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Effects of Prenatal Exposure to Phenobarbital on Verbal Abilities in School-Aged Children

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EFFECTS OF PRENATAL EXPOSURE TO PHENOBARBITAL ON VERBAL ABILITIES IN SCHOOL-AGED CHILDREN

A Thesis Presented

By

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ABSTRACT

EFFECTS OF PRENATAL EXPOSURE TO PHENOBARBITAL ON VERBAL ABILITIES IN SCHOOL-AGED CHILDREN

August 2020

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Phenobarbital is one of the oldest medications used for the treatment of epilepsy. Although its use has declined in many developed nations, phenobarbital is still a first-line treatment in several developing countries across the globe. If possible, current American Academy of Neurology guidelines advise against the use of phenobarbital during pregnancy due to an increased risk for structural malformations. However, less is known about the risk that prenatal exposure to phenobarbital poses to the cognitive and behavioral development of the child. Adams et al (in progress) have shown that, in comparison to demographically

matched controls, children prenatally exposed to phenobarbital for the treatment of maternal epilepsy have a significant reduction in general mental ability and verbal intelligence. In this paper, we aim to further explore the impact of prenatal exposure to phenobarbital on verbal abilities by examining performance on individual verbal subtests within the original testing battery. The performance of children that were prenatally-exposed to phenobarbital and demographically matched controls was compared on a selection of verbal subtests from the Wechsler Intelligence Scale for Children, 3rd Edition (WISC-III), the Stanford-Binet Intelligence Scales, 4th Edition (SB-IV), and the Wechsler Memory Scale, Revised (WMS-R). Maternal intelligence was assessed with the Wechsler Abbreviated Intelligence Scale, Revised (WAIS-R). Initial analyses were conducted to explore the effects of sex and treatment on verbal performance. However, no significant effects of sex nor any interaction effects were found, so sex was removed from later analyses. ANCOVA controlling for maternal intelligence confirmed a significant reduction in general mental ability and verbal intelligence in the sample of children prenatally exposed to phenobarbital. Multivariate ANCOVA controlling for maternal intelligence revealed a significant effect of exposure group on verbal performance across measures. Follow-up analyses revealed that children prenatally exposed to phenobarbital performed significantly worse than controls on the Vocabulary and Arithmetic subtests from the WISC-III and on a test of Story Memory from the WMS-R. Combined with previous findings by Adams et al., the results of this study further support an effect of prenatal exposure to phenobarbital on children's verbal abilities.

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CHAPTER 1

INTRODUCTION

According to the World Health Organization (WHO), there are approximately 50 million people living with epilepsy worldwide (World Health Organization, 2019). It is estimated that 25% of individuals living with epilepsy are women of childbearing age (Pennell et al., 2012) and in the United States alone, approximately three to five per 1000 births are to a woman with epilepsy (Harden et al., 2009). Historically, understanding the risks that maternal epilepsy during pregnancy poses to the fetus has been challenging, as it has been difficult to disentangle the risks associated with the disorder from the risks caused by exposure to antiepileptic medications (Holmes et al., 2001). Some researchers originally suggested that structural teratogenic effects seen in infants born to women with epilepsy were likely due to inheritance of genetic differences associated with maternal epilepsy (Gaily, Granstrom, Hiilesma, & Brady, 1988). However, a growing body of literature began to amass that compared the outcome of infants exposed to antiepileptic medications *in utero* with infants of women with well-managed, untreated epilepsy and healthy controls. These studies found that, while infants exposed to antiepileptic medications often exhibited physical anomalies, infants of women with untreated epilepsy did not differ from controls (Nulman, Scolnick, Chitaya, Farkas, & Koren, 1997; Holmes, Rosenberger, Harvey, Khosbin, & Ryan., 2000; Holmes et al., 2001). In general, these studies, along with others, suggested a link between antiepileptic medications and structural abnormalities and established the older

antiepileptic medications, including carbamazepine, phenytoin, valproate, and phenobarbital, as teratogens (Hill, Verniaud, Horning, McCulley, & Morgan, 1974; Hanson & Smith, 1976; Seip, 1976; Jones, Lacro, Johnson, & Adams, 1989; Jones & Chambers, 1992; Nulman et al., 1997; Holmes et al., 2000; Holmes et al., 2001). In addition, more severe teratogenic effects were reported in infants exposed to treatment at higher doses and polytherapy treatment regimens (Holmes et al., 2001; Harden et al., 2009).

For many women with epilepsy, discontinuation of medication during pregnancy is not an option due to risks of maternal physical injury, mortality, or effects on infant birth weight or prematurity (Battino & Tomson, 2007; Chen, Chiou, Lin, & Lin, 2009). Therefore, management of treatment must aim to maintain or decrease seizure frequency while also considering the risk that exposure to antiepileptic drugs poses to the fetus. Indeed, in order to reduce the risk of congenital malformations and cognitive impairment, the American Academy of Neurology (AAN) has recommended monotherapy at the lowest effective dose and cautions against the use of polytherapy during pregnancy (Harden et al., 2009).

First discovered in 1912, phenobarbital is one of the oldest medications that has been used to effectively treat epilepsy (López-Muñoz, Ucha-Udabe, & Alamo, 2005). Phenobarbital exerts its primary anti-seizure effect through allosteric binding to the $GABA_A$ receptor, leading to a prolonged influx of chloride and hyperpolarization of the postsynaptic membrane (Rogawski & Porter, 1990). Treatment with phenobarbital has been associated with adverse side effects in patients, including sedation, changes in mood, and impaired cognition (Kwan & Brodie, 2004). When compared to healthy controls, patients with general epilepsy who were treated with phenobarbital monotherapy showed adverse cognitive side effects, including slowed motor movements, slower processing speed, and impaired attention

(Manni et al., 1993). Furthermore, in a double-blind randomized trial in which patients with partial and secondary generalized tonic-clonic seizures were assigned to a treatment group, almost 50% of patients receiving phenobarbital withdrew from the study due to reported toxicity (Mattson et al., 1985). Due to these adverse side effects and poor tolerability by some patients, in the United States and many other developed nations, phenobarbital prescriptions have decreased in favor of newer antiepileptic medications (Kwan & Brodie, 2004; Brodie & Kwan, 2012), including during pregnancy (Meador et al., 2018). However, due to its low cost and high efficacy, phenobarbital remains a first-line treatment for epilepsy in low- and middle-income countries (World Health Organization, 2019).

During pregnancy, phenobarbital readily crosses the placenta and enters the fetal chamber (Melchior, Svensmark, & Trolle, 1967). Because phenobarbital has been associated with an increased risk of cardiac and oral cleft malformations (Hernández-Díaz et al., 2012), current guidelines for treatment during pregnancy advise against the use of phenobarbital if possible (Harden et al., 2009). However, in many nations, phenobarbital remains one of the only treatment options (Kwan & Brodie, 2004), and while it has been established to have structural teratogenic effects, less is known about how prenatal exposure to phenobarbital impacts the cognitive and behavioral development of the offspring.

Some of the earliest evidence to suggest a neuroteratogenic risk of phenobarbital has come from rodent models of gestational and neonatal exposure to the drug. Early research established that gestational exposure to phenobarbital from mid-to-late gestation resulted in decreased brain weight in exposed pups and reductions in cerebellar Purkinje cells and hippocampal pyramidal cells (Yanai, Rosselli-Austin, & Tabakoff, 1979). Gestational exposure has also resulted in behavioral alterations at several timepoints in development

(Christensen, Gonzalez, & Rayburn, 2004). As pups, exposed mice demonstrated fewer vocalizations following maternal separation and a greater startle response to a stimulus. As adults, phenobarbital-exposed offspring showed decreased motor activity, increased startle to a stimulus, increased anxiety-like behavior, and impaired learning on a tube runway.

Because the neonatal period from days 1 to 10 in rodents is estimated to align with the third trimester in humans (Clancy, Finlay, & Darlington, 2007), models of neonatal exposure to phenobarbital have also been used to inform the neuroteratogenic risk of the medication. Similar to gestational exposure, treatment with phenobarbital from the first to third week resulted in reduced brain weight in pre-weaning (Schain & Watanabe, 1975) and adolescent mice (Yanai & Bergman, 1981). The total area of the cerebellum and of the dentate gyrus of the hippocampus were also reduced in mice treated with phenobarbital, and there was a reduction in cerebellar Purkinje and granular cells and hippocampal pyramidal and granule cells (Yanai & Bergman, 1981). Within the cerebellum, there were lasting decreases in dendritic spine densities of Purkinje cells (Yanai & Iser, 1981).

Noting the reduction in total brain weight and in specific populations of neurons in phenobarbital-exposed animals, several groups have attempted to uncover possible mechanisms of action, including impaired neurogenesis and increased apoptosis. Following in utero phenobarbital exposure from mid- to late-gestation, there was decreased proliferation of Purkinje cells, pyramidal cells, and cells of the cortex in young offspring (Yanai, Woolf, Feigenbaum, 1982). Decreased proliferation was also seen throughout the brain following neonatal treatment with phenobarbital during the first week of life and resulted in decreased production of neurons at P15 (Stefovska et al., 2008). By adulthood, phenobarbital treated mice showed impaired spatial memory and structural abnormalities in the hippocampus and cingulate cortex (Stefovska et al., 2008). Combined, the results of these studies suggest that

phenobarbital exposure during gestation and neonatal periods impairs cell proliferation and neurogenesis and that these early alterations result in structural and functional brain abnormalities that persist into later life.

In addition to impairing the birth of new cells, research has also suggested that neonatal treatment with phenobarbital induces widespread apoptosis throughout the developing brain. Treatment with phenobarbital throughout the first month of life resulted in increased apoptosis throughout the brain, including in the hippocampus, thalamic nuclei, subiculum, amygdala, hypothalamus, caudate nucleus, nucleus accumbens, and globus pallidus (Bittigau, Sifringer, & Ikonomidou, 2003). Acute treatment with phenobarbital in pups also induced apoptosis in the striatum, ventral thalamus, and lateral thalamus, and these results were amplified by polytherapy treatment combined with lamotrigine (Katz, Kim, Gale, & Kondratyev, 2007).

Combined, studies conducted in rodent models suggest that phenobarbital exposure during periods of early brain development disrupts key developmental processes, including neurogenesis and apoptosis, and results in widespread structural changes in the brain. In addition to the previously described behaviors, rodents exposed to phenobarbital during the gestational or neonatal periods have also shown behavioral alterations in adulthood, including deficits in spatial learning and memory (Yanai, 1989; Forcelli et al., 2012; Stefovska et al., 2008), impaired learning in a passive avoidance paradigm (Frankel et al., 2016; Gutherz et al., 2014), decreased motor activity and coordination (Christensen et al., 2004; Forcelli et al, 2012), impaired cued fear conditioning (Forcelli et al., 2012), increased or decreased anxiety-like behavior (Christensen et al., 2004, Forcelli et al., 2012, Frankel et al, 2016), and impaired prepulse inhibition (Forcelli et al., 2012; Gutherz et al, 2014). Given

the structural changes that are seen in early development, several researchers have hypothesized the link between early brain development and the behavioral changes seen at later ages. Several behaviors, including spatial learning and memory and passive avoidance have been attributed to early changes in hippocampal structure (Stevofska et al., 2008; Frankel et al., 2016). Furthermore, Gutherz et al. (2014) hypothesized that deficits in passive avoidance could also be linked to early apoptosis in the frontal cortex, leading to impairments in executive functioning. Forcelli et al. (2012) considered early apoptosis in the amygdala, striatum, and nucleus accumbens as a possible explanatory factor for impaired prepulse inhibition in phenobarbital-exposed animals.

Overall, as illustrated above, although it is unclear how specific effects seen in rodents are related to clinical outcomes in humans exposed to phenobarbital *in utero*, studies in rodents raise concern about the possible lifelong neuroteratogenic effects of phenobarbital exposure in early development. Despite this fact, however, less is known about cognitive and behavioral outcomes in infants and children prenatally exposed to phenobarbital.

To date, several cohorts in North America and Europe have been established to study how prenatal exposure to monotherapy with antiepileptic medications impacts the neuropsychological functioning of the child at various ages in development, and these cohorts have been pivotal in classifying the neuroteratogenic risk of antiepileptic medications, such as phenytoin, carbamazepine, and valproic acid (Bromley & Baker, 2017). Unfortunately, however, due to its frequent use in polytherapy regimens and to its declining use in developed nations, phenobarbital has not been included as a primary medication of interest in these studies (Lutes, Borchelt, Janulewicz, & Adams, 2018). Of the limited studies that do exist for phenobarbital, the majority suggest that prenatal exposure to phenobarbital is

not associated with adverse effects on neuropsychological functioning in toddlers (Thomas et al., 2008) or children (Dean et al, 2002; Thomas, Sukumaran, Lukose, Geourge, & Sarma, 2007). One study, however, found poor performance in spelling and arithmetic for children prenatally exposed to phenobarbital (van der Pol, Hadders-Algra, Huisjes, & Touwen, 1991), and lowered verbal performance has been reported in adult men exposed to phenobarbital during the prenatal period (Reinisch, Sanders, Mortensen, & Rubin, 1995). The methodological rigor of these studies is questioned, however, by sample sizes less than 20 per exposure group (van der Pol et al., 1991; Dean et al., 2002; Thomas et al., 2007; Thomas et al., 2008), lack of a comparison to a control group (Thomas et al., 2008), and failure to adjust for possible confounding variables, such as maternal education and measures of maternal mental ability (IQ scores) (van der Pol et al., 1991, Reinisch et al., 1995; Dean et al., 2002; Thomas et al., 2007; Thomas et al., 2008).

Understanding the need for more research in this area, Adams et al. (manuscript in progress) assessed the cognitive performance of 34 phenobarbital-exposed children and 34 unexposed control children that were matched for maternal age at delivery, maternal socioeconomic status (including education), gender, and age at testing. Research on phenytoin-exposed and carbamazepine-exposed children and their unexposed matches was conducted in parallel and is not reported herein. Following adjustment for matching, siblings, and maternal IQ, in comparison to their matched controls, children in the phenobarbital-exposed group exhibited a significant reduction in verbal IQ (VIQ) and full-scale IQ (FSIQ). Because performance IQ (PIQ) did not differ significantly due to phenobarbital exposure, reductions in full-scale IQ were interpreted as largely resulting from impaired performance in verbal areas.

In order to further explore the profile of cognitive strengths and weaknesses in children prenatally exposed to phenobarbital, the current paper examines performance on individual verbal subtests in the neuropsychological battery for a subset of subjects from Adams et al. (in progress).

CHAPTER 2

METHODS

Recruitment of Subjects

Recruitment of subjects was conducted in two phases. During Phase I (1983-1993), the primary source of recruitment, women were recruited from a surveillance study being conducted at five maternity hospitals in the Boston area (Holmes, Harvey, Brown, Hayes, & Khoshbin, 1994; Holmes et al., 2001). During Phase II (1996-2000), additional subjects were recruited through referrals from neurologists, pediatricians, and obstetricians/gynecologists as well as through medical record evaluations by a large health maintenance organization in the Boston area. Flyers, newspaper and radio advertisements, and referrals from other subjects in the study were also used to recruit subjects from the community.

Selection of Subjects

Selection for participation in the study was dependent upon inclusion criteria, exclusion criteria, and the ability to match between groups. Women were excluded from participation in the study if they experienced one or more tonic-clonic seizures during pregnancy, had exposure to polytherapy during pregnancy, or if they were exposed to a known teratogen. Seizure and treatment histories were determined through medical record review by a neurologist and exposure to a teratogen was assessed by a teratologist at Massachusetts General Hospital. For inclusion in the study, both parents of the child had to demonstrate normal intelligence on the Raven's Progressive Matrices Test. If qualified for

participation in the study, maternal intelligence was also assessed using the Weschler Abbreviated Intelligence Scale (WAIS).

Participants were excluded from the study if the child was not a singleton birth, if the child had a postnatal history of illness or injury that could impact neuropsychological functioning, if the child had a visual or auditory impairment, or if English was not the child's first language. Evaluation of the presence of any of these criteria in children was assessed through a review of medical records, a parental questionnaire, and auditory and visual screening tasks.

If a subject met participation criteria, matching was then conducted in order to balance the maternal and child demographics of exposed and unexposed participants. Children from the phenobarbital exposure group were matched with an unexposed control child. Matching criteria included maternal age at child's birth, maternal socioeconomic status (including education), child age at testing, and child sex.

Neuropsychological Assessments

Children in the original study were administered a comprehensive neuropsychological screening battery that included the Wechsler Intelligence Scale for Children, Third Edition (WISC-III) and select subtests from the Stanford-Binet Intelligence Scale, Fourth Edition (SB-IV) and the Wechsler Memory Scale-Revised (WMS-R). All assessments were conducted by a trained psychometrist that was blind to exposure group.

The WISC-III is a standardized assessment that provides composite scores of verbal intelligence (VIQ), performance intelligence (PIQ) and full-scale intelligence (FSIQ). Verbal subtests that were administered include Information (a test of general factual knowledge), Arithmetic (a test of mental computation given verbal instructions), Vocabulary (a test of

word knowledge), and Digit Span (a test of verbal short-term memory). Composite scores have a mean of 100 and standard deviation of 15. Subtests have a mean score of 10 and a standard deviation of 3. Both the VIQ and FSIQ were scored according to prorating criteria described in the WISC-III manual (Wechsler, 1991).

The SB-IV is a standardized intelligence assessment comprised of 15 subtests that provide an assessment of cognitive ability in verbal and non-verbal areas. In this study, children were administered the Sentence Memory subtest as a measure of verbal memory. This subtest has a mean scaled score of 50 and a standard deviation of 8 (Thorndike, Hagen, & Sattler, 1986).

The WMS-R is a standardized memory assessment comprised of thirteen subtests from which five index scores can be derived (Wechsler, 1987). To assess verbal memory abilities, children were administered a task of immediate-recall story memory.

In addition to the assessment of children's mental abilities, maternal intelligence was assessed using the Wechsler Abbreviated Intelligence Scale-Revised (WAIS-R). The WAIS-R provides an assessment of VIQ, PIQ, and FSIQ. Composite scores have a mean of 100 and standard deviation of 15 (Wechsler, 1981).

Statistical Analyses

To account for possible variance attributed by sibling relationships within the sample, every effort was made to utilize one child per family and for the first-born child to be the one included in analyses. There were two exceptions made, however, in order to provide demographic, age, and gender matching and to protect sample size. In the unexposed control group, there were two sets of siblings (4 subjects) that were matched to phenobarbital-

exposed subjects. To balance the possible impact of sibling-based variance in the exposed group, two sets of phenobarbital-exposed siblings were also retained.

The effectiveness of our matching was assessed by using two-tailed independent samples t-tests to examine demographic characteristics of the exposed and unexposed groups. Because this paper addresses a subset of phenobarbital-exposed children that were included in the work by Adams et al., we were interested in confirming that our sample showed a similar pattern of performance on the WISC-III as seen in the full sample (Adams et al., in progress). Therefore, performance on the FSIQ, VIQ, and PIQ were examined using univariate analysis of covariance in which exposure group and sex were included as factors and maternal FSIQ was included as a covariate.

The effects of prenatal exposure to phenobarbital on verbal abilities were assessed using multivariate analysis of covariance (ANCOVA), in which exposure group and sex were included as factors and maternal FSIQ was included as a covariate. For this analysis, scores on all verbal subtests in the battery were included as dependent variables. These subtests included Information, Arithmetic, Vocabulary, and Digit Span from the WISC-III, Sentence Memory from the SB-IV, and Immediate Recall Story Memory from the WMS-R. Upon finding a significant multivariate effect, univariate ANCOVAs controlling for maternal FSIQ were conducted to determine which specific dependent variables contributed to the significant multivariate effect.

All analyses were conducted using IBM SPSS Statistics 27 for Macintosh, and all figures were created using GraphPad Prism 8. Significance was assessed at $p < 0.05$.

Approval for Work with Human Subjects

This study was approved by the Massachusetts General Hospital's Human Studies Committee and the University of Massachusetts Institutional Review Board. All parents provided written informed consent, and if a child was fourteen years of age or older, they were also asked to provide written assent prior to participating in their evaluation. No families withdrew from the study once consent was obtained.

CHAPTER 3

RESULTS

As shown in Table 1, the study population consisted of 26 phenobarbital (PB)-exposed subjects and 26 unexposed controls. Demographic variables, such as age at testing, maternal age at birth, and maternal education did not differ statistically between the two groups (Age at Testing: $t(50) = -0.030$, $p = 0.976$), Maternal Age: $t(48) = 0.676$, $p = 0.502$), Maternal Education: $t(49) = 0.161$, $p = 0.873$). Likewise, maternal FSIQ was similar between the two groups ($t(49) = -0.893$, $p = 0.376$).

Unadjusted means and standard deviations for performance on the neuropsychological measures are presented in Table 2. In general, for both exposure groups, mean scores on all measures were consistent with performance in the average range or higher. A determination of the number of children performing two standard deviations or more below the mean revealed one subject from the PB-exposed group and no subjects from the control group.

In initial confirmatory analyses, univariate ANCOVAs controlling for maternal FSIQ were conducted to examine the effects of phenobarbital exposure and sex on individual composite scores of the WISC-III. There was a significant effect of Exposure Group on FSIQ ($F(1,46) = 4.467$, $p = 0.040$, $\eta 2p = 0.089$) and VIQ ($F(1,46) = 5.889$, $p = 0.019$, $\eta 2p = 0.113$), but no effect on PIQ ($F(1,46) = 2.009$, $p = 0.163$, $\eta 2p = 0.042$). There were no significant effects of Sex (FSIQ: $F(1, 46) = 1.598$, $p = 0.213$, $\eta 2p = 0.034$; VIQ: $F(1,46) = 0.502$,

p = 0.482, η2p = 0.011; PIQ: F(1,46) = 3.174, p = 0.081, η2p = 0.065) and no Exposure Group by Sex interaction (FSIQ: F(1,46) = 0.072, p = 0.790, η2p = 0.002; VIQ: F(1,46) = 1.188, p = 0.281, η2p = 0.025 ; PIQ: F(1,46) = 0.615, p = 0.437, η2p = 0.013. Therefore, further analyses were conducted with Sex removed from the model. As shown in Table 3 and Figure 1, ANCOVA controlling for maternal FSIQ revealed a significant effect of phenobarbital exposure on FSIQ (F(1,48) = 4.967, p = 0.031, η2p = 0.094) and VIQ (F(1,48) = 7.255, p = 0.010, η2p = 0.131), while PIQ (F(1,48) = 1.688, p = 0.200, η2p = 0.034) did not significantly differ between the two groups.

After confirming decreased verbal performance on the WISC-III in our sample of PB-exposed subjects, our primary interests were in determining if there was a difference across verbal measures in our neuropsychological battery, and if so, which specific areas of verbal cognition differed between groups. In initial analyses, multivariate ANCOVA controlling for maternal FSIQ was conducted to examine the effects of phenobarbital exposure and sex on the aggregate group of verbal subtests. As shown in Table 4, the analysis revealed a significant effect of Exposure Group (F(6,39) = 2.584, Wilks' Λ = 0.716, p = 0.033, η2p = 0.284). There was no significant effect of Sex (F(6,39) = 0.847, Wilks' Λ = 0.885, p = 0.542, η2p = 0.115) and no Exposure Group by Sex Interaction (F(6,39) = 1.01, Wilks' Λ = 0.865, p = 0.433, η2p = 0.135), so analyses were conducted with Sex removed from the model. The adjusted means by Sex and Exposure Group are shown in Appendix A.

Multivariate ANCOVA controlling for maternal FSIQ revealed a significant effect of exposure group on verbal measures (F(6, 41) = 2.647, Wilks' Λ = 0.721, p = 0.029, η2p = 0.279). As shown in Table 5 and Figure 2, follow-up univariate between-group analyses revealed no individually-significant differences in the scores of the PB-exposed and control

children on Information ($F(1,46) = 2.522$, $p = 0.119$, $\eta 2p = 0.052$), Digit Span ($F(1,46) = 1.233$, $p = 0.273$, $\eta 2p = 0.026$), or Sentence Memory ($F(1,46) = 1.744$, $p = 0.193$, $\eta 2p = 0.037$). However, PB-exposed children performed significantly worse than controls on Vocabulary ($F(1,46) = 4.743$, $p = 0.035$, $\eta 2p = 0.093$), Arithmetic ($F(1,46) = 11.502$, $p = 0.001$, $\eta 2p = 0.200$), and Immediate Recall Story Memory ($F(1,46) = 4.397$, $p = 0.042$, $\eta 2p = 0.087$).

CHAPTER 4

DISCUSSION AND CONCLUSION

In a previous study, Adams et al. (in progress) found that children of women with epilepsy that were prenatally exposed to phenobarbital monotherapy demonstrated significantly lower verbal intelligence and full-scale intelligence on the WISC-III than unexposed children born to women without epilepsy and matched for demographic variables. Here, we have examined the effects of phenobarbital on specific verbal abilities assessed by a group of verbal subtests selected from the WISC-III, the SB-IV, and WMS-R. We have shown that, when compared to demographically matched, unexposed children, phenobarbital-exposed children performed significantly worse on a group of verbal subtests. Univariate examination of performance on specific measures revealed significant effects on performance on a test of word knowledge (Vocabulary), a test of mental arithmetic (Arithmetic), and a test of verbal memory (IR-Story Memory).

Although the research investigating the effects of prenatal exposure to phenobarbital on neurodevelopment is limited, our findings are consistent with current reported research. Reinisch et al. (1995) retrospectively ascertained 33 adult men who had been exposed to phenobarbital monotherapy *in utero* for reasons other than epilepsy (e.g. hypertension, preeclampsia, eclampsia, sedation). When evaluated on the Wechsler Abbreviated Intelligence Scale (WAIS), phenobarbital-exposed men demonstrated significantly lower than predicted verbal intelligence. Although other antiepileptic medications, such as

phenytoin, carbamazepine, valproic acid, and lamotrigine, exert their antiepileptic effects through different initial mechanisms than phenobarbital, several researchers have also reported a weakness in verbal abilities during at least one developmental timepoint in prenatally exposed children (Meador et al., 2011, 2012; Nadebaum et al., 2001; Baker et al., 2015). These common findings among different antiepileptic medications merit further investigation to better understand possible common mechanisms, final common pathways, or vulnerabilities.

The primary finding in this study was that, in comparison to controls, children prenatally exposed to phenobarbital showed a significant overall weakness on measures of verbal performance, with specific weaknesses on a test of mental arithmetic, story recall, and vocabulary. Van der Pol et al. (1991) conducted neurodevelopmental follow-up on 12 prospectively ascertained children that had been prenatally exposed to phenobarbital monotherapy for maternal epilepsy. When compared to demographically matched control children, the phenobarbital-exposed group had significantly more children that performed poorly on standardized tests of spelling and arithmetic, though the specific measures were not described. Phenobarbital-exposed children in our study also demonstrated lower performance on a test of vocabulary knowledge and had an impaired ability to immediately recall stories that they had heard. To our knowledge, this is the first report of impaired performance on these subtests in phenobarbital-exposed children.

It is unclear why children performed worse on Vocabulary, Arithmetic, and Story Memory, while other verbal subtests, such as Information, Digit Span, and Sentence Memory were not significantly impacted. Nicholson and Alcorn (1993) have discussed cognitive abilities that influence high or low performance on subtests of the WISC-III. According to

their assessment, performance on the Arithmetic subtest of the WISC-III relies on verbal output ability, focused attention, ability to perform simple math, short-term memory, low distractibility, and a good educational background. In their assessment, performance on the Vocabulary subtest is also dependent on verbal output abilities and a good educational background, but also requires an understanding of culture. Ernst, Warner, Morgan, Townes, Eiler, and Coppel (1986) conducted a factor analysis of the Weschler Memory Scale and found that focused attention and concentration abilities appear to be important factors in determining performance on the story memory component of the assessment. Because phenobarbital exposed subjects in this study performed similarly to controls on three subtests, it is unlikely that generalized verbal output abilities are the primary contributor to the differences in performance between groups. Due to the importance of attention and low distractibility for performance on the Arithmetic and Story Memory subtests, however, it is possible that the verbal working memory and attentional abilities required for these subtests challenged the abilities of children prenatally exposed to phenobarbital. To further understand the specific profile of cognitive strengths and weaknesses in phenobarbital-exposed children, future studies should include a neuropsychological battery that also contains robust measures of working memory abilities, attention, and distractibility.

Although verbal abilities in humans are unable to be directly studied in rodent models, the neuroteratogenic risk of phenobarbital can also be informed by animal models of gestational and neonatal exposure to the drug. Few studies have examined behavioral outcomes in pups or adolescent rodents, but behavioral alternations have been observed in adult rodents exposed to phenobarbital during periods of early brain development. Of interest to our study, Gutherz et al. (2014) reported that adult rats who were treated with

phenobarbital in the neonatal period exhibited poor performance on a task of passive avoidance. Although passive avoidance has been reported to be a hippocampal-dependent behavior (Frankel et al., 2016), Gutherz et al. (2014) also hypothesized that deficits in passive avoidance could be linked to early apoptosis in the frontal cortex, leading to deficits in executive functioning in phenobarbital-exposed animals. Significant apoptosis in the frontal cortex of P7 pups has been reported following acute exposure to phenobarbital in the neonatal period (Bittigau, Sifringer, & Iknonomidou, 2003). Additionally, Bittigau, Sifringer, & Ikonomidou (2003) also reported significant apoptosis following phenobarbital exposure in two brain regions reported to be part of the brain network for verbal working memory in a pediatric population (Yang et al., 2015), including the frontal cortex and cingulate cortex. Currently, a direct link between research in animals and behavioral outcome in humans has not been established, but more focused studies in animals at different developmental time points will be pivotal to informing the risk that early phenobarbital exposure poses to the development of the brain and behavior.

Although the animal literature suggests a neuroteratogenic risk of phenobarbital, currently, it is unclear exactly how research in animal models relates to neuropsychological outcomes in children that are exposed to phenobarbital in utero. However, Meador & Loring (2016) have suggested a correlation between outcomes of apoptotic models of exposure to antiepileptic medications and cognitive outcome in children. In an animal model of neonatal phenobarbital-induced apoptosis, widespread programmed cell death was seen at plasma concentrations of 25-35 μg/mL, a level stated to be of therapeutic relevance in human infants administered phenobarbital (Bittigau, Sifringer, & Ikonomidou, 2003). In a small sample of newborns exposed to phenobarbital in utero, average peak plasma concentration was 16.1

μg/mL, but levels as high as 21.6 μg/mL were reported in some infants (Zuppa et al., 2011). Although the mean phenobarbital concentration in prenatally exposed infants is thought to be less than the concentration seen in Bittigau, Sifringer, & Iknomidou (2003), it is unknown if lower concentrations of phenobarbital may result in apoptosis in light of potentially increased vulnerability of the developing brain at earlier stages. However, due to the possible association between drug-related apoptosis in animals and cognitive outcome in humans, Meador & Loring (2016) have suggested that the United States Food and Drug Administration require preclinical testing with apoptotic models to inform the neuroteratogenic risk of new antiepileptic medications prior to their use in humans.

The results of our study add to the limited amount of literature exploring the cognition of children prenatally exposed to phenobarbital. Specifically, our results suggest decreased performance on a test of vocabulary, decreased mental arithmetic abilities, and impaired verbal memory for stories in comparison to control children. Our study was limited by small sample size, lack of confidence in the consistency of classifications of types of maternal epilepsy, and incomplete information on doses of phenobarbital used throughout pregnancy. In order to truly understand the full risk posed by prenatal exposure to phenobarbital, future studies should examine dose-response relationships and risk of teratogenicity by length of exposure, when possible. Despite these limitations, however, strengths of our study include careful matching of exposed and control subjects on important demographic variables; exclusion of pregnant women who had seizures during pregnancy, polytherapy with other anticonvulsant medications, or exposures to other known teratogens; use of standardized neuropsychological assessments conducted by examiners that were blinded to exposure group: and statistical adjustment for maternal intelligence.

Although phenobarbital use has declined in developed, industrial nations, for many parts of the developing world there is a choice between treatment with phenobarbital or no treatment at all (Kwan & Brodie, 2004). Combined with the results of Adams et al. (in progress), this research suggests an effect of prenatal exposure to phenobarbital on verbal abilities, specifically vocabulary knowledge, mental arithmetic, and immediate recall verbal memory, that is consistent with previously published literature. Combined with animal literature that provides mechanistic insight into morphological and functional changes that occur in response to early phenobarbital exposure, these studies suggest that use of phenobarbital during pregnancy poses a neuroteratogenic risk to the developing infant. Future studies should aim to further explore neurodevelopmental outcomes in larger samples of children prenatally exposed to phenobarbital monotherapy. Specific areas of research could include exploring dose-response relationships, exposure durations, and outcomes related to cognitive abilities, such as memory and attention. Furthermore, research should be conducted to explore alternative efficacious and low-cost treatments for use in pregnant women with epilepsy in developing nations.

Table 1 Demographic Characteristics of Study Population by Exposure Group[a]

Children	Control (n = 26)	PB-Exposed (n = 26)
Age at Testing, Years	9.8 (2.7)	9.7 (2.7)
Age Range, Years	6.0-15.8	6.6-16.3
Sex (M:F)	11:15	11:15
Mothers		
Age at Child's Birth, Years	32.5 (4.9)	33.3 (4.2)
Education, Years	15.9 (2.4)	16.0 (2.8)
Full Scale IQ[b]	114.3 (12.8)	110.8 (15.1)

[a] Values are presented as mean (standard deviation)
[b] Mean for the control group is based on 25 subjects.

Table 2 Unadjusted Scores[a] on Neuropsychological Measures

WISC-III	Control (n = 26)	PB-Exposed (n = 26)
Full Scale IQ	113.12 (11.17)	102.88 (16.62)
Verbal IQ	115.19 (9.80)	103.88 (16.68)
Performance IQ	108.88 (13.27)	101.62 (16.13)
Information	12.96 (3.07)	11.31 (3.70)
Vocabulary	13.12 (2.92)	11.08 (3.53)
Arithmetic	12.31 (2.92)	9.12 (3.16)
Digit Span	11.23 (2.34)	10.46 (2.79)
WMS-R[b]		
Immediate Recall Story Memory	10.58 (3.45)	8.67 (3.41)
SB-IV[b]		
Sentence Memory*	54.17 (6.78)	52.13 (8.29)

[a]Values are presented as mean (standard deviation)
[b] Means are based on 24 subjects per group.

Table 3 Adjusted Means for Composite Scores of the WISC-III Following Univariate ANCOVA Examining the Effect of Exposure Group While Controlling for Maternal FSIQ

Composite Score	Control Adj. Mean (95% CI)	Control Adj. Mean (95% CI)	F value (p-value)
Full Scale IQ	111.88 (106.53 - 117.24)	103.54 (98.29 - 108.79)	4.967 **(0.031)**
Verbal IQ	114.46 (109.14 - 119.78)	104.44 (99.23 - 109.66)	7.255 **(0.010)**
Performance IQ	107.28 (101.76 - 112.81)	102.27 (96.85 - 107.68)	1.688 (0.200)

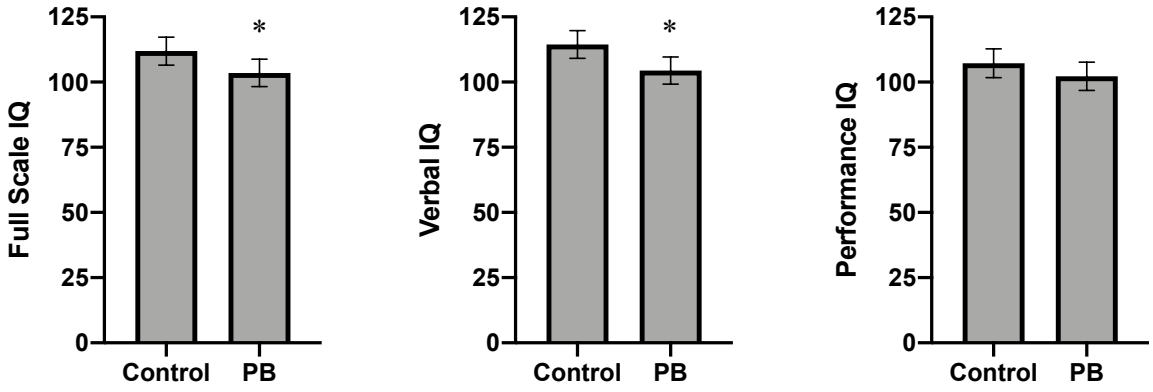

Figure 1 Mean Composite Scores on the WISC-III by Exposure Group. Vertical bars represent the 95% CI. Data were analyzed with univariate ANCOVA and adjusted for maternal FSIQ, * $p < 0.05$.

Table 4 Adjusted Means for Performance on Neuropsychological Measures Following Multivariate ANCOVA to Examine the Effects of Exposure Group and Sex on Verbal Abilities. Means are adjusted for maternal FSIQ.

Verbal Subtest	Control Adj. Mean (95% CI)	PB-Exposed Adj. Mean (95% CI)	F value (p-value)
Information	12.59 (11.25 – 13.94)	11.25 (9.89 – 12.61)	1.994 (0.165)
Vocabulary	12.88 (11.58 – 14.19)	10.92 (9.59 – 12.24)	4.493 **(0.40)**
Arithmetic	12.07 (10.79 – 13.36)	9.21 (7.90 – 10.52)	9.805 **(0.003)**
Digit Span	11.13 (10.09 - 12.17)	10.51 (9.45 - 11.56)	0.720 (0.401)
Sentence Memory	54.45 (51.50 – 57.40)	52.19 (49.19 – 55.18)	1.170 (0.285)
Immediate Recall Story Memory	10.92 (9.54 - 12.31)	8.69 (7.28 - 10.10)	5.173 **(0.028)**

Table 5 Adjusted Means for Performance on Neuropsychological Measures Following Multivariate ANCOVA to Examine Effects of Exposure Group on Verbal Abilities. Means are adjusted for maternal FSIQ.

Verbal Subtest	Control Adj. Mean (95% CI)	PB-Exposed Adj. Mean (95% CI)	F value (p-value)
Information	12.72 (11.42 - 14.02)	11.25 (9.93 - 12.58)	2.522 (0.119)
Vocabulary	12.96 (11.70 - 14.23)	11.00 (9.71 - 12.29)	4.743 **(0.035)**
Arithmetic	12.14 (10.90 - 13.39)	9.14 (7.88 - 10.41)	11.502 **(0.001)**
Digit Span	11.29 (10.27 - 12.32)	10.49 (9.44 - 11.53)	1.233 (0.273)
Sentence Memory	55.11 (52.12 - 58.11)	52.30 (49.24 - 55.35)	1.744 0.193
Immediate Recall Story Memory	10.76 (9.41 - 12.11)	8.75 (7.37 - 10.13)	4.397 **(0.042)**

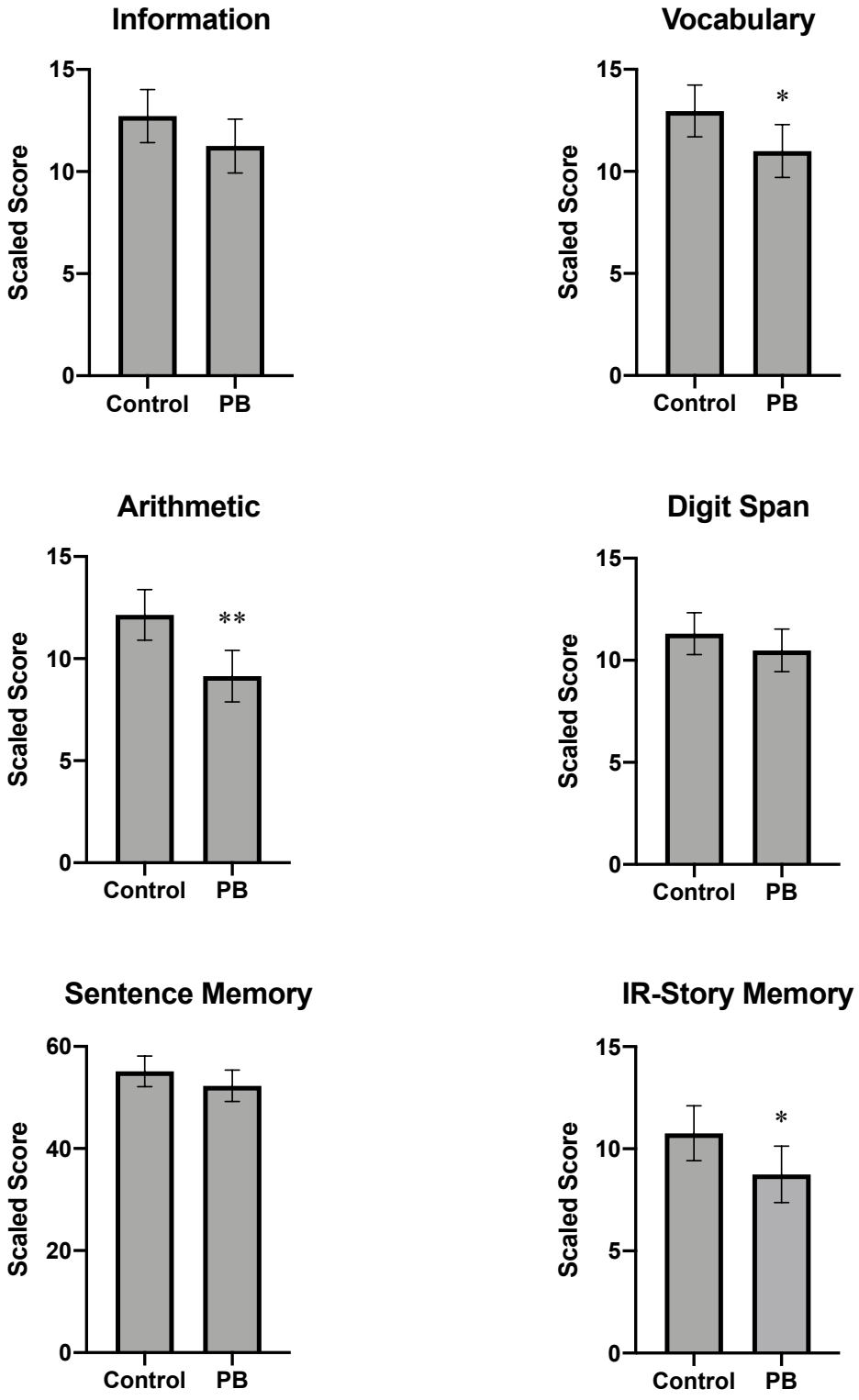

Figure 2 Mean Performance on Verbal Subtests of the Neuropsychological Battery by Exposure Group. Vertical bars represent the 95% CI. Data were analyzed with univariate ANCOVA and adjusted for maternal FSIQ, * $p < 0.05$, ** $p < 0.01$.

APPENDIX A. ADJUSTED MEANS FOR PERFORMANCE ON VERBAL MEASURES BY SEX AND EXPOSURE GROUP FOLLOWING MULTIVARIATE ANCOVA.

Means are adjusted for maternal FSIQ. Phenobarbital-exposed children performed significantly worse on verbal measures than control children ($p < 0.05$). There was no effect of sex ($p > 0.05$) or an interaction effect ($p > 0.05$).

Verbal Subtest		Control Adj. Mean (95% CI)	PB-Exposed Adj. Mean (95% CI)	F value (p-value)
Information	Females	13.22 (11.52 - 14.91)	11.26 (9.51 - 13.01)	--
	Males	11.97 (9.90 - 14.05)	11. 237 (9.16 - 13.3)	--
	Total	12.59 (11.25 - 13.94)	11.25 (9.89 - 12.61)	1.994 (0.165)
Vocabulary	Females	13.29 (11.64 - 14.94)	11.40 (9.70 - 13.11)	--
	Males	12.48 (10.46 - 14.50)	10.43 (8.40 - 12.46)	--
	Total	12.88 (11.58 - 14.19)	10.92 (9.59 - 12.24)	4.493 **(0.04)**
Arithmetic	Females	12.41 (10.78 -14.04)	8.81 (7.13 - 10.49)	--
	Males	11.74 (9.75 - 13.73)	9.62 (7.62 - 11.61)	--
	Total	12.07 (10.79 - 13.36)	9.21 (7.90 - 10.52)	9.805 **(0.003)**
Digit Span	Females	11.94 (10.62 - 13.25)	10.38 (9.03 - 11.74)	--
	Males	10.33 (8.72 – 11.94)	10.63 (9.02 - 12.24)	--
	Total	11.13 (10.09 - 12.17)	10.51 (9.45 - 11.56)	0.720 (0.401)
Sentence Memory	Females	57.77 (54.05 - 61.50)	52.85 (49.00 - 56.70)	--
	Males	51.13 (46.56 - 55.69)	51.52 (46.94 - 56.10)	--
	Total	54.45 (51.50 - 57.40)	52.19 (49.19 - 55.18)	1.170 (0.285)
Immediate Recall Story Memory	Females	10.13 (8.38 - 11.88)	9.03 (7.22 - 10.84)	--
	Males	11.72 (9.58 - 13.86)	8.35 (6.20 - 10.50)	--
	Total	10.92 (9.54 - 12.31)	8.69 (7.28 - 10.10)	5.173 **(0.028)**

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