1. Introduction

Malnutrition has long been known to alter growth and energy metabolism contributing to impaired brain function (Casper, 2004; Wauben and Wainwright, 1999). Developing organisms have a particular sensitivity to the nutritional status of the mother both in utero and during the perinatal period. Recently, interest in the role of nutritional deprivation during development has revived because of studies showing how early environmental factors (such as nutritional status) influence the phenotype of genetic disorders via interesting and often unpredictable gene–environment interactions (Casper, 2004; Wauben and Wainwright, 1999). These studies are of particular significance for public health because many children in both developed and developing countries live in extreme poverty with concomitant malnutrition that could influence brain development (de Vries, 1999).
The mechanisms of interaction between nutritional factors and genetic regulation in neural development are far from clear. Nutritional status can affect the availability of substrates necessary for processes including DNA methylation, cellular signaling, and neurotransmission (Zeisel, 1981; Zeisel and Blusztajn, 1994). Furthermore, it is likely that the lack of available substrates during critical periods in neural development could result in dramatic and long-lasting consequences in brain and behavior that could exacerbate a pre-existing genetic defect.

Neuronal development, which classically includes neurogenesis, migration, maturation, and synapse refinement (Rao and Jacobson, 2005), begins in utero and continues vigorously in the early postnatal period and, to a more limited extent, throughout life. These processes are not only genetically regulated but also clearly susceptible to environmental manipulation. For example, nutritional manipulations during critical periods of neural development can alter neuropathology and phenotype in several developmental disorders (Table 1). In toddlers, iron deficiency anemia in the first 2 years of life is associated with impaired mental and psychomotor development, but can be reversed by dietary iron supplementation (Idjradinata and Pollitt, 1993). Abnormalities in maternal homocysteine/folate metabolism may contribute to Down syndrome in the offspring (Scala et al., 2006; Sheth and Sheth, 2003). However, folate supplementation around conception decreases likelihood and may decrease severity of cognitive deterioration when it does occur (Barkai et al., 2003; Eskes, 2006; Thiel and Fowkes, 2005; Scala et al. (2006) and Sheth and Sheth (2003)).

Disorders with metabolic defects that can be reversed by dietary supplementation include anemia in early life (iron deficiency), Down syndrome (abnormalities in maternal homocysteine/folate metabolism), autism (Omega-3 fatty acid deficiency), and Rett syndrome (choline deficiency).

### Table 1: Interaction between nutritional environmental factors and developmental disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Nutritional deficit/environmental factor</th>
<th>Effect of deprivation/supplementation</th>
<th>Citations</th>
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<tbody>
<tr>
<td>Anemia in early life</td>
<td>Iron deficiency</td>
<td>Impaired mental and psychomotor development, can be reversed by dietary iron supplementation</td>
<td>Idjradinata and Pollitt (1993)</td>
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<tr>
<td>Down syndrome</td>
<td>Abnormalities in maternal homocysteine/folate metabolism</td>
<td>Folate deficiency may contribute to increased occurrence of disorder. Folate supplementation near conception decreases likelihood and may decrease severity of cognitive deterioration when it does occur</td>
<td>Barkai et al. (2003); Eskes (2006); Thiel and Fowkes (2005); Scala et al. (2006) and Sheth and Sheth (2003)</td>
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<tr>
<td>Autism</td>
<td>Omega-3 fatty acid</td>
<td>Deprivation not associated with occurrence, but supplementation decreases irritability, aggression and hyperactivity</td>
<td>Amminger et al. (2007) and Bell et al. (2004)</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>Choline</td>
<td>Disorder associated with decreased cholinergic activity. Perinatal supplementation improves both anatomical and behavioral symptoms in mouse models of the disorder</td>
<td>Nag and Berger-Sweeney (2007) and Ward et al. (2008)</td>
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</table>

The severity of Rett syndrome depends on the type (point, missense, or nonsense) and location of the mutation within the MeCP2 gene, both of which vary among individuals (Lee et al., 2001). Males with mutations of MeCP2 on their single X-chromosome produce no functional protein. Despite the nature of the mutation, such cases are almost always fatal early in development (Moretti and Zoghbi, 2006). In heterozygous females, the severity of the phenotype also depends on X-chromosome inactivation (Chae et al., 2004; Hammer et al., 2002). In every female cell, one of the two X chromosomes is randomly inactivated; therefore, the number of cells expressing mutated MeCP2 differs among individuals. Those with a higher number of cells expressing mutated MeCP2 may have more severe phenotypes than those favoring normal MeCP2 expression (Amir et al., 2000; Huppke et al., 2006).

Various knockout mice have been produced which serve as good models of Rett (Chen et al., 2001; Guy et al., 2001; Pelka et al., 2006; Shahbazian et al., 2002). While the genetic modifications differ, phenotypic and anatomical observations in all of these models are reminiscent of those observed in human Rett. Motor deficits are apparent in all, whereas anxious behaviors (Pelka et al., 2006; Shahbazian et al., 2002; Stearns et al., 2007), deficits in social interaction (Shahbazian et al., 2002), and cognition (Pelka et al. (2006); Stearns et al., 2007) are reported only in some. Interestingly, although MeCP2 expression is turned on early in development, recent studies suggest that certain neuroanatomical and behavioral symptoms can be reversed in mice in which MeCP2 expression is activated later in development (Giacometti et al., 2006).
2007; Guy et al., 2007). If the symptoms of RTT are reversible by Mecp2 reactivation, the neurons underlying the behavioral deficits may not be permanently damaged. Using other means to support or enhance neural functioning, such as the postnatal epigenetic treatments described here, may be able to improve some of the symptoms of the disorder. Although these treatments cannot hope to replace the functioning of the damaged gene, they may support neurodevelopment in other ways, allowing for more normal, if not completely normal, anatomical and behavioral phenotypes.

It has been suggested that many symptoms of RTT are due to alterations in neurotransmitter systems. Studies in human RTT report decreased choline acetyltransferase (ChAT) activity, a cholinergic marker, in the basal forebrain, basal ganglia and midbrain tegmentum (Kitt and Wilcox, 1995; Wenk, 1995; Wenk et al., 1991), and altered expression of GABA and glutamate receptors (Blue et al., 1999a; Johnston et al., 2005; Medrihan et al., 2007). GABA receptor density, as measured by autoradiography, is increased in the caudate of young girls with RTT (Blue et al., 1999a) but lower in the brain stem of Mecp2 knockout mice (Medrihan et al., 2007). Alterations in the glutamate receptor compliment are more complicated. AMPA and NMDA densities appear to be elevated in young RTT patients, but are significantly reduced in number, compared with normal controls, as the patients age (Blue et al., 1999a,b). In contrast, in one mouse model of RTT, AMPA and NMDA densities are no different from control in mice of any age, although there is an alteration in the proportional subunit makeup of the NMDA receptors (Asaka et al., 2006). It would stand to reason that boosting the function of the cholinergic, GABAergic and/or glutamatergic systems might very well ameliorate some of the symptoms of RTT. In this review, we focus on environmental manipulations that may be used to enhance the cholinergic system.

3. Dietary choline supplementation as a possible treatment of behavioral deficits in Rett syndrome

In a number of species, the central cholinergic system mediates cognitive and motor functions (Pepeu and Giovannini, 2004; Woolf, 1991). In rodents, choline supplementation during perinatal development leads to measurable long-term improvements in both cholinergic function as well as improvements on cognitive tasks. Rat pups are particularly sensitive to maternal levels of choline between embryonic day (ED) 12–17 and postnatal day (PD) 16–30. With supplementation during both critical periods, rats show improved performance on visuospatial memory tasks, and decreased hippocampal ChAT activity (Meck et al., 1989). In mice, choline supplementation during PD 1–16 affects retention of a passive avoidance task, increases locomotor activity in females, and decreases ChAT activity in the hippocampus (Ricceri and Berger-Sweeney, 1998). The decrease in ChAT activity in choline-supplemented rodents is consistent with reports that show decreased acetylcholinesterase (AChE) activity and increased acetylcholine (ACh) release (Blusztajn et al., 1998; Cermak et al., 1998; Koppen et al., 1997); suggesting higher intrasynaptic ACh concentrations and dwell times, possibly leading to enhanced cholinergic neurotransmission (Cermak et al., 1998). Other studies suggest that choline supplementation has optimal effects on improving performance on a spatial memory task when administered over both the pre- and postnatal periods (Meck et al., 1988). This perinatal period may be particularly susceptible to intervention by choline supplementation, because both the fetus and neonate utilize a large quantity of choline in growth-related membrane synthesis and biochemical pathways required for survival (Zeisel, 1981). Additionally, the perinatal period overlaps with critical developmental periods of several brain regions (Bayer et al., 1993), including the hippocampus and cerebellum, which play critical roles in learning and memory (Eichenbaum, 2004; Manns and Eichenbaum, 2006), and motor function (Bastian, 2006; Boyden et al., 2004), respectively. We have shown that choline supplementation from PD 1 to 22 in Mecp2 mutant mice has task-specific positive effects on behavior (Nag and Berger-Sweeney, 2007). In hemizygous males, perinatal choline supplementation significantly improves motor coordination as measured by rotor-rod performance at 5 weeks of age. At this age, heterozygous females do not exhibit a motor coordination deficit and perinatal choline supplementation does not significantly improve their performance. At 6–7 months of age, heterozygous females display motor coordination deficits compared to their age-matched wild-type control littermates (61.46 ± 4.80 and 98.82 ± 6.77 s), which was significant by genotype [F(1, 67) = 22.61, p < 0.001]. These deficits were not significantly improved with perinatal choline supplementation [F(1, 67) = 1.16, p = 0.29]. It may be that the effects of choline supplementation are not long lasting, or a higher dose and/or longer treatment regime is required. The results in hemizygous males nevertheless, provide the first evidence that a currently approved dietary supplement is potentially useful as a therapeutic agent for RTT, and suggest an important interaction between this genetic disorder and perinatal nutritional status.

4. Possible mechanisms through which choline may alter behavior

The nutritional status of RTT patients has not been examined carefully. However, feeding difficulties and abnormalities of the digestive tract in many RTT individuals have been noted (Isaacs et al., 2003; Lotan and Zysman, 2006), which may contribute to deficiencies in essential nutrients. Choline levels, measured in children and adults with RTT, are reportedly comparable to controls (Gokcay et al., 2002; Hashimoto et al., 1998; Khong et al., 2002), or elevated (Horska et al., 2000; Naidu et al., 2001); however, the profiles of choline during development are still unknown. In light of the possibility of nutritional deficiencies in RTT patients in conjunction with our findings that early perinatal choline improves motor performance in Mecp2 hemizygous male offspring, dietary supplementation makes sense as a palliative treatment to counteract behavioral deficits apparent in RTT.

We do not know the specific mechanism by which choline supplementation improves behavior, or even whether nutritional deficits exist and underlie the behavioral problems apparent in RTT. There are nevertheless several possible mechanisms by which this nutritional supplement could improve behavioral parameters in this developmental disorder (Fig. 1).

![Fig. 1. Depicted are several potential mechanisms through which choline supplementation improves motor coordination in Mecp2+/- mice.](image-url)
4.1. Choline availability may change the morphology and structure of neurons

One of the most consistent anatomical abnormalities in humans with RTT is the specific reduction in size of brain regions associated with motor and cognitive learning, which may contribute to some of the behavioral deficits. Similar to these studies in humans (Reiss et al., 1993; Subramaniam et al., 1997), we observe significant reductions in amygdalar, striatal, and hippocampal volumes in Mecp2 hemizygous male mice as compared to controls (Stearns et al., 2007). Reduced volumes in these regions may be due to reduced neuronal size and/or dendritic aborizations, both of which have been noted in human (Armstrong et al., 1995; Kaufmann et al., 2000) and mouse (Chen et al., 2001). Choline availability may be capable of improving behavioral deficits by improving the integrity of neuronal membranes to allow an increase in the size of neurons and their dendritic arbors.

Choline is a component of membrane phospholipids, and its availability during critical periods affects the structural integrity of cell membranes, and impacts the cells’ activity, function, and size. In rats, choline supplementation between ED 12 and 17 increases neuronal cell size in the medial septum/diagonal band (Williams et al., 1998), and alters the structure and function of hippocampal pyramidal cells (Li et al., 2004). In contrast, rats given choline-deficient diets between ED 11 and 17 have a smaller cross-sectional area of cholinergic neurons (McKeon-O’Malley et al., 2003).

We examined whether increases in brain volumes in Mecp2 mutant mice after postnatal choline supplementation may explain improved performance on some behavioral tasks. While neuronal maturation occurs predominantly during embryonic development, it continues into the postnatal period in the hippocampus and cerebellum (Bayer et al., 1993) and may be enhanced by increased choline availability. Data in our laboratory using longitudinal magnetic resonance imaging (MRI) show that postnatal choline supplementation increases the whole brain volume of heterozygous female mice to normal levels from the earliest time point measured (PD 21; Ward et al., 2008). These anatomical improvements precede the eventual emergence of motor coordination deficits in the female mice (Stearns et al., 2007). This volume recovery is maintained throughout the study (until PD 42). Choline treated hemizygous male mice showed some recovery at all time points (PD 21, PD 35, and PD 42), but because of high levels of variability in the data, these increases never reached statistical significance. Although perinatal choline supplementation has a greater effect on the cognitive abilities and brain development of male than on female mice (Williams et al., 1998), here postnatal choline supplementation appears more able to support more normal brain development in female mice. This apparent sex difference may reflect the chimeric nature of the heterozygous females, in which some cells express normal MeCP2 protein. The presence of a population of healthy cells may enable the female brains to take better advantage of choline supplementation than the male brains. Further increases in brain volume in hemizygous males may result if higher doses of choline, earlier (prenatal) supplementation, or continued supplementation past PD 21 are used.

4.2. Choline availability may alter the function of cholinergic neurons

Individuals with RTT have decreased ChAT activity (Wenk and Mobley, 1996), suggesting that cholinergic transmission itself is impaired. ChAT synthesizes the neurotransmitter acetylcholine and is a phenotypic marker for cholinergic neurons (Dobransky and Rylett, 2005).

Nutritional availability during perinatal periods can cause long-term adaptations in metabolism in adulthood. This type of adaptation, termed ‘metabolic imprinting’, occurs in the cholinergic system as well as in other physiological systems. For example, rats supplied a protein-free diet during the first 10 days of lactation show decreased insulin secretion and increased insulin sensitivity, as an adaptive response, at 1 year of age (de Souza Caldeira Filho and Moura, 2000). Additionally, in adulthood, lipogenic capacity in liver and in adipose tissues (Patel et al., 1993), as well as thyroid function (Passos et al., 2002; Phillips et al., 1993), appear to depend on maternal nutritional status during lactation.

Metabolic imprinting by choline availability during development results in changes in cholinergic neurotransmission in adulthood (Blusztajn et al., 1998; Meck and Williams, 2003). Choline availability in rats during ED 11–17 alters the turnover of choline and ACh in the hippocampus at PD 17 and 27 (Blusztajn et al., 1998). In adult rats that have been prenatally choline-supplemented, ACh turnover and choline recycling is slow while the evoked release of ACh is high (Blusztajn et al., 1998; Cermak et al., 1998). In view of this theory, it is possible that postnatal choline supplementation in Mecp2 hemizygous males improves behavior (Nag and Berger-Sweeney, 2007) by allowing increased synthesis and release of ACh in adulthood. Analysis of ChAT and AChE activities, and ACh release in these mice may provide insight into changes in cholinergic neurotransmission after choline supplementation.

4.3. DNA methylation

Methylation at CpG dinucleotides in genomic DNA is a fundamental epigenetic mechanism for controlling gene expression in vertebrates. Proteins with a methyl-CpG-binding domain (MBD), including MeCP2, can bind to single methylated CpGs. Upon binding, additional proteins are recruited that modify histone proteins, thereby forming compact, inactive chromatin. This process represses transcription, and is a method for mediating gene silencing (Fig. 2; Fan and Hutnick, 2005; Roloff et al., 2003).

The effective control of gene silencing by proteins such as MeCP2 may be hindered by the lack of available DNA methylation sites. Several dietary components such as methionine, folate, vitamins B2, B6, B12, selenium, betaine, choline, and zinc and the polyamines, can all influence the availability of methyl groups for DNA methylation. Varying the levels of any of the substrates that feed into this methylation pathway could increase or decrease DNA methylation efficiency (Van den Veyver, 2002). For example,
methyl-deficient diets cause hypo-methylation of hepatic DNA, leading to hepatocarcinogenesis (Bhave et al., 1988) and decreased hepatic levels of S-adenosylmethionine (Zeisel et al., 1989), a primary methyl donor. Methyl-supplemented diets, on the other hand, increase DNA methylation and have been shown to improve the health of agouti mice offspring (Cooney et al., 2002; Wolff et al., 1998).

The involvement of MeCP2 in methylation-specific transcriptional repression suggests that abnormalities associated with RTT may result from dysregulated gene expression (Renieri et al., 2003) which could be exacerbated by inappropriate DNA methylation. As methyl group availability is dependent on diet, dietary manipulation seems plausible as a means to improve the phenotype in this disorder. While several nutrients are likely candidates for inclusion in methyl-rich diets, the multifunctional roles of choline, including its ability to serve as a methyl donor after oxidation to betaine (Van den Veyer, 2002; Zeisel, 2006), make it an attractive supplement. It is possible that choline supplementation shifts DNA methylation from MeCP2 to the other proteins capable of methylation (Nag and Berger-Sweeney, 2007).

4.4. Transmembrane signal transduction

Early nutritional environment could also influence adult neuronal functioning through permanent modulation of the genetic regulatory machinery, which alters protein function. Specifically, neurotransmission can directly or indirectly initiate cascades of second-messenger molecules within the postsynaptic cell, which in turn modulate the activity of regulatory protein kinases. Kinases alter the function of other proteins by phosphorylating specific residues. Mitogen activated protein kinase (MAPK) in particular, plays a role in long-term potentiation (Soderling and Derkach, 2000; Sweatt, 2001), a putative mechanism underlying learning and memory, as well as various memory tasks (Atkins et al., 1998; Berman et al., 1998; Blum et al., 1999; Selcher et al., 1999). Prenatal choline supplementation in rats results in increased activity-dependent phosphorylation of MAPK, and of one of its targets, the transcription factor c-AMP-response element binding protein (CREB), in adulthood (Mellott et al., 2004). Conversely, prenatal choline deprivation leads to decreased activity induced phosphorylation of MAPK and CREB (Mellott et al., 2004). By influencing the responsiveness of regulatory cascades, early dietary choline levels may influence the effect of many aspects of an animal’s environment on gene expression far outside the actual window of supplementation.

5. Concluding remarks

We have seen in our own studies that the positive effects of choline supplementation are task-specific (Nag and Berger-Sweeney, 2007), while others have shown critical periods exist in which nutritional supplementation can have long-term effects on behavior (Meck et al., 1988, 1989; Ricceri and Berger-Sweeney, 1998). Future studies examining a range of nutritional supplements over various time periods, including adulthood, may allow insight into the extent to which diet may improve the health and behavior in individuals with RTT and other neurological disorders.

In addition to nutritional supplementation, other environmental variables, such as exposure to enriched housing (Leggio et al., 2005) can lead to positive, long-term effects on behavior and neuropathology. Rearing mice and rats in environmentally enriched housing, effects brain structure and function, by enhancing neurogenesis, gliogenesis, synaptogenesis and angiogenesis; by stimulating the activity of several neurotransmitter systems; and by increasing the expression of growth factors (Frick and Fernandez, 2003; van Praag et al., 2000). Additionally, environmental enrichment improves the behavioral phenotype in mouse models of other diseases. For example, enrichment slows the onset of motor decline in Huntington’s disease (Hockly et al., 2002) and improves cognitive performance in mouse models of Alzheimer’s disease (Arendash et al., 2004; Jankowsky et al., 2005). Preliminary data in our laboratory suggests that exposure to an enriched environment may also ameliorate some of the motor deficits in MeCP2 hemizygous male mice (Nag and Berger-Sweeney, unpublished observations). It may be interesting to investigate whether a combination of nutritional supplementation and environmental enrichment provides optimal beneficial effects on behavior and neuroanatomy.

It is clear that diet and enriched housing can have long-term consequences on behavior by altering gene expression, neurotransmission and/or neuronal structure. While these environmental factors are important for healthy development of all fetuses and children, they may be more critical when an underlying genetic disorder is present. Future studies examining the interaction between genetics and environment factors may offer insights to the mechanisms underlying the behavioral deficits, as well as potential avenues for palliative treatments.

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