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Sustained Attention in Adult Mice is Modulated by Prenatal Choline Availability

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Our laboratory has discovered that alterations in choline availability to the developing rat fetus lead to long-term changes in spatial and temporal memory function across the lifespan and associated changes in the septo-hippocamal system. The current study was undertaken to determine if performance on an attention task, believed to be relatively independent of septo-hippocampal function, was modified by changes in choline availability prior to birth. A sustained attention task was developed for mice that includes all the features of the 2-choice signal-detection procedure initially applied to rats (McGaughy & Sarter, 1995). 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. 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.

Numerous studies have shown that the amount of choline ingested by pregnant rats has dramatic effects on the brain and behavioral development of their offspring (Meck & Williams, in press). For example, during embryonic days (ED) 12-17 supplementation with approximately 4.5 times the amount of choline found in standard laboratory diets results in improved adult performance on spatial navigation (e.g., Meck et al., 1989; Meck et al., 1988; Meck & Williams, 1997a) and interval timing tasks (e.g., Meck & Williams, 1997b). Choline deficiency during the same developmental time frames produces impairments in adulthood for some, but not all of these behavioral measures (e.g., Meck & Williams, 1999). These changes in cognitive function are accompanied by alterations in the anatomy, electrophysiology, and neurochemistry of the septo-hippocampal pathway. Recent studies have shown that the modification of choline availability from ED 11-17 alters the timing of mitosis, apoptosis, and the early commitment to neuronal differentiation by progenitor cells in regions of the fetal hippocampus and septum (Albright et al., 1999). In young rats, acetylcholine turnover in the hippocampus is reduced by a short period of prenatal choline supplementation and accelerated by a similar period of choline deficiency (Cermak et al., 1998), suggesting changes in the organization of synaptic function as a result of prenatal choline availability. The effects on brain and behavior of modifying prenatal choline availability are long lasting. Prenatal supplementation leads to increases in

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the size and alterations in the shape of medial septal cholinergic neurons (Williams et al., 1998), and a lowered threshold for eliciting long-term potentiation (LTP; Jones et al., 1999; Pyapali et al., 1998), while prenatal choline deficiency has the opposite effects. Thus far, it appears that alterations in prenatal choline availability impact functioning of basal forebrain cholinergic neurons as well as efferent neurons involved in hippocampal LTP. These electrophysiological results are consistent with our findings that prenatal choline availability alters performance in spatial navigation tasks that rely heavily on the intact functioning of the septo-hippocampal pathway (e.g., Olton & Pappas, 1977).

Recently, the long-term effects of prenatal choline supplementation and deficiency have been examined using behavioral tasks with significant attentional components (e.g., Meck & Williams, 1997c). In contrast to a variety of memory processes, some forms of attention appear to be sensitive to alteration of basal forebrain cholinergic projections to neocortical regions (see Sarter & Bruno, 2000 for a review). For our purposes, attention can be broadly defined as detecting, orienting, and becoming alerted to stimuli (e.g., Posner & Peterson, 1990). There are several distinct forms of attention that have been investigated: selective attention, divided attention, and sustained attention (e.g., Muir, 1996). Selective attention is the ability of an animal to attend to a specific stimulus while ignoring others. Divided attention, in contrast, represents the capacity of an animal to distribute attentional processing across multiple stimuli, sometimes in two or more modalities. Sustained attention, also known as vigilance, is required to respond to stimuli that are difficult to detect (i.e., stimuli that occur infrequently, unpredictably, and/or at low intensities) over extended periods.

The effects of modifying the perinatal choline supply on the ability of adult rats to divide attention between different temporal criteria (e.g., 15-s and 30-s signal durations) has been examined using a task that requires the subject to time auditory and visual signals simultaneously (Meck & Williams, 1997b). When tested as young adults, the timing accuracy was equally good for supplemented, deficient, and control rats; however, when trained again at 24 months of age, the supplemented rats were significantly more accurate than the control or deficient groups, especially on the 30-s signal. An interesting finding was that prenatal choline-deficient rats were more attentive to the 15-s signal, on par with the supplemented group, while attending much less to the 30-s signal. It appears that aged rats that were choline deficient during ED 12-17, fail to divide their attention between the different temporal criteria. Behavioral compensation appears to have occurred such that selective attention is given to the shorter signal at the expense of the longer signal.

To date, few studies have examined the selective, sustained, or divided attention abilities of mice. One reason for the paucity of research on this topic may be the suggestion that mice do not perform as well as rats on complex operant tasks (e.g., McNamara et al., 1996) and do not press response levers as readily as rats (e.g., Crawley, 2000). For example, a task that reliably measures attentional vigilance of rats (McGaughy & Sarter, 1995) has been substantially modified for use with mouse subjects (McDonald et al., 1998). In the version of this task developed for rats, subjects are required to attend to infrequent stimuli (e.g., visual cues) of a variable duration, and respond to the presence or absence of a stimulus by pressing one of two levers. In a typical trial, a visual cue is turned on for 0.025,

0.05, or 0.5-s or not turned on at all. After a 2-s delay, the levers are extended and the subject responds with a press of one lever if a cue was present, and the press of the other lever if the cue was absent. To examine sustained attention in mice, this task was modified from a two-choice signal-detection procedure to a go/no-go procedure. Mice are required to withhold responses for some trials, rather than respond on every trial. This modification substantially alters the task such that both impulsivity and attention are measured rather than signal detection alone. Performance differences may then result from optimization of behavior rather than stimulus processing (Bushnell, 1997). Furthermore, it is not clear whether the neural systems that have been identified as critically important for sustained attention in rats (McCaughy & Sarter, 1995) are also essential for performance of this go/no-go task in mice.

The present study had two goals. The first goal was to develop a sustained attention procedure for mice that had all the features of the classic paradigm developed by McGaughy and Sarter (1995). Our second goal was to examine whether alterations in choline availability early in development would modify sustained attention. While several experiments have shown robust effects of prenatal choline supplementation and deficiency on spatial memory tasks in rats, these results have only recently been generalized to mice (Mohler et al., 1998). Initial studies have shown that C57BL/6J mice that were supplemented with choline during prenatal development showed improved spatial memory when trained as adults on a radial-arm maze task. Evidence that sustained attention is also influenced by prenatal choline manipulations would support our earlier findings that brain changes due to this nutritional manipulation likely extend beyond the septo-hippocampal system and may include cholinergic projections to neocortex (see Meck & Williams, in press).

Method

Subjects

In Experiment 1, timed-pregnant C57BL/6J mice were ordered from Jackson Laboratories (Bar Harbor, Maine) to arrive in our laboratory on ED 8. Beginning on ED 9, half of the dams was fed a control diet containing 1.1 g/kg choline chloride and half was given a diet that was deficient in choline. Each diet is based on the AIN-76A diet (Dyets Inc., Bethlehem, Pennsylvania), with choline chloride substituted for choline bitartrate for the control diet and no added choline for the deficient diet. Dams consumed control or deficient diets until they gave birth on ED 19. Litter sizes ranged between 6-8 pups, and no treatment effects on pups' weights or general health at birth were evident. Pups were toe-clipped at birth to indicate treatment group and cross-fostered to newly parturient dams receiving the control diet to eliminate potential effects of choline deficiency on milk or maternal behavior. Litters were mixed-treatment groups of all male mice with approximately 6 pups/litter. All pups were weaned at postnatal day (PD) 30, and housed 3-4 mice per cage (28 cm x 28 cm x 17.75 cm) with a light:dark cycle of 12:12 hours (lights on at 07:30 h). From birth throughout the duration of the experiment, all mice were fed the control diet. Until training began at 2 months of age, mice had free access to food and water.

In Experiment 2, C57BL/6 mice obtained from the Jackson laboratories were bred in our laboratory. Pregnant dams were supplemented from conception to birth by feeding with Dyets formula AIN76A with 4.5 g/kg choline chloride or were given the control diet containing 1.1 g/kg choline chloride. Pups were toe-clipped at birth to indicate treatment group and cross-fostered in litters of mixed treatment and sex to mothers receiving the control diet, and weaned at PD 30. Again, no significant differences in pups' body weights or general health were evident. After weaning, mice were housed as described above. Before training on the attention task that began at 10 months of age,

mice had been tested on a radial-arm maze task for 33 sessions, but received free access to the control diet for several weeks before the start of sustained attention training.

Apparatus

The experimental apparatus consisted of 12 operant boxes (Model ENV-307A, Med Associates, St. Albans, Vermont) housed in sound-attenuating chambers (Model ENV-021M; Med Associates). The dimensions of each operant box were 21.59 x 17.78 x 12.70 cm. The ceiling, side walls, and door of each box were made from clear Plexiglas. The front and back walls were stainless-steel panels and the floor was made of stainless-steel bars. The front wall of each box contained left and right retractable levers; a food cup was located between the levers; and a cue light was located directly above the food cup. The back wall of each box contained a house light (14-W, 100 mA) directed towards the ceiling.

Procedure

Two weeks before the start of behavioral training, mice were placed on a restricted feeding schedule in which they received approximately 2.0 g/day of food. This was adjusted on a day to day basis to maintain mice at 80-85% of their free-feeding body weight. Two days prior to testing, mice were fed only with the food pellets they would receive during training (Purina 5001 diet; Noyes Precision Pellets, Research Diets, New Brunswick, New Jersey). In order to get mice to perform this signal-detection task, some modifications of the standard procedures used with rats were made in order to account for the small size and appetite of mice. Specifically, we began training with a 2-s visual cue compared to the 1-s cue typically used in the rat paradigm. In addition, we doubled the intertrial interval (ITI) in order to give mice more time to consume the food reward. The latter change is necessary because mice receive 0.02 g of food/trial compared to 0.09 g of food/trial for rats; this is as much as 0.1% of a mouse's body weight while rats typically receive 0.02%.

Pre-training. The mice were trained for 8 consecutive daily sessions. Each session consisted of lever training in which any lever response yielded one food pellet (0.02 g). A session would start with only the left lever extended. After 10 lever presses, the left lever was retracted and the right liver was extended. The session ended after one hour, or when mice had pressed each lever 30 or more times. On the first day of training only, levers were also retracted and extended every minute at which time one pellet was delivered into the food cup. If a mouse did not lever press, peanut butter was placed on each lever to encourage contact with the lever. After 8 days of lever-press training, mice were moved to Level 1 of signal-detection training.

Level 1 Training. Trials consisted of a cue light being turned on for 2 s (signal) or not being turned on for 2 s (nonsignal), after which the levers were extended for 4 s during which time mice could respond by pressing the appropriate lever. A trial was terminated by a response (classified as a Hit, Miss, False Alarm, or Correct Rejection; see below) or by the lapse of 4 s without a response (Omission). As shown in Figure 1, a correct response to a signal trial is a Hit, an incorrect response to a signal trial is a Miss, a correct response to a nonsignal trial is a Correct Rejection, and an incorrect response to a nonsignal trial is a False Alarm. The next trial occurred after a random ITI (mean = 24 ± 6 s). Lever assignments were randomized such that approximately half of the mice were trained to respond to signal trials by pressing the left lever and the remaining mice were trained to respond to signal trials by pressing the right lever. If a mouse responded by pressing the incorrect lever a correction trial was given with the same stimulus presentation as the previous trial. After three incorrect responses on correction trials, mice received a forced trial in which only the appropriate lever was extended for a maximum of 90 s. Each daily session lasted for 1 h. After 5 sessions of performing at 70% correct responses or better on both signal and nonsignal trials, the mice were moved to Level 2 of signal-detection training.

Level 2 Training. The following changes were made to the procedure described above. Mice received visual cues of three different durations (0.025, 0.05, or 0.5 s) randomly selected for presentation on each trial. In addition, the use of correction trials was discontinued. After 7 days of 70% correct or better performance on both signal and nonsignal trials the experiment was terminated.

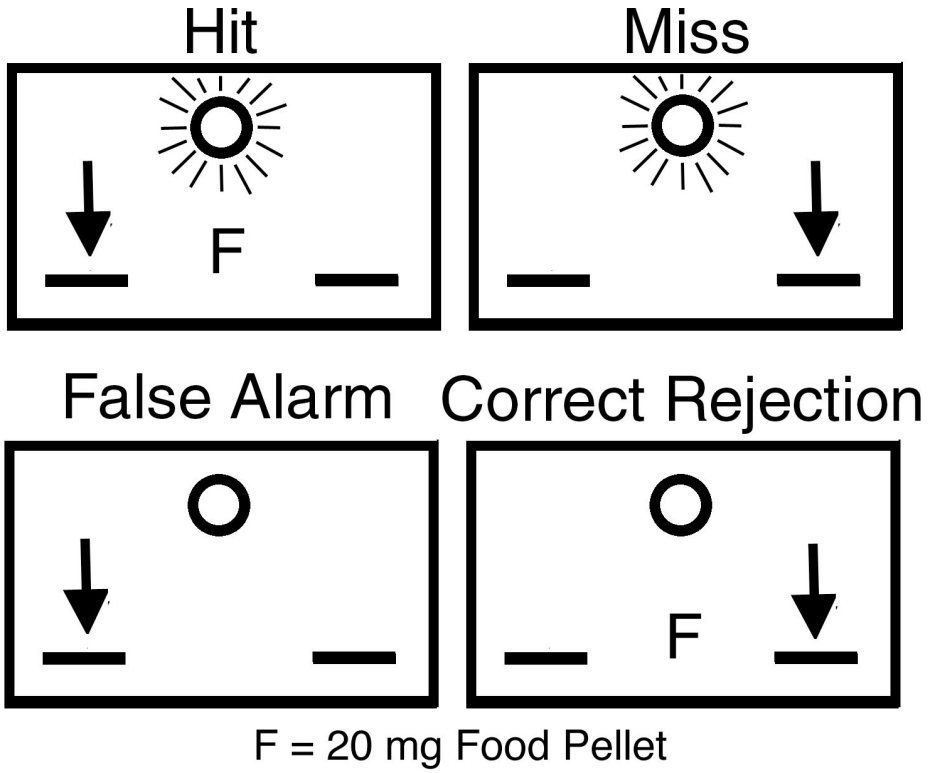

Figure 1. Diagram of the types of possible responses during the signal detection task. Only a Hit and a Correct Rejection resulted in a food reward. When mice did not respond to the extended levers on a given trial, an Omission, was counted.

Data Analysis. In order to equate the behavioral performance of each mouse, the data set analyzed was the 14-day period before criterion performance was reached for each subject. In the case of mice that did not reach the criterion for a particular level of training, the last 14 days of training before the experiment was terminated were analyzed. Hits, Misses, Correct Rejections, and False Alarms were individually tabulated. A percent correct score for signal trials [Hits/(Hits+Misses)] and nonsignal trials [Correct Rejections/(Correct Rejections+False Alarms)] along with a percentage of omissions (Omissions/Total Number of Trials) was calculated for each session and overall data were analyzed in this form. For Level 1, correction trials and forced trials were excluded from data analysis.

To examine whether performance remained consistent across an entire session, a difference score was calculated by taking the raw number of Hits, Correct Rejections, or Omissions from the first block of trials and subtracting the number of Hits, Correct Rejections, or Omissions from the last block of trials. Data were blocked as follows. Due to the variable number of trials in Level 1 (as a result of variations in the number of correction trials), the first half of a session was compared with the second half of a session (approximately 50 trials in each block). Level 2 had a fixed number of trials over the course of a 1-h session (126 trials), so the data were divided into three blocks of 42 trials each to provide a more refined analysis of the time course of performance. The difference score was calculated by subtracting the 3rd block from the 1st block. All data were statistically examined with an analysis of variance with the α level set at 0.05 unless otherwise noted.

Results

Experiment 1

The results of this experiment indicate that prenatal choline deficiency does not impair the overall ability of adult mice to properly detect visual cues. However, choline deficiency does significantly decrease the ability of mice to maintain attention throughout an entire 1-h session as evidenced by decreased Correct Rejections and increased overall Omissions late in the session compared to early in the session.

Level 1. As can be seen in the upper panel of Figure 2, during the last 14 days of training on Level 1 of the signal-detection task, both deficient and control mice responded with the same degree of accuracy to signal and nonsignal trials. Control mice made Hits to 73.7% (± 4.0 SEM) of signal trials compared to 77.4% (± 2.2) for choline-deficient mice. Similarly, control mice made Correct Rejections for 74.2% (± 3.2) of nonsignal trials compared to 73.6% (± 1.7) for choline-deficient mice. Each group omitted responses to approximately one third of the trials. There were no statistical differences in any of these measures, $F < 1$.

In contrast, deficient mice performed more poorly on the signal detection task during the second half of the session (see lower panel of Figure 2). Specifically, choline-deficient mice made significantly fewer correct responses to signal trials than control mice (Hits), $F(1, 8) = 8.99$, and nonsignal trials (Correct Rejections), $F(1, 8) = 10.16$, in the last 50 trials compared to the first 50 trials of the session. In addition, choline-deficient mice failed to respond (Omissions) more often than control mice on both signal, $F(1, 8) = 25.23$, and nonsignal trials, $F(1, 8) = 36.56$. Despite the significant decrease in performance during the last half of the session, 4 out of 5 choline-deficient mice and 3 out of 6 control mice were eventually able to reach criterion performance (70% or more correct responses to both signal and nonsignal trials for 5 consecutive days). Of the mice that advanced to Level 2, there were no significant group differences in the number of days required for the mice to reach criterion $F < 1$.

Level 2. As with Level 1 of training, both choline-deficient and control mice responded with the same degree of accuracy to signal and nonsignal trials, and omitted a response on less than one third of the signal trials when the data are averaged over the last 14 sessions, $F < 1$ (see upper panel of Figure 3). Choline-deficient mice made fewer omissions to nonsignal trials than control mice, $F(1, 5) = 4.17$. The ability of both groups to detect the visual cues was highly dependent on the duration of the signal, $F(3, 15) = 20.84$, but treatment (choline-deficient vs. control) did not interact with signal duration, $F < 1$. On trials with the 0.025-s visual cue, control mice made Hits 75.4% (± 4.3) of the time, while choline deficient mice made Hits 71.7% (± 3.7) of the time. For the 0.05-s visual cue, control mice responded correctly on 78.0% (± 2.6) of the trials while the choline-deficient group responded correctly to 74.32% (± 2.6) of the signal trials. On the longest stimulus

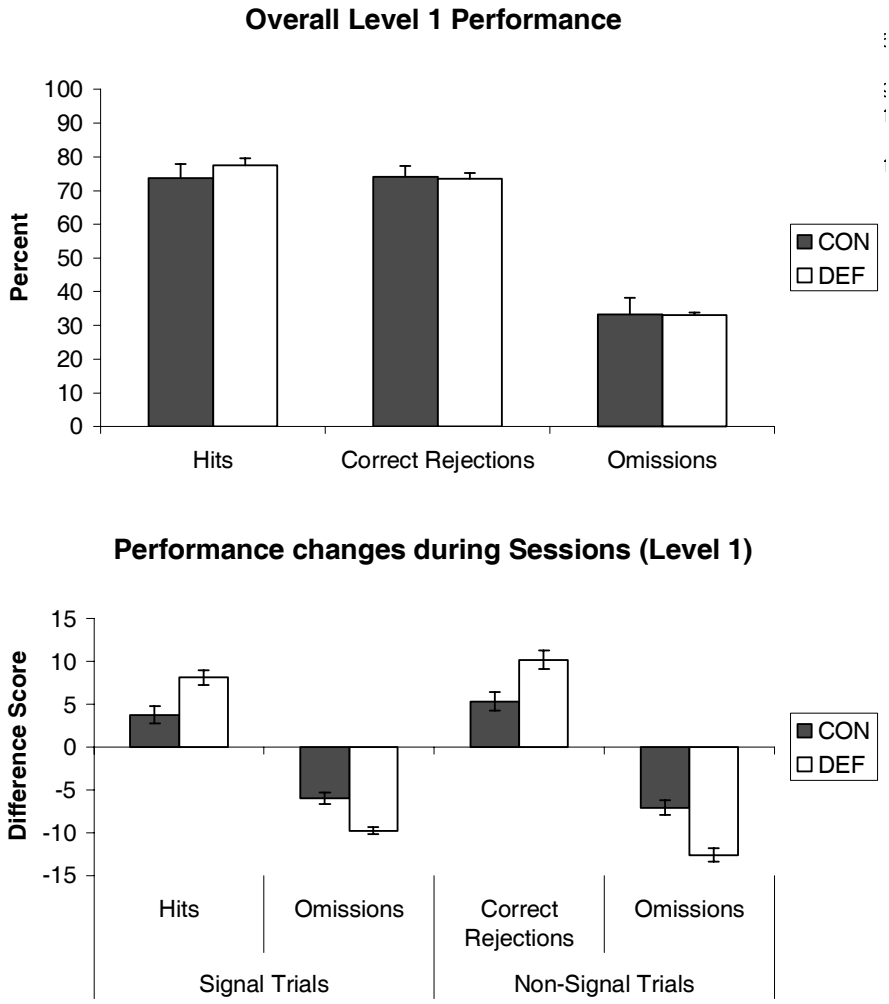

Figure 2. Prenatal choline-deficient (open bars) and control (filled bars) mice were trained on a sustained attention task with a 2-s visual cue (Level 1). Overall performance (upper panel) and difference in performance from the first to second half of the session (lower panel) were assessed. The difference score was calculated as performance (e.g., Hits, Correct Rejections, Omissions) on the first half of the session minus performance on the second half of the session.

duration (0.5 s), control mice made Hits on 90.0% (± 2.6) of trials while the choline-deficient group responded correctly to 87.4% (± 1.78) of the signal trials. The performance of each group was also similar on nonsignal trials with control mice making 80.3% (± 2.0) of correct rejections vs. 74.6% (± 2.1) for the choline-deficient group.

When performance during the first block of trials was compared to the last block of trials, choline-deficient mice were once again making more errors late in the session compared to the performance of control mice that were more consistent from early to late trials (see lower panel of Figure 3). Specifically, choline-

deficient mice made significantly fewer correct rejections to nonsignal trials than control mice, $F(1, 5) = 12.29$. Choline-deficient mice also showed significant performance decrements during the last block of trials that for several other measures as well: Correct responses (Hits) on signal trials, $F(1, 5) = 4.13$, Omissions on signal trials, $F(1, 5) = 4.13$, and Omissions on nonsignal trials, $F(1, 5) = 6.20$.

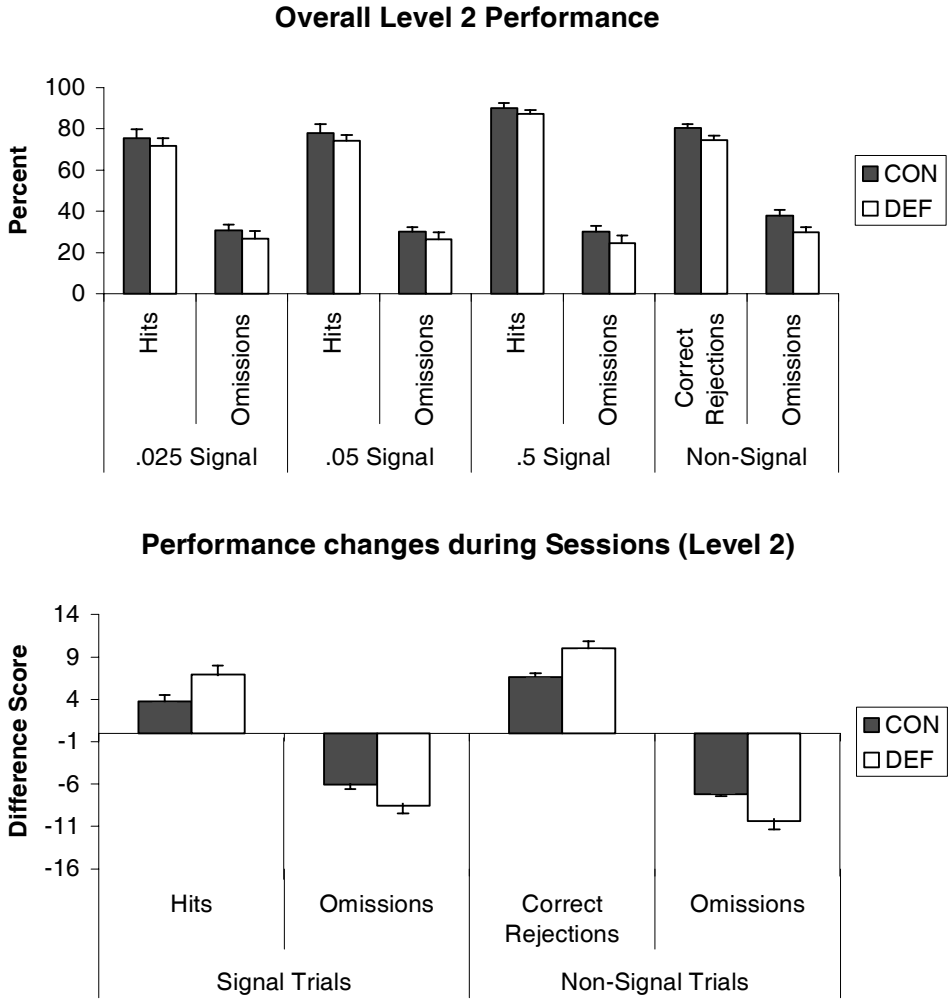

Figure 3. Prenatal choline-deficient (open bars) and control (filled bars) mice were trained on a sustained attention task with 3 visual cue durations (0.025 s, 0.05 s, 0.5 s) or no signal (Level 2) and overall performance (upper panel) and the difference in performance between the early and late trials in a session (lower panel) were assessed.

Experiment 2

The results of the second experiment indicate that prenatal choline supplementation significantly improves the overall ability of adult mice to properly detect visual cues compared with control mice. This improvement was especially evident when the attentional demands of the task were increased by shortening the

duration of the visual cues. Prenatal choline supplementation did not, however, increase the ability of mice to sustain attention throughout the 1-h session.

Level 1. During the last 14 days of training on Level 1 of the signal-detection task, the performance of choline-supplemented and control mice did not differ significantly in any measure, *Fs*(1, 9) $\leq$ 2.50. Control mice responded correctly to 69.6% (± 5.7 SEM) of signal trials compared to 74.3% (± 8.5) for choline-supplemented mice. Similarly, control mice responded correctly to 75.7% (± 2.7) of nonsignal trials compared to 63.1% (± 5.8) for the choline-supplemented mice. Each group omitted responses on less than one third of the trials (see upper panel of Figure 4).

When performance during the first half of the session was compared with performance during the second half, again there were no significant differences between control and choline-supplemented mice as shown in the lower panel of Figure 4, *F*s(1, 9) $\leq$ 2.50. Five out of 6 control mice and 4 out of 5 choline-supplemented mice were able to reach criterion (70% or more correct responses to both signal and nonsignal trials for 5 consecutive days) and move to the next level of training. Of the mice that advanced to Level 2, there were no significant group differences in the number of days to reach criterion $F(1,7) < 1.0$, $p > 0.05$.

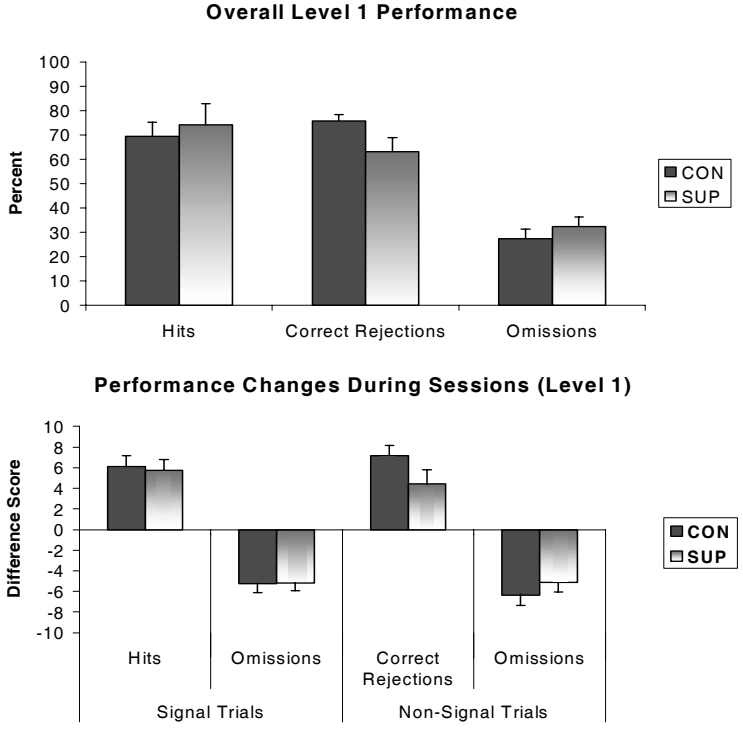

Figure 4. Prenatal choline-supplemented (shaded bars) and control (filled bars) mice were trained on a sustained attention task with a 2-s visual cue (Level 1). Overall performance (upper panel) and difference in performance from the first to second half of the session (lower panel) were assessed.

Level 2. During the last 14 days of training on Level 2, choline-supplemented mice were able to detect the presence of visual cues significantly more accurately than controls. Choline-supplemented mice made significantly more correct responses to the signal trials (Hits), than did control mice, $F(1, 7) = 6.50$, as shown in the upper panel of Figure 5. On trials with the 0.025 s stimulus duration, control mice made Hits 55.9% (± 3.7) of the time compared to 76.2% (± 7.3) for the choline-supplemented group. For the 0.05 s stimulus duration, control mice responded correctly 59.7% (± 5.3) of the time while the choline-supplemented group responded correctly to 78.7% (± 6.6) of the signal trials. When the longest stimulus duration (0.5 s) was used, control mice made Hits on 73.6% (± 4.9) of trials while the choline-supplemented group responded correctly to 90.3% (± 4.9) of the signal trials.

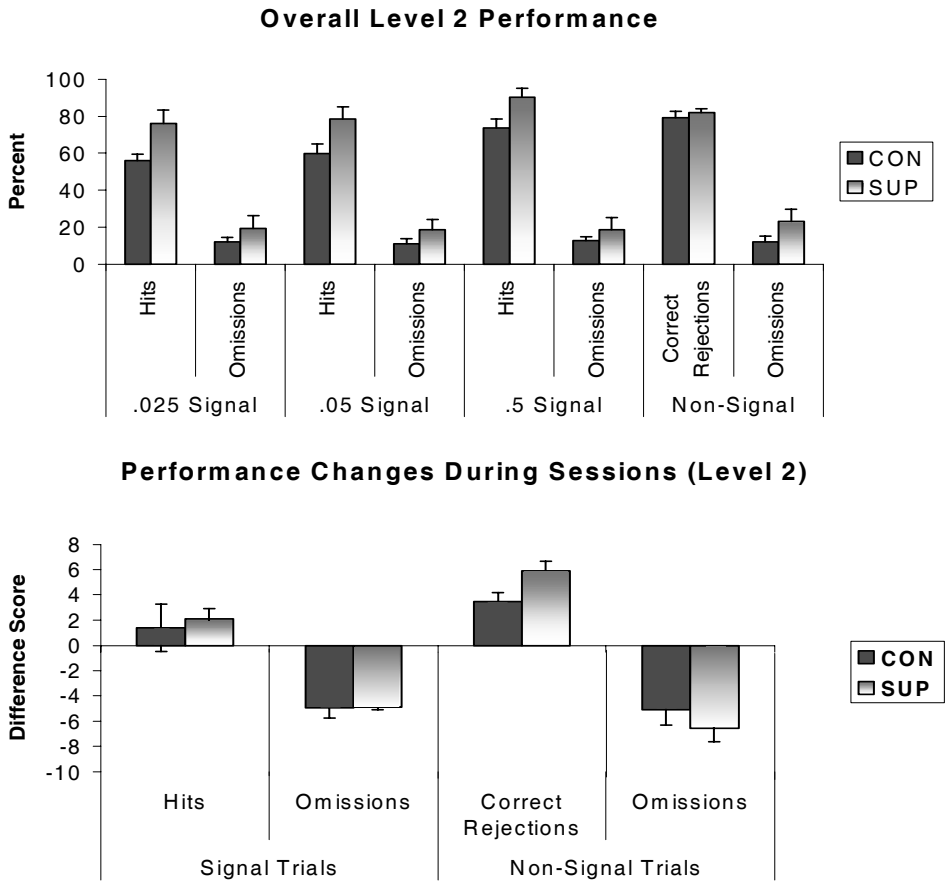

Figure 5. Prenatal choline-supplemented (shaded bars) and control (filled bars) mice were trained on a sustained attention task with 3 visual cue durations (0.025 s, 0.05 s, 0.5 s) and no signal (Level 2) and overall performance (upper panel) and the difference in performance between the early and late trials in a session (lower panel) were assessed.

The ability of both treatment groups to detect the visual cue was dependent on the duration of the signal, $F(3, 21) = 26.36$, with a significant interaction between treatment and signal duration, $F(3, 21) = 21.3$. This interaction is the result of significantly worse performance by control mice on signal trials compared to choline-supplemented mice, and relatively similar performance between the two groups on nonsignal trials. Control mice made 79.1% (± 3.6) of correct rejections, while choline-supplemented mice made 81.8% (± 6.8) of correct rejections. When performance during the first block of trials was compared with the last block of trials, control mice made significantly more correct responses to nonsignal trials, $F(1, 7) = 6.22$, as shown in the bottom panel of Figure 5.

Discussion

The results of these studies indicate that C57BL/6J mice can reliably perform a 2-choice signal-detection procedure using task dynamics and a training regimen described by McGaughy and Sarter (1995) for use in rats. To accommodate species and size differences between rats and mice, we increased the salience of the signal by changing the signal length from 1 s to 2 s on the first level of training, doubled the inter-trial interval to allow mice more time to eat food pellets, and decreased the overall number of trials from 162 to 126 because mice get sated more rapidly than rats. Mice in our studies showed signal detection performance and decreases in performance during a session quite similar to what has previously been described in rats (see McGaughy & Sarter, 1995). At this time, we do not know whether mice and rats are also similar in their ability to reach criterion at every level of training. In our study, group differences in the ability to reach criterion (move to the next level of training) was of interest and therefore all mice were included in our analysis. In contrast, most rat studies have examined drug or lesion effects on performance using only rats that have reached a set criterion, prior to the treatment.

Importantly, the current study demonstrates that prenatal choline availability influences the performance of adult mice on this task. Prenatal choline deficiency significantly impaired the ability of mice to sustain attention throughout a 1-h session as evidenced by decreased Hits and increased Omissions during the second half of trials on Level 1 training. A similar effect was observed on Level 2, although only a decrease in nonsignal correct responses was statistically significant. We also found that prenatal choline supplementation enhanced the ability of mice to detect visual signals, but did not alter their ability to maintain attention over several trials. Taken together, these data support the view that choline availability during prenatal development alters sustained attention in adulthood. These data complement and extend previous work from our laboratory indicating that modifications of the prenatal choline supply also alter the rats' ability to divide attention (Meck & Williams, 1997b).

One seemingly paradoxical finding from the current study is that choline-deficient mice perform significantly more poorly than control mice later in sessions, but have overall performance that is not significantly different from controls. The explanation for this oddity is that choline-deficient mice are actually somewhat more accurate than control mice during the first block of trials. This pattern of results is quite similar to the performance of deficient and control rats on a ra-

dial-arm maze task. We have found that when trials are run once a day, choline-deficient rats are able to perform as accurately or somewhat more accurately than control rats; however, when their cognitive resources are taxed by massing several trials in a row, the spatial navigation performance of choline-deficient rats suffers, while control rats maintain performance (Meck & Williams, 1999).

In addition, hippocampal slices from rats made choline deficient prenatally show increased acetylcholine breakdown, high-affinity choline uptake, and acetylcholine synthesis compared to slices from control rats, but show decreased potassium-evoked acetylcholine release (Cermak et al., 1998). These findings suggest that acetylcholine neurons in choline-deficient animals have been organized prenatally such that they can maintain sufficient acetylcholine for normal function, but they cannot sustain these levels for an extended period of time with repeated stimulation. These synaptic changes may be the reason why choline-deficient mice may initially perform as well as (or even better than) controls, but would be unable to sustain attention after several dozen trials. In support of this idea, the ability of choline-supplemented mice to attend to signal trials significantly better than controls may also be the result of comparable changes in the ability of cholinergic neurons to sustain prolonged acetylcholine release (Meck & Williams, in press). For example, in hippocampal slices from choline-supplemented rats, breakdown, reuptake, and synthesis of acetylcholine are slowed while potassium-evoked release yields significantly higher levels of acetylcholine (Cermak et al., 1998). This higher quantal release of acetylcholine may explain the enhancements in detecting visual signals observed in choline-supplemented mice.

Previous studies examining cholinergic manipulation of sustained attention have used mainly acute drug challenges. For example, rats treated with the muscarinic antagonist scopolamine and trained on a sustained attention task showed decrements in sustained attention similar to that observed in our choline-deficient mice (Bushnell et al., 1997). Specifically, these scopolamine-treated rats began a session performing moderately well and then performance declined over trials. Because the drug was injected only 15 min before the session began, it may be that cholinergic receptors are increasingly blocked as the session continued. If choline-deficient mice have decreases in acetylcholine release, this would produce the same outcome as decreases in cholinergic postsynaptic activity as a result of scopolamine administration.

Improvements in sustained attention after administration of some cholinergic agonists have also been reported. While physostigmine (McGaughy et al., 1998), and pilocarpine (Bushnell et al., 1997) do not appear behaviorally effective, modest to sizeable enhancements in sustained attention have been observed after treatment with nicotine (Bushnell et al., 1997) and the nicotinic receptor agonist ABT-418 (McGaughy et al., 1999). If the enhanced performance observed in our choline-supplemented mice is a result of increased quantal release of acetylcholine, this data would mirror the improved performance seen after increases in cholinergic postsynaptic activity resulting from nicotinic agonists. Nonetheless, the precise relation of prenatal choline manipulations to acute drug treatments with cholinergic agonists and antagonists remains to be determined.

Several alternative explanations for the findings reported here deserve comment. The performance of the 10-month old control mice used in Experiment 2 was not as accurate as the performance of the 2-month old control mice in

Experiment 1. We do not know why this was the case, but we do not believe that this difference in control performance between the first and second experiment compromised our study. There were several methodological differences between Experiments 1 and 2. The mice in Experiment 1 were the offspring of mice that were pregnant during shipping, while the mice in Experiment 2 were bred and born in our laboratory. In contrast to findings previously reported (e.g., Meek et al., 2000), however, the mice in the present studies that were offspring of dams that had been shipped during pregnancy had better performance on the attention task (i.e., control mice from Experiment 1) than those that were not shipped (i.e., control mice from Experiment 2). This suggests that prenatal stress during shipping alone is unlikely to account for the differences in performance between control groups. Another explanation that we consider somewhat more likely is that the differences in control performance may be the result of aging, since the mice in Experiment 2 were 8 months older than the mice in Experiment 1. The beneficial effect of prenatal choline supplementation may have been to prevent an age-related decline in attention. In support of this hypothesis is our finding that when rats are trained on a radial-arm maze task from 2 months to 26 months of age, choline supplementation prevents rats from showing a normal age-related decline in spatial memory (Meck & Williams, in press). Prenatal choline supplementation also prevents age-related declines in a simultaneous temporal processing task (Meck & Williams, 1997). Although rodents are not usually considered "old" at 10 months of age, C57BL/6 mice do show age-related deposits of fibrillar material in the hippocampus, piriform cortex, and other regions during this time frame, suggesting some neurodegeneration (Jucker et al., 1994).

The age differences of the mice used in Experiments 1 and 2 also make direct comparisons of the choline-supplemented and choline-deficient mice difficult. The results of Experiment 1 suggest that prenatal choline deficiency causes mice to respond well initially and then to decrease attention over the course of a 1-h session. The results of Experiment 2 suggest that prenatal choline supplementation improves the ability of mice to detect signals, but has no impact on their ability to sustain attention over time. One possible interpretation of these findings is that choline supplementation only enhances performance on a signal-to-signal basis and does not facilitate performance over time. However, it is also possible that the cognitive performance of choline-supplemented mice changes as they age along with changes in cholinergic neurochemistry. A direct comparison of choline-supplemented and choline-deficient subjects of the same age is needed to resolve this issue.

It is also possible that prenatal choline availability alters hunger and satiety mechanisms in adult mice. Alterations in these systems may affect performance on our attention task because mice are able to receive more than half of their daily caloric intake from the pellets provided as food reward in this task. Although prenatal choline deficient mice made more omissions than controls, they also made fewer correct responses on both signal and nonsignal trials. If alterations in motivation alone determined performance on this task, one might expect to see an increase in omissions later in a session as mice became sated, without a corresponding decrease in correct responses. While it is still possible that accuracy would also decline as mice become sated, similar effects might be expected in all groups, yet we only saw decreased accuracy in the deficient group. Thus while

choline-induced differences in hunger and satiety mechanisms may explain these data, in other studies of prenatal choline treated rats tested on a variety of spatial and temporal memory tasks, we have not seen evidence of treatment-induced differences in food-seeking behaviors (Meck et al., 1987; Meck & Williams, 1997b, 2002)

These data, along with previous findings showing that simultaneous temporal processing is improved by choline supplementation in rats (Meck & Williams, 1997) and that prenatal choline supplementation prevents MK-801-mediated toxicity in the cingulate/retrosplenial cortex (Guo-Ross et al., 2002), support our view that brain changes due to this early nutritional manipulation likely extend beyond the septo-hippocampal system and may include cholinergic projections to neocortex (see Meck & Williams, in press). Specifically, alterations of prenatal choline supply may affect cholinergic projections of the nucleus basalis to the cortex, which have been shown to be necessary for performance of this sustained attention task (e.g., McGaughy & Sarter, 1998; McGaughy et al., 1996).

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