain conditions increasing the availability of choline or lecithin (precursors for acetylcholine) may enhance cholinergic (Ch) transmission [28]. Moreover, when Ch neurons are physiologically active, the rate at which they synthesize and release acetylcholine varies directly with the amount of free choline available to them [10,16]. Because Ch neurons are important for both short- and long-term memory processes in a variety of species [8,11], the findings that choline availability modified Ch transmission stimulated research on the behavioral effects of modifying choline intake during adulthood and old age.

Although increasing the amount of choline or lecithin can improve performance on certain behavioral tasks in mature subjects [8,9,72,83], attempts to improve memory performance in aged humans or animals have not generally succeeded—for review see Ref. [8,11]. It has been suggested that one reason for this failure in geriatric subjects may be that the aged brain is unable to incorporate extra amounts of choline into acetylcholine, as reportedly occurs in younger brain tissue [9]. It is also possible that it may be necessary to improve other factors in aged brains before substantial increases in presynaptic Ch effects are obtained with precursor loading. For example, although normal Ch activity is dependent upon intact oxidative metabolism, it is known that several parameters that reflect energy production are decreased in the aged central nervous system [82,102,104]. Further, although the conversion of choline into acetylcholine occurs more readily under conditions of increased neuronal activity, recent evidence suggests the activity of certain Ch pathways progressively decreases with age [42,73]. Thus, either of these (or similar) factors could contribute to a situation in the aged brain that would prohibit extra choline from being effectively utilized for the synthesis of additional acetylcholine, and in turn, explain the negative results obtained with precursor studies in aged animals and humans.

One way to compensate for these age-related behavioral deficits would be to administer abundant amounts of choline not to mature or aged animals, but to young animals during pre- and postnatal stages of development. As was described above, dietary intake of choline by the mother contributes to high serum choline concentrations in the neonatal rat [113] and the neonatal blood–brain barrier readily transports choline into the brain [86]. It is possible that organizational effects produced by the short-term administration of choline during neonatal development may alter the course of brain development, produce long-term modification of attention and memory processes that remain functional throughout adulthood, and aid in the lessening or prevention of age-related cognitive impairments.

5. Memory enhancing effects of perinatal choline supplementation

We have reported that choline supplementation during both pre- and postnatal development has long-term facilitative effects on spatial navigation that extend well into adulthood [80,81]. And, these effects have been replicated and extended in important ways by other laboratories [96,101,106–109].

Memory processes were investigated using a 12-arm radial maze food-searching task, with eight baited and four unbaited arms. This task includes both episodic memory (also referred to by Ref. [89] as 'working memory') and reference memory components [89]. During each test, on the first approach to each baited arm the rat ought to choose that arm and get the food from the end of it. In all subsequent approaches during that day's test, the rat should not select a previously chosen arm because the food has been removed. Consequently, the rat must remember each choice for the duration of each test, but at the end of the test the rat ought to forget these choices so that they will not interfere with its performance in subsequent tests. Thus, errors made to previously visited arms constitute a measure of the reliability of episodic memory (i.e. memory for individual events). There are also reference memory components of the radial arm maze task. These include information about the fact that food is found on the ends of the arms, about the motor coordination that is required to stay on the arms, and particularly which set of arms in the maze are consistently baited and which are consistently not baited.

Choline supplementation during two sensitive periods of early development (see below) led to a significant reduction in both the number of episodic memory errors and the number of reference memory errors made both during acquisition and at steady-state performance, even when rats were trained daily for 12–16 weeks. This behavioral facilitation observed in the spatial memory task was due, in part, to an increase in memorial capacity and a reduction in proactive interference. These changes in memory allowed for greater discrimination and less generalization among spatial locations both within and between test trials for rats treated perinatally with choline. Additional experimental evidence for choline-induced improvement of spatial memory has been obtained from two strains of rats (Long-Evans and Sprague-Dawley) and can be outlined as follows.

Perinatal choline treated rats show a constant improvement in visuospatial memory scores compared to control rats as a function of training that is proportional to the memory set size employed [80,81]. The fact that a constant percentage improvement in choice performance is observed at all stages of training strongly implicates a memory effect rather than a change in learning rate parameters.

Perinatal choline-supplemented rats are able to retain a larger number of items in working memory at any one time than control rats. This claim is supported by the finding that choline-treated rats are able to visit a greater number of different visuospatial locations before losing track of selected locations and thereby repeating one already visited [80,81]. In addition, rats given perinatal choline supplementation have an increased threshold for applying a chunking strategy in the radial-arm maze when arms can be clustered by food type [76]. That is, when the maze arms are baited by stable patterns of food of different hedonic values (i.e. four sunflower seed arms, four rice puff arms, four standard food pellet arms), rats are able cluster or 'chunk' their arm choices by food type. Chunking strategies are employed by rats when normal memory capacity has been exceeded by task demands, and its implementation by adult subjects can be used to index memory capacity and efficiency on the radial-arm maze task as a function of perinatal choline availability.

Perinatal choline-supplemented rats, in contrast to control rats, show little or no proactive interference as a function of massed trials in a visuospatial memory task [78,95]. This observation supports the claim that perinatal choline supplementation leads not only to increased memory capacity, but also to increased memory precision allowing for greater discrimination among trials. Otherwise, greater proactive interference would be expected for choline-supplemented rats because of their increased memory capacity and ability to retain a larger number of items.

Behavioral data indicate separate pre- and postnatal sensitive periods for choline-induced facilitation of visuospatial memory [79]. Two prenatal and five postnatal supplementation periods spanning ED 6 to PD 75, as well as control treatments, have been evaluated. The results show choline supplementation improved visuospatial memory only when given during the ED 11–17 and the PD 16–30 time frames. The first sensitive period occurs during the same time period when Ch neurons in the basal forebrain are undergoing neurogenesis [100]. During the second, later time period, neurons in the basal forebrain undergo remodeling, and the dentate gyrus, thalamus, and other related brain regions dendritic undergoes dendritic growth and synaptogenesis [49].

It is of particular interest that no facilitation of memory processes was observed when choline was supplemented during the week or so after birth. There are several potential reasons for this 'off period' of choline sensitivity. Even when newly parturient rats are consuming a standard diet, choline levels in pup blood and brain are exceptionally high because choline and choline metabolites in mother's milk are at their highest levels immediately after birth [119,121]. Thus, pups may already be consuming sufficient choline from mother's milk in the week or so after birth, and our supplemented choline diet may not add to this already saturated system. An alternative hypothesis is that choline supplementation is only effective during times when specific brain systems (i.e. the cholinergic system) is undergoing phases of development when extra choline can be used to alter synaptic functioning. Further evidence in support of this hypothesis will be described later in this review.

6. Age-related decline in cognition

One of the most consistent findings in the gerontological literature on cognition is an age-related decline in attention, spatial learning and memory abilities [43]. These deficits have been noted in tasks that require people to accurately

find their way around in either artificial or real environments [19,38,60,112] and in tasks that require rats to locate a place or places to find reward using distal visual environmental cues for accurate navigation [5,7,34,35,42,47]. Because the brain areas necessary for proper performance of spatial tasks have been reasonably well characterized in the rat [4,6,7], this species has been an excellent model in which to test potential therapeutic strategies for ameliorating cognitive aging deficits [36]. In fact, correlations have been obtained between spatial behavior in mature and aged rats and a number of anatomical, neurochemical, and electrophysiological changes in the hippocampus and frontal cortex [4,47,58,81,105]. Recent work has extended these correlations to attentional mechanisms [99,103].

There have been several successful attempts to alter spatial memory and attention in old rats. One strategy has been to inject fetal cell suspensions into the hippocampus of old animals in an attempt to enhance cholinergic function of the hippocampus and as a consequence, improve their performance on learning and memory tasks [27,40,41]. The data from these studies have been extremely encouraging. Old animals have been shown to improve markedly in their ability to locate accurately a submerged, hidden platform in a Morris water pool after such a graft. However, because the extent of septal cell ingrowth and extent of behavioral recovery were not well correlated, the possibility exists that diffuse transmitter release might play an important role in this recovery process. For this and other reasons, major efforts continue in the exploration of chemical therapeutic intervention methods that target the cholinergic, monoaminergic, hormonal, and other systems with the goal of improving a variety of cognitive processes in either an organizational (long-term effect produced by a transient treatment) or activational (short-term effect occurring only in the presence of the drug treatment) manner [5,9,10,93].

7. Effects of perinatal choline availability on age-related declines in spatial memory

The effects of perinatal choline treatment on Ch activity and memory function appear to be long-lasting. As part of a long-term study designed to evaluate the longevity of choline-induced memory improvements, rats given control, prenatal choline-supplementation (SUP), or prenatal choline-deficiency (DEF) treatments have been observed in both cross-sectional and longitudinal designs until 27 months of age [13,79]. In contrast to the CON and DEF rats that showed impaired performance with age, SUP rats did not exhibit any age-related decline in choice performance when tested on a 12-arm radial maze between the ages of two and 27 months. For the cross-sectional study, all 12 of the maze arms were baited and the number of episodic memory errors that occurred during a trial as a function of the serial (choice) position are shown for the aged (26 months) rats in Fig. 1. These data represent the first 15

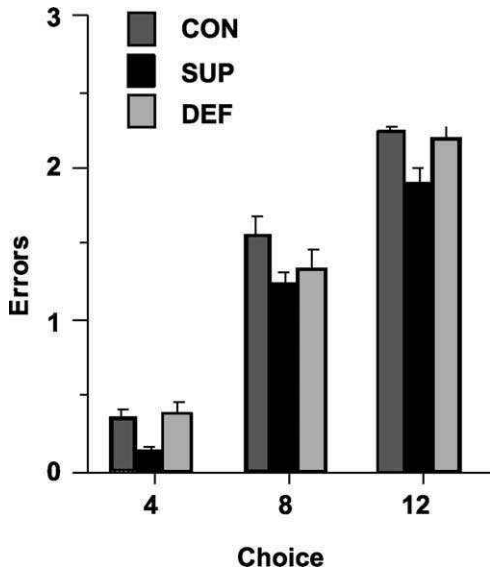

Fig. 1. Cross-sectional study. Mean (± S.E.M.) number of working memory errors on the 12-arm radial maze as a function of choices (1–4, 5–8, and 8–12) and treatment. Rats treated prenatally (ED 11–17) with choline supplementation (SUP), choline deficiency (DEF), or untreated controls (CON) trained for 15 consecutive sessions at 26 months of age. Rats were experimentally naïve at the beginning of this training phase. An ANOVA conducted on the cumulative errors indicated a significant effect of treatment, $F(2, 36) = 6.1$, $p < 0.01$. Fisher PLSD tests indicated significant differences between the CON vs. SUP and the SUP vs. DEF comparisons, $p's < 0.05$.

sessions of exposure to maze task for these aged rats (males and females combined, $n = 10$). In addition to observing how error rates change as a function of the serial (choice) position within a trial, the total number of errors prior to locating all baited maze arms can be examined. For this measure, the SUP group showed significantly fewer errors than did either the DEF or CON groups.

For the longitudinal study, the number of choices required to locate all baited arms (choices to criterion) as a function of the mean age during the 30 consecutive daily sessions are shown in Fig. 2. Male ($n = 10$/group) and female ($n = 10$/group) rats were trained with the mixed-baiting paradigm of baited and unbaited arms described previously. This procedure discourages the development of response algorithms and also provides measures of both episodic and reference memory. These data indicate that SUP rats (both males and females, combined on Fig. 2) show little, if any, age-related decline in performance of this spatial task even at 27 months of age. In contrast, the CON and DEF treatment groups display significant declines in choice accuracy as a function of age.

Following an initial stage of baseline training rats were assessed on a massed-trials procedure used to evaluate the degree of proactive interference as a function of sequential trials ($n = 5$) being rapidly presented within a single daily session. These data are shown in Fig. 3 and indicate that SUP rats exhibit a significant reduction in proactive interference compared with control rats. DEF rats, although

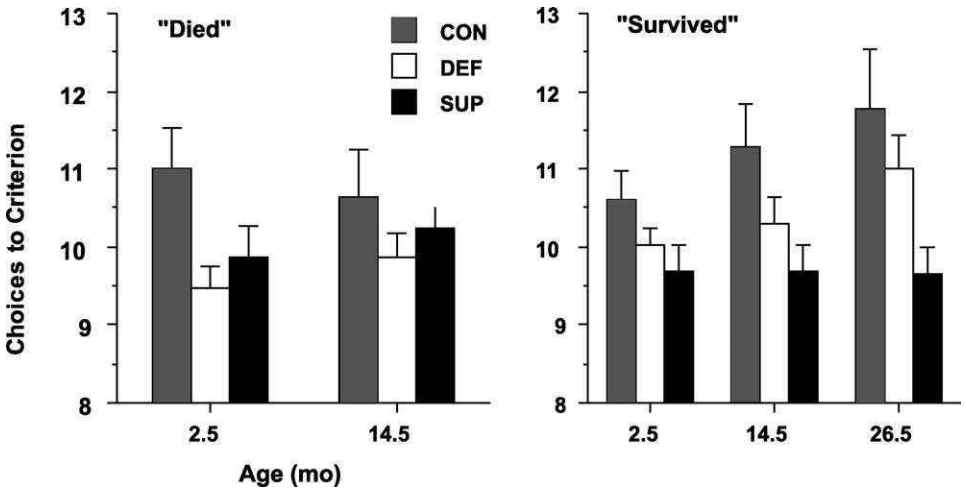

Fig. 2. Longitudinal study. Mean (± S.E.M.) choices to criterion on the 12-arm radial maze as a function treatment of treatment and age. Rats treated prenatally (ED 11–17) with choline supplementation (SUP), choline deficiency (DEF), or untreated controls (CON) were behaviorally evaluated for 30 consecutive sessions at different ages. Data for rats that died prior to the third test phase are plotted in the left panel. Data for rats that completed all three phases of training are plotted in the right panel. These data indicate significant improvements in choice performance for both male and female rats (data combined) given prenatal choline supplementation at all ages and an age-related decrease in choice performance only for the CON and DEF treatment groups, p's < 0.01. Although rats in the DEF treatment group displayed rates of age-related decline similar to rats in the CON group, their overall choice accuracy was reliably better than CON rats.

they initially show better performance than CON rats, show the highest levels of proactive interference as subsequent trials are massed. This result indicates that DEF rats are much more susceptible to the effects of interfering stimuli when attention and memory processes are stressed than CON rats. In contrast, SUP rats show dramatically reduced levels of proactive interference under these test conditions.

Finally, a retention-interval procedure was used in which a varying delay or retention interval was inserted between the fourth and fifth correct choice during a single daily trial.

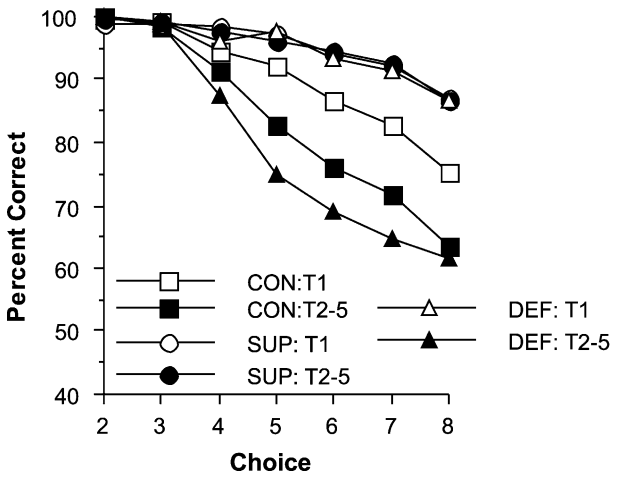

Fig. 3. Mean percent correct for massed-trials performance on the 12-arm radial maze as a function of the choice sequence for Choices 2–8 during Trial (T) 1 and the mean of T's 2–5. Rats treated prenatally (ED 11–17) with choline supplementation (SUP), choline deficiency (DEF), or untreated controls (CON) were behaviorally tested as aged adults. SUP rats exhibit a large reduction in proactive interference compared with CON rats. DEF rats on T1 exhibit reliably better performance than CON rats, however, they also show the highest levels of proactive interference as trials are massed, p's < 0.05.

These delays ranged between 1.25 and 10 h, during which time the rat was placed in its home cage on the cart used to transport them to the test room. After the delay the rats were replaced on the maze, which was baited in the same manner

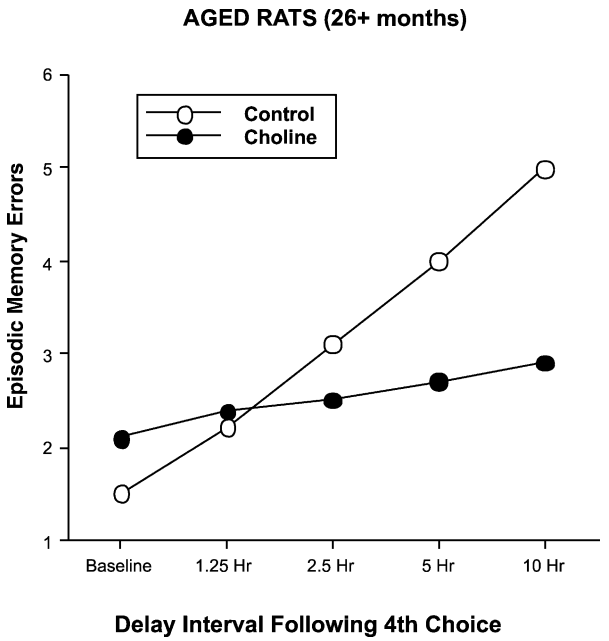

Fig. 4. Mean 12-arm radial maze choice performance during a retention interval-test as indexed by episodic memory errors (arm repeats) for prenatally choline-treated rats and untreated control rats, reveal that prenatal choline treatment rescues the age-related decline in memory retention seen in untreated rats. Data are taken from a longitudinal study in which male rats received 30 consecutive training sessions beginning at 9 and 26 months of age. Baseline training refers to trials without the insertion of a delay interval. An ANOVA indicated significant main effects of treatment and delay interval as well as a significant treatment × delay interval interaction: $F(1, 12) = 7.65$, $p < 0.02$, $F(4, 48) = 12.38$, $p < 0.001$, and $F(4, 48) = 4.28$, $p < 0.01$, respectively.

in which they had left it, and were allowed to complete the trial. Performance during this phase of the experiment was evaluated relative to the baseline levels of performance during which no delay was interposed between choices. The results from this retention-interval procedure revealed significant treatment effects consistent with previous findings by showing a reduction in the rate of forgetting for the SUP rats as shown in Fig. 4.

8. Effects of perinatal choline availability on attention and cognitive function

The ability to perform tasks requiring attention and memory declines with aging in rodents, non-human primates and humans; however, the central mechanisms responsible for senescent cognitive decline are not well understood [30,31,99]. In the interval timing literature, probability of attention, $p(A)$, to the relevant stimulus dimension has proven to be a useful measure for analyzing situations where animals are not always attentive to signal duration. Although the information-processing model described by Gibbon, Church, and Meck [48] characterizes many trials in a time discrimination task, on some trials the animal appears to respond independently of the signal duration. Quantitative fits of performance are often improved if it is assumed that, with some probability, the animal responds on the basis of signal duration in a temporally graded fashion and, on the remainder of the trials, the animal responds at a relatively constant rate without respect to signal duration. On these 'inattentive' trials the animal selects the response on the basis of some constant bias. In temporal generalization tasks, most of the between-subject variance can be accounted for by parameters representing the probability of attention and responsiveness given inattention [26].

Perinatal choline-supplemented rats demonstrate a greater degree of temporal control for a lever-press response in peak-interval timing procedures compared to control rats [75]. This conclusion is supported by the observation of a discrete number of multiple response states (e.g. 3 or 4) that are regulated in a hierarchical manner for choline-supplemented rats, in contrast to the lower number of response states (e.g. 1 or 2) typically exhibited by control rats. Because the transition from one response state to another results in an increased rate of responding with a progressive reduction in variability, a 'temporal cascade' model of interval timing can be used to describe the data set obtained from choline-supplemented rats [48] Basically, these findings indicate that a larger number of independent response thresholds are maintained by choline-supplemented rats compared to control rats. These response thresholds may be considered independent, in the sense that as the criterion time approaches, each successive threshold has a smaller associated variance measure. This type of hierarchical control of timing behavior allows rats to predict with greater accuracy, the exact temporal location of the target being tracked.

8.1. Simultaneous temporal processing

Simultaneous temporal processing (STP) can be studied in situations where different signal modalities (e.g. auditory, tactile, and visual cues) are each paired with a unique temporal criterion (e.g. 15, 30, or 60 s) and are presented concurrently in an asynchronous fashion with random onset times. In most experiments, these stimuli are presented in a hierarchical manner where the shorter signal durations are embedded within the longer signal durations [70,90]. Presumably the $p(A)$ to each of these multiple signals is free to vary independently, thus allowing for the calculation of the probabilities that animals are attending to one, two, or all three of the concurrently elapsing signal durations. Under normal testing conditions animals (e.g. rats) are apparently incapable of attending to all three signal durations simultaneously on every trial. The data indicate that attention is allocated in a hierarchical manner with the $p(A)$ decreasing with the order of stimulus onset—which is also correlated with signal duration due to the fact that the shorter signal durations are embedded within the longer signal durations. Meck [70] demonstrated that the $p(A)$ to each of three concurrently presented signal durations could be increased in a proportional manner by the administration of vasopressin neuropeptide. That is, attention to a particular signal increased, not by an absolute amount, but by an amount proportional to the distance between the saline treatment performance and the assumed asymptotic level of performance [$p(A) = 1.0$]. This proportional result implies that the rate of signal processing by the rat's attentional mechanisms was increased by drug administration. Increasing the speed of mental operations would be expected to be advantageous in that more operations per unit of time can be executed without overloading the system. Such an increase in speed might lead to an enhancement in the ability to detect multiple signals while at the same time maintaining the temporal integration processes associated with each signal. Such a result can be shown to produce proportional increases in the $p(A)$ to each of the signals being attended [70]. Moreover, recent work [71] has indicated that cortical cholinergic systems are involved attending to multiple signals in parallel. In this context, it is important to note that the duration of a signal is a dynamic rather than a static stimulus property. Because of the demonstrated independence of the timing processes for the different signals presented in STP procedures, previous investigators have proposed that multiple temporal integration processes operate in parallel and that attention can be shifted among these different pacemaker/accumulators at a relatively high rate in order to control the necessary response states [48]. Consequently, investigations have explored the possibility that the behavioral enhancement observed in tasks that measure visuospatial and temporal memory in perinatally

choline supplemented rats may be accounted for, in part, by alterations in attentional processing mechanisms. In particular, the relationship between timing multiple stimuli concurrently and the allocation of attentional resources as a function of perinatal choline supplementation or deficiency was examined. Some of these results are presented in Fig. 5, showing age-related declines in STP, which are reduced in rats that had been treated with prenatal choline supplementation and exacerbated in rats that had experienced prenatal choline deficiency.

The variables of major interest in our recent experiments [77] have been choline availability during prenatal development and the age of the rats during behavioral evaluation. Age-related discrepancies in the content of temporal memory have been observed for aged rats trained on variants of the peak-interval timing procedure similar to the one used in this study [73]. These effects included an increase in peak time and a broadening of the response function as rats aged from 10 to 30 months of age. The data shown in Fig. 5 also reveal an increase in peak time that interacted with age and prenatal treatment condition. CON rats demonstrated a small but significant increase in peak time as a function of age that was proportional (11.4 ± 0.9%) to the signal durations being timed. DEF rats demonstrated a similar, but larger, increase in peak time as a function of age that was also proportional (17.6 ± 1.1%) to the signal durations being timed. In contrast, SUP rats did not show any reliable changes in peak time as a function of aging for either signal duration. These results are reminiscent of the finding that systemic injections of arginine vasopressin to 10–13 month old rats prevented age-related discrepancies in the content of temporal memory and the associated increases in sodium-dependent high affinity choline uptake in the frontal cortex when they became aged (27–30 months) [73].

Although there was a significant treatment effect on peak rate, with SUP rats demonstrating the lowest response rates, peak rate did not change in any straight-forward fashion as a function of aging. The changes that were observed involved an interaction among signal duration, age, and treatment such that CON and DEF rats exhibited an age-related decline in peak rate for the 15-s signal duration while SUP rats exhibited an increase in peak rate for the 15-s signal as a function of age. In contrast, these trends were either diminished or reversed for the 30-s signal, thereby leading to a modest, but significant, interaction [77].

As outlined above, the results obtained for the $p(A)$ response measure are arguably the most interesting. Investigators have previously examined the major components of attentional processes (i.e. perceptual sensitivity, response criterion, and processing capacity) in order to determine whether they are relevant to the investigation of the neuronal basis of age-related changes in cognitive abilities [99]. The current results indicated that in our STP task rats show more temporal control for the shorter signal (15-s signal) than for the longer signal (30-s signal). This is most likely due to the fact that the 15-s signal indicates a shorter delay to reinforcement than the 30-s signal and hence is more highly valued or preferred. This difference in temporal control exerted by the two signals provided an opportunity to observe interactions between different signal durations as a function of prenatal choline availability and age. The results indicated that for the 15-s signal, both the SUP and the DEF rats allocate significantly higher levels of attention (as indexed by degree of temporal control) to the 15-s signal than the CON rats. When examining the results

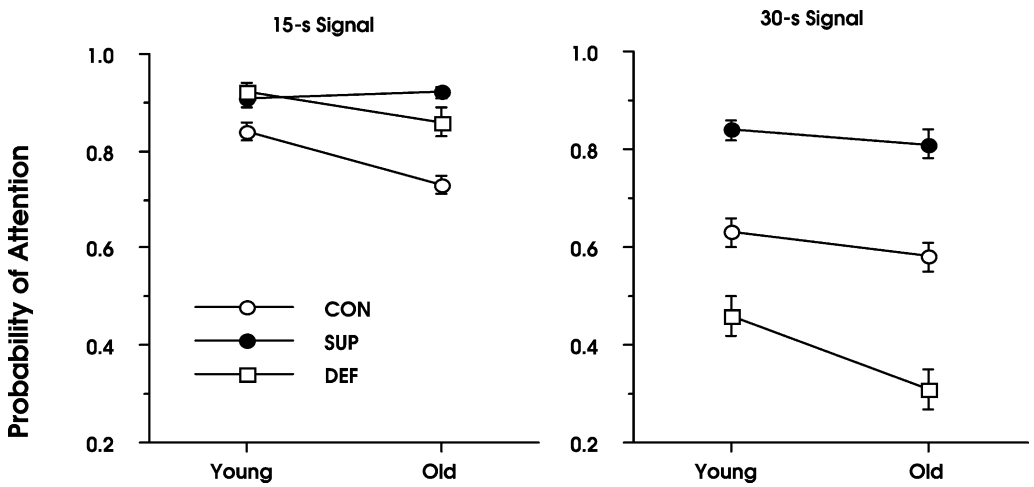

Fig. 5. Mean (±S.E.M.) probability of attention, $p(A)$, as a function of prenatal treatment and age at time of behavioral evaluation (young = 2–4 months, old = 24–26 months) for the 15-s signal (left panel) and the 30-s signal (right panel). Rats treated prenatally (ED 11–17) with choline supplementation (SUP), choline deficiency (DEF), or untreated controls (CON) were behaviorally evaluated for 30 consecutive sessions on a peak-interval timing procedure using 15 and 30-s temporal criteria. A repeated measures ANOVA with signal duration nested within age indicated significant main effects of treatment, signal duration, and age; $F(2,27) = 42.78$; $p < 0.0001$, $F1,27 = 324.24$; $p < 0.0001$, and $F(1,27) = 54.08$; $p < 0.0001$, respectively. The age × treatment and signal duration × age × treatment interactions were also significant; $F(2,27) = 9.28$; $p < 0.001$ and $F(2,27) = 3.98$; $p < 0.05$, respectively. In contrast, the signal duration × age interaction was unreliable; $F(1,27) = 0.77$; $p > 0.05$.

for the 30-s signal, a similar pattern is observed for the SUP and CON rats, while the DEF rats allocate significantly less attention to the 30-s signal. This loss of temporal control in the DEF rats suggests a failure of divided attention. In addition, the $p(A)$ to both the 15 and 30-s signals declines reliably with age for both the CON and DEF rats, but not for the SUP rats. Furthermore, this age-related decline is exacerbated for the DEF rats when they are timing the 30-s signal.

The proposed explanation for these changes in memory and attention is an alteration in processing speed for the brain regions that contribute to the temporal control of behavior. If it were the case that prenatal choline supplementation increased the speed of information processing, then one might expect to observe proportional increases in the probability of attention being divided among multiple stimuli. In addition, prenatal choline supplementation is associated with a reduction in the age-related decline of temporal control and attentional processes. In contrast, prenatal choline deficiency leads to an apparent decrease in processing speed and forces DEF rats to selectively attend to stimuli rather than process them in parallel by dividing attention among relevant events. Consequently, an increase in attention to the primary signal is observed concomitant with a large decrease in attention to the secondary signal. These effects are also associated with an acceleration of the age-related decline of temporal control and attentional processes as rats that were choline deficient during ED 12–17 reach 24–26 months of age.

8.2. Conditioned stimulus processing

Deficits in attention and the depth and speed of processing have been considered as prime sources for the age-related decrements in animal and human memory [30,31]. Contemporary learning theorists have suggested that exposure to a sequence of conditioned (CS) and unconditioned (UCS) stimuli can alter the attentional processing of future events that reliably predict reinforcement [64,92]. Animals will attend to good predictors of reinforcement, and will not attend to less reliable cues or to cues that offer redundant information. In addition, animals will attend to cues that deviate from what is expected or predicted based upon their past conditioning history. Therefore, subjects possess the ability to increment as well as decrement attentional resources to differential processing of environmental cues.

Lesions of the hippocampus or its cholinergic inputs from the medial septum/vertical limb of the diagonal band (MS/VDB) have been shown to disrupt the normal decreases in conditioned stimulus processing that are dependent upon attentional mechanisms. Evidence of this effect includes an absence of latent inhibition in lesioned animals as well as a lack of reduced processing of a cue that had been a consistent predictor of another conditioned stimulus in a serial conditioning procedure used to study increments in attentional processing of visual cues [12,24,25]. In contrast, other research with these same tasks has provided evidence of another complementary system that controls incremental (as opposed to decremental) processing. This system includes the central nucleus of the amygdala (CN) and its projections to the nucleus basalis magnocellularis/substantia innominata (nBM/SI). Lesions of the CN or of cholinergic neurons in the nBM/SI spare a subject's ability to decrement attention to a conditioned stimulus that is presented repetitively in the absence of an unconditioned stimulus (e.g. latent inhibition) but eliminate the incremental processing that typically occurs when stimulus parings are altered in a serial conditioning procedure [24,52]. It has been suggested that the role of the hippocampus in decremental processing may be similar to the function of the CN in incremental processing by regulating activity in the cortical targets of the MS/VDB (e.g. cingulate cortex via its efferent projections to the septum [45,46]).

As indicated above, Holland and Gallagher [52,53] provide evidence that these two attentional pathways are separate and can be experimentally manipulated. These investigators used a serial conditioning procedure first described by Ref. [116], in which the accuracy of a light CS in predicting the occurrence of another CS, a tone, (rather than it accuracy in predicting the US) was manipulated. As Holland and Gallagher [52] indicate, this procedure has three training phases and is somewhat complicated, but its complexity permits ruling out a variety of less interesting accounts of the results. Their data suggest that lesions to the CN disrupt incremental attentional processing in a serial conditioning procedure. Phase 1 of this procedure entails the presentation of a light, followed by a sound, which is then followed by the delivery of food only on half of the trials. Therefore, the light reliably predicts the sound and because it represents redundant information concerning the availability of food, attentional resources allocated to the light should decrease as the rat habituates to its repeated presentation. Phase 2 of the experiment is the same as Phase 1 for half of the rats (Non-Shift group). For the remaining rats (Shift group), however, on half of the trials the light is followed by the sound, which is followed by the delivery of food (as in Phase 1), but on the other half of the trials, the light is presented alone. On these trials, the light no longer reliably predicts the sound and in so doing deviates from what is expected from Phase 1. As a result, attentional processing to the light should increase because it has lost its predictive value. During Phase 3 of the experiment, conditioning to the light is examined by presenting the light followed by the delivery of food on all trials. If attentional processing (and therefore associative ability) has been lost to the light, the light/food association should take longer to condition, however, if an increase in attentional processing has taken place, the light/food association should be acquired faster. For shifted control rats, the association that light reliably predicts the delivery of food is attained quickly, while the association takes

longer to condition in unshifted rats. Shifted rats with lesions in the CN, however, fail to show enhanced conditioning to the light, suggesting that lesions to this part of the amygdala disrupt increments in attentional processing. In fact, the CN-lesion rats showed the opposite pattern of data from the control rats. Not only did the reduction in the light cue's predictive accuracy fail to enhance attention to that cue, but the rats apparently attended to the light even less after light → tone → food, light → nothing training than after light → tone → food, light → tone → nothing training in Phase 2. This outcome was anticipated by the investigators due to the absence of the change in controlled processing predicted by Pearce and Hall [92]. 'Several investigators have shown that more habituation and latent inhibition occur to a cue presented alone than to one presented in compound with another stimulus [51,62]. Without the countervailing tendency of enhanced processing prompted by reductions in the light's predictive accuracy, the effects of decremental changes in attention that also normally occur as a result of simple non-reinforced presentations of the light would be unmasked. Consequently, rats in the CN-lesion Shift group would display less conditioning than those in the CN-lesioned Non-Shift group' (Holland and Gallagher [52], p. 251).

In a recent series of experiments, the effects of prenatal choline supplementation and deficiency on changes in the ability to increase attention to a conditioned stimulus (light-CS) when its relationship to another cue (tone-CS) is altered in a serial conditioning procedure were studied [74]. Adult male Sprague-Dawley rats (3–6 months) that had been given SUP, CON or DEF diets from ED 11–17 were tested on the attentional procedure of Holland and Gallagher [57] described above. CS-related behaviors (e.g. rearing, head-jerking, and food-cup entries) did not differ among any of the treatment groups during Phases 1 and 2. In Phase 3, all rats received light-CS–US pairings and the acquisition of conditioning to the light-CS cue was indexed by head entries into the food cup. The results from Phase 3 of training are presented as CON vs. SUP and CON vs. DEF comparisons in Figs. 6 and 7, respectively.

The similarity of the CON/Shift, SUP/Shift, and SUP/Non-Shift data shown in Fig. 6 suggests either that SUP rats did not decrement attention to the light-CS during Phases 1 and 2 of training or that the Shift condition was ineffective in increasing attention to the light-CS during Phase 3. Because CS-related behaviors of SUP rats did not differ from CON rats during Phases 1 and 2 (p's > 0.05), tests of latent inhibition will be necessary in order to evaluate whether or not SUP rats are impaired in their ability to decrement attention to unpredictive signals. Furthermore, the similarity of the CON/Non-Shift and DEF/Non-Shift data shown in Fig. 7 in comparison to the opposite effects observed for the CON/Shift and DEF/Shift rats suggests that DEF rats were unable to increment attention to the light-CS during Phase 3 of training thus leaving decremental mechanisms unopposed. Because

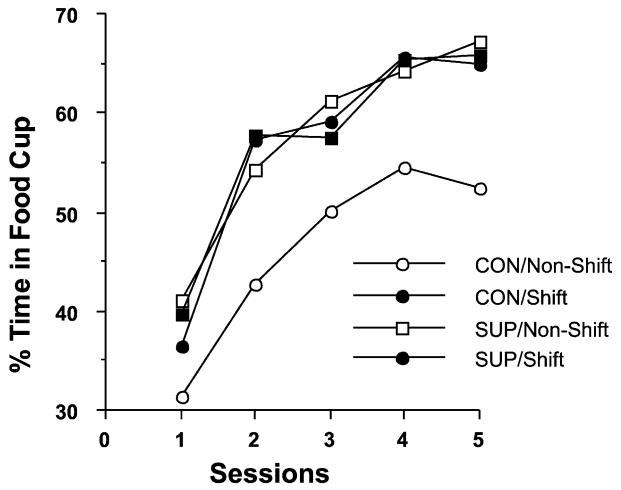

Fig. 6. Rats treated prenatally (ED 11–17) with choline supplementation (SUP) and untreated controls (CON) were behaviorally evaluated for their responding to the light CS during Phase 3 of training. Mean percent time in food cup is plotted as a function of trial type (Non-Shift vs. Shift) and session blocks for each treatment group (see text for explanation of procedure). The main effects of treatment and sessions were found to be significant (p's < 0.05) as well the treatment × trial-type interaction: $F(1, 28) = 5.17$, $p < 0.05$.

CS-related behaviors of DEF rats did not differ from CON rats during Phases 1 and 2 (p's > 0.05), tests of latent inhibition should show either normal or enhanced ability to decrement attention to unpredictive signals.

Overall, CON/Shift rats exhibited significantly more food-cup behavior during the presentation of light-CS compared to CON/Non-Shift rats, replicating earlier findings [52] and suggesting an increase in the associative value of the light-CS in the Shift condition. Rats in both

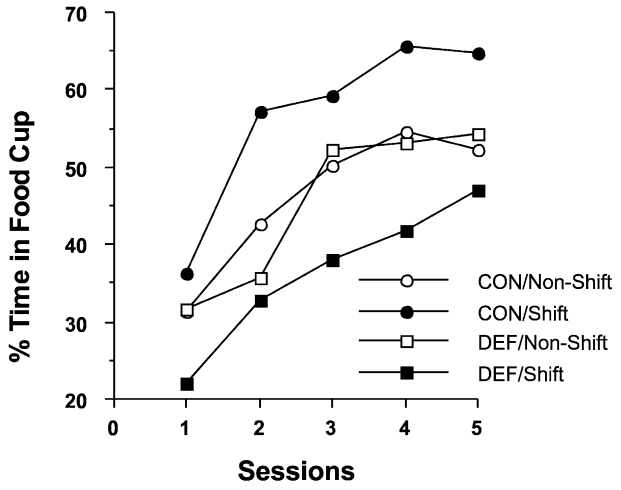

Fig. 7. Rats treated prenatally (ED 11–17) with choline deficiency (DEF) and untreated controls (CON) were behaviorally evaluated for their responding to the light CS during Phase 3 of training (see text for details of procedure). Mean percent time in food cup is plotted as a function of trial type (Non-Shift vs. Shift) and session blocks for each treatment group. The main effects of treatment and sessions were found to be significant (p's < 0.01) as well the treatment × trial-type interaction: $F(1, 28) = 6.22$, × $p < 0.02$.

the SUP/Shift and the SUP/Non-Shift groups showed enhancement of the associability to light-CS in a manner similar to the CON/Shift rats. In contrast, DEF/Shift rats showed significantly less food-cup behavior than the DEF/Non-Shift rats.

Interestingly, the behavioral data from choline-deficient rats are virtually identical to the pattern of results obtained by Holland and Gallagher [52] for rats with CN lesions. In addition, the behavioral data obtained from the choline-supplemented rats is very similar to the pattern of results obtained by Baxter, Holland, and Gallagher [12] for rats with 192 IgG-saporin lesions of Ch neurons in the medial septum/vertical limb of the diagonal band. Consequently, the major questions are: (1) is there an interaction between perinatal choline availability and aging as a function of conditioned stimulus processing? (2) Do these different prenatal treatment groups show comparable effects on tests of latent inhibition? (3) Does choline-deficiency impair the ability to increment conditioned stimulus processing by altering processing in the CN? (4) Is the apparent similarity of the choline-supplemented rats to rats with lesions of hippocampal Ch input the result of increased attentional processing and a greater degree of light–tone association or a basic disruption of decremental conditioned stimulus?

One way to determine the basis for these intriguing results is to analyze the sequential conditioning preparation for the strength of light–tone associations; something not done in earlier work because of the assumption that such associations are non-existent or very weak. In addition, it is critical to examine decrements in conditioned stimulus processing directly by assessing the strength of latent inhibition rather than inferring disruption of decrements in conditioned stimulus processing from the sequential conditioning procedures that are specifically designed to assess the effects of increments in conditioned stimulus processing.

Recently we have examined the effects of prenatal choline availability on another form of attention (e.g. sustained attention) [84]. This type of attention can be indexed using a signal detection task in which the probability of hits, misses, and false alarms can be monitored as a function of signal intensity (e.g. length), trial rate, and signal predictability [65–68]. The study was undertaken, in part, to determine if performance this task, believed to be independent of hippocampus modulation, was modified by changes in choline availability prior to birth. Prenatal choline deficiency significantly impaired the ability of adult mice to sustain attention to a brief visual cue throughout a session as evidenced by decreased ‘Hits’ and increased ‘Omissions’ during the second-half of trials. In contrast, prenatal choline supplementation enhanced the ability of mice to detect visual cues but did not alter their ability to maintain attention throughout a session [84]. These data support the view that the effects of alterations in choline availability on brain anatomy, physiology, and function likely extend beyond the septo-hippocampal system that modulates spatial memory. In the case of the sustained attention task, this likely includes cholinergic projections from the basal forebrain to neocortex.

9. Metabolic imprinting by the availability of choline perinatally

At present, the neurochemical mechanisms by which choline supplementation in utero leads to an improvement in memory are not known. As presented in the Section 1, choline serves several biological functions as the precursor of the neurotransmitter, acetylcholine (ACh) [16]; the precursor of PC and sphingomyelin which are structural phospholipids in biological membranes; the precursor of two signaling lipids, sphingosylphosphocholine and platelet-activating factor; and as a methyl donor after its oxidization to betaine [14]. It is possible that choline availability in utero affects one or more of these functions during brain development, resulting in changes in the brain’s organization. Because the period of ED 11–17 coincides with the peak of cell division and apoptosis in the developing brain, this explanation is likely. Indeed, choline supplementation during ED 11–17 stimulates cell division in the embryonic brain (assessed immunohistochemically on ED 18 following the injection of pregnant dams on E16 with the DNA precursor bromodeoxyuridine [110], while choline deficiency during this period increases the rate of apoptosis (observed on ED 18) in hippocampus and septum [56,110], two brain regions involved in memory processing.

Another possible explanation for the long-term actions of prenatal choline availability on brain function is that prenatal choline status causes long-term adaptations in choline metabolism, resulting in changes in cholinergic neurotransmission. Long-term adaptations of fat and carbohydrate metabolism to the availability of these substances during the pre-weaning period have been observed previously [91]. This type of adaptation has been termed ‘metabolic imprinting’. In order to determine whether ‘metabolic imprinting’ for choline occurs, pregnant rats were fed varying amounts of choline during the period E11–17 and several indices of ACh synthesis, degradation and release, as well as the activity of a PC-hydrolyzing enzyme phospholipase D (PLD) were examined in the hippocampus of the offspring, approximately one month after termination of treatment [14]. PLD was chosen because choline liberated from PC by PLD can be used for ACh synthesis [15,59]. The results showed that prenatal choline availability altered choline and ACh turnover in the hippocampus. Prenatally choline-deficient rats displayed elevations in AChE and ChAT activities, and increased synthesis of ACh from choline transported by high-affinity choline uptake (HACU). These effects were concomitant with reductions in hippocampal ACh content and a relative inability to sustain depolarization-evoked ACh release. Taken together, these findings indicate that in the hippocampus of prenatally

choline-deficient rats ACh turnover is accelerated (i.e. there is more rapid synthesis, degradation, and choline reutilization by HACU as indicated by the high specific radioactivity of newly-synthesized ACh). In contrast, prenatally choline-supplemented animals showed less pronounced changes in their hippocampal cholinergic system, however the direction of those changes was consistent with the above model. AChE and ChAT activities, and ACh synthesized from choline transported by HACU, were lowest in prenatally choline-supplemented rats. However, depolarization-evoked ACh release was highest in these animals. The latter result, together with the reduced AChE activity, suggests that intrasynaptic ACh concentrations and dwell times are increased, possibly resulting in enhanced cholinergic neurotransmission. The observations that ACh turnover in prenatally choline-supplemented animals is relatively slow (as indicated by low specific radioactivity of ACh newly-synthesized from exogenous choline), but that cholinergic neurotransmission is well maintained (as evidenced by robust ACh release), suggest that the pool of choline used for the synthesis of ACh in these animals may include that stored in membrane PC [111,113], and may be generated by the hydrolysis of PC catalyzed by PLD [15,59]. Consistent with the latter possibility, hippocampal PLD activity was two-fold higher in prenatally choline-supplemented rats relative to control and prenatally choline-deficient animals and may modify the development of the hippocampal cholinergic system [21,22,54]. However, it is not yet clear if this PLD is in fact involved in supplying choline for ACh synthesis in hippocampal cholinergic nerve terminals.

In future work it will be particularly important to relate the behavioral effects of perinatal choline availability to the proposal that altering the cholinergic system with acute experimental manipulations (e.g. adulthood choline supplementation or deficiency) may reveal interactions between age and perinatal choline availability. Because the activities of the macromolecules involved in cortical and hippocampal ACh turnover appear to be imprinted by choline availability during early development, cholinergic neurotransmission in the adult may be differentially sensitive to the availability of choline in adulthood, based on its supply in utero [14]. This view suggests that elevated dietary choline in adult animals that were prenatally choline-deficient would result in large increases of ACh synthesis, whereas it might have a lesser effect in prenatally choline-supplemented animals. Conversely, choline deficiency in adult rats that were prenatally choline-supplemented might result in a large reduction of ACh synthesis concomitant with a depletion of their PC pool. Thus, prenatal choline status would impart differential vulnerability to the choline supply in adulthood. This would allow us to use aging as an intervening variable in experiments designed to evaluate changes in the vulnerability of neuronal mechanisms involved in visuospatial memory and conditioned stimulus processing as a function of choline supplementation and deficiency in adulthood [99].

10. Summary

Supplemental choline, administered during critical periods of development, permanently alters brain function in a manner that produces long-term facilitation of visuospatial memory, lowered thresholds for hippocampal long-term potentiation [57,85,94] correction of the cognitive deficits associated with fetal alcohol syndrome [109], and reduced susceptibility to seizures [117]. During development, large changes in the availability of choline to tissues normally occur because of variations in dietary intake of choline and because of changes in the metabolism of choline. Ensured availability of choline may be important in the neonate, as competing demands for this amine are especially high. Choline is needed for the formation of important membrane phospholipids (i.e. PC and sphingomyelin). In particular, brain growth, which is extremely rapid in the neonate, uses large amounts of choline for membrane biosynthesis. Neonatal rat brain efficiently extracts choline from blood and increased serum choline in the neonatal rat is associated with an increase in brain choline concentration.

Age-related learning and memory deficits have been well documented using animal models and in humans. To date, no effective treatment exists to prevent cognitive deterioration in old age. The findings reviewed here show that choline supplementation during the second half of gestation constitutes such a treatment. We hypothesize that choline supplementation during vulnerable periods of early development may alter brain organization in such a way that it has more ‘cognitive reserve’ that can be used as the brain ages and loses connectivity. In addition, the time frames for behavioral sensitivity to perinatal choline availability may correspond to prenatal periods of neurogenesis and postnatal periods of dendritic remodeling of cholinergic neurons in the basal forebrain. Additional studies aimed at further delineating the physiological mechanisms that distinguish the critical periods of development, and the extent and nature of the interaction between them, should advance our understanding of the neurobiology of memory capacity and precision. It will be critically important to determine if our observations obtained with a rat model are applicable to humans (see [63]). Human infants are exposed to diets with varying levels of free choline and other choline compounds. Postnatally, human infants may drink breast milk or a milk substitute and infant formulas vary widely in their choline content [119]. Our studies indicate that alterations in the dietary availability of a single nutrient, choline, can alter brain development and have lifelong effects on cognitive function.

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