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Prenatal choline supplementation increases sensitivity to time by reducing non-scalar sources of variance in adult temporal processing

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Abstract

Choline supplementation of the maternal diet has a long-term facilitative effect on timing and temporal memory of the offspring. To further delineate the impact of early nutritional status on interval timing, we examined effects of prenatal-choline supplementation on the temporal sensitivity of adult (6 mo) male rats. Rats that were given sufficient choline in their chow (CON: 1.1 g/kg) or supplemental choline added to their drinking water (SUP: 3.5 g/kg) during embryonic days (ED) 12–17 were trained with a peak-interval procedure that was shifted among 75%, 50%, and 25% probabilities of reinforcement with transitions from 18s → 36s → 72s temporal criteria. Prenatal-choline supplementation systematically sharpened interval-timing functions by reducing the associative/non-temporal response enhancing effects of reinforcement probability on the *Start* response threshold, thereby reducing non-scalar sources of variance in the left-hand portion of the Gaussian-shaped response functions. No effect was observed for the *Stop* response threshold as a function of any of these manipulations. In addition, independence of peak time and peak rate was demonstrated as a function of reinforcement probability for both prenatal-choline supplemented and control rats. Overall, these results suggest that prenatal-choline supplementation facilitates timing by reducing impulsive responding early in the interval, thereby improving the superimposition of peak functions for different temporal criteria.

Keywords

Developmental nutrition; Timing and time perception; Temporal memory; Response thresholds; Weber's law; Impulsivity; $\alpha 7$ nicotinic acetylcholine receptors

1. Introduction

Previous research has shown that adult rats treated prenatally with supplemental choline, a selective agonist of $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ -nAChR – Alkondon et al., 1997, 1999; Guseva et al., 2006), exhibit enhanced attention and precision in the temporal control of responding when trained in a peak-interval (PI) timing procedure (Meck and Williams

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1997a, b). This enhancement can be related to an increase in rats' sensitivity to the inhibitory effects of a signaled delay to reinforcement and the associated resetting of an internal clock by the delivery of reinforcement (Matell and Meck 1999; Cheng et al. 2006). The question remains as to whether the increased temporal sensitivity exhibited by adult rats given prenatal choline supplementation differentially affects variance in clock, memory, or response thresholds (e.g., Buhusi, 2003; Cheng, 1992; Gibbon and Church 1984, 1992; Church et al. 1991). For example, Church et al. (1994) reported that rats trained on the PI procedure in which a high state of responding is centered around the criterion time are considerably more variable in the time at which they start responding (S1) than in the time at which they stop responding (S2) – synonymous with the “giving in” and “moving on” times in models of optimal foraging (e.g., Brunner et al. 1996; Kacelnik and Brunner 2002). Whether or not these differences in S1 and S2 response thresholds reflect separate mechanisms for determining when to start and stop responding is an open question (see MacDonald et al., 2007). Recently it was shown that S1 times in the PI procedure are relatively unaffected by systemic methamphetamine administration while the S2 times are shifted proportionately leftward in a dose-dependent manner, consistent with a change in clock speed (Gooch et al., 2007; Matell et al., 2006). Although such dissociation between drug-induced alterations in S1 versus S2 times has been previously reported (Abner et al., 2001), the basis for such a discrepancy between control mechanisms for the initiation and termination of this behavioral sequence remains unclear. However, it has recently been demonstrated that the initiation of responding on temporal reproduction procedures is influenced by both a low variability, temporally controlled process, and a high-variability, temporally uncontrolled process whose influence diminishes with extended training (Matell and Portugal, 2006). It is conceivable that under normal circumstances a treatment's ability to produce a leftward shift in response initiation times is masked by the larger relative variability in these S1 times compared to S2 times (see Matell et al., 2006). Consequently, it is important to determine to what extent S1 and S2 response thresholds can be selectively altered as a function of treatments such as prenatal choline availability and shifts in reinforcement density.

Scalar Expectancy Theory (SET) as initially proposed by Gibbon (1977) and later developed into an information-processing theory by Gibbon et al. (1984) attempts to specify sources and forms of variance in timing (Church et al., 1994; Church et al., 1991; Gibbon, 1991; Gibbon and Church, 1984, 1992). Extensions of SET include a clock stage comprised of a pacemaker that generates pulses that flow through an on/off switch into an accumulator. Upon delivery of reinforcement, the accumulated pulses (e.g., clock reading) are stored in reference memory as a single occurrence in a distribution of clock readings to be used in future decision processes. Response initiation is achieved by the on-line comparison between a randomly chosen value from reference memory and the current (continually updated) value in the accumulator. The comparison is an evaluation of whether the ratio of the discrepancy between the current time value and the reference memory value to the same reference memory value is below a particular response threshold. This ratio comparison, combined with normal variability in the clock and/or memory and/or decision stage, leads to both the scalar property and the nearly Gaussian response form observed in PI functions (Church et al., 1991, 1994; Meck and Angell, 1992; Meck and Church, 1984).

In a discrete-trials fixed-interval (FI) procedure, a signal is turned on and the first response after the fixed interval has elapsed (e.g., 30 s) delivers reward, terminates the signal, and ends the trial. Although responses on an individual trial are well described by a single transition from a low response rate to a high response rate about 2/3 of the way into the FI (Schneider, 1969), a scallop-like pattern is observed when responding is averaged over many trials. The PI procedure is a variant of this FI procedure, in which unreinforced “probe” trials are added (Buhusi and Meck, 2006; Paule et al., 1999; Roberts, 1981; Sandstrom, 2007), thereby allowing observation of the entire temporal response form. On these probe trials, the discriminative

stimulus remains on for two to three times the normal FI duration and operant responses have no consequence. These probe trials allow an observation of both the initiation and termination of the participant's responses. Averaging across trials in this procedure produces a Gaussian-shaped response function that "peaks" very close to the criterion time. The single-trial data from this procedure are similar to that from the FI procedure, but with 2 transitions (low rate to high rate to low rate – see Church et al. 1994; Meck, 1988; Rakitin et al., 1998). The PI procedure has been widely used for studying timing due to the finding that changes in response rate can be dissociated from changes in response timing, i.e., peak time and peak rate are independent measures of responding (Roberts, 1981, 1993). Because motivational manipulations appear to affect response rate and not response timing, this procedure has been considered a relatively "pure" indication of a participant's perception of time. As such, the ability to match the Gaussian-shaped response functions obtained from this procedure when the data are plotted on a linear time scale has been used as a criterion to evaluate the validity of interval-timing models involving selective effects on attention, clock, memory, and decision stages (Gibbon et al., 1997; Meck, 1983, 1996, 2005; Penney et al., 1996).

The $\alpha 7$ -nAChR has been localized in the hippocampus and the basal ganglia where it plays a neuromodulatory role by enhancing the release of dopamine and glutamate (Azam et al., 2002; Dajas-Bailador and Wonnacott, 2004; Risso et al., 2004). Various ligands have been identified as selective agonists and antagonists for the $\alpha 7$ -nAChR. In the adult rat, choline activates $\alpha 7$ receptors with a potency that is 10-fold lower than observed for ACh without any detectable effect at $\alpha 4$, $\alpha 2$, $\beta 2$, or $\beta 4$ ACh receptor subtypes (Papke et al., 1996). In the mammalian brain, $\alpha 7$ -nAChRs constitute one of the predominant nAChR subtypes and nicotine application to the ventral tegmental area, paired with postsynaptic stimulation, contributes to a form of long-term potentiation, an effect attributed to presynaptic $\alpha 7$ -nAChRs on glutamatergic afferents (Jones and Wonnacott, 2004; Mansvelder and McGehee, 2000; Rousseau et al., 2005; Song et al., 2005). Of relevancy to the present study is the observation that prenatal choline supplementation increases the sensitivity of adult rats to the clock-speed enhancing effect of nicotine (Meck and Williams, 1997a), thus suggesting the possibility that prenatal choline supplementation can alter $\alpha 7$ -nAChR function in adulthood (e.g., Cheng et al., 2006; Fayuk and Yakel, 2004; Guseva et al., 2006; Morley et al., 1977; Stevens et al., 2007).

There is a variety of behavioral and neurobiological evidence supporting the view that rats given prenatal-choline supplementation express increased memory capacity and are capable of forming more enduring memories in adulthood (Loy et al., 1991; Meck et al., 1988, 1989; Mellott et al., 2004; Montoya et al., 2000; Williams et al., 1998). For example, recent studies have shown that prenatal-choline supplementation can elevate the baseline level of hippocampal neurogenesis in adult rats, a phenomenon that is considered to be facilitative of certain types of memory (e.g., Glenn, et al., 2007). It has also been demonstrated that prenatal-choline supplementation protects against seizure-induced memory deficits (Holmes, et al., 2002; Yang, et al., 2000). In terms of neuroplasticity, adult hippocampal slices from prenatally choline supplemented rats exhibit a lower threshold for the induction of long-term potentiation compared with slices from control rats (Jones et al., 1999; Pyapali et al., 1998). Furthermore, perinatal choline supplementation can alter behavior and neurochemistry following a variety of developmental disorders, including the alleviation of behavioral abnormalities associated with fetal alcohol syndrome in Sprague-Dawley rats (Thomas et al., 2000, 2004, 2007; Wagner and Hunt, 2006), attenuation of some of the motor deficits observed in a *Mecp2*^{lox} mouse model of Rett syndrome (Nag and Berger-Sweeney, 2007), and the improvement of sensory gating in a DBA/2 mouse model of schizophrenia that exhibits reduced numbers of hippocampal $\alpha 7$ nicotinic receptors (Stevens et al., 2007). Consequently, it was of interest in the present study to investigate the effects of prenatal choline supplementation on the implementation of S1 and S2 response thresholds involved in duration discrimination tasks

such as the PI procedure due to the potential sensorimotor enhancing actions of $\alpha 7$ nicotinic receptor agonists during early development (Meyer et al., 1998).

2. Results

2.1. Baseline Fixed-Interval (FI) 18s Training

Because the FI-18s condition was unique to the first phase of training (i.e., this was the only 100% FI condition) and the PI-18s condition was the first to parametrically examine shifts in reinforcement probability these two conditions were analyzed separately (see Cheng et al., 2007a). Mean proportion of maximum response rate as a function of signal duration for FI-18s Sessions 1–3 and Sessions 13–15 for the CON and SUP treatment groups are displayed in Fig. 1 – panels a and b, respectively. In addition, the mean S1 Rate Index for individual FI-18s sessions (Sessions 1–15) is displayed in Fig. 1, panel c. An ANOVA comparing the mean S1 Rate Index for a session block comprised of the first three sessions of FI-18s training versus a session block comprised of the last three sessions indicated non-significant effects of Treatment, $F(1,13) = 3.01$, $p > 0.05$ and Session Block, $F(1,13) = 1.58$, $p > 0.05$; whereas the Treatment $\times$ Session Block interaction was significant, $F(1,13) = 4.86$, $p < 0.05$. An additional ANOVA conducted using all sessions indicated a non-significant effect of Treatment, $F(1,13) = 1.95$, $p > 0.05$; whereas significant effects were observed for both Session, $F(14,182) = 2.44$, $p < 0.01$, and the Treatment $\times$ Session interaction, $F(14,182) = 1.84$, $p < 0.05$.

2.2. Peak-Interval (PI) 18s training with shifts in reinforcement probability

A repeated-measures ANOVA for the mean S1 Rate Index for PI-18s training as a function of Treatment and Reinforcement Probability indicated significant main effects of Treatment, $F(1,13) = 4.85$, $p < 0.05$, Reinforcement Probability, $F(2,26) = 13.52$, $p < 0.001$, and the Treatment $\times$ Reinforcement Probability interaction, $F(2,26) = 6.26$, $p < 0.01$. A second repeated-measures ANOVA for the mean S2 Rate Index for PI-18s training as a function of Treatment and Reinforcement Probability indicated significant main effects of Reinforcement Probability, $F(2,26) = 13.52$, $p < 0.001$, whereas non-significant effects were observed for Treatment, $F(1,13) = 4.85$, $p > 0.05$ and the Treatment $\times$ Reinforcement Probability interaction, $F(2,26) = 6.26$, $p > 0.05$. These effects for PI-18s training are illustrated by the mean proportion of maximum response rate plotted in Figs. 2–3 as well as the scalar property illustrated in Fig. 4, the mean S1 and S2 rate indices plotted in Fig. 5, and the session by session peak time, peak rate, and S1 rate index measures plotted in the upper panels of Fig. 6.

2.3. Peak-Interval (PI) 18s, PI-36s, and PI-72s training with shifts in reinforcement probability

An omnibus $2 \times 3 \times 3$ repeated measures ANOVA for the S1 rate index found a significant main effect of Treatment, $F(1,13) = 4.63$, $p < 0.05$ as well as significant effects of the Reinforcement $\times$ Duration, $F(4,52) = 5.13$, $p < 0.01$ and the Treatment $\times$ Reinforcement Probability interactions, $F(2,26) = 3.63$, $p < 0.05$ were observed. In contrast, non-significant main effects of Duration, $F(2,26) = 3.1$, $p > 0.05$; Reinforcement Probability, $F(2,26) = 0.04$, $p > 0.05$ as well as the Treatment $\times$ Duration, $F(2,26) = 1.13$, $p > 0.05$ and the Treatment $\times$ Duration $\times$ Reinforcement Probability interactions, $F(4,52) = 0.81$, $p > 0.05$ were observed. The significant effects were primarily the result of the S1 rate index for the PI-72s training increasing as a function of reinforcement probability, with the S1 rate index for PI-36s training remaining unchanged and the S1 rate index for PI-18s training decreasing as a function of reinforcement probability.

An omnibus $2 \times 3 \times 3$ repeated measures ANOVA for the S2 rate index found non-significant main effects of Treatment, $F(1,13) = 0.28$, $p > 0.05$; Duration, $F(2,26) = 3.1$, $p > 0.05$; Reinforcement Probability, $F(2,26) = 0.04$, $p > 0.05$; as well as the Treatment $\times$ Duration, $F(2,26) = 1.13$, $p > 0.05$ and the Treatment $\times$ Duration $\times$ Reinforcement Probability interactions,

$F(4,52) = 0.81$, $p > 0.05$. In contrast, significant effects of the Reinforcement $\times$ Duration, $F(4,52) = 5.13$, $p < 0.01$ and the Treatment $\times$ Reinforcement Probability interactions, $F(2,26) = 3.63$, $p < 0.05$ were observed. These effects were the result of the S2 rate index for the PI-72s showing a larger effect of reinforcement probability than the PI-18s or PI-36s conditions.

The major trends for the omnibus ANOVAs for peak time and peak rate as a function of training duration (18, 36, and 72s), reinforcement probability (25, 50, and 75%) and treatment (CON and SUP) are illustrated in terms of the mean proportion of maximum response rate in Figs. 2–4 and the individual measures of peak time, peak rate, S1 and S2 response ratios in Figs. 5–7.

Repeated measures ANOVAs conducted on the peak rate and peak time measures for the PI-18s training conditions under which reinforcement probability varied systematically from 75% $\rightarrow$ 50% $\rightarrow$ 25% FI trials in a repeated sequence across sessions revealed a highly significant effect of Session sequence on peak rate, $F(10,130) = 17.48$, $p < 0.0001$ – with a significant linear trend, $F = 133.61$, $p < 0.0001$; whereas the effects of Treatment $F(1,13) = 0.16$, $p > 0.05$ and the Treatment $\times$ Session interaction, $F(10,130) = 0.53$, $p > 0.05$ were non-significant. In contrast, no significant effects were observed for any factors of the peak time measure; Treatment $F(1,13) = 0.06$, $p > 0.05$; Session $F(1,10) = 1.03$, $p > 0.05$; and the Treatment $\times$ Session interaction $F(10,130) = 0.21$, $p > 0.05$. These effects of reinforcement probability on peak time and peak rate for PI-18s training are illustrated in the upper panels of Fig. 6.

Repeated measures ANOVAs conducted on the peak rate and peak time measures for the PI-36s training conditions under which reinforcement probability varied systematically from 75% $\rightarrow$ 50% $\rightarrow$ 25% FI trials in a repeated sequence across sessions revealed a highly significant effect of Session sequence on peak rate, $F(10,130) = 18.39$, $p < 0.0001$ – with a significant linear trend, $F = 150.11$, $p < 0.0001$; whereas the effects of Treatment $F(1,13) = 0.02$, $p > 0.05$ and the Treatment $\times$ Session interaction, $F(10,130) = 0.72$, $p > 0.05$ were non-significant. In contrast, no significant effects were observed for any factors of the peak time measure; Treatment $F(1,13) = 0.31$, $p > 0.05$; Session $F(1,10) = 1.09$, $p > 0.05$; and the Treatment $\times$ Session interaction $F(10,130) = 0.68$, $p > 0.05$. These effects of reinforcement probability on peak time and peak rate for PI-36s training are illustrated in the lower panels of Fig. 6.

Repeated measures ANOVAs conducted on the peak rate and peak time measures for the PI-72s training conditions under which reinforcement probability varied systematically from 75% $\rightarrow$ 50% $\rightarrow$ 25% FI trials across sessions revealed a highly significant effect of Session sequence on peak rate, $F(10,130) = 17.54$, $p < 0.0001$ – with a significant linear trend, $F = 207.1$, $p < 0.0001$; whereas the effects of Treatment $F(1,13) = 0.15$, $p > 0.05$ and the Treatment $\times$ Session interaction, $F(10,130) = 0.69$, $p > 0.05$ were non-significant. In contrast, no significant effects were observed for any factors of the peak time measure; Treatment $F(1,13) = 0.23$, $p > 0.05$; Session $F(1,10) = 0.6$, $p > 0.05$; and the Treatment $\times$ Session interaction $F(10,130) = 0.64$, $p > 0.05$. These effects of reinforcement probability on peak time and peak rate for PI-72 training are illustrated in Fig. 7.

2.4. Transition from Peak-Interval (PI) 18s to PI-36s training

An ANOVA conducted on peak time as a function of the first 10 sessions of PI-36s training following the transition from PI-18s training indicated a significant effect of Sessions, $F(9,117) = 4.18$, $p < 0.001$, but non-significant effects of Treatment, $F(1,13) = 0.75$, $p > 0.05$ and the Treatment $\times$ Session interaction, $F(9,117) = 1.02$, $p > 0.05$ – indicating that the CON and SUP groups acquired the PI-36s criterion at approximately equal rates.

2.5. Transition from Peak-Interval (PI) 36s to PI-72s training

An ANOVA conducted on peak time as a function of the first 10 sessions of PI-72s training following the transition from PI-36s training indicated a significant effect of Sessions, $F(9,117) = 3.79$, $p < 0.001$, but non-significant effects of Treatment, $F(1,13) = 0.89$, $p > 0.05$ and the Treatment $\times$ Session interaction, $F(9,117) = 1.14$, $p > 0.05$ – indicating that the CON and SUP groups acquired the PI-72s criterion at approximately equal rates.

3. Discussion

Overall, prenatal choline supplemented (SUP) male rats trained on 18s, 36s, and 72s PI procedures were more sensitive to time than control (CON) male rats – in agreement with earlier findings reported by Meck and Williams (1997a) for CON and SUP male rats trained on a PI-20s procedure. In the present study, however, the “normative” timing of CON rats in the PI procedure displayed disruptive effects that occurred as a function of reinforcement probability and the delay to reward indicated by the signal used to initiate and sustain timing on FI and probe trials. These disruptive effects involved an increase in responsiveness that was most prominent near the beginning of a trial and restricted to the left-hand portion of the peak functions. These “impulsive” responses were systematically related to reinforcement probability which interacted with the programmed time of reinforcement and prenatal choline treatment, i.e., for the PI-18s procedure there was a positive relationship between reinforcement probability and degree of disruption reflected in the lowered levels of the S1 rate index for CON rats, but not for SUP rats. Neither group of rats displayed a reliable relation between their S1 rate index and reinforcement probability for the PI-36s procedure. In contrast, for the PI-72s procedure there was a negative relationship between reinforcement probability and degree of disruption in the S1 rate index for SUP rats, but not for CON rats.

The asymmetrical levels of responsiveness in the S1 rate index as a function of the programmed time of reinforcement are most clearly observed when the probability of reinforcement is held constant at 50% and the 18, 36, and 72-s peak functions are plotted on a relative time scale (Fig. 4). In this case, the non-scalar source(s) of variance contributed by impulsive responding (presumably related to the “associative” properties of the signal and its relation to reinforcement probability and delay to reward) systematically reduced the degree of superimposition observed in the left-hand tails of the response distributions for CON rats, but were virtually non-existent in SUP rats who exhibit excellent superimposition of their timing functions. The result of this effect was a nesting of the left-hand portion of the Gaussian-shaped peak functions for CON rats with the PI-72s function exhibiting the sharpest ascent, followed by the PI-36s function, and the PI-18s function exhibiting the least amount of temporal control.

In comparison to the significant Treatment $\times$ Reinforcement Probability interaction for the S1 rate index, the effect of reinforcement probability on the S2 rate index appears to be more straight-forward. A positive relationship was observed between these two variables across all three programmed times of reinforcement (see Fig. 5). Although lower S2 rate indices were observed for the PI-72s functions than for the PI-18s and PI-36s functions, there were no significant differences as a function of prenatal choline treatment. Overall, these effects are in agreement with the observations of Church et al. (1994) for the preceding effect of food or nonfood trials on the setting of the S1 and S2 response thresholds for the current probe trial.

The present results also provide the most extensive support to-date for the claim of independence between peak time and peak rate in the PI procedure as first reported by Roberts (1981). This independence for both CON and SUP rats was evident from the lack of a significant effect of training sessions on peak time as a function of the variation in reinforcement probability which showed a large and systematic session effect on peak rate with a highly significant linear trend for repeated shifts from 75% $\rightarrow$ 50% $\rightarrow$ 25% reinforcement probability

at each of the 18-s, 36-s, and 72-s temporal criteria for both CON and SUP rats. The lack of co-variation in peak time and peak rate indicates that these measures represent different measures of performance and that the motivational mechanisms controlling response rate in CON and SUP rats can be separated from the timing mechanisms controlling the start (S1) and stop (S2) response thresholds necessary for the determination of peak time (see Buhusi and Meck, 2005; Matell and Meck, 2000, 2004; Matell et al., 2003, 2006; Meck and Benson, 2002; Meck, 2006a, c). These data parallel the feedback effects observed in PI timing in humans where normal participants are relatively insensitive to changes in the probability of feedback (Hinton and Meck, 2004; Rakitin et al., 1998), but individuals given dopamine receptor blockers (Lustig and Meck, 2005) or adults with attention-deficit/hyperactivity disorder taken off of their dopaminergic/nicotinic medication (Levin et al., 1996; Meck, 2005) exhibit significant impairments in the accuracy and precision of their timing performance when given feedback following 25% of the trials as opposed to 75% of the trials.

In contrast to previous findings obtained from differential reinforcement of low rates (DRL) schedules of reinforcement (Cheng et al., 2007b), the transitions from 18s → 36s → 72s did not reveal any significant differences between the CON and SUP rats (Fig. 6). The earlier finding of impaired response efficiency for SUP male rats compared to CON male rats during the transition to a longer temporal criterion suggests that the reinforcement contingencies associated with the DRL procedure prevented SUP rats from coming into contact with a new (longer) temporal criterion due to their persistence in responding at the previous temporal criterion. In contrast, because there are no penalties for premature responding in the PI procedure, initial persistence at the previous criterion doesn't preclude obtaining reward at the new (longer) criterion on the current trial – thus allowing CON and SUP rats to make the transition from 18s → 36s → 72s at virtually equivalent rates (although note the initial persistence at 18s for the SUP rats in the transition from 18s → 36s in the upper panel of Fig. 6 during the first session). In agreement with the current results, however, SUP rats trained on a series of 18-s, 36-s, and 72-s DRL procedures exhibited significantly lower levels of “impulsive” burst responding than CON rats.

Although the present results are most directly related to possible interactions of prenatal choline and $\alpha 7$ -AChRs in the striatum (Cheng et al., 2007; Meck, 2006b; Mobley et al., 1985; Morley et al., 1977) it is also important to note that prenatal choline supplementation leads to increases in NGF levels in the frontal cortex and hippocampus (Sandstrom et al., 2002). These results suggest that increased NGF levels throughout development may contribute to higher $\alpha 7$ -nAChR densities and influence corticostriatal circuits involved in adult timing behavior (e.g., Kawai et al., 2002; Matell and Meck, 2004; Summers et al., 2007).

In summary, the current results indicate a “purer”, more symmetrical and scalar form of timing performance for the SUP rats than for CON rats. This prenatal choline-induced enhancement of temporal control is due, in part, to a reduced level of contamination by impulsive responses related to reinforcement probability and the delay to reward signaled by the onset of a conditioned stimulus that also serves to initiate the timing of the interval (see Cheng, 1992; Cheng et al., 2006, 2007a, b; Church et al., 1991; Fantino et al., 1979). The observation that the “impulsive” responses of CON rats diminished as a function of lowered reinforcement probability and had less of an impact on the timing of longer intervals is consistent with the results reported for the PI procedure by Portugal & Matell (2007) and is in agreement with the finding that the initiation of responding in probe trials following a reinforced trial in the PI procedure is less sensitive to time than responding in probe trials preceded by another probe trial for shorter durations (e.g., 15 s) than for longer durations (e.g., 30 and 60 s) as reported by Church et al. (1994). These findings also raise the intriguing possibility that laboratory rodents raised on standard diets do not receive optimal levels of choline and other nutrients during early brain develop (e.g., Blusztajn, 1998; Blusztajn et al., 1998; Zeisel, 2000a, b,

2005; Zeisel and Blusztajn, 1994; Zeisel and Niculescu, 2006). As a consequence, such animals may not perform in the most advantageous manner on a variety of behavioral tasks requiring response inhibition, divided attention, and the flexible use of memory strategies across the lifespan (e.g., McCann et al., 2006; Meck, 2002, 2007c; Meck et al., 1986; Meck and Williams, 1997b, 1999b, 2003).

4. Experimental procedures

4.1. Animals, housing, and nutrition

Eight litters of timed-pregnant Sprague-Dawley Crl:CD(SD)BR-CD strain rats (Charles River Laboratories, Stone Ridge, NY) were used to generate two groups of prenatally treated male rats (n=16): Control (CON – n=8) and Choline-Supplemented (SUP – n=8). Upon arrival at our laboratory the pregnant dams were given a modified AIN-76A purified diet (Dyets Inc., Bethlehem, PA) with 1.1g/kg choline chloride substituted for choline bitartrate and water *ad libitum*.

Prenatal choline supplementation was given by adding a 25 mM (3.5 g/kg) choline solution (choline chloride from Sigma, St. Louis, MO, USA) during embryonic days (ED) 11–17 via saccharine sweetened drinking water (50 mM). Based on water consumption, the average choline dose for supplementation of the dams during gestation was approximately 250 mg/kg/day. Dams of the offspring to later be included in the CON treatment group received saccharine sweetened drinking water with no added choline. There were no significant differences in the amount of diet or water consumed by the dams in the different treatment groups, p 's < 0.05. At birth, all pups were randomly divided among untreated foster-mothers (10 pups/litter). All pups were weaned at post-embryonic days (PD) 25 after which rats were housed in pairs and given free access to the modified AIN-76A diet and water.

Behavioral training began at approximately 6 mo of age. Two weeks prior to the start of training, the rats were placed on a 24-hr food deprivation schedule during which they were fed approximately 12 g/day of the purified diet during a single daily feeding in order to maintain them at 80–85% of their free-feeding weights throughout the experiment. A light-dark cycle of 12:12 hr was maintained in the vivarium with fluorescent lights on from 7:00–19:00 EST.

4.2. Apparatus

All experimental data were collected in 8 operant-conditioning chambers constructed of an aluminum front/back walls and two plexiglass slide walls and ceiling. The floor was constructed of stainless-steel parallel bars with a drop tray underneath. Three response levers were evenly spaced across the front wall of the chamber, 10 cm above the grid floor. During the behavioral session, only the left lever was effective, while the central lever served as a control lever (i.e., always extended), and the right lever was always retracted. A pellet dispenser delivered 45-mg food pellets (Research Diets, Inc., New Brunswick, NJ) to a food cup located in the middle of the front wall and below the central levers. A 6-W houselight was mounted on the front wall of the chamber above the central lever and served as the signal to begin a trial. Each operant-conditioning chamber was housed inside a light and sound insulated box, and was equipped with a ventilation fan and an eyepiece viewer for observation. An IBM-PC compatible computer attached to a MED-PC interface box was used to control the experimental equipment and record the behavioral responses.

4.3 Behavioral Procedures

4.3.1. Pretraining (Sessions 1–5)—All rats received five sessions of combined magazine and lever training. During these sessions, a food pellet was delivered contingent on the press of the left lever or was automatically delivered once a min for 60 min. This daily training

session ended after the rat received 60 food pellets. The houselight illuminated the chamber upon completion of the session.

4.3.2. Baseline Fixed-Interval (FI) 18s Training (Sessions 6–20)—Sessions began with the illumination of the houselight. An 18-s discrete-trials fixed-interval (FI) procedure was scheduled for presses on the left lever. The first response after 18 s was reinforced with a 45 mg food pellet and the houselight was turned off. A random inter-trial interval (ITI) with a mean of 57 s and a range of 41–110 s immediately followed the reinforced response. All sessions lasted approximately 2 hrs.

4.3.3. Baseline Peak-Interval (PI) 18s Training (Sessions 21–30)—These sessions were identical to those of FI training with the exception that a non-reinforced “probe” trial was added to the trial types randomly selected for presentation on each trial so that the session contained a random mixture of 50% FI trials and 50% probe trials. These non-reinforced probe trials lasted for a minimum of 45 s plus a randomly selected amount of time with a mean of 17 s and a range of 1–70 s.

4.3.4. Peak-Interval (PI) 18s training with shifts in reinforcement density (Sessions 31–70)—During this phase of training, rats underwent a sequence of shifts in reinforcement density that was repeated twice. The sequence began with a random mixture of 75% FI/25% probe trials for 5 sessions followed by 50% FI/50% probe trials, 25% FI/75% probe trials, and 50% FI/50% probe trials. Each of these segments lasted for 5 sessions for a total of 40 sessions.

4.3.5. Transition from Peak-Interval (PI) 18s to PI-36s training (Sessions 71–80)—Following PI-18s training rats were given 10 sessions of standard PI-36s training (50% FI/50% probe trials). During the PI-36s training (the same hereafter), the mean length of the probe trials was extended to 90 s, plus the same range of a randomly selected interval as described above.

4.3.6. Peak-Interval (PI) 36s training with shifts in reinforcement density (Sessions 81–120)—During this training phase, rats again went through the same pattern of shifts in reinforcement density, as described above for PI-18s training.

4.3.7. Transition from Peak-Interval (PI) 36s to PI-72s training (Sessions 121–134)—For the final training phase of the experiment, 14 sessions of standard PI-72s training (50% FI/50% probe trials) were conducted, with the mean length of the probe trials extended to 180 s, plus the same range of a randomly selected interval as described above.

4.3.8. Peak-Interval (PI) 72s training with shifts in reinforcement density (Sessions 122–137)—During this phase of training, rats underwent a sequence of shifts in reinforcement density. The sequence began with a random mixture of 75% FI/25% probe trials for 5 sessions followed by 50% FI/50% probe trials for 6 sessions, and 25% FI/75% probe trials for 5 sessions – for a total of 16 sessions.

4.4 Data analysis

In the PI training sessions, peak time was defined as the time of the rat’s maximal response rate during a probe trial while that maximal response rate determines the peak rate. Peak time serves as a measure of when the subject maximally expects reinforcement to occur and it has been used to assess the accuracy of time perception while peak rate reflects the motivational state of the subject (see Church et al., 1994; Gibbon et al., 1984; Meck and Church, 1987a, b; Meck et al., 1987). In order to determine peak time and peak rate, the number of responses in

each 1-s interval during the signal presentation was recorded and summed over trials for each session. Next, mean response rate as a function of time since signal onset was calculated for each rat for each session. From the mean response rate functions, the peak time was estimated in the following manner: (a) the number of responses in a successive 4-s interval following each second was counted, (b) the interval with the maximum number of responses was determined, and (c) the middle of that interval was defined as peak time. If more than one interval satisfied the above criterion, the median value of these intervals was defined as the time of the peak rate on that trial. The peak rate was obtained by taking the mean response rate during the selected 4-s interval(s). This “sliding” window method was applied to the 18, 36, and 72-s PI training sessions in the same manner with the exception that the length of the sliding window was proportionally increased from 4 → 8 → 16-s respectively for each of the different FI values.

In addition to the measure of temporal accuracy (i.e., peak time), the measure of temporal precision (i.e., sensitivity) was also calculated in the current study. The S1 and S2 rate indices were determined by taking the response rate in a specified interval just prior to (S1) or after (S2) the observed peak time as a ratio of overall response rate within the first or second half of the trial. For example, the S1 rate index for the PI-18s procedure was defined by the response rate occurring during the 6-s period just prior to the observed peak time (e.g., seconds 13–18) divided by the overall response rate for the first 18 s of the trial (e.g., seconds 1–18). Similarly, the S2 rate index was defined by the response rate occurring during the 6-s period just after the observed peak time (e.g., seconds 19–24) divided by the overall response rate during the 18 s following the 18-s criterion (e.g., seconds 19–36). This method was applied to FI-18s training (in this case, only S1 was calculated), PI-36s, and PI-72s training sessions in the same manner except that the length of the interval defining the numerator was proportionally increased from 6 → 12 → 24s, respectively.

A criterion level of responding was set for all conditions such that rats had to respond at least 15 resp/min in order to be included in the analysis. One rat from the SUP group was excluded from the study using this response criterion. The p value was set at a minimum of 0.05 for all statistical analyses.

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Fig. 1.

Mean proportion maximum response rate plotted as a function of time (s) in the FI-18s signal for the first 3 sessions of 100% FI training (panel a – sessions 1–3) and the last 3 sessions of FI training (panel b – sessions 13–15) for adult rats in the control (CON – open squares) and prenatal choline supplemented (SUP – closed squares) treatment conditions. The mean ($\pm$ SEM) S1 Rate Index as a function of the individual training sessions is shown in panel c for CON and SUP rats.

Fig. 2.

Mean proportion maximum response rate for control (CON – open symbols) and prenatal choline supplemented (SUP – closed symbols) rats plotted as a function of time (s) in the PI-18s signal averaged over the blocks of sessions in which either a 25%, 50%, or 75% reinforcement probability was in effect (e.g., percentage of FI trials).

Fig. 3.

Mean proportion maximum response rate for control (CON – open circles) and prenatal choline supplemented (SUP – closed circles) rats plotted as a function of time (s) in the PI-18s, PI-36s, and PI-72s signal averaged for blocks of sessions in which either a 25% (top panel), 50% (middle panel), or 75% (bottom panel) reinforcement probability (Rf Probability) was in effect.

Fig. 4.

Mean proportion maximum response rate for control (CON – left panel) and prenatal choline supplemented (SUP – right panel) rats plotted as a function of normalized time (s) in the 18s, 36s, and 72s PI conditions. The scalar property of interval timing is identified when response functions having different temporal criteria superimpose when plotted on a relative/normalized time scale (Gibbon et al., 1984).

Fig. 5. Mean ($\pm$ SEM) *start* (S1) rate index (left panel) and *stop* (S2) rate index for control (CON – open symbols) and prenatal choline supplemented (SUP – closed symbols) rats. Data are expressed as a response rate ratio for the high rate of responding either just before (S1) or just after (S2) the time of reinforcement divided by the average rate of responding in either the first half (S1) or the second half (S2) of the probe trials for the PI-18s, PI-36s, and PI-72s conditions as a function of 25%, 50%, or 75% reinforcement probability (e.g., percentage of FI trials).

Fig. 6. Mean ($\pm$ SEM) peak time (s), peak rate (resp/min) and S1 rate index (ratio) as a function of sessions during which reinforcement probability shifted from 75% $\rightarrow$ 50% $\rightarrow$ 25% FI trials before transitioning to a new temporal criterion. Data for the PI-18s (upper panels) and PI-36s (lower panels) conditions are plotted for control (CON – open squares) and prenatal choline supplemented (SUP – closed squares) rats.

Fig. 7. Mean ($\pm$ SEM) peak time (s), peak rate (resp/min) and S1 rate index (ratio) as a function of sessions during which reinforcement probability shifted from 75% $\rightarrow$ 50% $\rightarrow$ 25% FI trials. Data for the PI-72s condition are plotted for control (CON – open squares) and prenatal choline supplemented (SUP – closed squares) rats.