Alteration of Neurotrophins in the Hippocampus and Cerebral Cortex of Young Rats Exposed to Chlorpyrifos and Methyl Parathion

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Exposure to either chlorpyrifos (CPS) or methyl parathion (MPS) results in the inhibition of acetylcholinesterase and leads to altered neuronal activity which normally regulates critical genes such as the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). The effects of postnatal exposure to CPS and MPS on the expression of messenger RNA (mRNA) and protein levels for NGF and BDNF were investigated in the frontal cerebral cortex (cortex) and hippocampus of rats. Oral administration of CPS (4.0 or 6.0 mg/kg), MPS (0.6 or 0.9 mg/kg), or the safflower oil vehicle was performed daily from postnatal day 10 (PND10) through PND20. Exposure induced significant effects on growth and cholinesterase activity. Increased NGF protein levels were observed in the hippocampus but not the cortex on PND20 with some reduction occurring on PND28 in both regions. These changes did not correlate with the changes in NGF mRNA. BDNF mRNA was increased in both regions on PND20 and PND28, whereas BDNF protein levels were increased on PND20. On PND12, c-fos mRNA, a marker of neuronal activation, was increased in both regions. Total BDNF protein was increased in the hippocampus but decreased in the cortex. No changes in NGF protein were observed. These results indicate that repeated developmental OP exposure during the postnatal period alters NGF and BDNF in the cortex and the hippocampus and the patterns of these alterations differ between regions.

Key Words: neurotrophins; chlorpyrifos; methyl parathion; developmental; organophosphate insecticide.

The neurotoxic effects observed following developmental organophosphorus (OP) insecticide exposure in experimental animals have led to increased concerns about the possible neurotoxic effects of these compounds on children (Eskenazi et al., 2007; Rauh et al., 2006). OP insecticide exposure of the general population is likely to occur in many scenarios due to the widespread use of these compounds in agricultural and commercial settings in the U.S., in the control of vector-borne diseases in tropical areas, and in gardening worldwide. Because children have a greater likelihood of exposure through food intake (National Research Council, 1993) and contact with contaminated surfaces than adults (Zartarian et al., 2000), significant attention has been placed on understanding the mechanisms underlying the neurotoxic effects of OP exposure during development. In addition, juvenile animals are known to be more sensitive to acute exposures and some toxicological end-points are more affected in juveniles than adults following subchronic exposure to OPs. These factors support the increased concern about OP exposure during development.

Among OPs, the phosphorothionates chlorpyrifos (CPS) and methyl parathion (MPS), stand out because of their heavy use. The acute toxicity of CPS and MPS is attributed to the inhibition of the enzyme acetylcholinesterase (AChE, acetylcholine hydrolase, EC 3.1.1.7) by their reactive metabolites chlorpyrifos-oxon and methyl paraaxon, respectively. Because AChE hydrolyzes the neurotransmitter acetylcholine (ACh), its inhibition allows more ACh molecules to bind to the nicotinic and muscarinic acetylcholine receptors which are present in the nervous system from the earliest periods of fetal development. Overstimulation of the ACh receptors by the presence of excessive ACh in the synapse has been shown to alter the pharmacokinetics of receptor activation. Downstream signaling pathways activated by muscarinic receptors have been the focus of current research for several reasons. First, loss of cholinergic transmission is one of the hallmark signs of neurodegeneration observed in Alzheimer’s patients (Bartus et al., 1982), and, second, muscarinic receptor activation is directly linked to cellular pathways involved in learning and memory processes such as long-term potentiation (Burgard and Sarvey, 1990). Although AChE inhibition is considered the main toxicological end-point for acute high dose exposures to OPs, it does not seem to correlate with the adverse effects observed after long-term, low-dose OP exposure. Delayed neurotoxic effects have been reported in neonatal animals exposed to CPS at levels which do not produce systemic toxicity and behavioral effects have been observed after substantial recovery of AChE activity (Campbell et al., 1997; Levin et al., 2001, 2002). Such studies have led to the hypothesis that many OP-mediated developmental effects are not related to AChE inhibition. However, the developmental toxicity of OPs may be due in part to indirect consequences of the disruption of cholinergic activity.
In addition, the relationship between long-term exposure to OPs and AChE inhibition is not clear because of the development of tolerance. In vitro studies have shown that acute treatment of hippocampal slices with AChE inhibitors induces a transient early phase of enhanced excitability that is followed by a delayed phase of suppressed neuronal activity (Kaufe et al., 1998). Decreased neuronal activity following hyperexcitation is more than likely a compensatory effort, such as receptor desensitization, to recover normal patterns of activity. In vivo, repeated exposure results in the down-regulation of muscarinic receptors which has been observed following developmental OP exposure (Betancourt and Carr, 2004; Richardson and Chambers, 2004; Tang and Chambers, 1999; Tang et al., 2003). Both the short-term suppression of neuronal activity and the long-term alteration of muscarinic receptors have the potential to change expression of the cholinergically regulated genes via alterations in muscarinic receptor-dependent pathways, yet the possible consequences of such alterations following OP exposure are not well understood.

The neurotrophins are a family of related proteins involved in the development of several neuronal populations in both the central and peripheral nervous systems. In the central nervous system, the neurotrophins, nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), have trophic actions on the cholinergic system including cholinergic marker expression. Both neurotrophins are regulated by the cholinergic system during development (da Penha Berzaghi et al., 1993). Our previous studies demonstrated that CPS exposure during the early postnatal period (PND1–7) decreased both NGF messenger RNA (mRNA) and protein in the rat forebrain (Betancourt and Carr, 2004; Betancourt et al., 2006). These earlier studies focused on the first postnatal week in rats which is developmentally equivalent to that of third trimester human fetal brain (Reinis and Goldman, 1980). The present study investigated the effects of repeated oral exposure to either CPS or MPS from PND10 through PND20 on NGF and BDNF mRNA and protein concentration in the hippocampus and the frontal cerebral cortex. The exposure period was selected because it is more reflective of an exposure during the childhood period in humans and targets the developmental period when NGF and BDNF normally began to change to reach adult levels in the rat. The brain areas selected are important for memory and cognition and possess the highest NGF and BDNF protein concentration in the brain during development (Hofer et al., 1990). The dosages of CPS (4.0 and 6.0 mg/kg) selected are above and below a dosage of CPS (5.0 mg/kg) previously reported to induce changes in neurospecific proteins following exposure to rats in a similar age range (Garcia et al., 2003). The dosages of MPS used were those which would give similar levels of ChE inhibition as the dosages of CPS.

OP exposure during postnatal development has been reported to alter neuronal cholinergic activity and cholinergic activity has been shown to induce transcription of immediate early genes (IEG) such as the transcription factor c-fos in several areas of the brain (Bernard et al., 1993). AChE inhibitors have been shown to induce c-fos in hippocampal slices (Kaufe et al., 1998). Given the IEG nature of BDNF (Lauterborn et al., 1993) and the regulation of the neurotrophins by cholinergic activity (da Penha Berzaghi et al., 1993), c-fos expression was determined as a marker of neuronal activation (Sagar and Sharp, 1993) along with NGF and BDNF protein levels at a point early during the exposure period to determine if changes in neurotrophin levels occur simultaneously with neuronal activation.

**MATERIALS AND METHODS**

**Chemicals.** CPS (> 99% purity) was a generous gift from DowElanco Chemical Company (Indianapolis, IN). Analytical grade MPS (> 97% purity) was supplied by Dr Howard Chambers (Department of Entomology and Plant Pathology, Mississippi State University). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO).

**Animals.** Adult male and female Sprague–Dawley rats [CD IGS] were obtained from Charles River Laboratories (Wilmington, MA) and used for breeding. Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility in a temperature controlled environment (22 ± 2°C) with a 12-h dark–light cycle with lights on between 0700 and 1900. LabDiet rodent chow (Richmond, IN) and tap water were freely available during the experimentation. The day of birth was designated as postnatal day 0 (PND0). Individual pups of each sex were assigned to each treatment within each litter. All procedures were approved in advance by the Mississippi State University Institutional Animal Care and Use Committee.

**Animal treatments.** CPS and MPS were dissolved in safflower oil and administered orally at a volume of 0.5 ml/kg body weight. Rats were treated daily from PND10 through PND20. The size of the litter was adjusted as much as possible in order to obtain litters of the same size (10 pups) and even distribution of male and female pups within each litter. The treatment groups were control (safflower oil); low dosage of CPS (CPS-Low, 4 mg/kg); high dosage of CPS (CPS-High, 6 mg/kg); low dosage of MPS (MPS-Low, 0.6 mg/kg); and high dosage of MPS (MPS-High, 0.9 mg/kg). In addition, a small number of animals were treated with the high dosages only from PND10 through PND12 and select parameters were determined at that age.

The dosages used in this study were designed to induce inhibition of ChE to further understand the potential risk of exposure in children. As stated in the Introduction, the dosages were based on the previous work of Garcia et al. (2003) who used 5 mg/kg CPS. In addition, previous studies have used dosages as high as 5 mg/kg and claimed that this dosage falls within the range of estimated fetal and neonatal exposures from environmental exposure or home use of CPS (Aldridge et al., 2005a). However, it is unclear if these dosages are within the range of exposure levels in children. Most likely, CPS and MPS exposure in children will occur at levels lower than those used in this study with the exception of situations which involve high levels of contamination.

Daily body weights for pups were recorded prior to treatment. Rats were euthanized by decapitation on PND12 and 20 (4 h after the last exposure to either CPS or MPS) and on PND28 (1 week after the last exposure to either CPS or MPS). The brain was rapidly removed and dissected on ice to obtain the hippocampus and the frontal cerebral cortex. Tissues were weighed, frozen immediately on dry ice, and maintained at −80°C until processed as described below. All dissection instruments and surfaces used for tissue dissection were previously washed and rinsed with RNaseZap solution (Ambion, Austin, TX), and distilled water.
Cholinesterase analysis. The frontal cerebral cortex and hippocampus were homogenized at 40 mg/ml in cold 0.05M Tris–HCl buffer (pH 7.4 at 37°C) in a glass mortar using a Wheaton motorized tissue grinder and a Teflon pestle. For CPS, ChE activity was determined spectrophotometrically using a modification (Chambers and Chambers, 1989) of Ellman et al. (1961). For MPS, ChE activity was determined using a continuous ChE method similar to that of Ellman et al. (1961). Both methods used acetylthiocholine as the substrate and 5,5′-dithiobis-(2-nitrobenzoic acid) as the chromogen. Protein concentrations were quantified with the Folin phenol reagent using bovine serum albumin as a standard (Lowry et al., 1951).

Real-time PCR. Total RNA isolation, RNA quantification and quality assessment from the hippocampus and the frontal cerebral cortex were performed as previously described in Betancourt et al. (2006). 18S ribosomal RNA was used as the internal standard for the duplex quantitative reverse-transcriptase PCR (Q-PCR). Primers and probes for 18S and NGF were the same as those used in our previous study (Betancourt et al., 2006) and, for BDNF, were as published by Wu et al. (2004). Similar to the reference and target genes, primers and probes for c-fos (sense primer: GGACAGGCTTTTCTC- TACTACATT; antisense primer: TTGGCAGCTAGAGACGGACAGA; and probe: CCCCAGCAGCTTCTTCCACGAT) were designed to span intron–exon boundaries (Molecular Beacon 2.0 software; Biosoft International, Palo Alto, CA). All primers were synthesized by MWG Biotech (High Point, NC), and all probes were synthesized by Sigma Genosys (The Woodlands, TX). Primer, probe, and template concentrations were optimized for 18S, c-fos, NGF, and BDNF individually followed by optimization in duplex PCRs (iCycler; Biorad, Hercules, CA; Platinum Quantitative RT-PCR ThermoScript One-Step System; Invitrogen, Carlsbad, CA).

Q-PCR for rat c-fos, NGF, and BDNF mRNA levels was performed. Each PCR was done in triplicate in a final reaction volume of 50 μl. The thermal cycler conditions for the RT reaction were 65°C, 30 min; 95°C, 5 min; and 40 cycles of 95°C, 15 s; 65°C, 1 min. In all experiments, samples containing no template were included as negative controls. Standard curves of both the reference gene (18S) and each target gene were used to calculate the PCR efficiency for each gene (X-fold dilutions from 1:10 to 1:1000). The threshold cycle values (Ct values) of the target gene were normalized to the Ct values of 18S to correct for template variation. From the normalized mean Ct values for each target gene, the fold change was calculated using the method of $2^{(C_{t} \text{ (control)} - C_{t} \text{ (treated)})/ \text{PCR efficiency}}$. Within each gene and age, statistical analysis was performed as described below and 95% confidence intervals were determined.

Neurotrophin quantification. The frontal cerebral cortex and hippocampus were suspended in six volumes of lysis buffer (100mM Tris–HCl, 1M NaCl, 2% BSA, 4mM ethylene-diaminetetraacetic acid, 0.2% Triton X-100, 0.02% sodium azide, 0.1 μg/ml pestatin A, 5 μg/ml aprotinin, 0.5 μg/ml antipain, 167 μg/ml benzamidine, 5.2 μg/ml phenylmethanesulphonylfluoride) (Zettler et al., 1996). Tissues were homogenized in a glass mortar using a Wheaton motorized tissue grinder and a Teflon pestle, and centrifuged at 14,000 × g for 20 min at 4°C. The supernatant was removed, placed in 1.5-ml microcentrifuge tubes, and maintained at −80°C until assay.

Acid treatment of NGF and BDNF samples. Acid treatment of the samples before determination has been shown to release receptor-bound complexes of neurotrophins that cannot be accessed by antibodies (Zettler et al., 1996). This procedure has been extensively used to avoid underestimation of receptor-bound neurotrophins and it was optimized in our study using control samples from the frontal cerebral cortex and hippocampus. These preliminary experiments showed that acid treatment of hippocampal and cortical samples increased the amount of detectable BDNF but not of NGF (data not shown). Therefore, BDNF protein was determined in both non-acid-treated (unbound) and acid-treated (total) samples, whereas NGF protein was determined only in non–acid-treated samples. Samples were acidified by the addition of 1 μl of 1N HCl per 50 μl of diluted sample. The pH was confirmed to be less than 3.0 and the samples were incubated for 15 min at room temperature, following neutralization by the addition of 1 μl of 1N NaOH per 50 μl of sample. The pH was confirmed to be approximately 7.6.

NGF and BDNF enzyme-linked immunosorbent assay. NGF and BDNF protein levels were measured by enzyme-linked immunosorbent assay (ELISA) using the DuoSet kit for rat β-NGF, and the DuoSet kit for human BDNF (R&D Systems, Minneapolis, MN). Optimal conditions for tissue concentration, lysis buffer, and acid treatment of the samples were determined for each neurotrophin.

Statistical analysis. Statistical analysis was performed by analysis of variance using the Mixed procedure (Littell et al., 1996) to determine significant sex, treatment, and sex × treatment interactions. Subsequent mean separation was performed by the Least Significant Difference method. The criterion for significance was set at $p \leq 0.05$.

RESULTS

Initial experiments included all five treatment groups with daily administration of chemicals beginning on PND10 and continuing for 10 days with sacrifice on PND20 and PND28. Following determination of ChE specific activity and NGF and BDNF mRNA and protein levels in these PND20 and PND28 samples, the exposure paradigm was repeated with the high dosages only and animals were sacrificed on PND12 and select parameters were determined at that age. Statistical analysis determined a significant sex × treatment interaction for body weight and data are presented with sexes separated. However, statistical analysis of the mRNA, protein, and ChE data did not yield any significant sex differences or any sex × treatment interactions at any age. Therefore, data from males and females were pooled for analysis and data are presented as combined males and females.

In females, CPS-High significantly reduced body weights at all ages with some recovery by PND28 (Fig. 1A). CPS-Low significantly reduced body weight on PND20 and PND28. With MPS-High, body weight was significantly reduced on PND20 and PND28. There were no effects of MPS-Low on body weight in females. In males, CPS appeared to have greater effects with respect to reduction of body weights than did MPS (Fig. 1B). Significant reductions were present with both dosages of CPS at all four ages but no significant effects of MPS were observed at any age.

ChE specific activity was significantly reduced in both the hippocampus and the frontal cerebral cortex (cortex) with all treatment groups at all ages. On PND12, CPS-High induced greater inhibition than did MPS-High regardless of brain region (Figs. 2A and B). By PND20, there was less inhibition with CPS-High but similar/greater inhibition with MPS-High in both brain regions as compared with the level of inhibition on PND12. At this age, all treatment groups of both compounds yielded comparable levels of inhibition with each brain region. By PND28, substantial recovery had occurred with all treatment groups in both brain regions. In contrast to the
pattern of inhibition at PND12, the MPS treatment groups yielded greater inhibition than did the CPS treatment groups on PND28.

In the hippocampus, NGF mRNA was significantly decreased by CPS-High and MPS-High on PND20, whereas on PND28, these two treatments significantly increased NGF mRNA (Fig. 3A). NGF protein was significantly increased with the high dosages of both CPS and MPS on PND20 (Fig. 3B). On PND28, NGF was significantly decreased with MPS-Low and MPS-High but, although not statistically significant, both dosages of CPS decreased NGF by 10–11%. In the cortex, less pronounced effects were observed with only CPS-High significantly increasing NGF mRNA on PND20 and no significant effects with any treatment on PND28 (Fig. 4A). A nonsignificant increase in NGF protein was observed with MPS-High only on PND20 (Fig. 4B). On PND28, NGF was significantly decreased with both CPS-High and MPS-High with greater effects observed with CPS. Overall, the changes in NGF protein observed did not correlate with the effects at the level of NGF mRNA.

As indicated in the Methods section, acid treatment of samples did alter the levels of measurable BDNF and ELISA was conducted on both non–acid-treated samples (unbound of free BDNF) and acid-treated samples (bound and unbound BDNF or total BDNF). Acid-treatment of the samples demonstrated greater changes in BDNF protein than the non–acid treatment of the samples in both the hippocampus and the frontal cerebral cortex.

BDNF mRNA levels were much more responsive to the exposure than were NGF mRNA levels. In the hippocampus, BDNF mRNA was significantly increased with all treatment groups on PND20 (Fig. 5A). This statistically significant increase in BDNF mRNA was still detectable on PND28 with the high dosages of both compounds, although it was lower than that at PND20. The unbound fraction of BDNF protein in the hippocampus on PND20 was increased with all dosages of both compounds but only CPS-High was statistically significant (Fig. 5B). However, unbound BDNF, although lower, were statistically similar to control levels by PND28. Total BDNF protein in the hippocampus on PND20 was significantly increased by the high dosages of both compounds (Fig. 5C). On PND28, total BDNF protein was significantly reduced with CPS-Low only but all treatments were lower than control levels.

In the cortex, all treatments except CPS-Low significantly increased BDNF mRNA on PND20, whereas on PND28, a significant increase was observed with all four treatments (Fig. 6A). On PND20 and PND28, MPS-Low demonstrated greater effects than did CPS-Low. Unbound BDNF protein was significantly increased with both CPS-High and MPS-High on PND20 but no significant effects were observed on PND28 (Fig. 6B). Total BDNF protein was significantly increased in the high dosages of CPS and MPS on PND20 with greater effects observed with MPS (Fig. 6C). However, by PND28, total BDNF was significantly increased only with MPS-Low.
while all other treatments were increased (> 25%) above control levels.

The increase in BDNF protein observed on PND20 suggests that this increase could be the result of increased neuronal activation induced by the OP treatment. Thus, additional samples were obtained from rats treated with the high dosages only on PND12, 4 h following that day’s treatment. NGF protein levels and BDNF protein levels were determined in the hippocampus and the cortex, as was ChE as reported earlier. In the PND12 hippocampus, NGF levels were not significantly different from controls but both unbound and total BDNF protein were significantly increased with high dosages of both CPS and MPS with greater effects observed on total BDNF than on unbound BDNF (Fig. 7A). However, in the cortex, NGF levels were decreased ~9–11% in both treatment groups, whereas no changes in unbound BDNF were observed on PND12 (Fig. 7B). However, total BDNF was significantly decreased with the high dosages of both CPS and MPS. Also on PND12, the mRNA levels of the transcription factor c-fos, which can be considered a marker of cellular activation, were significantly increased in both the hippocampus and cortex with the high dosages of both CPS and MPS with greater effects observed in cortex with CPS (Fig. 8).

**DISCUSSION**

The results of this study demonstrate that late preweanling exposure to CPS and MPS has the potential to induce significant changes in the expression of NGF and BDNF mRNA expression and protein levels in the hippocampus and frontal cerebral cortex. As stated earlier, the dosages of CPS used in this study were above and below a dosage of CPS (5.0 mg/kg) previously reported to induce neurotoxicity in rats treated daily from PND11–14 without inducing overt systemic toxicity, weight loss, or mortality (Song et al., 1997; Campbell et al., 1997; Johnson et al., 1998; Dam et al., 1999, 2000; Garcia et al., 2003). However, our results do not agree with these previous studies in that we observed a significant reduction in growth with both dosages of CPS in males and CPS-High in females after only 3 days of treatment on PND13 (measured prior to that day’s exposure). Differences in the route of administration may be the basis for the differences between the present study (oral gavage in oil) and previous studies (subcutaneous in dimethyl sulfoxide). Another interesting observation is that more effects of MPS appeared in females, whereas males appeared to be more affected by CPS. However, this was observed only with respect to body weight because no other sex differences were observed in any other parameter studied with either compound.

The different dosages of CPS and MPS did not yield a dose–response inhibition of ChE activity when measured on PND20 or PND28. Because ChE was measured at times following multiple repeated exposures, it is possible that a dose–response pattern of inhibition may have occurred at an earlier time and was merely not detectable at the time of sampling. Unfortunately, we did not perform a time course for the initial
pattern of inhibition of ChE and, in addition, it is possible that the pattern of inhibition of ChE could differ following additional exposures in a repeated exposure scenario. Therefore, it would not be wise to make direct correlations between ChE inhibition and the changes in neurotrophin expression and protein levels.

With respect to NGF, the changes in mRNA expression did not consistently result in a corresponding change in protein suggesting that the expression of NGF mRNA and the production of NGF protein are regulated differently at these ages. This differs from what we observed in neonatal rats (Betancourt and Carr, 2004; Betancourt et al., 2006) but agrees with previous studies showing differential NGF mRNA and protein regulation in the adult rat hippocampus (Rossner et al., 1997). Conflicting reports concerning NGF mRNA expression following cholinergic stimulation with both increased mRNA levels (da Penha Berzaghi et al., 1993) and decreased mRNA levels (Seo et al., 2002) being reported. Given that ours was a repeated exposure paradigm and sampling did not occur until well into the exposure, multiple neurotransmitter systems, many of which can influence NGF expression, would have been activated as a result of the initial cholinergic excitation and the patterns of the expression of mRNA and the levels of protein could be reflective of input from multiple systems, both excitatory and inhibitory. Thus, the actual basis for the differential changes in NGF mRNA and protein levels is not clear. It could be that the OP treatment itself is merely causing some type of alteration in the translation of NGF mRNA or in the stability of NGF mRNA and/or protein. However, the trend toward a decrease in NGF protein levels observed on PND28, which is 8 days following cessation of exposure, may be indicative of a persistent decrease in NGF protein levels. Future studies are needed to clarify these issues.

Both BDNF mRNA levels and protein levels, especially in the high dosages, were increased on PND20 but a similar
pattern was not present on PND28 where mRNA was still elevated but few effects were observed on protein levels. On PND20, the high dosages of both CPS and MPS increased the unbound fraction of BDNF protein (non–acid treatment) but induced even larger increases in total BDNF (acid treatment) suggesting that a significant portion of the OP-induced BDNF protein resides in a bound state. In addition, although unbound levels of BDNF were increased to a similar extent in the hippocampus and cortex, a larger increase in total BDNF (acid treatment) was present in the cortex than the hippocampus with the high dosages of both compounds.

The increased levels of BDNF protein was an unexpected finding and differed from what we previously observed in neonatal rats (Betancourt and Carr, 2004). As stated earlier, BDNF exhibits an IEG nature (Lauterborn et al., 1993) and the increased BDNF protein levels may be the result of OP-induced cellular excitation. Using c-fos expression as a marker, neuronal excitation was clearly evident in both brain regions on PND12. These data agree with previous studies reporting upregulation of c-fos following OP insecticide exposure (Dam et al., 2003). Although the increase in c-fos expression was paralleled by a significant increase in BDNF protein in the hippocampus, a similar pattern was not observed in the cortex on PND12. Although this further suggests that these two regions respond differently following a repeated OP exposure, the increased BDNF levels on PND20 in both regions suggest that there is ultimately a similar response. However, the pattern of onset and magnitude of this response...
varies between regions and detection of differences may depend on the time of sampling following exposure.

It is known that BDNF expression is linked to cAMP response element binding protein (CREB) phosphorylation which then activates the CRE in the rat BDNF promoter (West et al., 2001). CREB phosphorylation is the point of convergence of several signaling pathways that raise intracellular Ca$^{2+}$ concentration including activation of muscarinic and nicotinic receptors which would occur during an OP exposure. Previous results have also demonstrated that CPS can increase CREB phosphorylation in cortical and hippocampal neuronal cultures (Schuh et al., 2002) and it would interesting, in future studies, to determine if a similar phosphorylation of CREB occurs in vivo.

Not many parameters demonstrated significant differences between CPS and MPS treatment. Those observed were confined to the cortex with none observed in the hippocampus. For example, different effects were observed between CPS and MPS in NGF protein on PND28 (Fig. 4B), in BDNF mRNA on PND20 and PND28 (Fig. 6A), and in total BDNF protein on PND20 (Fig. 6C). In other words, no obvious pattern was observed with respect to neurotrophin levels or expression. With respect to c-fos mRNA expression, CPS-High induced greater expression than did MPS-High on PND12 (Fig. 9). This difference in c-fos expression on PND12 could be attributed to CPS causing more cholinergic excitation than MPS since on PND12, the amount of ChE inhibition was higher in the CPS treated group than in the MPS treated group. These data suggest that although inhibition levels were similar on PND20 following repeated exposures, differences in the level of inhibition exist at earlier time points in the exposure period. Because it is known that different OPs exhibit differential pharmacokinetic and pharmacodynamic behavior with respect to metabolism (Karanth et al., 2004), time to peak ChE inhibition (Chambers and Carr, 1993), and inhibition and recovery dynamics of ChE activity (Chambers, 1992; Wilson et al., 1992), this emphasizes the importance of determining the level of ChE inhibition at multiple time points in a repeated OP exposure scenario. Overall, these effects suggest that the cortex has the potential to respond distinctly to different OPs with respect to some parameters.

Overall, developmental OP exposure altered the expression and protein levels of neurotrophins. The change in neurotrophin levels could merely be a response to increased cellular stress, including metabolic stress (Lindvall et al., 1992). In addition, neurotrophins have been implicated in neuroprotection (Holgado-Madurga et al., 1997; Kume et al., 2000; Tabakman et al., 2006) and the changes may be a protective mechanism because exposure of neuronal cell cultures to CPS.
has been shown to induce apoptosis (Garcia et al., 2001; Caughlan et al., 2004). Overall, the exact mechanism behind the changes in neurotrophin levels is not clear. The increased BDNF protein levels in the hippocampus appear to be related to the OP-induced cellular excitation but this may or may not be true for the cortex. However, it may be that the response of the cortex is slower than that of the hippocampus such that BDNF mRNA levels may have been increased in the cortex and not yet translated into protein at the time of our sampling.

A key feature of all structures in the developing central nervous system is the presence of endogenous large-scale spontaneous activity (Feller, 1999). This type of endogenous activity has been suggested to have an important role in the activity-dependent wiring of neuronal circuits (Rivera et al., 2005). It is clear that an OP exposure not only disrupts this ongoing spontaneous activity but can also induce changes in the expression of activity-regulated genes. BDNF and NGF are important in activity-dependent development (Pesavento et al., 2000; Tartaglia et al., 2001) and their availability during development is highly regulated. Thus, abnormal levels of neurotrophins could induce negative long-term effects. In transgenic mice overexpressing BDNF, cognitive function deficits in a passive avoidance task (Croll et al., 1999) and anxiogenic effects (Govindarajan et al., 2006) have been reported. Our laboratory has observed deficits in a passive avoidance task and anxiogenic effects in an elevated plus maze following developmental exposure to OPs (Johnson et al., 2006). Others have also observed effects in the elevated plus maze (Aldridge et al., 2005b). In addition, it has been proposed that increased BDNF levels during brain development may be involved in the etiology of autism (Tsai, 2005) and that pesticide exposure could be an environmental factor involved in the pathology of this developmental disorder (D’Amelio et al., 2005; Keller and Persico, 2003). Thus, it is possible that alteration of neurotrophin levels during brain maturation could be of pivotal importance in the developmental neurotoxic effects of OP insecticides.

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