August 2015

Estrogen-Sensitive Learning is not Affected by Combination Ethinyl Estradiol and Levonorgestrel Oral Contraceptive Use

Darlene F. Ficco
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A Dissertation Presented

by

DARLENE F. FICCO

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

MAY 2015

Psychological and Brain Sciences
ESTROGEN-SENSITIVE LEARNING IS NOT AFFECTED BY COMBINATION ETHINYL ESTRADIOL AND LEVONORGESTREL ORAL CONTRACEPTIVE USE

A Dissertation Presented by DARLENE F. FICCO

Approved as to style and content by:

Neil Berthier, Chair
Nancy Forger, Member
Sandra Petersen, Member
Heather Richardson, Member

Harold Grotevant, Department Head Psychological and Brain Sciences
DEDICATION

In loving memory of Renée, Britain, and Mark.
ACKNOWLEDGMENTS

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ABSTRACT

ESTROGEN-SENSITIVE LEARNING IS NOT AFFECTED BY COMBINATION ETHINYL ESTRADIOL AND LEVONORGESTREL ORAL CONTRACEPTIVE USE

MAY 2015

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Directed by: Dr. Neil Berthier

Two studies were conducted to explore the cognitive effects of combination ethinyl estradiol and levonorgestrel contraceptive use during late adolescence and young adulthood. Three groups of females, naturally cycling, active pill phase, and hormone-free interval phase, were tested on a battery of estrogen-sensitive, i.e., place learning and word generation, and estrogen-insensitive, i.e., map drawing, mental rotation, digit span, story recall, and object recall, tasks. Study 2 was conducted as a means to replicate the findings observed in Study 1 and to manipulate task difficulty and sensitivity. Two measures of mood were administered, and salivary estradiol levels at time of testing were assayed. Findings from both studies do not suggest mood or endogenous estrogen effects on cognition. Additionally, findings from both studies suggest no cognitive effects of combination ethinyl estradiol and levonorgestrel contraceptive use. The large sample size of Study 2, \( n = 65 \), indicates sufficient power to detect statistical differences. These studies do not provide evidence of either cognitive detrimental or beneficial effects of combined oral contraceptive use during either late adolescence or young adulthood. Post-hoc analyses with a nondepressed subsample, however, suggest potential sparing of estrogen-sensitive place learning by active pill phase females relative to hormone-free interval and naturally cycling females during periods of low endogenous estrogens.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGEMENTS</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1. LITERATURE REVIEW</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Sex and the Human Brain</td>
<td>1</td>
</tr>
<tr>
<td>Sex and Gonadal Hormone Cognitive Differences</td>
<td>2</td>
</tr>
<tr>
<td>Organizational Effects</td>
<td>2</td>
</tr>
<tr>
<td>Activational Effects</td>
<td>3</td>
</tr>
<tr>
<td>The Adolescent Period of Brain and Cognitive Development</td>
<td>4</td>
</tr>
<tr>
<td>Brain Development</td>
<td>4</td>
</tr>
<tr>
<td>Sex and Adolescent Brain Development</td>
<td>4</td>
</tr>
<tr>
<td>Cognitive and White Matter Development</td>
<td>5</td>
</tr>
<tr>
<td>Hormones and Adolescent Brain Development</td>
<td>6</td>
</tr>
<tr>
<td>Hormonally Atypical Populations</td>
<td>6</td>
</tr>
<tr>
<td>Congenital Adrenal Hyperplasia</td>
<td>6</td>
</tr>
<tr>
<td>Delayed Pubertal Onset</td>
<td>7</td>
</tr>
<tr>
<td>Hormonal Contraceptives</td>
<td>7</td>
</tr>
<tr>
<td>History and Prevalence of Hormonal Contraceptive Use</td>
<td>8</td>
</tr>
<tr>
<td>Mechanisms of Action</td>
<td>8</td>
</tr>
<tr>
<td>Effects on Endogenous Gonadal Hormones</td>
<td>9</td>
</tr>
<tr>
<td>Metabolic Effects</td>
<td>9</td>
</tr>
<tr>
<td>Neuroanatomical Effects</td>
<td>10</td>
</tr>
<tr>
<td>Modulation of Cognitive Functioning</td>
<td>11</td>
</tr>
<tr>
<td>Mood and Affect</td>
<td>11</td>
</tr>
<tr>
<td>Motor and Perception</td>
<td>11</td>
</tr>
<tr>
<td>Memory</td>
<td>12</td>
</tr>
<tr>
<td>Language</td>
<td>12</td>
</tr>
<tr>
<td>Spatial Processing</td>
<td>12</td>
</tr>
<tr>
<td>Place Versus Response Learning</td>
<td>13</td>
</tr>
<tr>
<td>Multiple Navigation Processes</td>
<td>13</td>
</tr>
<tr>
<td>Early Findings</td>
<td>13</td>
</tr>
<tr>
<td>Contemporary Findings</td>
<td>14</td>
</tr>
<tr>
<td>Estrogen Effects on Spatial Learning</td>
<td>16</td>
</tr>
</tbody>
</table>
Summary .................................................................................................................. 17
Overview of the Proposed Studies ............................................................................ 17
Predictions .................................................................................................................. 18

2. METHOD .................................................................................................................. 19

Subjects ..................................................................................................................... 19
Setting ........................................................................................................................ 19
Tasks ........................................................................................................................... 19

Virtual Navigation Task (VNT) .................................................................................. 19
Map Drawing .............................................................................................................. 21
Word Generation ....................................................................................................... 21
Mental Rotation Task ............................................................................................... 21
Nonspatial Control Tasks .......................................................................................... 22
Object Recall ............................................................................................................. 22
Mood Measures ....................................................................................................... 23

Hormone Assessment ............................................................................................... 23

3. RESULTS ................................................................................................................ 26

Mood Measures ........................................................................................................ 27
Mood and Cognitive Performance Correlations ....................................................... 30

Mood and Dual-Solution Learning ......................................................................... 30
Mood and Place Learning ......................................................................................... 31
Mood and Map Drawing ......................................................................................... 31
Mood and Mental Rotation ....................................................................................... 32
Mood and Object Recall ......................................................................................... 32
Mood and Word Generation ..................................................................................... 32
Mood and Digit Span ............................................................................................... 32
Mood and Story Recall ............................................................................................ 33

Salivary 17β-Estradiol and Cognitive Performance .................................................. 33

Correlations .............................................................................................................. 33

Salivary 17β-estradiol and Mood ............................................................................. 34
Salivary 17β-estradiol and Dual-Solution Learning ............................................... 34
Salivary 17β-estradiol and Place Learning ............................................................. 34
Salivary 17β-estradiol and Map Drawing ............................................................... 35
Salivary 17β-estradiol and Mental Rotation ........................................................... 35
Salivary 17β-estradiol and Object Recall ............................................................... 36
Salivary 17β-estradiol and Word Generation ........................................................ 36
Salivary 17β-estradiol and Digit Span .................................................................. 36
Salivary 17β-estradiol and Story Recall ................................................................. 36

Estrogen-Sensitive Learning ................................................................................. 37

Place Learning ........................................................................................................ 37

Place Learning and Dual-Solution Learning .......................................................... 37
Place Learning and Map Drawing .......................................................................... 39
Place Learning and Mental Rotation .................................................................... 40
Place Learning and Object Recall ...................................................................... 41
Place Learning and Word Generation .................................................................. 42

viii
<table>
<thead>
<tr>
<th>Task Combination</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place Learning and Digit Span</td>
<td>42</td>
</tr>
<tr>
<td>Place Learning and Story Recall</td>
<td>42</td>
</tr>
<tr>
<td>Word Generation</td>
<td>43</td>
</tr>
<tr>
<td>Word Generation and Map Drawing</td>
<td>44</td>
</tr>
<tr>
<td>Word Generation and Mental Rotation</td>
<td>44</td>
</tr>
<tr>
<td>Word Generation and Object Recall</td>
<td>44</td>
</tr>
<tr>
<td>Word Generation and Digit Span</td>
<td>44</td>
</tr>
<tr>
<td>Word Generation and Story Recall</td>
<td>45</td>
</tr>
<tr>
<td>Estrogen-Insensitive Learning</td>
<td>46</td>
</tr>
<tr>
<td>Dual-Solution Learning</td>
<td>46</td>
</tr>
<tr>
<td>Dual-Solution Learning and Map Drawing</td>
<td>47</td>
</tr>
<tr>
<td>Dual-Solution Learning and Mental Rotation</td>
<td>48</td>
</tr>
<tr>
<td>Dual-Solution Learning and Object Recall</td>
<td>49</td>
</tr>
<tr>
<td>Dual-Solution Learning and Digit Span</td>
<td>50</td>
</tr>
<tr>
<td>Dual-Solution Learning and Story Recall</td>
<td>51</td>
</tr>
<tr>
<td>Map Drawing</td>
<td>51</td>
</tr>
<tr>
<td>Map Drawing and Mental Rotation</td>
<td>51</td>
</tr>
<tr>
<td>Map Drawing and Object Recall</td>
<td>52</td>
</tr>
<tr>
<td>Map Drawing and Digit Span</td>
<td>52</td>
</tr>
<tr>
<td>Map Drawing and Story Recall</td>
<td>52</td>
</tr>
<tr>
<td>Mental Rotation</td>
<td>52</td>
</tr>
<tr>
<td>Mental Rotation and Object Recall</td>
<td>53</td>
</tr>
<tr>
<td>Mental Rotation and Digit Span</td>
<td>53</td>
</tr>
<tr>
<td>Mental Rotation and Story Recall</td>
<td>53</td>
</tr>
<tr>
<td>Object Recall</td>
<td>54</td>
</tr>
<tr>
<td>Object Recall and Digit Span</td>
<td>54</td>
</tr>
<tr>
<td>Object Recall and Story Recall</td>
<td>54</td>
</tr>
<tr>
<td>Digit Span</td>
<td>54</td>
</tr>
<tr>
<td>Digit Span and Story Recall</td>
<td>55</td>
</tr>
<tr>
<td>Story Recall</td>
<td>55</td>
</tr>
<tr>
<td>Post-Hoc Analyses</td>
<td>56</td>
</tr>
<tr>
<td>Oral Contraceptive Cognitive Effects in Depressed versus Nondepressed Subsamples</td>
<td>56</td>
</tr>
<tr>
<td>Depressed Subsample</td>
<td>56</td>
</tr>
<tr>
<td>Nondepressed Subsample</td>
<td>57</td>
</tr>
</tbody>
</table>

4. DISCUSSION ................................................................................................................. 59

Combined Oral Contraceptive Use and Mood Effects ................................................................ 60
Combined Oral Contraceptive Use and Endogenous 17β-Estradiol Effects .................................. 62
Intra-Study and Inter-Study Reliability: Examination of Task Correlations ............................ 62
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Study 1 Sample Sizes, Medians, and Interquartile Ranges</td>
<td>68</td>
</tr>
<tr>
<td>2. Study 1 Means and Standard Deviations</td>
<td>69</td>
</tr>
<tr>
<td>3. Study 1 Comparison of Group Medians</td>
<td>70</td>
</tr>
<tr>
<td>4. Study 2 Sample Sizes</td>
<td>71</td>
</tr>
<tr>
<td>5. Study 2 Medians and Interquartile Ranges</td>
<td>72</td>
</tr>
<tr>
<td>6. Study 2 Means and Standard Deviations</td>
<td>73</td>
</tr>
<tr>
<td>7. Study 2 Comparison of Group Medians</td>
<td>74</td>
</tr>
<tr>
<td>8. Spearman Nonparametric Correlations Across Groups for Study 1</td>
<td>75</td>
</tr>
<tr>
<td>9. Spearman Nonparametric Correlations Across Groups for Study 2</td>
<td>76</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Subject groupings, self-reported menstrual or pill phase, and expected hormone status at time of testing</td>
<td>18</td>
</tr>
<tr>
<td>2. Low and high standard controls across the three assays</td>
<td>24</td>
</tr>
<tr>
<td>3. $17\beta$-Estradiol levels by group for Study 1 (a) and Study 2 (b)</td>
<td>25</td>
</tr>
<tr>
<td>4. Boxplots of Z-Transformation Scores for All Factors in Study 1 (a) and Study 2 (b)</td>
<td>27</td>
</tr>
<tr>
<td>5. CES-D scores for all subjects in Studies 1 and 2</td>
<td>28</td>
</tr>
<tr>
<td>6. Frequency distribution of CES-D scores for subjects in Studies 1 (a) and 2 (b)</td>
<td>29</td>
</tr>
<tr>
<td>7. CES-D scores for subjects by group in Study 1 (a) and Study 2 (b)</td>
<td>30</td>
</tr>
<tr>
<td>8. Scatterplots of (a) Study 1 and Study 2 (b) Place Errors and CES-D scores</td>
<td>31</td>
</tr>
<tr>
<td>9. Scatterplots of salivary $17\beta$-estradiol and (a) Study 1 Place Latency (b) Study 1 Place Errors (c) Study 2 Place Average Latency, and (d) Study 2 Place Average Errors</td>
<td>35</td>
</tr>
<tr>
<td>10. Scatterplots of (a) Study 1 Dual-Solution and Place Latency (b) Study 1 Dual-Solution and Place Errors (c) Study 2 Dual-Solution and Place Latency, and (d) Study 2 Dual-Solution and Place Errors</td>
<td>38</td>
</tr>
<tr>
<td>11. Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Map Drawing Errors</td>
<td>40</td>
</tr>
<tr>
<td>12. Scatterplots of (a) Study 1 Place Latency and Mental Rotation Items (b) Study 1 Place Errors and Mental Rotation Items (c) Study 2 Place Latency and Mental Rotation Items, and (d) Study 2 Place Errors and Mental Rotation Items</td>
<td>41</td>
</tr>
<tr>
<td>13. Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Object Recall</td>
<td>42</td>
</tr>
<tr>
<td>14. Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Story Recall</td>
<td>43</td>
</tr>
<tr>
<td>15. Scatterplots of (a) Study 1 Word Generation and Digit Span (b) Study 2 Word Generation and Digit Span</td>
<td>45</td>
</tr>
<tr>
<td>16. Scatterplots of (a) Study 1 Word Generation and Story Recall, and (b) Study 2 Word Generation and Story recall</td>
<td>46</td>
</tr>
<tr>
<td>17. Scatterplots of (a) Study 1 Dual-Solution Latency and Map Drawing Errors (b) Study 1 Dual-Solution Errors and Map Drawing Errors (c) Study 2 Dual-Solution Latency and Map Drawing Errors, and (d) Study 2 Dual-Solution Errors and Map Drawing Errors</td>
<td>48</td>
</tr>
<tr>
<td>18. Scatterplots of (a) Study 1 Dual-Solution Latency and Mental Rotation Items (b) Study 1 Dual-Solution Errors and Mental Rotation Items (c) Study 2 Dual-Solution Latency and Mental Rotation Items, and (d) Study 2 Dual-Solution Errors and Mental Rotation Items</td>
<td>49</td>
</tr>
<tr>
<td>19. Scatterplots of (a) Study 1 Dual-Solution Latency and Objects Recalled (b) Study 1 Dual-Solution Errors and Objects Recalled (c) Study 2 Dual-Solution Latency and Objects Recalled, and (d) Study 2 Dual-Solution Errors and Objects Recalled</td>
<td>50</td>
</tr>
<tr>
<td>20. Boxplots from Exploratory Analyses with a Subsample of Nondepressed Study 2 Subjects</td>
<td>58</td>
</tr>
</tbody>
</table>
Correlations between sex and cognitive ability have been observed in the domains of learning, memory, attention, visuo-spatial processing, language comprehension and production, and motor and perceptual skills (Hampson, 2002; Kimura, 2000; Kimura & Hampson, 1994). The most consistent sex differences are found in the spatial domain, notably in tasks involving mental rotation, object location memory, and navigation. Performance differences between the sexes are reliable, but are often small, whereas differences within a sex, i.e., females compared with other females (Nelson, 2005, pp. 204). Curiously, both cognitive differences within and between the sexes may be explained by gonadal hormones. Natural fluctuations in estrogens and progesterone occur on a monthly and daily basis as well as across the lifespan, e.g., increase peri- and post-puberty; decline post-menopause. Sex, menstrual cycle phase, and 17β-estradiol have been shown to correlate with strategy use and cortical recruitment during navigation (Grön, Riepe, Tomczak, Spitzer, & Wunderlich, 2000; Harrison et al., n.d.; Saucier et al., 2002; Zurkovsky, Brown, & Korol, 2006).

Much of our understanding of estrogen- and progesterone-mediated cognitive differences stems from research with nonhuman animals and hormone replacement therapy in menopausal women (for review see Gibbs, 2006; for review see Spencer, Waters, Romeo, Wood, Milner, et al., 2008). Studies conducted with healthy young women (Mordecai, Rubin, & Maki, 2008) and transsexuals (Sommer et al., 2008) also suggest estrogen- and progesterone-mediated cognitive differences. Endogenous levels of estrogens and progesterone have been shown to influence brain development during the adolescent period (for review see Blakemore & Choudhury, 2006; Peper et al., 2009), and there is growing evidence of exogenous gonadal hormone, such as hormonal contraceptives and cross-sex steroid treatment, effects on cognition through suppression of exogenous hormones (Mordecai et al., 2008; Sommer et al., 2008).

Despite extensive documentation of the physiological effects of hormonal contraceptives (Rapkin, Sorger, & Winer, 2008; Riveria, Yacobson, & Grimes, 1999), little is understood or at least documented concerning potential neuroanatomical and neurofunctional effects of these widely prescribed drugs. Furthermore, the majority of hormonal contraceptive clients are between 15 and 30 years of age (Mosher, Martinez, Chandra, Abma, & Willson, 2004), a notable period of brain and cognitive development (for review see Lenroot & Giedd, 2010). The current
study is designed with two aims in mind. First, to replicate the findings of previous work concerning sex, estrogens, and place learning during virtual navigation. Second, to explore the possible cognitive effects of hormonal contraceptive use during late adolescent and young adult development.

**Sex and Gonadal Hormone Cognitive Differences**

A variety of cognitive sex differences have been documented throughout the lifespan, from the preschool years (McGuinness & Morley, 1991; Voyer, Voyer & Bryden, 1995) well into the elder years (Hampson, 2002; Kimura & Hampson, 1994). Disagreement as to the presence, extent, and origins of sex as a source of variation in human cognition has existed in experimental psychology since the beginning of the 20th century (for review of early experiments see Woolley, 1914). More agreement exists among contemporary psychologists in regard to the presence of reliable domain-specific cognitive sex differences (Hampson, 2002; Kimura, 2000; Kimura & Hampson, 1994); however, the extent to which these differences are behaviorally significant is still an area of contention. Sex and gonadal hormone differences in behavior are often discussed in terms of organizational and activational effects, which are described briefly in the following sections.

**Organizational Effects**

Sex differences in neuroanatomy and behavior that are typically long-lasting and permanent are referred to as organizational effects. Sexually dimorphic organizational effects in neuroanatomy typically result from differences in gonadal hormones and often occur during pre- and neonatal development. Recent neuroimaging evidence, however, suggests that sex- and hormone-mediated brain organization may occur as late as adolescence (for review on sex differences see Lenroot & Giedd, 2010). In human adolescents, for example, sex differences in gray matter volume correlate with estrogen and testosterone levels (Peper et al., 2009).

Organizational effects of gonadal hormones often present as sex differences in cortical lateralization and recruitment in adults (Grön, Riepe, Tomczak, Spitzer, & Wunderlich, 2000; Hampson, 2002; Kimura, 1983; Shaywitz et al., 1995). Several studies with children, however, suggest that these organizational sex differences appear early in development, prior to activational effects of hormones associated with puberty and gonadal activation and may account for the organizational effects observed during adulthood.

Research conducted in the mid-1970s suggests right hemisphere specialization for spatial processing in 6-year-old male, but not age-matched female, children (Witelson, 1976). More recently, Hahn, Jansen, and Heil (2010)
observed hemisphere asymmetry between preschool boys and girls using event-related potentials (ERPs) that where synced with a task requiring mental rotation of letters. These researchers, like Witelson before, observed right hemisphere specialization in spatial processing for pre-school boys but not girls. A study with 8- to 10-year-old children suggested a male advantage in spatial learning during a virtual Morris Water Maze task (Newhouse, Newhouse, & Astur, 2007). Presuming their subjects to be prepubescent, Newhouse and colleagues concluded that this male advantage is attributable to organizational hormone effects independent of activational hormone effects; however, this conclusion is questionable considering the absence of quantifiable hormonal data confirming the prepubescent status of participants in this study. Furthermore, gonadal hormones have been shown to circulate peripubertally, contributing to the onset of puberty (as reviewed in Bay, Andersson, & Skakkebaek, 2004), and may be present in prepubescent children. Consequently, activational effects of gonadal hormones should not be ignored as potential mediating factors of observed pre- and peri-pubertal cognitive sex differences, nor should the effects of hormonal contraceptive use during this and other periods of brain development.

**Activational Effects**

Transient, rarely permanent, neurofunctionally-based sex differences in cognitive behavior, that result from variation in gonadal hormone levels are referred to as activational effects. Activational effects have been observed in multiple domains of cognition including motor control and perception (for reviews see Cameron, 2001 and Hampson & Kimura, 1988), spatial learning (Broverman et al., 1981; Grön et al., 2000; Saucier et al., 2002), language abilities (Hampson, 1990), verbal fluency and memory (Kugaya et al., 2003; Rosenberg & Park, 2002), working memory (Grigorova, Sherwin, & Tulandi, 2006; Shaywitz et al., 1999) and memory (Hampson, 2002; Kimura & Hampson, 1994; Lawton, 2010; Maki & Resnick, 2000).

Activational effects of gonadal hormones often present as cognitive differences between the sexes (Grön et al., 2000; Maki & Resnick, 2000; Saucier et al., 2002); however, they may also appear as cognitive differences within a sex. Human (Harrison et al., n.d.) and nonhuman animal (Korol, Malin, Borden, Busby, & Couper-Leo, 2004) research has identified menstrual and estrus cycle phase differences in strategy preference and efficacy during spatial processing, which support a compensatory explanation for sexual and hormonal dimorphic cognitive differences within and between the sexes. Prenatal organizational and pubertal activational effects of sex hormones and gene expression may result in permanent neuroanatomical sex differences that in turn compensate for reproductive cyclical fluctuations in gonadal hormones leading to only minimal behavioral differences between the
sexes (de Vries, 2004). For example, a fMRI study of virtual navigation revealed marked sex differences in cortical recruitment during navigation despite minimal behavioral sex differences in navigation (Grön et al., 2000).

If a compensatory explanation of gonadal hormones and cognition is accurate, then gonadal hormones are necessary for effective neurofunctioning. If gonadal hormones are necessary for effective neurofunctioning and hormonal contraceptives suppress gonadal hormones, then hormonal contraceptives may interfere with optimal neuronal functioning. This line of reasoning calls for further research on the cognitive effects of hormonal contraceptives.

The Adolescent Period of Brain and Cognitive Development

Brain Development

Brain development during adolescence may be a second period of steroid-mediated cortical organization. Brain growth during periadolescence is characterized by superfluous increases in neurons, synapses, and receptors (Andersen, 2003; De Bellis et al., 2001), rendering adolescence a period of experience-expectant brain development. Animal research suggests that exogenous steroid-hormone administration may not elicit adult-like reproductive behaviors in adolescent rodents; however, these exogenous hormones may still organize the adolescent rodent brain (for review see Sisk & Foster, 2004). If the latter is correct, then hormonal contraceptives may also organize the adolescent human brain possibly interfering with optimal neuronal functioning during this and later periods of human development.

Sex and Adolescent Brain Development

Some of the earliest research on adolescent development involved psychomotor measures similar to those used widely today, such as finger tapping, manual dexterity, digit span and verbal fluency tasks (Woolley, 1915). Using these psychomotor measures, Woolley identified small differences between the sexes but greater variation within the sexes, particularly for females. This finding is in line with a century of riddling cognitive sex research.

Nonhuman primate research conducted in the mid-1970s, involving orbito-prefrontal lesions, elicited performance impairments on object reversal in male but not female 2.5-month-old rhesus macaques (Goldman, Crawford, Stonkes, Galkin, & Rosvold, 1974). Testing in the same animals, at 12 months of age, produced similar results; however, testing at 18 months of age produced performance impairments in both males and females. These findings suggest protracted orbito-prefrontal development in female but not male nonhuman primates (Goldman et
al., 1974). Human studies also suggest protracted white matter development throughout adolescence and young adulthood in females but not males (De Bellis et al., 2001; Groeschel, Vollmer, King, & Connelly, 2010). However, several neuroimaging studies suggest protracted cortical development for males compared to females (for review see Lenroot & Giedd, 2010). Additional findings concerning sexually dimorphic human white matter development are also mixed. Some DTI studies report region-specific differences in white matter development between the sexes (Rubia, Hyde, Halari, Giampietro, & Smith, 2010), while others observe age but not sex differences in white matter development (Asato, Terwilliger, Woo, & Luna, 2010).

Interestingly, a comprehensive MRI study of healthy young and older adults (age range 26 - 51 years) revealed that the most notable sex differences in human neuroanatomy correspond with cortical regions identified in developing nonhuman animals as containing the highest numbers of gonadal hormone receptors, e.g., hippocampus and amygdala (Goldstein et al., 2001). This suggests a potential role of gonadal hormones in brain development and the imperative to determine potential organizational effects of hormonal contraceptive use during periods of brain development.

Cognitive and White Matter Development

In children and young adolescents, aged 8 to 18 years, working memory and reading comprehension development was positively correlated with white matter development in the superior frontal and parietal (working memory) and left temporal (reading ability) lobes (Nagy, Westerberg, & Klingberg, 2004). An ensuing study identified global cognitive correlations between white matter development and IQ. Positive correlations, in females, have been observed between white matter and IQ, while negative correlations between white matter and IQ have been observed in males (Schmithorst, 2008). Hänggi and colleagues (2008) also found sexually dimorphic correlations between neuroanatomy and visuospatial cognition, as tested using a block design task from the WAIS-R. Visuospatial cognition was positively correlated predominantly with parietal white matter in males, and in parietal and superior temporal gray matter in females. These sexually dimorphic differences between gray/white matter volumes and cognitive performance may be interpreted as increased female relative to male reliance on specific intra- versus inter-regional processes; however, this interpretation conflicts with behavioral and neuroimaging studies, with older adolescents and adults, that suggest greater bilateral activation in females compared to males (Hampson, 2002; Kimura, 1983; Shaywitz, Shaywitz et al., 1995). More recently, Bava and colleagues (2010) observed positive correlations between performance on a variety of cognitive measures, such as
backward digit span, letter fluency, and block design, and white matter development during middle adolescence, i.e., 16- to 20-years-old. Additional research is required to interpret these developmental cortical sex differences both in terms of behavioral relevance and sensitivity to gonadal hormones.

**Hormones and Adolescent Brain Development**

Recent neuroimaging studies have identified correlations between circulating gonadal hormone levels and gray matter volume in adolescent males and females, suggesting that gonadal hormones continue to organize the human brain during adolescence. In young adolescents, 8- to 15-years-old, testosterone levels were negatively correlated with hippocampal and left parietal volumes, whereas serum estrogen was positively correlated with parahippocampal (Neufang et al., 2009) and mid-occipital gyrals, mid-frontal, and inferior-temporal volumes and negatively correlated with prefrontal, orbito-frontal, parietal, and mid-temporal volumes (Peper et al., 2009). This age group represents the youngest adolescents but still implicates gonadal hormones in cortical development.

Additional relationships between circulating gonadal steroids and gray matter volume may be observed in older adolescents who experience greater concentrations of gonadal hormones than younger adolescents. Indeed, a recent MRI study conducted with older adolescents in their twenties, revealed that, in both males and females, 17β-estradiol levels were positively correlated with gray matter volumes in the left superior parietal gyrus, while progesterone and testosterone where negatively correlated with gray matter in right temporal pole (progesterone) and left inferior frontal gyrus (testosterone) regions (Witte, Savli, Holik, Kasper, & Lanzenberger, 2010). Again, these findings suggest a role for gonadal hormones in brain development, and the imperative to determine potential organizational effects of hormonal contraceptives.

**Hormonally Atypical Populations**

**Congenital Adrenal Hyperplasia**

Congenital adrenal hyperplasia (CAH) is a genetic disorder that results in atypical levels of steroid hormones, e.g., cortisol, androgens, estrogens. Females with severe cases of CAH present with masculinized genitalia at birth, whereas females with less severe cases may not be diagnosed until pubertal milestones are missed, e.g., failure to develop secondary sex characteristics or experience menarche.

Considering the atypically high level of androgen exposure that results from CAH one may expect the brains and behavior of CAH females to resemble that of males more so than females; however, there are mixed
findings concerning CAH and cognitive abilities (Berenbaum, 2001). Additionally, it is important to use caution when ascribing sex or hormone differences found in samples including CAH females, since females with the severest forms of CAH typically possess genital characteristics at birth that distinguish them from ‘typical girls.’ Gender assignment and differential treatment begins prenatally and continues throughout development (Beal, 1994, pp.31 - 49) and it is possible that CAH females are expected to be less feminine and are treated differently than same sex siblings without the disorder. Therefore, differences in behavior between CAH females and non-CAH females may be attributable to gender as well as hormone effects.

**Delayed Pubertal Onset**

Additional research has explored manipulations of gonadal hormone levels in adolescents who experienced delayed pubertal onset, most often as a result of Turner’s Syndrome, for females, and Constitutional Delay, for males (Liben et al., 2002). A male performance advantage on spatial tasks was observed in this population; however, correlations between gonadal hormone levels and cognitive performance, for either sex, were not. It is important to note that the hormones manipulated and measured in this study were exogenous hormones. These synthetic hormones, while clearly physiologically influential, may not have the same cognitive effects as endogenous gonadal hormones (Mordecai et al., 2008; Sommer et al., 2008).

**Hormonal Contraceptives**

Place and response learning, as demonstrated in subsequent sections, rely on brain structures and cognitive processes that continue to develop throughout adolescence and young adulthood. Hippocampal-dependent place and striatal-dependent response learning have been shown to be improved (place learning) or impaired (response learning) by estrogens, thus making adolescence a second window of opportunity for gonadal hormones to organize brain areas associated with place and response learning. Hormonal contraceptives suppress endogenous estrogens (Mordecai et al., 2008) and could interrupt the necessary estrogenic mechanisms for typical brain and place learning development; however, ethinyl estradiol, the active estrogen in hormonal contraceptives, is an estrogen-receptor agonist (Blair et al., 2000) and may in fact enhance estrogen-sensitive cognitive processes, i.e., place learning. The following sections review the literature pertaining to the neuromechanisms and cognitive effects of hormonal contraceptives.
History and Prevalence of Hormonal Contraceptive Use

In 1957 the first US approved hormonal contraceptive, Enovid, did not include “contraceptive” in its title, and was only approved to treat “gynecological disorders” such as menstrual depression and discomfort. Enovid was later approved for use as a contraceptive in 1960 (White-Junod & Marks, 2002). In 1961, reports of harmful physical side effects, e.g., blood clots, began to emerge (White-Junod & Marks, 2002); however, currently prescribed hormonal contraceptives have minimal adverse physiological side effects when usage guidelines are followed and the health status and age of the patient is considered (Kiley & Hammond, 2007; Rapkin, Sorger, & Winer, 2008; Sondheimer, 2008).

As of 2004, 53% of 15- to 19-year-old females in the United States reported oral contraceptive use (Mosher, Martinez, Chandra, Abma, & Willson, 2004); however, this estimate does not account for females who use intrauterine, e.g., Mirena, intramuscular, e.g., Depo Provera, intravaginal, e.g., NuvaRing, or transdermal, e.g., Ortho Evra, forms of hormonal contraceptives. In 2009, approximately 251 out of 326 (77.1%) polled undergraduate females, at just one university, reported hormonal contraceptive use (Brunner Huber & Ersek, 2009). At these frequencies, if hormonal contraceptives do influence neuroanatomy and functioning they have the ability to do so in epidemic proportions.

Mechanisms of Action

The effects of hormonal contraceptives may be fast acting. Some hormonal contraceptives, such as drospirenone 3mg/ethinyl estradiol 20µg, reach peak concentration levels within one to two hours after administration and a steady state after eight days (Rapkin, Sorger, & Winer, 2008). Hormonal contraceptives prevent pregnancy and suppress endogenous hormones primarily by acting on the hypothalamus-pituitary-gonadal axis (HPG-A). When hormonal contraceptives reach peak concentration, negative feedback loops detect high levels of estrogens and progestins—synthetic and/or endogenous if in a phase other than menses. This increase, in estrogens and progestins, signals inhibition of pituitary production and release of luteinizing (LH) and follicle stimulating (FSH) hormones. Low levels of LH and FSH are observed within seven days of hormonal contraceptive administration, inhibit ovarian release of estrogens and progesterone, and prevent follicle development. Inhibited follicle development prevents the formation of corpora lutea, secondary sources of progesterone. Within one day of active pill cessation, LH and FSH levels return to normal and new follicles begin to develop (Rivera, Yacobson, & Grimes, 1999). Hormonal contraceptives that use shorter hormone-free intervals (HFI; i.e., inactive pill phases) or
provide low-dose ethinyl estradiol during the HFI result in greater inhibition of HPG-A activity and follicle development as determined by lower levels of FSH and endogenous 17β-estradiol (Reape, DiLiberti, Hendy, & Volpe, 2008; Vandever et al., 2008). These effects, while desirable for the purpose of preventing pregnancy, contribute to chronically diminished endogenous gonadal hormones, the cognitive consequences of which have yet to be definitely determined.

Effects on Endogenous Gonadal Hormones

Currently, the Food and Drug Administration has approved three synthetic estrogens and twelve synthetic progestins for contraceptive use in the US (for a nearly complete list see: Rapkin, Sorger, & Winer, 2008). The physiological effects of hormonal contraceptives are well documented (Aronson, 2006); however, the neurophysiological effects are not as well understood. What has been documented concerning hormonal contraceptives and cognition is discussed in the following sections.

Hormonal contraceptives have been shown to suppress endogenous 17β-estradiol (Griksiene & Ruksenas, 2011; Klipping, Duijkers, Trummer, & Marr, 2008; Komnenich, Lane, Dickey, & Stone, 1978; Mishell, Thorneygroft, Nakamura, Nagata, & Stone, 1972; Mordecai et al., 2008), progesterone (Griksiene & Ruksenas, 2011), and testosterone (Mordecai et al., 2008) levels to those levels observed during the menses and early follicular phase, when 17β-estradiol and progesterone are lowest. Ethinyl estradiol alone has been shown to exert neuroprotective effects against excitotoxins in healthy young ovariectomized female rats (Picazo, Becerril-Montes, Huidobro-Perez, & Garcia-Segura, 2010); however, to the my knowledge no other findings as to the neuromechanisms of hormonal contraceptives have been reported.

Metabolic Effects

Perhaps even less understood than the neuromechanisms of hormonal contraceptives are potential individual differences in the metabolism of these medications. To my knowledge, few studies, and only two published recently, have addressed this issue. The estrogens and progestins in hormonal contraceptives are differentially metabolized. Ethinyl estradiol “ranges in bioavailability” (Goldzieher, 1989) as a result of first-pass effects of metabolism in the liver and intestines. Variability in bioavailability exists even after adjusting for body weight and controlling for diet during trial periods. Less variability has been observed in a commonly used synthetic progestin, levonorgestrel (Goldzieher, 1989). At the 50µg dose, ethinyl estradiol plasma and urinary levels over a
24-hour period vary considerably between females in developing versus developed cultures, as well as between western and eastern cultures (as discussed in Goldzieher, 1989). While a 50µg dose is higher than what is currently prescribed, data on the bioavailability of currently prescribed synthetic progestins, estrogens, and their respective doses, is not available.

In 2005, Brunner Huber and Hogue published findings suggesting a potential mediating factor of obesity on hormonal contraceptive efficacy. After adjusting for age and “race/ethnicity,” among other demographic co-variables, obesity was reported to double the occurrence of unintended pregnancy among hormonal contraceptive users. Unfortunately, Brunner Huber and Hogue do not report on potential age or ethnic differences in hormonal contraceptive efficacy; however, only two years later Brunner Huber and Toth (2007) published findings that did not support associations between obesity and hormonal contraceptive failure after adjusting for age, “race/ethnicity,” and other demographic variables.

It is not possible, from the early studies reviewed in Goldzieher (1989), to determine which aspects of culture, ethnicity, and/or geographical location account for these population differences. Research exploring potential individual differences in hormonal contraceptive metabolism is required in order to safely prescribe hormonal contraceptives and minimize potentially adverse neurophysiological effects, which may result from variability in drug bioavailability. This is especially important considering hormonal contraceptive administration does not follow the well-documented recommendations for safe hormone replacement treatment concerning dose, i.e., pulsatile versus continuous, regimen, i.e., estrogen followed by progesterone, and duration, i.e., acute versus chronic (Blaustein, 2008).

**Neuroanatomical Effects**

Hormonal contraceptive use has been shown to correlate positively with region-specific gray matter volume (Pletzer et al., 2010), and may mimic short term morphometric increases observed over the menstrual cycles of naturally cycling females (De Bondt et al., 2013). Hormonal contraceptive use has been shown to correlate with greater gray matter volume in the prefrontal cortex, parahippocampal and fusiform gyri (Pletzer et al., 2010), as well as areas implicated in emotional processes, such as the anterior cingulate cortex, which has been shown to negatively correlate with 17β-estradiol levels for naturally cycling, but not hormonal contraceptive, females (De Bondt et al., 2013). Increased anterior cingulate cortex activity, and greater emotional regulation, has been observed in low estrogen situations (for review see De Bondt et al., 2013), which may explain instances of decreased negative
affect associated with hormonal contraceptive use (Herzberg, Draper, Johnson, & Nicol, 1971; Paige, 1971). These findings suggest that hormonal contraceptives, through suppression of endogenous estrogens, may exert neuroprotective effects, particularly concerning the processing of emotional information.

**Modulation of Cognitive Functioning**

**Mood and Affect**

Early research exploring the effects of hormonal contraceptives on mood and affect suggests higher incidents (Nilsson & Almgren, 1968), lower incidents (Herzberg, Draper, Johnson, & Nicol, 1971), and no connection between incident rate of depression and hormonal contraceptive use (for review see Oinonen & Mazmanian, 2002). Ensuing research using daily ratings of mood and affect, over extended periods of time, found decreased variability and negative affect in hormonal contraceptive users compared to nonusers (Paige, 1971). Rouse (1978) identified age as a mediator of the relationship between hormonal contraceptive use and depression, with users younger than 20-years-old experiencing increased negative affect, whereas users older than 30-years-old reported less negative affect. Hormonal contraceptives with higher dosages of ethinyl estradiol and synthetic progesterone have been correlated with greater incidences of negative mood (Deijen, Duyn, Jansen, Klitsie, & 1992).

To account for the beneficial effects of hormonal contraceptives on mood, beyond amelioration of physical discomfort, Oinonen and Mazmanian (2002) suggest an interplay between estrogen and monoamine oxidase levels that results in increased serotonin levels, which translates to decreased negative affect and greater mood stability. Increased negative affect and depressive mood, however, have been cited as the primary reasons for discontinuation of hormonal contraceptives, which may contribute to a ‘survivor effect’ and over representation of beneficial hormonal contraceptive mood effects in the literature (Kutner & Brown, 1972; Oinonen & Mazmanian, 2002).

**Motor and Perception**

Hormonal contraceptive use has been correlated with improved fine motor coordination, as measured by performance on the grooved pegboard task, and faster acquisition of conditioned startle responsivity, as measured by the pairing of an auditory stimulus and air-puffs to the eye (Beck et al., 2008). In an eye-tracking study, females using oral contraceptives initially looked and continued looking longer at contextual content and away from sexual content of static images as compared with naturally cycling females and males (Rupp & Wallen, 2007). This finding
is particularly interesting as it suggests top-down, i.e., hormone-driven, rather than bottom-up, i.e., stimulus-driven, perceptual effects associated with hormonal contraceptive use.

**Memory**

Females in the Baltimore Longitudinal Study of Aging (BSLA) who received estrogen replacement treatment, prior to study onset as part of their own health regimens, demonstrated better visual memory at study onset and two years later, when compared to healthy age-matched females not using estrogen replacement treatments (Resnick, Metter, & Zonderman, 1997). The findings of the BLSA, along with multiple other human and nonhuman animal studies, suggests a neuroprotective role of estrogens in healthy aging females (for review see Spencer, Waters, Romeo, Wood, Milner, & McEwen, 2008) despite the controversial findings of the Women’s Health Initiative Memory Study (Shumaker et al., 2003). Surprisingly, improved verbal, but not visual, memory was found in hormonal contraceptive users when compared with naturally cycling females (Mordecai et al., 2008). Mordecai and colleagues reported no difference in verbal fluency, mental rotation, or attention between hormonal contraceptive users and naturally cycling females. Likewise, Vranić and Hromatko (2008) published findings that suggest no affect of hormonal contraceptives on working memory.

**Language**

In a fairly recent study, hormonal contraceptive use was associated with poorer performance on a verbal fluency task (Griksiene & Ruksenas, 2011), which is somewhat surprising considering previous research that failed to find correlations between 17\(\beta\)-estradiol levels and performance on verbal fluency tasks (Hampson, 1990; Mordecai et al., 2008). Cortisol-induced verbal memory impairments, however, have been observed in naturally cycling but not hormonal contraceptive females (Kuhlman & Wolf, 2005). This finding suggests that hormonal contraceptive use may preserve memory through reduced sensitivity to acute elevations in cortisol levels, and in the bigger picture, stress. This finding exemplifies the necessity of considering hormonal milieu when studying the neuromechanisms of psychiatric disorders as well as typical cognitive behaviors.

**Spatial Processing**

A fairly recent study suggests that hormonal contraceptive effects on mental rotation abilities may vary in a generation-specific manner (Wharton et al., 2008). Hormonal contraceptive generations differ in terms of the
androgenic effects of the synthetic progestins they contain, with third and new generation hormonal contraceptives producing fewer androgenic effects than second generation hormonal contraceptives, and new generation contraceptives producing anti-androgenic effects. Wharton and colleagues observed that naturally cycling females demonstrated better mental rotation abilities than females using new generation hormonal contraceptives. Similarly, Griksiene and Ruksenas (2011) observed mental rotation impairments with third and new generation hormonal contraceptive use, suggesting androgenic and not estrogenic effects of hormonal contraceptives on some spatial abilities, such as the male-advantaged mental rotation task. Future studies should consider contraceptive generation effects.

**Place versus Response Learning**

**Multiple Navigation Processes**

Navigation processes are typically categorized based on the spatial information attended to during navigation and are popularly referred to as “place and response” or “allocentric and egocentric” learning in rodent and human studies, respectively. Place and allocentric learning often refer to learning that involves the formation of a cognitive map that contains reference information about the relations and distances between objects and is characterized by learning independent of external behaviors, particularly motor responses (Levy et al., 2005; White & McDonald, 2002). This type of learning may also be accomplished by orienting in space based on distal cues, coordinate systems, and/or the geometric qualities of an environment (Chabanne et al., 2004; Iglói et al., 2009; Nedelska et al., 2012; Saucier et al., 2002; van Gerven et al., 2012). Conversely, response and egocentric learning refer to learning that involves memory of a sequence of movements or motor responses, sometimes linked with proximal cues or salient landmarks (Doeller et al., 2008; Hawley et al., 2012; Spritzer et al., 2013; White & McDonald, 2002). This type of learning is also referred to as habit or familiar route learning.

**Early Findings**

The earliest dissociation between navigation strategies in spatial learning is perhaps traced to an experiment by Tolman, Ritchie, and Kalish (1946). Tolman and colleagues (1946) trained 16 male rats to retrieve a food reward, in a simple T-maze, using either a place or response strategy. Of the eight rats trained to use a place strategy, all eight reached criterion within only eight training sessions. Of the eight rats trained to use a response strategy, three never reached criterion, while five did, but only after shifting to a place strategy. These findings led Tolman et al. to
conclude that “place-learning is simpler than response-learning.” More recent studies suggest that place learning may be “simpler than response-learning,” but in a sex- and hormone-sensitive manner.

**Contemporary Findings**

In a forced strategy real-world navigation task, navigational instructions were presented in two forms: as Euclidean directions, which would elicit place learning; or as a list of landmark cues, which would elicit response learning (Saucier et al., 2002). Saucier and colleagues observed that females in the place condition, i.e., Euclidean directions, committed significantly more errors and required more time to completion than females, and males, in the response condition, i.e., landmark cues, as well as males in the place condition. There were no sex differences in the response condition or condition effects on male navigation. The results of this study suggest that males and females differ in their ability to utilize different cognitive strategies during spatial tasks, with males succeeding in both place learning and response learning, whilst females are more successful in response learning.

A more recent study observed similar findings in wayfinding. Undergraduate students entering late adolescence, i.e., 17.7 - 20.9 years of age, were asked to verbally describe the route from one common campus location to a second (Cherney, Brabec, & Runco, 2008). In line with Saucier and colleagues females reported more landmarks suggesting a preference for a response strategy while males reported more euclidian directions suggesting a preference for a place strategy.

A study with children and young adolescents suggests that these sex-specific preferences for place versus response strategies are present early in development. A trend towards a strong preference for and advantage with response learning demonstrated by greater recall in landmark cues in females than males is present as early as nine-years-old. This preference and advantage becomes statistically significant by 12 years of age (Choi & Silverman, 2003). A female advantage in object and location memory present during this developmental period suggests that memory processes may contribute to the observed female advantage in response learning (Choi & Silverman, 2003). Interestingly, males in this sample who demonstrated a response strategy preference performed more poorly on non-navigational measures of spatial processing, e.g., water level task, than males who demonstrated a place strategy preference.

These findings suggest that distinct cognitive processes, e.g., object memory, reference processing, may underlie the varying components of spatial learning, e.g., navigation, perception, mental rotation, and that these cognitive processes vary in their efficacy to produce successful performance on spatial learning tasks. Differences in
the ability to effectively utilize these distinct cognitive processes also appear to vary by sex; however, further research is required to test the viability of these hypotheses. Furthermore, the age at which these sex differences in place versus response preference and efficacy emerges, i.e., 12 years, coincides with the onset of puberty and increased levels of circulating gonadal hormones, suggesting potential activational hormone effects. Indeed, several rodent models and at least one human study provide such evidence.

Healthy adolescents and young adult males and females, aged 17 - 31 years, were tested on a dual-solution virtual navigation task that could be solved using either place or response learning (Harrison et al., n.d.). Females were tested either two days before ovulation when estrogens are rising, i.e., preovulatory females, or two-three days after menstruation when estrogens are low, i.e., menses females. Neither sex nor hormone differences were observed during the dual-solution training; however, when landmark cues were removed during a probe trial, i.e., forced place learning, menses females performed more poorly than both preovulatory females and males. No differences were observed between males and preovulatory females. Consider these findings, which suggest estrogen facilitation of place learning for females, in light of the previously discussed findings by Saucier and colleagues (2002), who observed a significant interaction between sex, i.e., male vs. female, and navigation condition, i.e., place vs. response, with females in the place condition experiencing the greatest navigation impairment. If estrogens are necessary for successful female place learning, one might suspect that these females in the place condition may have had low levels of estrogens at time of testing, therefore experiencing the most disruption to successful navigation. Additional research with nonhuman animals lends credence to this hypothesis.

Rodent models of place and response learning support sexually dimorphic strategy preference and efficacy in spatial learning in addition to the mediating role of gonadal hormones. On a T-maze task, gonadally intact female rats demonstrated a place strategy preference during proestrus, when gonadal hormone levels are high, and a response strategy preference during estrus, when gonadal hormone levels are low (Korol, Malin, Borden, Busby, & Couper-Leo, 2004). Differences in navigation abilities were not observed between the two estrus phases. These findings suggest that a) gonadal hormones mediate place versus response strategy preference in spatial learning, and b) different strategies during spatial learning may have emerged to compensate, i.e., eliminate performance advantages, for naturally occurring fluctuations in gonadal hormones. The first hypothesis is supported by the findings that ovariectomy, in healthy young rats, has been shown to impair place but not response learning, while estrogen replacement treatment enhanced place but not response learning (Davis, Jacobson, Aliakbari, Mizumori, 2005; Korol & Kolo, 2002). However, additional research is necessary to evaluate the second hypothesis.
Collectively, these findings suggest a) sex- and hormone-mediated strategy preference and efficacy, b) dissociative neuromechanisms for place and response learning, c) and, estrogen facilitation of place but not response learning. Future studies of gonadal hormone levels in pre- and peri-pubescent children are necessary to determine whether the strategy preference and efficacy sex differences observed during these developmental periods are the result of organizational effects, activational effects, or a combination of both (Choi & Silverman, 2003; Newhouse, Newhouse, and Astur, 2007).

**Estrogen Effects on Place and Response Learning**

Place and response learning have been shown to rely predominately on independent and dissociated neural pathways. Place learning is supported by hippocampal structures, whereas response learning is supported by the striatum (for review see White & McDonald, 2002). There is evidence, however, that place and response learning often occur in parallel, in tandem, and with overlapping neural activity in both the hippocampus and striatum (Jacobson et al., 2012; Marchette et al., 2011).

Intrahippocampal estrogen administration has been shown to improve place, but not response, learning (Zurkovsky, Brown, Boyd, Fell, & Korol, 2007) possibly due to estrogen-suppression of GABAergic inhibitory synaptic activity (Murphy et al., 1998 as reviewed in Gibbs, 2006; for review see Zurkovsky, 2009). Intrahippocampal administration of a GABA_A agonist, in gonadally intact young female rats, resulted in increased preference for a response strategy during spatial learning (McElroy & Korol, 2005). Conversely, intrastriatal estrogen infusions impaired striatal-dependent response learning while sparing place learning, possibly through decreased dopaminergic and glutamatergic activity (for review see Zurkovsky, 2009). Furthermore, estrogen is believed to increase hippocampal activity via increased brain-derived neurotrophic factor expression (for review see Baudry et al., 2013). The net result of these estrogenic effects is increased hippocampal activity and improved performance on hippocampal-dependent cognitive tasks, such as place learning.

Like estrogen treatments, progesterone treatments have been shown to improve place learning in ovariectomized female rats; however, estrogen plus progesterone treatments have been shown to impair place learning (for reviews see Korol, 2004 and Baudry et al., 2013). Recent findings suggest that progesterone exerts antagonistic estrogenic effects on synaptic plasticity. Estrogens are believed to influence hippocampal activity via increased brain-derived neurotrophic factor (BDNF) expression, which progesterone has been shown to reverse, possibly through downregulation of estrogen receptors (for review see Baudry et al., 2013). These findings raise
important concerns about the cognitive effects of combined oral contraceptives, which include both estrogens and progestins.

**Summary**

Hormonal contraceptive use is prevalent among American females during a critical period of brain development. Scant research is available pertaining to the neural effects of hormonal contraceptives and considerable controversy surrounds the cognitive effects of hormonal contraceptives. Nonhuman animal research on gonadal hormones, brain development, and spatial processing provides a theoretical framework that suggests an integral role of endogenous estrogens on hippocampal-dependent place learning. Combination oral contraceptive use could impair cognitive functioning, as demonstrated in rodent models. Alternatively, hormonal contraceptives could impair cognition through suppression of endogenous estrogen, which could result in impaired estrogen-sensitive learning; however, ethinyl estradiol, is an estrogen-receptor agonist, which could spare estrogen-sensitive learning. Additional research is needed to understand the relationship between combination contraceptives and cognition. The present studies were designed to explore the cognitive effects of combined oral contraceptive use during late adolescence and young adulthood.

**Overview of the Present Studies**

Two studies were conducted to explore the cognitive effects of combined oral contraceptive use during late adolescence and young adulthood. Both Study 1 and Study 2 compared performance on a battery of estrogen-sensitive and -insensitive tasks between three groups of females: one group of naturally cycling females, and two groups of combined oral contraceptive, i.e., ethinyl estradiol and levonorgestrel or one of its derivatives (Appendix C), females tested either during active or inactive, i.e., sugar, pill phases. Study 2 was conducted to provide intra-study reliability through replication of Study 1, and in order to manipulate the difficulty of the estrogen-sensitive navigation task. Naturally cycling females were tested around the menses phase when estrogens and progesterone are low. Combined oral contraceptive females tested during the active pill phase were tested on pill days 15 - 17 when endogenous hormones are low and exogenous hormones are elevated, or during days 1 - 2 of the hormone free interval (sugar pill) phase when exogenous hormones are low and endogenous hormones, while rising, are also lower than mid-cycle menstrual phase levels (Figure 1). Menstrual and pill phase were determined based on self-report of last menstruation or current pill number, and salivary 17β-estradiol levels at time of testing were measured.
Figure 1. Subject groupings, self-reported menstrual or pill phase, and expected hormone status at time of testing.

Predictions

Considering ethinyl estradiol’s high affinity for estrogen-receptors it is possible that ethinyl estradiol, acting as an estrogen-receptor agonist, will enhance estrogen-sensitive learning; however, considering the detrimental effects of combined estrogen and progestin treatments observed in rodent models (for reviews see Korol, 2004 and Baudry et al., 2013), it is also possible that combined contraceptive use may impair estrogen-sensitive learning. Consequently, several predictions are plausible. First, if endogenous estrogen is necessary for enhanced estrogen-sensitive learning, then the three groups, tested during low estrogen phases, should not differ in performance on estrogen-sensitive tasks, such as place learning and word generation. Second, if ethinyl estradiol acts as an estrogen receptor agonist and mimics endogenous estrogen effects, then females in the active pill phase may show enhanced estrogen-sensitive learning in comparison to both menses phase naturally cycling and hormone-free interval females. Lastly, if combination estrogen and progesterone treatment is cognitively detrimental, as suggest by the rodent literature, then active pill phase females are predicted to demonstrate impaired cognitive performance in comparison with naturally cycling, and possibly even hormone-free interval, females.
CHAPTER 2

METHOD

Subjects

Subjects were recruited from the University of Massachusetts Amherst through the Psychology Department’s on-line research recruitment site SONA. Interested subjects self-selected based on eligibility requirements posted on the SONA site. Eligibility was confirmed at time of testing (Appendices A, B, and C). Thirty females, approximately ten per group, provided data for Study 1. An additional 65 females, 19 - 23 per group, were recruited for replication purposes and provided data for Study 2. Combined oral contraceptive subjects included females who use a combination contraceptive containing ethinyl estradiol and levonorgestrel or one of its metabolites (Appendix C). Females in the combined oral contraceptive groups were tested either during the hormone free interval phase, i.e., placebo pill days 1 - 2, or during the active pill phase, i.e., pill days 15 - 17. Females in the active pill phase were tested approximately one to five hours after taking their contraceptive pills. A control group of naturally cycling females were tested during the menses phase, i.e., days 0 - 6. All subjects were native English speakers, right-hand dominate, reported normal or corrected to normal vision, and were free of medical conditions and chronic illnesses that are treated with hormones or affect hormone levels. Subjects reported at least three consecutive menstrual cycles and were not pregnant or breast feeding for at least three months prior to testing. Subjects were compensated with extra credit towards participating psychology courses.

Setting

All testing occurred in a small comfortably lit room in an academic building. Subjects were tested individually by female researchers who remained in the room during the entire 90 minute session. All tasks, with the exception of the virtual navigation task, were presented as paper and pen or verbal response. All instructions were delivered verbally. Subjects were allowed to adjust the distance between themselves and the computer according to their own comfort. Testing occurred weekdays during regular University operating hours, i.e., 8:00 am - 6:00 pm.

Tasks

Virtual Navigation Task (VNT)

The VNT is a spatial navigation paradigm consisting of three parts: a familiarization trial, three (Study 2) to five (Study 1) dual-solution training trials, and one (Study 1) to two (Study 2) place learning probe trials. Subjects
were naive to the place probe trial(s) during training so as not to bias them toward a place learning strategy during training. These trials were presented in simulated three dimensions using a virtual reality software package (Virtus Walkthrough Pro). Subjects pressed the up, down, left, and right arrow keys on a standard computer keyboard to move through the virtual environment. Movement in the virtual environment was limited to walking forward or backward using the up and down arrow keys respectively, and turning, but not walking, left and right using the left and right arrow keys. Doors in all three mazes were opaque and prevented subjects from seeing through doorways into other rooms. Subjects completed a familiarization trial in a four-room practice maze which afforded acclimation to the appearance of and movement through the virtual environment. Subjects advanced to the dual-solution training and place probe trials after indicating that they were comfortable with the task.

The familiarization trial was followed by the dual-solution training trials. Subjects in Study 1 completed five dual-solution training trials, with both proximal landmarks and geometric cues available, followed by one place probe trial in which landmarks were removed. Few subjects in Study 1 experienced difficulty navigating the probe trial, and in an effort to minimize potential floor effects, subjects in Study 2 completed only three dual-solution training trials. Additionally, a second place probe trial was added to Study 2 to minimize accurate navigation based on random door selection. The second probe trial immediately followed the first, and place latency and errors were averaged across the two probe trials. To further minimize reliance on response learning during the place probe trial, subjects in Study 2 completed the probe trials in reverse, beginning in the last rather than the first room.

The virtual navigation maze consisted of eight rooms identical in size, shape, and color. Rooms were scaled to the perspective of 10-foot high ceilings with an area of 50 x 50 feet. The observer’s perspective from eye level was 5-feet above ground. Each room contained four identical doors consistent in size, shape, and color. Wall and door color was consistent throughout all eight rooms. In each room, two doors opened to adjoining rooms and two opened to closets, with the exception of the first and eighth rooms which contained three closets. The wall color inside the closets was consistent throughout the maze and was distinct from the color of the walls in the rooms. Unique groupings of virtual furniture in each room were available as proximal landmark cues in the dual-solution training trials.

Subjects were allowed as much time as necessary to navigate through the maze during both training and probe trials. Trial latency was recorded as the time between movement onset and entrance into the last room. Trial errors were recorded as the total number of doors opened. The experimenter recorded latency using an electronic
timer and marked door choices on a paper floor plan. Subjects were instructed to minimize latency and errors within each trial by minimizing the number of inaccurate door choices.

**Map Drawing**

A map drawing task was included to assess learning of the virtual environment. Subjects were given a white piece of paper with an 8 x 8 black grid (16 cm x 9 cm) and were instructed to use the grid to create a floor plan of the virtual environment by assigning 8 of the 64 squares in the grid a number that represented each of the eight rooms. The map drawing task was scored by tallying the accurate number of transitions from one room to another. For each of the rooms, only one door led to the subsequent room, i.e., one door led from room one to room two, one led from room two to room three, and so forth. Subjects were scored based on their transitions from one room to the next, permitting subjects to receive credit for each correctly positioned room despite errors in previous or subsequent positioning of rooms. This task was not timed.

**Word Generation**

The word generation task is a measure of verbal fluency, and was included as an estrogen-sensitive nonspatial control task. The word generation task consists of two parts, letters and categories, and each part consists of three trials for a total of six trials. In the first part of this task subjects were instructed to say aloud as many words as they could think of that began with a specific letter, i.e., A, R, and F, with one letter per trial. In the second part of this task subjects were instructed to name items belonging in a specific category, again with one category, i.e., animals, sports, and items of clothing, per trial. Correctly generated words or category items were tallied, and subjects were given 30 seconds to complete each trial.

**Mental Rotation**

The Revised Vandenberg & Kuse Mental Rotation Test MRT-A was included as a potentially estrogen-insensitive spatial task (Peters et al., 1995). This task consisted of two double-sided pages of 12 mental rotation trials per page. For each trial, subjects were shown a row of five geometric figures composed of uniform blocks. The first figure in the row was the target figure. To the right of the target figure were four other figures, two of which were rotated versions of the target figure and two of which were distractor items. Subjects were instructed that exactly two of the figures on the right were the same object as the target on the left, but had been rotated in space.
Subjects were required to identify both of the rotated targets for a given trial in order for that trial to be scored as correct. Subjects were given 12 minutes to complete this task and were alerted when they reached the halfway mark for time management purposes.

**Nonspatial Control Tasks**

Tasks measuring short-term and working memory were included as estrogen-insensitive nonspatial control tasks. These tasks were selected from the Revised Wechsler Memory Scale (Wechsler, 1987). The Revised Wechsler Memory Scale is an extensively normed and widely used neuropsychological battery. Short-term memory was assessed using the Wechsler Logical Memory Task, i.e., story recall. Subjects listened to a short story consisting of five sentences. Immediately upon completion of the story, subjects were asked to recall as many details from the story as possible. Subjects received one point for each correctly recalled story component from a predetermined list of 25 components.

Working memory was assessed using the Wechsler Digit Span Task, i.e., digit span. This task consisted of three parts: forward, backward, and sequential digit spans. For the forward digit span trials, the experimenter read a sequence of digits aloud to the subject, who was instructed to repeat the sequence, in the same order, immediately after the experimenter finished speaking. For the backward digit span trials, subjects were instructed to repeat the digit sequence in reverse order, and for the sequential digit span trials, subjects were instructed to repeat the digits in sequential order from lowest to highest. Each part consisted of 24 trials. The length of the number sequences increased by one digit every two or three trials. Each correctly repeated sequence was scored as one point, for a possible digit span score of 48 points.

**Object Recall**

An object recall task was administered after all other tasks as a means of assessing navigation strategy. Subjects who used a landmark-dependent navigation strategy during training were predicted to recall more virtual environment landmarks than subjects who used a landmark-independent strategy. Approximately 45 to 60 minutes elapsed between exposure to virtual maze objects and the object recall task. Subjects were given one minute to complete this task and received one point for each correctly recalled object.
Mood Measures

The Profile of Mood States (PoMS; McNair et al., 1971) is a self-report questionnaire designed to assess mood at time of testing. The Feelings Inventory (CES-D) is a measure of stable mood, and is adopted from Radloff (1977). The PoMS and CES-D were included to account for possible mood effects on cognition. PoMS subscales assessed anger, confusion, depression, fatigue, tension, and vigor.

Hormone Assessment

Salivary 17β-estradiol levels were measured at time of testing to account for endogenous estrogen effects. Saliva samples were processed with high sensitivity antibody enzyme immunoassay (ELISA) kits¹ that have a high affinity for salivary 17β-estradiol and a low affinity for salivary ethinyl estradiol. An exhaustive search failed to produce means or ranges for menses phase salivary 17β-estradiol as measured with ELISA kits. Therefore, salivary 17β-estradiol levels at time of testing cannot serve as an accurate predictor of menstrual cycle phase, but do provide a direct assessment of 17β-estradiol levels at time of testing.

Salivary 17β-estradiol levels were measured by collecting three passive drool saliva samples, approximately 30 minutes apart, over the 90 minute testing session. The three samples were combined to provide one session sample per subject. Standard recommendations and precautions for saliva sampling, as provided by Salimetrics, Inc., were followed. To summarize, subjects were asked to rinse their mouths with water 10 minutes prior to the first sample collection as well as to refrain from drinking fluids or consuming food, e.g., candy, gum, during the 90 minute testing session. Subjects used a short straw to deposit saliva in test vials. Vials were placed directly on ice for the remainder of the testing session and then stored at -80°F until processing. Saliva samples were processed over three individual assays. Low and high control values were comparable across all three assays suggesting high inter-assay reliability (Figure 2).

¹Salimetrics 101 Innovation Blvd., Suite 302 State College, PA 16803 USA (T) 814-234-7748
Mean salivary 17β-estradiol levels were comparable between Studies 1 (\(M = 1.96\) pg/mL, \(SD = .76\); Table 1) and 2 (\(M = 1.84\); \(SD = .66\); Table 5), \(F(1, 92) = .216, p > .05\). Salivary 17β-estradiol levels ranged from .79 to 3.54 pg/mL for Study 1, and from .50 to 2.97 pg/mL for Study 2. Thirteen salivary 17β-estradiol values, four in Study 1 and nine in Study 2, were lower than the lowest observed standard control (1 pg/mL). Data analyses, however, were not affected by these data points, which are included in all reported findings. Intra-assay coefficient of variation percentages were well below average values reported in the literature and assay kit (\(M = 1.10\%\), \(SD = .94\), Range: .03 - 6.3\%) suggesting highly precise processing of saliva samples.

Combined oral contraceptives suppress 17β-estradiol to levels comparable to or lower than those observed during the early follicular phase (days 2 - 4 of the menstrual cycle, when day 1 is the first day of menstruation; Klipping, Duijkers, Trummer, & Marr, 2008; Mordecai et al., 2008). Therefore, it is not surprising that our first wave of data collection revealed slightly higher salivary 17β-estradiol levels in our menses phase naturally cycling group (tested days 0 - 6 when day 0 is the first day of menstruation; \(M = 2.27\) pg/mL, \(SD = .73\)) than those observed in our hormone-free interval group (inactive pill days 1 - 2; \(M = 1.51\) pg/mL, \(SD = .52\), \(U = 23, p < .01\) (Figure 3a and Tables 1 and 3). No other group differences in salivary 17β-estradiol levels were observed for Studies 1 or 2 (Figure 3b and Tables 1, 3, 5, and 7).
Figures 3a and 3b. 17β-estradiol levels by group for Study 1 (a) and Study 2 (b). Lines and whiskers represent medians and 1.5 times the interquartile range or the maximum or minimum if no data point falls within 1.5 times the interquartile range. NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone-Free Interval Phase. *Denotes $p$-value < .05.
CHAPTER 3
RESULTS

A total of 117 subjects were tested across Studies 1 and 2. Fourteen subjects were excluded due to changes in the final protocol, equipment failure, or experimenter illness. Three subjects declined to complete all aspects of the study, and were excluded due to incomplete data. Lastly, five subjects disclosed neurological disorders midway through their testing sessions. For these five subjects, the experimenters deemed continuation of the session to be most inline with maintaining the subject’s well-being, but excluded their data from analyses. A total of 95 female subjects were included in data analyses: Study 1 total \( n = 30 \), naturally cycling \( n = 12 \), active pill \( n = 8 \), hormone-free interval \( n = 10 \); Study 2 total \( n = 65 \), naturally cycling \( n = 19 \), active pill \( n = 23 \), hormone-free interval \( n = 23 \) (Tables 1 - 9). Subject ages ranged from 18 to 26 years with a mean age of 20.36 years. Mean age did not differ between Study 1 (\( M = 20.47 \) years, \( SD = 1.17 \)) and Study 2 (\( M = 20.31 \) years, \( SD = 1.41 \)), \( F(1, 93) = .288, p > .05 \). Means and standard deviations are reported in Tables 2 and 6, for Studies 1 and 2 respectively.

Exploratory data analyses revealed deviations from normality and unequal variance for several variables in both Studies 1 (Figure 4a) and 2 (Figure 4b). Consequently, nonparametric tests were selected for all group comparisons in both studies. Nearly identical results were obtained when analyses were repeated with statistically stronger one-way analysis of variance tests. For each study, 13 Kruskal-Wallis \( H \) one-way analysis of variance tests were conducted and revealed significant effects for 17\( \beta \)-estradiol level, \( H = 6.22, p = .05 \), PoMS score, \( H = 8.70, p = .013 \), and CES-D score, \( H = 9.49, p < .01 \) in Study 1 only. A priori pairwise comparisons between each of the three groups were conducted for each of the 13 dependent variables and for each study.
Figures 4a and 4b. Boxplots of Z-Transformation Scores for All Factors in Study 1 (a) and Study 2 (b). Lines and whiskers represent medians and 1.5 times the interquartile range or the maximum or minimum if no data point falls within 1.5 times the interquartile range.
Note. Open circles indicate outliers and asterisks indicate extreme outliers.

A priori pairwise comparisons of task performance between each of the three groups were conducted individually for each study using independent Mann-Whitney U tests. Performance on the virtual navigation task was analyzed separately for the dual-solution training trials and place probe trials to detect differential hormone effects on dual-solution and place learning, respectively. For each study, 39 independent Mann-Whitney comparisons were conducted with group status, i.e., naturally cycling, active pill phase, and hormone free interval, as the independent variable and 13 dependent variables: salivary 17β-estradiol, dual-solution latency and errors, place latency and errors, map drawing errors, mental rotation accuracy, objects recalled, words generated, digit span, story recall, and two mood assessments, i.e., PoMS and CES-D. A few outliers were present for various factors; however, inclusion of these outliers did not alter results and all subjects who provided viable data were included in final analyses. Likewise, 16 subjects in Study 2 were found to be underweight, overweight, or obese according to their BMI values, however, inclusion of these subjects did not alter results and were not excluded from analyses.

**Mood Measures**

The two measures of affect, the Profile of Mood State (PoMS) and Feelings Inventory (CES-D), were included to account for mood effects. PoMS and CES-D scores correlated significantly in both Study 1, $r_s = .76$, $p < .01$ (Table 8), and Study 2, $r_s = .53$, $p < .01$ (Table 9), suggesting that subjects who scored higher on measures of negative steady state, i.e., CES-D score, also scored higher on measures of negative trait affect, i.e., PoMS score.
Four outliers, two per study, with high CES-D scores were identified (Figure 5); however, removal of these outliers did not affect results. Consequently, all subjects were included in analyses.

Figure 5: CES-D scores for all subjects in Studies 1 and 2. Horizontal reference line represents the clinical subthreshold score, i.e., 16, for depressive symptoms. Lines and whiskers represent medians and 1.5 times the interquartile range or the maximum or minimum if no data point falls within 1.5 times the interquartile range. Data points represent outliers.

Note. CES-D = Center for Epidemiologic Studies Depression [scale].

Four independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted, two per study, to detect group differences in mood trait, i.e., PoMS score, and depressive state, i.e., CES-D score. Twelve Mann-Whitney $U$ pairwise comparisons, six per study, were planned to compare PoMS and CES-D scores between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. Significant group effects on PoMS scores were observed in Study 1, $H = 8.70$, $p = .01$, but not in the larger Study 2, $H = .60$, $p > .05$. In Study 1, naturally cycling females exhibited significantly more negative affect ($Mdn = 79.00$, $IQR = 65.00$) than active pill females ($Mdn = 40.00$, $IQR = 20.00$), $U = 14.00$, $p < .01$ (Tables 1 and 3). No other significant group differences in PoMS scores were observed in Study 1: naturally cycling versus hormone-free interval, $U = 33.00$, $p > .05$; active pill versus hormone-free interval, $U = 22.00$, $p > .05$ (Tables 1 and 3). In Study 2, none of the three Mann-Whitney comparisons of PoMS scores were significant: naturally cycling versus active pill, $U = 192.00$, $p > .05$; naturally cycling versus hormone-free interval, $U = 191.50$, $p > .05$; active pill versus hormone-free interval, $U = 259.50$, $p > .05$ (Tables 5 and 7). Confidence intervals indicate that both studies were sufficiently powered to detect significant differences in PoMS scores (Tables 3 and 7).

The clinical subthreshold for depressive symptoms is a CES-D score of 16 or higher. Approximately half of subjects in Studies 1 ($Mdn = 13.00$, $IQR = 20.00$) and 2 ($Mdn = 14.50$, $IQR = 13.00$) scored below the clinical subthreshold for depression (Figures 6a and 6b; Tables 1 and 5).
A significant group effect on CES-D score was observed in Study 1, $H = 9.49$, $p < .01$. Study 1 naturally cycling females scored above the CES-D clinical subthreshold for depressive symptoms and reported significantly more depressive symptoms ($Mdn = 28.00$, $IQR = 19.00$) than both active pill ($Mdn = 8.00$, $IQR = 4.00$), $U = 15.50$, $p < .01$, and hormone free-interval females ($Mdn = 12.50$, $IQR = 11.00$), $U = 23.00$, $p = .01$, (Figure 7a; Tables 1 and 3). One low CES-D outlier was observed in the naturally cycling group of Study 1; however, removal of this outlier did not produce significant group differences between any groups on any cognitive tasks. Consequently, all 30 subjects in Study 1 were included in analyses.

Significant differences in CES-D scores were not observed between Study 1 active pill and hormone-free interval females, $U = 25.00$, $p > .05$ (Figure 7a; Table 3). A significant group effect on CES-D score was not observed in Study 2, $H = 1.42$, $p > .05$. Additionally, significant differences in CES-D scores were not observed between Study 2 naturally cycling ($Mdn = 15.00$, $IQR = 16.00$) and active pill females ($Mdn = 14.00$, $IQR = 14.00$), $U = 169.00$, $p > .05$, naturally cycling and hormone-free interval females ($Mdn = 14.00$, $IQR = 10.00$), $U = 178.50$, $p > .05$, or active pill and hormone-free interval females, $U = 241.00$, $p > .05$ (Figure 7b; Tables 5 and 7).

Confidence intervals indicate that both studies were sufficiently powered to detect significant differences in CES-D scores (Tables 3 and 7). As with Study 1, one high CES-D outlier was observed in the naturally cycling group of Study 2; however, removal of this outlier did not produce significant group differences between any groups on any cognitive tasks. Consequently, all 65 subjects in Study 2 were included in analyses.
Mood and Cognitive Performance Correlations

Forty nonparametric bivariate Spearman correlations, twenty per mood measure, were conducted between the two mood factors, i.e., PoMS and CES-D, and the ten cognitive factors, i.e., dual-solution and place latency and errors, map drawing, mental rotation, object recall, word generation, digit span, and story recall. Confidence intervals are not possible on Spearman’s rho; however, considering the large sample size of Study 2, \( n = 65 \), it is not likely that Study 2 was insufficiently powered to detect significant correlations. The following correlations suggest that mood did not correlate significantly with cognitive performance in either Study 1 or 2.

Mood and Dual-Solution Learning

Eight nonparametric bivariate correlations, four per study, between the two mood measures and dual-solution latency and errors were conducted to account for mood effects on dual-solution learning. PoMS scores did not correlate significantly with dual-solution latency in either Study 1, \( r_s = .23, p > .05 \) (Table 8), or Study 2, \( r_s = .21, p > .05 \) (Table 9). Likewise, PoMS scores did not correlate significantly with dual-solution errors in either Study 1, \( r_s = .23, p > .05 \) (Table 8), or Study 2, \( r_s = .16, p > .05 \) (Table 9). Similarly, CES-D scores did not correlate significantly with dual-solution latency in either Study 1, \( r_s = .03, p > .05 \) (Table 8), or Study 2, \( r_s = .11, p > .05 \) (Table 9), or with dual-solution errors in either Study 1, \( r_s = .07, p > .05 \) (Table 8), or Study 2, \( r_s = .10, p > .05 \) (Table 9).
Mood and Place Learning

Eight nonparametric bivariate correlations, four per study, between the two mood measures and place latency and errors were conducted to account for mood effects on place learning. PoMS scores did not correlate significantly with place latency in either Study 1, \( r_s = .09, p > .05 \) (Table 8), or Study 2, \( r_s = .04, p > .05 \) (Table 9). Likewise, PoMS scores did not correlate significantly with place errors in either Study 1, \( r_s = .08, p > .05 \) (Table 8), or Study 2, \( r_s = .08, p > .05 \) (Table 9). Similarly, CES-D scores did not correlate significantly with place latency in either Study 1, \( r_s = -.13, p > .05 \) (Table 8), or Study 2, \( r_s = .16, p > .05 \) (Table 9), or with place errors in Study 1, \( r_s = -.10, p > .05 \) (Table 8). A significant correlation between CES-D score and place errors was observed in Study 2, \( r_s = .31, p < .05 \) (Table 9), suggesting that subjects with higher depression scores committed more errors during the place trial. One high place error outlier was observed in Study 1 (Figure 8a), and one high place error outlier and two high CES-D outliers were observed in Study 2 (Figure 8b). Removal of the Study 1 outlier did not affect the correlation between place errors and CES-D score, \( r_s = -.01, p > .05 \). Removal of the high place error outlier in Study 2 eliminated the significant correlation in Study 2, \( r_s = .15, p > .05 \), and a similar elimination was observed with removal of the two CES-D outliers in Study 2, \( r_s = .13, p > .05 \). These findings suggest that the correlation between Study 2 place errors and CES-D score was driven by these outliers.

Figures 8a and 8b: Scatterplots of (a) Study 1 and Study 2 (b) Place Errors and CES-D scores. Note. For Study 2, place errors represents an average of two trials.

Mood and Map Drawing

Four nonparametric bivariate correlations, two per study, between the two mood measures and map drawing errors were conducted to account for mood effects on map drawing. PoMS scores did not correlate
significantly with map drawing in either Study 1, $r_s = -.03, p > .05$ (Table 8), or Study 2, $r_s = .19, p > .05$ (Table 9). Similarly, CES-D scores did not correlate significantly with map drawing in either Study 1, $r_s = -.09, p > .05$ (Table 8), or Study 2, $r_s = .03, p > .05$ (Table 9).

**Mood and Mental Rotation**

Four nonparametric bivariate correlations, two per study, between the two mood measures and mental rotation accuracy were conducted to account for mood effects on mental rotation accuracy. PoMS scores did not correlate significantly with mental rotation in either Study 1, $r_s = .10, p > .05$ (Table 8), or Study 2, $r_s = .05, p > .05$ (Table 9). Similarly, CES-D scores did not correlate significantly with mental in either Study 1, $r_s = .28, p > .05$ (Table 8), or Study 2, $r_s = .06, p > .05$ (Table 9).

**Mood and Object Recall**

Four nonparametric bivariate correlations, two per study, between the two mood measures and object recall accuracy were conducted to account for mood effects on object recall. PoMS scores did not correlate significantly with object recall in either Study 1, $r_s = .12, p > .05$ (Table 8), or Study 2, $r_s = -.001, p > .05$ (Table 9). Similarly, CES-D scores did not correlate significantly with object recall in either Study 1, $r_s = .07, p > .05$ (Table 8), or Study 2, $r_s = .03, p > .05$ (Table 9).

**Mood and Word Generation**

Four nonparametric bivariate correlations, two per study, between the two mood measures and word generation were conducted to account for mood effects on word generation. PoMS scores did not correlate significantly with word generation in either Study 1, $r_s = .09, p > .05$ (Table 8), or Study 2, $r_s = -.13, p > .05$ (Table 9). Similarly, CES-D scores did not correlate significantly with word generation in either Study 1, $r_s = .14, p > .05$ (Table 8), or Study 2, $r_s = -.06, p > .05$ (Table 9).

**Mood and Digit Span**

Four nonparametric bivariate correlations, two per study, between the two mood measures and digit span were conducted to account for mood effects on digit span. PoMS scores did not correlate significantly with digit
span in either Study 1, \( r_s = -0.05, p > .05 \) (Table 8), or Study 2, \( r_s = -0.18, p > .05 \) (Table 9). Similarly, CES-D scores did not correlate significantly with digit span in either Study 1, \( r_s = 0.05, p > .05 \) (Table 8), or Study 2, \( r_s = -0.14, p > .05 \) (Table 9).

Mood and Story Recall

Four nonparametric bivariate correlations, two per study, between the two mood measures and story recall were conducted to account for mood effects on story recall. PoMS scores did not correlate significantly with story recall in either Study 1, \( r_s = 0.06, p > .05 \) (Table 8), or Study 2, \( r_s = -0.14, p > .05 \) (Table 9). Similarly, CES-D scores did not correlate significantly with story recall in either Study 1, \( r_s = -0.08, p > .05 \) (Table 8), or Study 2, \( r_s = 0.03, p > .05 \) (Table 9).

Salivary 17\( \beta \)-Estradiol and Cognitive Performance

Correlations

Twenty-four nonparametric bivariate Spearman correlations, twelve per study, were conducted to account for endogenous 17\( \beta \)-estradiol effects. Correlations were conducted between salivary 17\( \beta \)-estradiol, the two mood measures, and the ten cognitive factors, i.e., dual-solution and place latency and errors, map drawing, mental rotation, object recall, word generation, digit span, and story recall. Confidence intervals are not possible on Spearman’s rho; however, considering the large sample size of Study 2, \( n = 65 \), it is not likely that Study 2 was insufficiently powered to detect significant correlations. The following correlations suggest that salivary 17\( \beta \)-estradiol correlated significantly with place performance in Study 1 alone. No other correlations between salivary 17\( \beta \)-estradiol and cognitive performance were observed in either Study 1 or 2.

Salivary 17\( \beta \)-Estradiol and Mood

Four nonparametric bivariate correlations, two per study, between salivary 17\( \beta \)-estradiol, PoMS, and CES-D scores were conducted to account for endogenous estrogen effects on mood. PoMS scores did not correlate significantly with salivary 17\( \beta \)-estradiol in either Study 1, \( r_s = 0.01, p > .05 \) (Table 8), or Study 2, \( r_s = 0.17, p > .05 \) (Table 9). Similarly, CES-D scores did not correlate significantly with salivary 17\( \beta \)-estradiol in either Study 1, \( r_s = 0.11, p > .05 \) (Table 8), or Study 2, \( r_s = 0.04, p > .05 \) (Table 9).
**Salivary 17β-Estradiol and Dual-Solution Learning**

Four nonparametric bivariate correlations, two per study, between salivary 17β-estradiol and dual-solution latency and errors were conducted to account for endogenous 17β-estradiol effects on dual-solution learning. Dual-solution latency did not correlate significantly with salivary 17β-estradiol in either Study 1, \( r_s = .12, p > .05 \) (Table 8), or Study 2, \( r_s = .04, p > .05 \) (Table 9). Likewise, dual-solution errors did not correlate significantly with salivary 17β-estradiol, in either Study 1, \( r_s = .04, p > .05 \) (Table 8), or Study 2, \( r_s = .07, p > .05 \) (Table 9).

**Salivary 17β-Estradiol and Place Learning**

Four nonparametric bivariate correlations, two per study, between salivary 17β-estradiol and place latency and errors were conducted to account for endogenous estrogen effects on estrogen-sensitive place learning. In Study 1, salivary 17β-estradiol was positively correlated with both place latency, \( r_s = .48, p < .01 \), and place errors, \( r_s = .49, p < .01 \) (Figures 9a and 9b; Table 8), suggesting that higher salivary 17β-estradiol was associated with longer latencies and more errors on the place probe trial. Four outliers were observed in Study 1 salivary 17β-estradiol, one high and one low, and two high outliers in both place latency and errors. The high salivary 17β-estradiol outlier was also a high place latency and error outlier. Removal of these outliers only slightly weakened the correlations between salivary 17β-estradiol and place latency, \( r_s = .40, p < .05 \), and place errors, \( r_s = .43, p < .05 \).

Significant correlations were not observed in the larger Study 2 between salivary 17β-estradiol and place latency, \( r_s = .13, p > .05 \), or place errors, \( r_s = .02, p > .05 \) (Figures 9c and 9d; Table 9). Considering the large sample size of Study 2, \( n = 65 \), it is not likely that Study 2 was insufficiently powered to detect significant correlations. Additionally, the lack of pre-existing evidence to support a correlation between higher 17β-estradiol and poorer place learning suggests that the correlation between salivary 17β-estradiol and place learning in Study 1 may not be reliable.
Salivary 17\(\beta\)-estradiol and Map Drawing

Two nonparametric bivariate correlations, one per study, between salivary 17\(\beta\)-estradiol and map drawing errors were conducted to account for endogenous estrogen effects on map drawing. Map drawing performance did not correlate significantly with salivary 17\(\beta\)-estradiol in either Study 1, \(r_s = .32, p > .05\) (Table 8), or Study 2, \(r_s = -.01, p > .05\) (Table 9).

Salivary 17\(\beta\)-Estradiol and Mental Rotation

Two nonparametric bivariate correlations, one per study, between salivary 17\(\beta\)-estradiol and mental rotation accuracy were conducted to account for endogenous estrogen effects on mental rotation. Mental rotation
performance did not correlate significantly with salivary 17β-estradiol in either Study 1, $r_s = -.28, p > .05$ (Table 8), or Study 2, $r_s = .22, p > .05$ (Table 9).

**Salivary 17β-Estradiol and Object Recall**

Two nonparametric bivariate correlations, one per study, between salivary 17β-estradiol and object recall accuracy were conducted to account for endogenous estrogen effects on object recall. Object recall did not correlate significantly with salivary 17β-estradiol in either Study 1, $r_s = .21, p > .05$ (Table 8), or Study 2, $r_s = .19, p > .05$ (Table 9).

**Salivary 17β-Estradiol and Word Generation**

Two nonparametric bivariate correlations, one per study, between salivary 17β-estradiol and word generation were conducted to account for endogenous estrogen effects on estrogen-sensitive word generation. Word generation scores did not correlate significantly with salivary 17β-estradiol in either Study 1, $r_s = -.02, p > .05$ (Table 8), or Study 2, $r_s = .01, p > .05$ (Table 9).

**Salivary 17β-Estradiol and Digit Span**

Two nonparametric bivariate correlations, one per study, between salivary 17β-estradiol and digit span were conducted to account for endogenous estrogen effects on digit span. Digit span did not correlate significantly with salivary 17β-estradiol, for either Study 1, $r_s = .21, p > .05$ (Table 8), or Study 2, $r_s = -.04, p > .05$ (Table 9).

**Salivary 17β-Estradiol and Story Recall**

Two nonparametric bivariate correlations, one per study, between salivary 17β-estradiol and story recall were conducted to account for endogenous estrogen effects on story recall. Story recall did not correlate significantly with salivary 17β-estradiol in either Study 1, $r_s = -.004, p > .05$ (Table 8), or Study 2, $r_s = -.12, p > .05$ (Table 9).
**Estrogen-Sensitive Learning**

**Place Learning**

Place learning was measured in terms of virtual maze probe trial errors and latency in Study 1; however, in Study 2, place latency and errors were averaged over two probe trials. Place latency and errors significantly correlated both in Study 1, $r_s = .94, p < .01$ (Table 8), and in Study 2, $r_s = .81, p < .01$ (Table 9), suggesting that subjects who required more time to complete the virtual maze also committed more errors while doing so.

Four independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted: one for each of the two dependent measures of place learning, i.e., latency and errors, once for Study 1 and once for Study 2. In Study 1, significant group effects were not observed for either place latency, $H = 3.59, p > .05$, or place errors, $H = 3.99, p > .05$. Likewise, in Study 2, significant group effects were not observed for either place latency, $H = .36, p > .05$, or place errors, $H = .62, p > .05$.

Twelve Mann-Whitney $U$ pairwise comparisons, six per study, were planned to compare place learning, i.e., latency and errors, between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the six Mann-Whitney $U$-tests revealed significant differences in place latency for naturally cycling versus active pill, $U = 34.50, p > .05$, naturally cycling versus hormone-free interval, $U = 31.50, p > .05$, or active pill versus hormone-free interval, $U = 33.00, p > .05$, or place errors for naturally cycling versus active pill, $U(1, 18) = 43.50, p > .05$, naturally cycling versus hormone-free interval, $U = 31.00, p > .05$, or active pill versus hormone-free interval, $U = 25.50, p > .05$ (Table 3). Likewise, for Study 2, none of the six Mann-Whitney $U$-tests revealed significant differences in place latency for naturally cycling versus active pill, $U = 184.00, p > .05$, naturally cycling versus hormone-free interval, $U = 203.00, p > .05$, or active pill versus hormone-free interval, $U = 226.50, p > .05$, or in place errors for naturally cycling versus active pill, $U = 183.50, p > .05$, naturally cycling versus hormone-free interval, $U = 281.00, p > .05$, or active pill versus hormone-free interval, $U = 214.00, p > .05$ (Table 7). Confidence intervals indicate that both studies were sufficiently powered to detect significant differences (Tables 3 and 7). These findings suggest that place performance did not vary with combined oral contraceptive use in either Study 1 or 2.

**Place and Dual-Solution Learning**

In Study 1, performance on the dual-solution training and place probe virtual navigation trials positively correlated both in terms of latency, $r_s = .73, p < .01$, and errors, $r_s = .57, p < .01$ (Figures 10a and 10b; Table 8),
suggesting that subjects who required more time and made more errors while learning the virtual maze also required
more time and made more errors during the place probe trial. These findings were not replicated in Study 2 for either
latency, $r_s = .14, p > .05$, or errors, $r_s = .12, p > .05$ (Figures 10c and 10d; Table 9).

Figures 10a - 10d: Scatterplots of (a) Study 1 Dual-Solution and Place Latency (b) Study 1 Dual-Solution and Place
Errors (c) Study 2 Dual-Solution and Place Latency, and (d) Study 2 Dual-Solution and Place Errors.
Note. For Study 2 place latency and errors represent an average of two trials. Dual-Solution represents training trial
five for Study 1 and training trial three for Study 2.

To address the absence of correlation between dual-solution and place learning in Study 2, three outliers in Study 2
were removed and nonparametric bivariate correlations were conducted again; however, removal of these outliers
did not produce significant correlations between Study 2 dual-solution and place latency or errors.

Subjects in Study 1 completed five dual-solution training trials and one place probe trial, whereas subjects
in Study 2 completed only three dual-solution training trials and two place probe trials. Consequently, Study 2
subjects may not have learned the maze as well as Study 1 subjects, which may account for the absence of
correlation between dual-solution and place learning in Study 2. To test this hypothesis, four Mann-Whitney $U$-tests
were conducted to compare dual-solution and place learning between Study 1 and 2 subjects. To compensate for the additional training experienced by Study 1 subjects, dual-solution trial three latency and errors were compared, rather than trial five for Study 1 and three for Study 2. Likewise, Study 2 average place latency and error scores were compared to the singular place latency and error scores of Study 1 subjects. Results indicate that both Study 1 and 2 subjects learned the virtual maze equally well in terms of dual-solution trial 3 latency, $U = 734.00, p > .05$, and errors, $U = 895.50, p > .05$; however, Study 2 subjects required significantly more time to complete the place probe trial ($Mdn = 114.75$ seconds, $IQR = 76.38$ seconds, Range: 52 - 354 seconds) than Study 1 subjects ($Mdn = 58.50$ seconds, $IQR = 33.00$ seconds, Range: 37 - 162 seconds), $U = 240.50, p < .001$. Furthermore, Study 2 subjects committed significantly more place errors ($Mdn = 13.50$, $IQR = 6.00$, Range: 8 - 57) than Study 1 subjects ($Mdn = 8.50$, $IQR = 3.00$, Range: 7 - 21), $U = 247.50, p < .001$. These differences suggest that probe performance was impaired in Study 2 compared to Study 1, and that dual-solution performance may not be an accurate predictor of probe performance in instances of decreased training and reversal of task demands, as was the case in Study 2.

**Place Learning and Map Drawing**

Four nonparametric bivariate correlations, two per study, between map drawing errors and place latency and errors were conducted to explore the relationship between place learning and map drawing. Study 1 place latency and map drawing errors correlated significantly, $r_s = .37, p < .05$, suggesting that subjects who required longer to complete the maze also committed more errors while creating a mental map of the maze (Figure 11a; Table 8). Study 1 place errors, however, did not correlate significantly with map errors, $r_s = .27, p > .05$ (Figure 11b; Table 8), nor did Study 2 place latency, $r_s = .12, p > .05$, or place errors, $r_s = .14, p > .05$ (Table 9). Two high place latency outliers were identified in Study 1; however, removal of these outliers only strengthened the correlation between Study 1 place latency and map drawing errors, $r_s = .46, p = .01$. One high place latency outlier was observed in Study 2; however, removal of this outlier did not produce a significant correlation between Study 2 place latency and map drawing errors, $r_s = .12, p > .05$. 
Figures 11a and 11b: Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Map Drawing Errors. Note. For Study 2, place latency represents an average of two trials. Removal of Study 1 outliers did not eliminate the correlation observed in Study 1 but not Study 2.

**Place Learning and Mental Rotation**

Four nonparametric bivariate correlations, two per study, between mental rotation accuracy and place latency and errors were conducted to explore the relationship between place learning and mental rotation. In Study 1, performance on the place probe trial negatively correlated with mental rotation both in terms of place latency, \( r_s = -.47, p < .01 \), and place errors, \( r_s = -.43, p < .05 \) (Figures 12a and 12b, Table 8), suggesting that subjects who required more time and committed more errors in the probe trial also committed more errors on the mental rotation task. These findings, however, were not replicated in Study 2 for either place latency, \( r_s = .03, p > .05 \), or place errors, \( r_s = -.09, p > .05 \) (Figures 12c and 12d; Table 9). Removal of the two Study 1 place latency outliers strengthened the correlation between Study 1 place latency and mental rotation, \( r_s = -.55, p < .01 \). Removal of one Study 2 place errors outlier did not produce significant correlations between Study 2 mental rotation and place latency, \( r_s = .01, p > .05 \), or place errors, \( r_s = -.06, p > .05 \).
Figures 12a - 12d: Scatterplots of (a) Study 1 Place Latency and Mental Rotation Items (b) Study 1 Place Errors and Mental Rotation Items (c) Study 2 Place Latency and Mental Rotation Items, and (d) Study 2 Place Errors and Mental Rotation Items.

Note. MRT = Mental Rotation Task. For Study 2, place latency and errors represent an average of two trials.

**Place Learning and Object Recall**

Four nonparametric bivariate correlations, two per study, between object recall accuracy and place latency and errors were conducted to explore the relationship between place learning and object recall. Study 1 place latency and object recall correlated significantly, $r_s = .40, p < .05$, suggesting that subjects who required more time to complete the place trial also recalled more items during object recall (Figure 13a; Table 8). Study 1 place errors, however, did not correlate significantly with object recall, $r_s = .32, p > .05$ (Figure 13b; Table 8), nor did Study 2 place latency, $r_s = .09, p > .05$ (Table 9), or place errors, $r_s = .04, p > .05$ (Table 9). Removal of the two place latency outliers did not affect the correlation in Study 1, $r_s = .11, p < .05$, nor did removal of the one place latency outlier in Study 2 produce significant correlations for either place latency, $r_s = .11, p > .05$ or place errors, $r_s = .03, p > .05$. 

41
Figures 13a and 13b: Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Object Recall. Note. For Study 2, place latency represents an average of two trials.

**Place Learning and Word Generation**

Four nonparametric bivariate correlations, two per study, between word generation and place latency and errors were conducted to explore the relationship between place learning and word generation. Place latency did not correlate significantly with word generation in either Study 1, \( r_s = -.31, p > .05 \) (Table 8), or Study 2, \( r_s = .06, p > .05 \) (Table 9). Similarly, place errors did not correlate significantly with word generation in either Study 1, \( r_s = -25., p > .05 \) (Table 8), or Study 2, \( r_s = .04, p > .05 \) (Table 9).

**Place Learning and Digit Span**

Four nonparametric bivariate correlations, two per study, between digit span and place latency and errors were conducted to explore the relationship between place learning and digit span. Place latency did not correlate significantly with digit span in either Study 1, \( r_s = .05, p > .05 \) (Table 8), or Study 2, \( r_s = .003, p > .05 \) (Table 9). Similarly, place errors did not correlate significantly with digit span in either Study 1, \( r_s = .03, p > .05 \) (Table 8), or Study 2, \( r_s = -.07, p > .05 \) (Table 9).

**Place Learning and Story Recall**

Four nonparametric bivariate correlations, two per study, between story recall accuracy and place latency and errors were conducted to explore the relationship between place learning and story recall. Study 1 place latency and story recall correlated significantly, \( r_s = -.38, p < .05 \), suggesting that subjects who required more time to
complete the place trial also recalled fewer story items (Figure 14a; Table 8). Study 1 place errors, however, did not correlate significantly with story recall, \( r_s = -0.23, p > 0.05 \) (Figure 14b; Table 8), nor did Study 2 place latency, \( r_s = 0.08, p > 0.05 \) (Table 9), or place errors, \( r_s = 0.04, p > 0.05 \) (Table 9). Removal of the two place latency outliers and one high story recall outlier eliminated the correlation in Study 1, \( r_s = -0.24, p > 0.05 \); however, removal of the one place latency outlier in Study 2 did not affect the correlations between story recall and place latency, \( r_s = 0.08, p > 0.05 \), or place errors, \( r_s = 0.10, p > 0.05 \).

Figures 14a and 14b: Scatterplots of (a) Study 1 and Study 2 (b) Place Latency and Story Recall. Note. For Study 2, place latency represents an average of two trials.

**Word Generation**

A word generation task was included as a nonspatial estrogen-sensitive task. Two Kruskal-Wallis \( H \) one-way analysis of variance tests were conducted, one for each study, to determine group effects on word generation. Significant group differences in word generation were not detected in either Study 1, \( H = 0.55, p > 0.05 \), or Study 2, \( H = 2.15, p > 0.05 \). Similarly, six a priori Mann-Whitney \( U \) pairwise comparisons revealed no significant differences in Study 1 for naturally cycling versus active pill, \( U = 43.50, p > 0.05 \), naturally cycling versus hormone-free interval, \( U = 54.00, p > 0.05 \), or active pill versus hormone-free interval, \( U = 31.50, p > 0.05 \) (Table 3), or in Study 2 for naturally cycling versus active pill, \( U = 173.50, p > 0.05 \), naturally cycling versus hormone-free interval, \( U = 162.50, p > 0.05 \), or active pill versus hormone-free interval, \( U = 260.50, p > 0.05 \) (Table 7). These findings suggest that word generation did not vary with combined oral contraceptive use in either Study 1 or 2.
**Word Generation and Map Drawing**

Two nonparametric bivariate correlations, one per study, between word generation and map drawing errors were conducted to explore the relationship between word generation and map drawing. Word generation did not correlate significantly with map drawing in either Study 1, $r_s = -.09, p > .05$ (Table 8), or Study 2, $r_s = -.16, p > .05$ (Table 9).

**Word Generation and Mental Rotation**

Two nonparametric bivariate correlations, one per study, between word generation and mental rotation accuracy were conducted to explore the relationship between word generation and mental rotation. Word generation did not correlate significantly with mental rotation in either Study 1, $r_s = .11, p > .05$ (Table 8), or Study 2, $r_s = .003, p > .05$ (Table 9).

**Word Generation and Object Recall**

Two nonparametric bivariate correlations, one per study, between word generation and object recall accuracy were conducted to explore the relationship between word generation and object recall. Word generation did not correlate significantly with object recall in either Study 1, $r_s = -.08, p > .05$ (Table 8), or Study 2, $r_s = .16, p > .05$ (Table 9).

**Word Generation and Digit Span**

Two nonparametric bivariate correlations, one per study, between word generation and digit span were conducted to explore the relationship between word generation and digit span. Word generation did not correlate significantly with digit span in Study 1, $r_s = .23, p > .05$ (Figure 15a; Table 8); however, with a larger sample size, the positive correlation in Study 1 was strengthened in Study 2, $r_s = .43, p < .01$ (Figure 15b; Table 9), suggesting that subjects who were able to generate more words also had longer digit spans. No word generation or digit span outliers were observed in Study 1; however, one high digit span and two high word generation outliers were identified in Study 2. Removal of the Study 2 digit span outlier only slightly weakened the correlation between word generation and digit span, but did not affect the significance of the relationship, $r_s = .36, p < .01$. Similarly, removal of the Study 2 word generation outliers only slightly weakened the correlation between word generation and digit span, but did not affect the significance of the relationship, $r_s = .39, p < .01$. The direction of the correlations were
consistent between Studies 1 and 2, consequently, it is unlikely that the significant correlations observed in Study 2 were the result of excessive statistical power.

Figures 15a - 15b: Scatterplots of (a) Study 1 Word Generation and Digit Span (b) Study 2 Word Generation and Digit Span.

**Word Generation and Story Recall**

Two nonparametric bivariate correlations, one per study, between word generation and story recall accuracy were conducted to explore the relationship between word generation and story recall. Word generation did not correlate significantly with story recall in Study 1, $r_s = .28, p > .05$ (Figure 16a; Table 8); however, with a larger sample size, the positive correlation in Study 1 was strengthened in Study 2, $r_s = .42, p < .01$ (Figure 16b; Table 9), suggesting that subjects who were able to generate more words were also able to recall more story details. No word generation outliers were observed in Study 1; however, two word generation outliers, one of which was also a high story recall outlier, were observed in Study 2. Removal of these outliers only slightly weakened the relationship between word generation, but did not affect the significance of the relationship, $r_s = .41, p < .01$. Furthermore, removal of one high story recall outlier identified in Study 1 significantly strengthened the correlation between Study 1 word generation and story recall, $r_s = .41, p < .05$. The direction of the correlations were consistent between Studies 1 and 2, consequently, it is unlikely that the significant correlations observed in Study 2 were the result of excessive statistical power.
Figures 16a - 16b: Scatterplots of (a) Study 1 Word Generation and Story Recall, and (b) Study 2 Word Generation and Story recall.

**Estrogen-Insensitive Learning**

**Dual-Solution Learning**

Learning during the dual-solution training trials was measured in terms of latency to complete the virtual maze and errors committed while doing so. Dual-solution latency and errors were highly correlated in Study 1, $r_s = .86, p < .01$ (Table 8), and Study 2, $r_s = .88, p < .01$ (Table 9), suggesting that subjects who required more time to complete the virtual maze also committed more errors while doing so. Four independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted: one for each of the two dependent measures of dual-solution learning, i.e., latency and errors, once for Study 1 and once for Study 2. In Study 1, significant group effects were not observed for either dual-solution latency, $H = 3.33, p > .05$, or dual-solution errors, $H = 1.76, p > .05$. Likewise, in Study 2, significant group effects were not observed for either dual-solution latency, $H = 3.33, p > .05$, or dual-solution errors, $H = 1.76, p > .05$.

Twelve Mann-Whitney $U$ pairwise comparisons, six per study, were planned to compare dual-solution learning, i.e., latency and errors, between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the six Mann-Whitney $U$-tests revealed significant differences in dual-solution latency for naturally cycling versus active pill, $U = 31.50, p > .05$, naturally cycling versus hormone-free interval, $U = 34.00, p > .05$, or active pill versus hormone-free interval, $U = 37.00, p > .05$, or place errors for naturally cycling versus active pill, $U = 34.00, p > .05$, naturally cycling versus hormone-free interval, $U = 45.50, p > .05$, or active pill versus hormone-free interval, $U = 38.50, p > .05$ (Table 3). Likewise, for Study 2, none of the six Mann-Whitney $U$-tests revealed significant differences in dual-solution latency for naturally cycling versus active pill,
U = 195.00, p > .05, naturally cycling versus hormone-free interval, U = 170.00, p > .05, or active pill versus hormone-free interval, U = 226.50, p > .05, or in dual-solution errors for naturally cycling versus active pill, U = 213.50, p > .05, naturally cycling versus hormone-free interval, U = 194.50, p > .05, or active pill versus hormone-free interval, U = 238.00, p > .05 (Table 7). These findings suggest that dual-solution performance did not vary with combined oral contraceptive use in either Study 1 or 2.

**Dual-Solution Learning and Map Drawing**

The map drawing task was included as a measure of maze learning. Four nonparametric bivariate correlations, two per study, between map drawing accuracy and dual-solution latency and errors were conducted to determine the relationship between map drawing and dual-solution learning. Subjects with lower dual-solution latency and errors were expected to commit fewer errors on the map drawing task. This pattern was observed in Study 1, both for map drawing errors and dual-solution latency, rs = .48, p < .01 (Figure 17a; Table 8), and map drawing and dual-solution errors, rs = .45, p < .05 (Figure 17b; Table 8). A similar pattern between map drawing and dual-solution latency, rs = .17, p > .05 (Figure 17c; Table 9), and dual-solution errors, rs = .10, p > .05 (Figure 17d; Table 9), was not observed in Study 2. Four outliers were observed and removed from Study 1; however, removal of these outliers did not eliminate the correlation between Study 1 map drawing and dual-solution latency, rs = .41, p < .05, or dual-solution errors, rs = .42, p < .05. Seven outliers were observed in and removed from Study 2; however, removal of these outliers did not produce significant correlations between Study 2 map drawing and dual-solution latency, rs = .17, p > .05, or dual-solution errors, rs = .8, p > .05. As previously reported, two Mann-Whitney U-tests were conducted comparing dual-solution learning in Study 1 versus Study 2, which suggested no difference in dual-solution learning between Study 1 and Study 2 subjects. Therefore, it is not likely that the lack of correlation between dual-solution learning and map drawing in Study 2 is the result of Study 2 subjects not learning the maze as well.
Four nonparametric bivariate correlations, two per study, between mental rotation accuracy and dual-solution latency and errors were conducted to explore the relationship between dual-solution learning and mental rotation. In Study 1, mental rotation accuracy negatively correlated with both dual-solution latency, $r_s = -.44$, $p < .01$, and errors, $r_s = -.49$, $p < .01$ (Table 8), suggesting that Study 1 subjects who required more time for and made more errors during completion of the dual-solution training trials also made more errors during the mental rotation task. These correlations, however, were not observed in the larger Study 2 for either mental rotation and dual-solution latency, $r_s = -.04$, $p > .05$ or errors, $r_s = -.05$, $p > .05$ (Table 9). Examination of scatterplots suggests that the correlations observed in Study 1 may have been driven by several longer latency outliers (Figures 18a -
18d). Removal of four outliers from Study 1 resulted in no significant correlations between mental rotation items and dual-solution latency, $r_s = -.24, p > .05$, or errors, $r_s = -.28, p > .05$.

![Scatterplots of Study 1 and Study 2](image)

**Figures 18a - 18d:** Scatterplots of (a) Study 1 Dual-Solution Latency and Mental Rotation Items (b) Study 1 Dual-Solution Errors and Mental Rotation Items (c) Study 2 Dual-Solution Latency and Mental Rotation Items, and (d) Study 2 Dual-Solution Errors and Mental Rotation Items.

Note. MRT = Mental Rotation Task. Dual-Solution represents training trial five for Study 1 and training trial three for Study 2.

**Dual-Solution Learning and Object Recall**

Four nonparametric bivariate correlations, two per study, between object recall accuracy and dual-solution latency and errors were conducted to explore the relationship between dual-solution learning and object recall. Object recall correlated positively with dual-solution latency, $r_s = .50, p < .01$, and errors, $r_s = .37, p < .05$ in Study 1 (Figures 19a and 19b; Table 8). These correlations, however, were not observed in the larger Study 2 for either dual-solution latency, $r_s = .03, p > .05$, or errors, $r_s = -.01, p > .05$ (Figures 19c - 19d; Table 9). Removal of the four
outliers in Study 1 eliminated the correlation between dual-solution errors and object recall, \( r_s = .25, p > .05 \); however, the correlation between dual-solution latency and object recall remained significant, \( r_s = .44, p < .05 \).

Figures 19a - 19d: Scatterplots of (a) Study 1 Dual-Solution Latency and Objects Recalled (b) Study 1 Dual-Solution Errors and Objects Recalled (c) Study 2 Dual-Solution Latency and Objects Recalled, and (d) Study 2 Dual-Solution Errors and Objects Recalled.

Note. Dual-Solution represents training trial five for Study 1 and training trial three for Study 2.

Dual-Solution Learning and Digit Span

Four nonparametric bivariate correlations, two per study, between digit span and dual-solution latency and errors were conducted to explore the relationship between dual-solution learning and digit span. Dual-solution latency did not correlate significantly with digit span in either Study 1, \( r_s = -.02, p > .05 \) (Table 8), or Study 2, \( r_s = -.01, p > .05 \) (Table 9). Similarly, dual-solution errors did not correlate significantly with digit span in either Study 1, \( r_s = .02, p > .05 \) (Table 8), or Study 2, \( r_s = -.04, p > .05 \) (Table 9).
**Dual-Solution Learning and Story Recall**

Four nonparametric bivariate correlations, two per study, between story recall accuracy and dual-solution latency and errors were conducted to explore the relationship between dual-solution learning and story recall. Dual-solution latency did not correlate significantly with story recall in either Study 1, $r_s = -.30, p > .05$ (Table 8), or Study 2, $r_s = .04, p > .05$ (Table 9). Similarly, dual-solution errors did not correlate significantly with story recall in either Study 1, $r_s = -.18, p > .05$ (Table 8), or Study 2, $r_s = .002, p > .05$ (Table 9).

**Map Drawing**

A map drawing task was included as an estrogen-insensitive measure of maze learning. Performance on the map drawing task was measured in terms of errors committed while recreating a floor-plan of the virtual maze. Two independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted, one per study, to detect group differences in map drawing abilities. Significant group effects on map drawing were not observed in either Study 1, $H = .95, p > .05$, or Study 2, $H = 1.13, p > .05$.

Six Mann-Whitney $U$ pairwise comparisons, three per study, were planned to compare map drawing errors between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the three Mann-Whitney $U$-tests revealed significant differences in map drawing for naturally cycling versus active pill, $U = 35.00, p > .05$, naturally cycling versus hormone-free interval, $U = 56.50, p > .05$, or active pill versus hormone-free interval, $U = 33.50, p > .05$ (Table 3). Likewise, for Study 2, none of the three Mann-Whitney $U$-tests revealed significant differences in map drawing errors for naturally cycling versus active pill, $U = 201.00, p > .05$, naturally cycling versus hormone-free interval, $U = 193.00, p > .05$, or active pill versus hormone-free interval, $U = 206.50, p > .05$ (Table 7). These findings suggest that map drawing ability did not vary with combined oral contraceptive use in either Study 1 or 2.

**Map Drawing and Mental Rotation**

Two nonparametric bivariate correlations, one per study, between map drawing and mental rotation accuracy were conducted to explore the relationship between map drawing and mental rotation. Map drawing errors and mental rotation accuracy correlated significantly in both Study 1, $r_s = -.54, p < .01$ (Table 8), and Study 2,
suggesting that subjects with better spatial rotational and working memory, as indicated by higher mental rotation accuracy, also made fewer errors when reproducing a mental map of the virtual environment.

**Map Drawing and Object Recall**

Two nonparametric bivariate correlations, one per study, between map drawing and object recall accuracy were conducted to explore the relationship between map drawing and object recall. Map drawing errors did not correlate significantly with object recall in either Study 1, $r_s = .23, p > .05$ (Table 8), or Study 2, $r_s = .10, p > .05$ (Table 9).

**Map Drawing and Digit Span**

Two nonparametric bivariate correlations, one per study, between map drawing and digit span were conducted to explore the relationship between map drawing and digit span. Map drawing errors did not correlate significantly with digit span in either Study 1, $r_s = .09, p > .05$ (Table 8), or Study 2, $r_s = -.03, p > .05$ (Table 9).

**Map Drawing and Story Recall**

Two nonparametric bivariate correlations, one per study, between map drawing and story recall accuracy were conducted to explore the relationship between map drawing and story recall. Map drawing errors and story recall significantly correlated in Study 1, $r_s = -.40, p < .05$ (Table 8); however, this correlation was not replicated in the larger Study 2, $r_s = -.22, p > .05$ (Table 9).

**Mental Rotation**

A mental rotation task was included as an estrogen-insensitive spatial task. Performance on the mental rotation task was measured in terms of correctly identified pairs. Two independent Kruskal-Wallis $H$ one-way analysis of variance tests, one per study, were conducted to detect group differences in mental rotation abilities. Significant group effects on mental rotation items were not observed for either Study 1, $H = .58, p > .05$, or Study 2, $H = 3.47, p > .05$.

Six Mann-Whitney $U$ pairwise comparisons, three per study, were planned to compare mental rotation performance between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For
Study 1, none of the three Mann-Whitney U-tests revealed significant differences in mental rotation abilities for
naturally cycling versus active pill, $U = 39.50, p > .05$, naturally cycling versus hormone-free interval, $U = 53.50, p
> .05$, or active pill versus hormone-free interval, $U = 34.00, p > .05$ (Table 3). Likewise, for Study 2, none of the
three Mann-Whitney U-tests revealed significant differences in mental rotation abilities for naturally cycling versus
active pill, $U = 156.00, p > .05$, naturally cycling versus hormone-free interval, $U = 172.00, p > .05$, or active pill
versus hormone-free interval, $U = 209.00, p > .05$ (Table 7). These findings suggest that mental rotation ability did
not vary with combined oral contraceptive use in either Study 1 or 2.

**Mental Rotation and Object Recall**

Two nonparametric bivariate correlations, one per study, between mental rotation accuracy and object recall
accuracy were conducted to explore the relationship between mental rotation and object recall. Mental rotation
performance did not correlate significantly with object recall in either Study 1, $r_s = -.23, p > .05$ (Table 8), or Study
2, $r_s = -.03, p > .05$ (Table 9).

**Mental Rotation and Digit Span**

Two nonparametric bivariate correlations, one per study, between mental rotation accuracy and digit span
were conducted to explore the relationship between mental rotation and digit span. Mental rotation performance did
not correlate significantly with digit span in either Study 1, $r_s = .08, p > .05$ (Table 8), or Study 2, $r_s = .12, p > .05$
(Table 9).

**Mental Rotation and Story Recall**

Two nonparametric bivariate correlations, one per study, between mental rotation accuracy and story recall
accuracy were conducted to explore the relationship between mental rotation and story recall. Mental rotation
performance did not correlate significantly with story recall in either Study 1, $r_s = -.12, p > .05$ (Table 8), or Study 2,
$r_s = -.18, p > .05$ (Table 9).

**Object Recall**

An object recall task was included as a measure of attention to landmarks during dual-solution training.
Performance on the object recall task was measured in terms of correctly recalled items from the virtual
Two independent Kruskal-Wallis $H$ one-way analysis of variance tests, one per study, were conducted to detect group differences in object recall. Significant group effects on object recall were not observed for either Study 1, $H = .89, p > .05$, or Study 2, $H = .21, p > .05$.

Six Mann-Whitney $U$ pairwise comparisons, three per study, were planned to compare object recall between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the three Mann-Whitney $U$-tests revealed significant differences in object recall for naturally cycling versus active pill, $U = 47.50, p > .05$, naturally cycling versus hormone-free interval, $U = 49.50, p > .05$, or active pill versus hormone-free interval, $U = 29.50, p > .05$ (Table 3). Likewise, for Study 2, none of the three Mann-Whitney $U$-tests revealed significant differences in object recall for naturally cycling versus active pill, $U = 206.00, p > .05$, naturally cycling versus hormone-free interval, $U = 204.50, p > .05$, or active pill versus hormone-free interval, $U = 234.50, p > .05$ (Table 7). These findings suggest that object recall did not vary with combined oral contraceptive use in either Study 1 or 2.

**Object Recall and Digit Span**

Two nonparametric bivariate correlations, one per study, between object recall accuracy and digit span were conducted to explore the relationship between object recall and digit span. Object recall did not correlate significantly with digit span in either Study 1, $r_s = .08, p > .05$ (Table 8), or Study 2, $r_s = .08, p > .05$ (Table 9).

**Object Recall and Story Recall**

Two nonparametric bivariate correlations, one per study, between object recall accuracy and story recall accuracy were conducted to explore the relationship between object recall and story recall. Object recall did not correlate significantly with story recall in either Study 1, $r_s = -.12, p > .05$ (Table 8), or Study 2, $r_s = .18, p > .05$ (Table 9).

**Digit Span**

A digit span task was included as an estrogen-insensitive measure of general intelligence. Performance on the digit span task was measured in terms of correctly repeated digit sequences. Two independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted, one per study, to detect group differences in digit span. Significant group effects on digit span were not observed for either Study 1, $H = 2.32, p > .05$, or Study 2,
Six Mann-Whitney U pairwise comparisons, three per study, were planned to compare digit span between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the three Mann-Whitney U-tests revealed significant differences in digit span for naturally cycling versus active pill, $U = 47.50, p > .05$, naturally cycling versus hormone-free interval, $U = 39.50, p > .05$, or active pill versus hormone-free interval, $U = 26.00, p > .05$ (Table 3). Likewise, for Study 2, none of the three Mann-Whitney U-tests revealed significant differences in digit span for naturally cycling versus active pill, $U = 170.50, p > .05$, naturally cycling versus hormone-free interval, $U = 149.00, p > .05$, or active pill versus hormone-free interval, $U = 246.50, p > .05$ (Table 7). These findings suggest that groups within a study did not vary in terms of digit span, and that digit span did not vary with combined oral contraceptive use in either study.

**Digit Span and Story Recall**

Two nonparametric bivariate correlations, one per study, between digit span and story recall accuracy were conducted to explore the relationship between digit span and story recall. Digit span did not correlate significantly with story recall for either Study 1, $r_s = -.02, p > .05$ (Table 8), or Study 2, $r_s = .15, p > .05$ (Table 9).

**Story Recall**

A story recall task was included as an estrogen-insensitive measure of general intelligence. Performance on the story recall task was measured in terms of correctly recalled story details. Two independent Kruskal-Wallis $H$ one-way analysis of variance tests were conducted, one per study, to detect group differences in story recall. Significant group effects on story recall were not observed for either Study 1, $H = 3.03, p > .05$, or Study 2, $H = .72, p > .05$.

Six Mann-Whitney $U$ pairwise comparisons, three per study, were planned to compare story recall between each of the three groups, i.e., naturally cycling, active pill, and hormone-free interval. For Study 1, none of the three Mann-Whitney $U$-tests revealed significant differences in story recall for naturally cycling versus active pill, $U = 31.00, p > .05$, naturally cycling versus hormone-free interval, $U = 48.00, p > .05$, or active pill versus hormone-free interval, $U = 22.00, p > .05$ (Table 3). Likewise, for Study 2, none of the three Mann-Whitney $U$-tests revealed significant differences in story recall for naturally cycling versus active pill, $U = 202.00, p > .05$, naturally cycling versus hormone-free interval, $U = 188.00, p > .05$, or active pill versus hormone-free interval, $U = 239.50, p > .05$.
(Table 7). These findings suggest that groups within a study did not vary in terms of story recall, and that story recall did not vary with combined oral contraceptive use in either study.

**Post-Hoc Analyses**

**Oral Contraceptive Cognitive Effects in Depressed versus Nondepressed Subsamples**

Considering the high incidence of depression in our subject pool, and the possibility of cognitive mood effects in our data, exploratory post-hoc analyses were conducted independently for two subsamples from Study 2: depressed and nondepressed subjects. Depressed subjects are defined as subjects with CES-D scores equal to or greater than 16, i.e., the subthreshold for clinical depression. Nondepressed subjects are those subjects who scored lower than 16 on the CES-D scale. Twenty-six, two for each of the thirteen dependent variables, Kruskal-Wallis $H$ one-way analysis of variance tests were conducted independently for each subsample. Additionally, Mann-Whitney $U$ pairwise comparisons were conducted for each of the 13 dependent variables in each of the subsamples, resulting in a total of 78 pairwise comparisons (39 per subsample). The smaller sample size of Study 1 did not permit for similar analyses from that subject pool.

**Depressed Subsample**

Removal of nondepressed subjects from Study 2 resulted in the following group sizes: naturally cycling $n = 9$, active pill phase $n = 9$, hormone-free interval $n = 8$. Thirteen Kruskal-Wallis $H$ one-way analysis of variance tests were conducted, one for each dependent variable: salivary 17β-estradiol, dual-solution latency and errors, place average latency and errors, map drawing errors, mental rotation accuracy, objects recalled, words generated, digit span, story recall, and two mood assessments, i.e., PoMS and CES-D. Additionally, 39 pairwise comparisons of task performance between each of the three quartiles on each of the 13 dependent variables were conducted using independent Mann-Whitney $U$ tests. Preliminary data analyses suggested one high place average latency outlier in the hormone-free interval group, one high place average errors outlier in the active pill phase group, and four outliers in the word generation task, three in the naturally cycling (two high, one low) and one (low) in the active pill phase group. Removal of these outliers did not alter the findings. No significant differences were observed for any of the 13 dependent variables, either at the group or pairwise level. These findings suggest that oral contraceptive effects are not associated with cognitive effects in a depressed subsample.
Nondepressed Subsample

Removal of depressed subjects from Study 2 resulted in the following group sizes: naturally cycling \( n = 8 \), active pill phase \( n = 10 \), hormone-free interval \( n = 13 \). Thirteen Kruskal-Wallis \( H \) one-way analysis of variance tests were conducted, one for each dependent variable: salivary 17\( \beta \)-estradiol, dual-solution latency and errors, place average latency and errors, map drawing errors, mental rotation accuracy, objects recalled, words generated, digit span, story recall, and two mood assessments, i.e., PoMS and CES-D. Additionally, 39 pairwise comparisons of task performance between each of the three groups on each of the 13 dependent variables were conducted using independent Mann-Whitney \( U \) tests. Preliminary data analyses suggested one high place average latency outlier in the active pill phase group, which was removed from further analyses. Removal of this outlier resulted in strengthened but otherwise unaltered findings, i.e., direction of differences remained unchanged.

Of the four estrogen-sensitive cognitive measures, i.e., place latency and errors (estrogen enhanced), word generation (estrogen enhanced), and mental rotation (estrogen impaired), significant differences were observed in place learning both in terms of place average latency, \( H = 8.60; p = .014 \), and place average errors, \( H = 8.58; p = .014 \). The only other significant difference to emerge at this level was on the digit span task, \( H = 6.14; p = .046 \).

Exclusion of depressed subjects resulted in enhanced place learning for active pill phase females both in terms of place average latency and errors. Active pill phase females required less time (\( Mdn = 84.50s; IQR = 39.25 \)) to complete the place probe trials than both naturally cycling (\( Mdn = 124.25s; IQR = 53.13 \)), \( U = 11.00; p = .015 \), and hormone-free interval females (\( Mdn = 135.00s; IQR = 87.00 \)), \( U = 19.00; p = .008 \) (Figure 20a). Furthermore, active pill phase females committed fewer errors on the place probe trials (\( Mdn = 10.25; IQR = 2.25 \)) than both naturally cycling (\( Mdn = 13.50; IQR = 3.88 \)), \( U = 10.00; p = .02 \), and hormone-free interval females (\( Mdn = 15.00; IQR = 8.25 \)), \( U = 16.50; p = .01 \) (Figure 20b). No differences were observed between naturally cycling and hormone-free interval females in either average place latency or errors. Significant differences in estrogen-enhanced word generation and estrogen-impaired mental rotation were not observed at this level. The only other significant difference to emerge in the nondepressed subsample was on the digit-span task, with hormone-free interval females demonstrating significantly longer digit spans (\( Mdn = 27.5; IQR = 3.00 \)) than naturally cycling females (\( Mdn = 23.50; IQR = 9 \)), \( H = 22.50; p = .014 \). These exploratory findings suggest enhanced place learning associated with oral contraceptive use in a subsample of nondepressed subjects.
Figures 20a and 20b: Boxplots from exploratory analyses with a subsample of nondepressed Study 2 subjects. Place average latency (a) and errors (b) for subjects by group in Study 2 nondepressed subsample. Lines and whiskers represent medians and 1.5 times the interquartile range or the maximum or minimum if no data point falls within 1.5 times the interquartile range. Data points represent outliers. Inclusion of outliers did not alter findings. *Denotes p-value < .05. **Denotes p-value significant at or below the .01 level.

Note. CES-D = Center for Epidemiologic Studies Depression [scale]; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone-Free Interval Phase.
CHAPTER 4
DISCUSSION

The present studies were designed to explore the cognitive effects of combined oral contraceptive use, specifically those containing ethinyl estradiol and levonorgestrel or its metabolites, during late adolescence and young adulthood. These studies are unique in that they included a considerably large sample size and are among only a few cognitive studies of hormonal contraceptives to address potentially confounding mood effects. This second point is of particular importance considering the exploratory findings revealed by post-hoc analyses, which suggest enhanced estrogen-sensitive place learning in nondepressed, but not depressed, individuals during active pill phases of combination oral contraceptive use relative to hormone-free interval phases and naturally cycling females. This last finding emphasizes the importance of considering mood effects in cognitive studies of hormonal contraceptives.

Blair and colleagues (2000) reported a high affinity of ethinyl estradiol for estrogen receptors, which suggests a potential for ethinyl estradiol containing oral contraceptives to enhance estrogen-sensitive learning. Nonhuman animal research, however, suggests that combination estrogen and progesterone treatments have detrimental cognitive effects (for reviews see Korol, 2004 and Baudry et al., 2013). Considering these findings, three predictions were made. First, if endogenous estrogen is necessary for enhanced estrogen-sensitive learning, then the three groups of women, all of whom have low estrogen levels at time of testing, should not differ in performance on estrogen-sensitive tasks, such as place learning and word generation. Second, if ethinyl estradiol acts as an estrogen receptor agonist and mimics endogenous estrogen effects, then females in the active pill phase may show enhanced estrogen-sensitive learning in comparison to both menses phase naturally cycling and hormone-free interval females. Lastly, if combination estrogen and progesterone treatment has detrimental cognitive effects, as the rodent literature suggests, then active pill phase females are predicted to demonstrate impaired cognitive performance in comparison with naturally cycling, and possibly even hormone-free interval, females. To test these hypotheses, performance on a battery of estrogen-sensitive and insensitive tasks was compared between two groups of females undergoing combination oral contraceptive treatment and a group of naturally cycling females. Present findings indicated no cognitive differences between naturally cycling and combined oral contraceptive females in the full powered Study 2. Differences were observed in the smaller Study 1, however, these differences were likely due to the very high incidence of depressive symptoms in Study 1 naturally cycling females.
Naturally cycling females were tested during the first seven days of menstruation. Females treated with combined oral contraceptives were tested either during active pill days 15 - 17 or during inactive pill days 1 - 2. Females in the combined oral contraceptive groups used an ethinyl estradiol and levonorgestrel, or a levonorgestrel metabolite, containing oral contraceptive. Data collection occurred over three academic semesters. Study 1 was conducted in the spring semester and Study 2 was conducted over a spring and fall semester separated by summer session. All subjects were tested once over a 90 minute session that included two estrogen-sensitive tasks, i.e., place learning and word generation, and five estrogen-insensitive tasks, i.e., map drawing, mental rotation, digit span, story and object recall. Two mood measures were administered to account for mood effects associated with fluctuations in gonadal hormones and/or menstrual discomfort. Salivary 17β-estradiol samples were collected to account for effects of endogenous estrogens on the estrogen-sensitive tasks. Ninety-five subjects were tested between Study 1 \((n = 30)\) and Study 2 \((n = 65)\) and confidence intervals indicate that both studies were sufficiently powered to detect combination oral contraceptive use effects on cognition. Surprisingly, place learning correlated positively with 17β-estradiol levels in Study 1, suggesting that subjects with higher 17β-estradiol committed more errors and required more time to complete the place learning task; however, these findings contradict previous findings and were not replicated in the full powered Study 2, suggesting that this unexpected relationship observed in Study 1 may not be reliable. To summarize, these studies do not suggest either a cognitively detrimental or beneficial effect of combined oral contraceptive use during late adolescence or young adulthood. Exploratory analyses, however, suggest that mood effects, particularly depression, may mask combined oral contraceptive sparing of estrogen-sensitive place learning during periods of low endogenous estrogens, as demonstrated by our nondepressed subsample.

**Combined Oral Contraceptive Use and Mood Effects**

Oral contraceptive use has been shown to decrease negative affect (Paige, 1971). Consequently, two mood measures, the Profile of Mood State (PoMS) and Feelings Inventory (CES-D), were included to account for possible mood mediated differences in cognitive performance. As expected, PoMS and CES-D scores positively correlated in both studies, indicting that subjects with higher negative affect at time of testing also exhibited higher depressive symptoms. No other correlations were observed between negative affect, i.e., PoMS score, and cognitive performance. Depressive state, i.e., CES-D score, positively correlated with place errors in Study 2; however, this correlation was eliminated with the removal of an influential outlier. No other correlations between depressive symptoms and cognitive performance were observed in either study. No group differences were observed in negative
affect, i.e., PoMS scores, at time of testing in either study; however, pairwise comparisons suggested that Study 1 naturally cycling females demonstrated more negative affect at time of testing than Study 1 active pill phase females. This difference was not observed in the larger Study 2. In both Studies, subjects in the combined oral contraceptive groups exhibited subthreshold depressive symptoms, i.e., CES-D scores below 16, as did Study 2 naturally cycling females. Study 1 naturally cycling females exhibited depressive symptoms above the clinical subthreshold, which may have contributed to mood-induced cognitive impairments for Study 1 naturally cycling females that masked cognitive differences between treatment groups in Study 1; however, significant differences were not observed in depressive symptoms or cognitive performance between treatment groups in the larger Study 2. Removal of four subjects, two per study, who exhibited above subthreshold depressive symptoms, did not alter the findings. Additionally, removal of one subthreshold Study 1 naturally cycling female also failed to produce significant cognitive differences between naturally cycling and contraceptive females.

Post-hoc analyses, however, suggest that mood effects may have masked contraceptive cognitive effects in that Study 2 subsamples of depressed and nondepressed subjects demonstrated different patterns of estrogen-sensitive place learning. Post-hoc analyses with a subsample of depressed subjects from the larger Study 2 produced findings similar to those reported for Study 1 and Study 2 in that combination oral contraceptive use was not associated with cognitive enhancements or detriments. Post-hoc analyses with a nondepressed subsample from Study 2, however, revealed sparing of estrogen-sensitive place learning in active pill phase females relative to hormone-free interval and naturally cycling females during periods of low endogenous estrogens. These exploratory findings stress the importance of considering mood effects in cognitive studies of oral contraceptives. These exploratory findings are in line with other research that suggests cognitive enhancements associated with oral contraceptive use, which are discussed in subsequent sections.

While not the focus of the present studies, it is interesting to note that recent research has shown that oral contraceptive use may in fact reduce negative affect and depressive tendencies by altering the way in which emotional memories are processed and recalled. For instance, oral contraceptive females have been shown to recall fewer emotionally, versus neutrally, charged details than naturally cycling females (Nielsen, Ertman, Lakhani, & Cahill, 2011), a pattern of behavior that is more male-like suggesting a masculinization of the brain with oral contraceptive use (Cahill & Van Stegeron, 2003 and Cahill, Gorski, Belcher, & Huynh, 2004 as reviewed in Nielsen et al., 2011).
Combined Oral Contraceptive Use and Endogenous 17β-Estradiol Effects

Salivary 17β-estradiol levels at time of testing were measured to account for endogenous 17β-estradiol effects on cognitive performance. Salivary 17β-estradiol levels were comparable between the two groups of combined oral contraceptive females in both studies. Additionally, salivary 17β-estradiol levels were comparable between Study 2 naturally cycling and contraceptive females; however, Study 1 naturally cycling females exhibited significantly higher salivary 17β-estradiol levels than Study 1 hormone-free interval females.

Salivary 17β-estradiol levels were positively correlated with place learning in Study 1, but not in Study 2, suggesting that Study 1 subjects with higher salivary 17β-estradiol required more time to complete the place trial and committed more errors while doing so. Such findings were unexpected considering rodent evidence of preferred place learning strategies during high estrogen estrus cycles, i.e., proestrus (Korol et al., 2004), and improved place but not response learning after estrogen infusions (Korol & Kolo, 2002). A high number of less accurate subjects, both in terms of latency and errors committed, were observed in Study 2 but not Study 1; however, removal of these less accurate Study 2 subjects did not produce the salivary 17β-estradiol and place performance correlations observed in Study 1. Additionally, considering the large sample size of Study 2, n = 65, it is unlikely that Study 2 was inadequately powered to detect significant correlations. Therefore, the unexpected positive correlation between salivary 17β-estradiol and place learning impairments observed in Study 1 may not be reliable. No other correlations were observed between salivary 17β-estradiol and cognitive performance in either study suggesting that endogenous 17β-estradiol effects were not a concern in these studies.

Intra-Study and Inter-Study Reliability: Examination of Task Correlations

Dual-Solution Learning Correlations

Dual-solution latency and errors positively correlated in both studies suggesting that more errors were committed by subjects who required more time to complete the dual-solution training trials. Similarly, dual-solution and place learning positively correlated in Study 1, suggesting that subjects who experienced more difficulty learning the dual-solution maze also experienced more difficulty solving the place probe trial. These correlations were not observed in the larger Study 2, in which the task demands were altered to minimize floor effects observed in Study 1. It is quite possible that decreasing the number of training trials and introducing the reverse navigation requirement in Study 2 minimized the transfer of learning strategies from the dual-solution training to the place probe trial. Study 1 subjects may have used comparable strategies for successful completion of both the training and
probe trials, i.e., response learning, whereas Study 2 subjects may have been forced to adopt a new strategy during the probe trial, i.e., place learning, thus reducing the predictability of performance from the training to the probe trial. Additionally, as future sections will demonstrate, increasing task demand in Study 2 may have disrupted the transference of learning between other tasks, i.e., map drawing, as well as between the dual-solution and place learning trials.

As with place learning, performance on the dual-solution trials positively correlated with map drawing errors in Study 1 but not Study 2. Removal of two Study 2 outliers failed to produce the correlations observed in Study 1. Whereas the negative correlation between dual-solution and mental rotation performance observed in Study 1, but not Study 2, was eliminated with the removal of influential outliers. Similarly, a Study 1 positive correlation between dual-solution errors and object recall performance, not observed in Study 2, was eliminated after the removal of influential outliers. No other correlations were observed for dual-solution performance, i.e., not with digit span or story recall, in either study.

**Place Learning Correlations**

Place latency and errors positively correlated in both studies suggesting that more errors were committed by subjects who required more time to complete the place probe trials. As with dual-solution learning, several place learning correlations were observed in Study 1 but not in Study 2. For example, place latency and map drawing errors were positively correlated in Study 1, but not in Study 2, and removal of outliers from either study did not alter these inconsistent findings. As previously discussed, place learning may be a more accurate predictor of spatial cognitive performance when task demands are minimized and a reversal component, i.e., navigate the place trial in reverse, is not introduced. Place errors did not correlate with map drawing errors in either study, and provides some indication that the place latency and map drawing correlation in Study 1 may be a statistical artifact. As with dual-solution learning, Study 1 place learning was negatively correlated with mental rotation performance and positively correlated with object recall, in terms of latency, but these correlations were not observed in Study 2. Removal of outliers did not alter these findings. As with dual-solution learning, place learning did not correlate with digit span. A negative correlation between place learning and story recall was observed in Study 1, but was eliminated with the removal of influential outliers. No correlations between place and story recall performance were observed in the larger Study 2.
Word Generation Correlations

Word generation performance did not correlate with map drawing, mental rotation, or object recall performance in either study. Word generation, a verbal fluency task, positively correlated with the two measures of general intelligence, i.e., digit span and story recall, in Study 2 but not in Study 1. Removal of several Study 2 outliers only slightly weakened the strength but not the significance of these correlations. A similar trend was observed in Study 1 word generation and digit span and story recall, in which case removal of outliers strengthened the correlation but did not change the significance level, suggesting that the correlations observed in the larger Study 2 are reliable correlations.

Map Drawing Correlations

Map drawing performance did not correlate with mental rotation, object recall, or digit span performance in either study. Study 1 map drawing performance did correlate with story recall performance in Study 1; however, this correlation was not replicated in the larger Study 2, and was not pursued as it is not a correlation of interest. No other correlations were observed between mental rotation, digit span, or story recall performance in either study.

Contraceptive Use and Cognitive Performance

Combined Oral Contraceptive Use and Estrogen-Sensitive Learning

Place Learning

Combination 17β-estradiol and progesterone treatments have been shown to impair place learning in rodent navigation models (for reviews see Korol, 2004 and Baudry et al., 2013), possibly through a disruption of estrogen modulated hippocampal activity. Estrogens are believed to influence navigational processes by increasing hippocampal brain-derived neurotropic factor (BDNF) expression, and progesterone has been shown to exert antagonistic estrogenic effects on hippocampal synaptic plasticity, possibly through downregulation of estrogen receptors in the hippocampi (for review see Baudry et al., 2013). Fortunately, we did not find evidence of disrupted place learning in the presence of an estrogen plus progesterone treatment, i.e., combined oral contraceptive use. Group effects were not observed on the estrogen-sensitive place probe trials, suggesting that combined oral contraceptive use neither impaired nor enhanced place learning in a virtual navigation task.

There are several possible explanations for why we did not replicate the detrimental effects of combination estrogen and progesterone treatment observed in rodents. Most apparent is the occasional lack of translation from
rodent to human models of cognition. Additionally, the rodents discussed above were gonadectomized, while the
gonadally intact subjects of the present studies may have been shielded from detrimental exogenous progestogenic
effects by the neuroprotective effects of endogenous gonadal hormones (Resnick, Metter, & Zonderman, 1997; for
review see Spencer et al., 2008). Furthermore, the present findings are limited to comparisons of cognitive
performance during periods of low estrogen. A different pattern of findings, such as impaired place learning, may be
detected when combined oral contraceptive use is compared with higher estrogen naturally cycling females.

Lastly, recent evidence suggests that hormonal contraceptives may exert their effects via cortical
recruitment more so than overt behavior. For example, hormonal contraceptive use has been shown to correspond
with increased blood oxygenation level-dependent (BOLD) response, particularly the right fusiform face area during
face processing, despite a lack of behavioral differences, i.e., eye movements, in other similar comparisons of
naturally cycling and hormonal contraceptive females (Marečková et al., 2012 Studies I and III). Indeed, comparison
of males, naturally cycling, and oral contraceptive females on number comparison and bisection tasks suggests oral
contraceptive use may produce behavioral patterns in oral contraceptive females characteristic of naturally cycling
females but accompanied by masculinized brain activation patterns (Pletzer, Kronbichler, Neurk, Kerschbaum,
2014).

**Word Generation**

In line with previous research, combined oral contraceptive use in the present study did not alter
performance on a word generation task in either Study 1 or 2 (Hampson, 1990; Mordecai et al., 2008). As previously
discussed, neuroimaging evidence suggests sub-behavioral differences between naturally cycling and oral
contraceptive females on a verb generation task despite a lack of behavioral differences (Rumberg et al., 2010), in
which case oral contraceptive use was associated with masculinized brain activation patterns. In summary, the
present findings suggest that combined oral contraceptive use may not affect estrogen-sensitive place learning or
word generation, at least not at the behavioral level and/or when oral contraceptive use is compared with low-
estrogen naturally cycling females.

**Combined Oral Contraceptive Use and Estrogen-Insensitive Learning**

As with previous research, combined oral contraceptive use in the present studies was not associated with
altered working memory (Vranić & Hromatko, 2008) or mental rotation performance (Mordecai et al., 2008;
Additionally, combined oral contraceptive use in the present studies was limited to females who use levonorgestrel, a second-generation progestin. Therefore it is not surprising that oral contraceptive use in the present studies failed to produce the mental rotation impairments observed with third and new, but not second, generation progestin use (Griksiene & Ruksenas, 2011; Wharton et al., 2008).

**Limitations**

Post-hoc measures of power, such as the Hodges-Lehmann estimate of median differences, suggest that these studies, with a combined sample of 95 subjects, were not insensitive to real estrogen effects on cognitive performance. These findings, however, are limited to comparisons between combined oral contraceptive and low-estrogen menstrual cycle phase females. Effects of combined oral contraceptive use may emerge when contraceptive females are compared to females tested during high-estrogen menstrual cycle phases.

An additional limitation may exist in the task chosen to measure place learning. Study 1 subjects reported low reliance on estrogen-sensitive place learning during the virtual navigation probe trial, relying instead on response or verbal strategies, such as rehearsing motor movements and labeling doors. This probe trial was an essential aspect of these studies and if this trial failed to tap into estrogen-sensitive place learning then the effectiveness of these studies to assess combined oral contraceptive effects on estrogen-sensitive place learning may have been significantly reduced. Reducing the number of training trials and introducing the reverse navigation requirement in Study 2 resulted in fewer reports of response and verbal navigation strategies during the probe trial. Additionally, it is reassuring that no relationships emerged between oral contraceptive use and performance on multiple cognitive tasks including an estrogen-sensitive word generation task.

**Conclusions**

Three groups of females, one naturally cycling and two combined oral contraceptives, were tested on a variety of estrogen-sensitive and -insensitive cognitive tasks over two waves of data collection. Naturally cycling females were tested shortly after menstruation and contraceptive females were tested during either the hormone-free interval or active pill phase. Saliva samples collected at time of testing indicated little to no difference in salivary 17\(\beta\)-estradiol, which suggests no endogenous estrogen effects. Similarly, two mood measures provided little to no evidence of overall mood effects. Post-hoc analyses, however, suggest that depression in particular may mask beneficial cognitive effects of oral contraceptives on estrogen-sensitive place learning. These findings are in line
with several previous studies that report little to no behavioral differences between naturally cycling and contraceptive females. Future research, however, should consider the masculinizing effects of combined oral contraceptives at the sub-behavioral level in terms of neuroactivational patterns even in the absence of behavioral differences as well as the potentially confounding effects of mood as demonstrated by our exploratory findings.
### Table 1

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<td>2.19 [1.08]</td>
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<tr>
<td>Digit Span</td>
<td>0.5</td>
<td>2.19 [1.08]</td>
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<tr>
<td>Story Recall</td>
<td>0.5</td>
<td>2.19 [1.08]</td>
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</tr>
</tbody>
</table>

Note. Mdn = Median; IQR = Interquartile Range; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; MRT = Mental Rotation Task; PoMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression Scale.
Table 2

<table>
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<th>Study 1 Means and Standard Deviations</th>
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<td>MRT</td>
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</tr>
<tr>
<td>Story Recall</td>
</tr>
<tr>
<td>PoMS</td>
</tr>
<tr>
<td>CES-D</td>
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<td>MRT</td>
</tr>
<tr>
<td>Digit Span</td>
</tr>
<tr>
<td>Story Recall</td>
</tr>
<tr>
<td>PoMS</td>
</tr>
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</table>

Note. SD = Standard Deviation; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; MRT = Mental Rotation Task; PoMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression [scale].

*Measured in seconds.*
### Table 3: Study 1 Comparison of Group Medians

<table>
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<tr>
<th>Factor</th>
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<th>UL 95% CI</th>
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<td>38.00</td>
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<td><strong>Place Latency</strong></td>
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<td>49.50</td>
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<tr>
<td><strong>Digit Span</strong></td>
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<td>41.50</td>
<td>53.50</td>
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<tr>
<td><strong>Story Recall</strong></td>
<td>31.00</td>
<td>25.00</td>
<td>37.00</td>
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<td><strong>PoMS</strong></td>
<td>14.00</td>
<td>10.00</td>
<td>18.00</td>
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<td><strong>CES-D</strong></td>
<td>15.50</td>
<td>11.50</td>
<td>19.50</td>
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</table>

**Note.** LL = 95% Confidence Interval Lower Limit; UL = 95% Confidence Interval Upper Limit; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; MRT = Mental Rotation Task; PoMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression Scale.


a Mann-Whitney Test, *p* < .05 not reported.
b Hodges-Lehmann Median Difference Estimate.
c Measured in seconds.
**Study 2 Sample Sizes**

<table>
<thead>
<tr>
<th>Study 2</th>
<th>Group</th>
<th>Comparison</th>
<th>n</th>
<th>NC</th>
<th>AP</th>
<th>HFI</th>
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<tr>
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<td></td>
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<td>NC vs. HFI</td>
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<td>AP vs. HFI</td>
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</tr>
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</table>

**Note.** NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval.

Hodges-Lehmann Comparison of Medians. Average sample size of two place trials.

- CES-D
- PoMS
- Story Recall
- Digit Span
- Word Comprehended
- Objects Reassembled
- MRT Items
- Line Errors
- Place Errors
- Place Learning
- Dual-Simulation Task Errors
- Dual-Simulation Task Imitacy
- 1TP-Imitated

Table 4 Sample Sizes
Table 5
Study 2 Medians and Interquartile Ranges

<table>
<thead>
<tr>
<th>Factor</th>
<th>Study 2</th>
<th>NC</th>
<th>AP</th>
</tr>
</thead>
<tbody>
<tr>
<td>17β-Estradiol (pg/mL)</td>
<td>1.86 [1.06]</td>
<td>2.09 [1.01]</td>
<td>1.54 [1.13]</td>
</tr>
<tr>
<td>Dual-Solution Trial 3 Latency</td>
<td>80.00 [57.00]</td>
<td>80.50 [7.00]</td>
<td>81.00 [36.00]</td>
</tr>
<tr>
<td>Dual-Solution Trial 3 Errors</td>
<td>9.00 [7.00]</td>
<td>9.00 [6.00]</td>
<td>9.00 [3.00]</td>
</tr>
<tr>
<td>Place Latency</td>
<td>114.75 [76.38]</td>
<td>117.75 [68.63]</td>
<td>108.75 [69.63]</td>
</tr>
<tr>
<td>Map Errors</td>
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<td>3.00 [3.00]</td>
<td>3.00 [3.00]</td>
</tr>
<tr>
<td>MRT Items</td>
<td>15.00 [9.00]</td>
<td>16.00 [7.00]</td>
<td>13.00 [11.00]</td>
</tr>
<tr>
<td>Objects Recalled</td>
<td>10.00 [4.00]</td>
<td>9.50 [4.00]</td>
<td>10.00 [4.00]</td>
</tr>
<tr>
<td>Words Generated</td>
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<td>67.00 [15.00]</td>
<td>72.50 [14.00]</td>
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<tr>
<td>Digit Span</td>
<td>27.00 [8.00]</td>
<td>24.50 [8.00]</td>
<td>26.50 [11.00]</td>
</tr>
<tr>
<td>Story Recall</td>
<td>14.00 [5.00]</td>
<td>13.00 [5.00]</td>
<td>14.00 [5.00]</td>
</tr>
<tr>
<td>PoMS</td>
<td>56.00 [34.00]</td>
<td>60.00 [43.00]</td>
<td>49.00 [24.00]</td>
</tr>
<tr>
<td>CES-D</td>
<td>14.50 [13.00]</td>
<td>15.00 [16.00]</td>
<td>14.00 [10.00]</td>
</tr>
</tbody>
</table>

Note. Mdn = Median; IQR = Interquartile Range; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; MRT = Mental Rotation Task; PoMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression Scale.

*a* Measured in seconds.

*b* Values reported based on average performance across two place trials.
### Study 2 Means and Standard Deviations

<table>
<thead>
<tr>
<th>Factor</th>
<th>Study 2</th>
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<tr>
<td></td>
<td>HFI</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CES-D</td>
<td>16.73 [1.18]</td>
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<tr>
<td>PoMS</td>
<td>59.73 [15.72]</td>
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<tr>
<td>Word Counted</td>
<td>12.52 [1.11]</td>
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<tr>
<td>MRT Items</td>
<td>12.94 [1.04]</td>
</tr>
<tr>
<td>Memory Errors</td>
<td>3.35 [0.72]</td>
</tr>
<tr>
<td>Place Memory Errors</td>
<td>12.72 [4.87]</td>
</tr>
<tr>
<td>Digit Span</td>
<td>12.96 [5.37]</td>
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<tr>
<td>Place Memory Errors</td>
<td>12.96 [5.37]</td>
</tr>
<tr>
<td>Digit Span</td>
<td>12.96 [5.37]</td>
</tr>
<tr>
<td>Place Memory Errors</td>
<td>12.72 [4.87]</td>
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<tr>
<td>Digit Span</td>
<td>12.96 [5.37]</td>
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<tr>
<td>Place Memory Errors</td>
<td>12.72 [4.87]</td>
</tr>
</tbody>
</table>

Note. M = Mean, SD = Standard Deviation. NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; DS = Dual-Solution; MRT = Mental Rotation Task; PoMS = Profile of Mood States; CES-D = Center for Epidemiologic Studies Depression Scale. Comparison sample sizes are presented in Table 3.

Values reported based on average performance across two place trials.
Table 7
Study 2 Comparison of Group Medians

<table>
<thead>
<tr>
<th>Factor</th>
<th>NC vs. AP</th>
<th>AP vs. HFI</th>
<th>NC vs. HFI</th>
<th>NC vs. AP</th>
<th>AP vs. HFI</th>
<th>NC vs. HFI</th>
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<tr>
<td>184.00 - 203.00</td>
<td>135.00 - 100.20</td>
<td>156.00 - 180.00</td>
<td>204.00 - 234.00</td>
<td>172.50 - 202.50</td>
<td>193.00 - 223.00</td>
<td>234.00 - 264.00</td>
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<tr>
<td>204.00 - 234.00</td>
<td>172.50 - 202.50</td>
<td>193.00 - 223.00</td>
<td>234.00 - 264.00</td>
<td>156.00 - 180.00</td>
<td>135.00 - 100.20</td>
<td>184.00 - 203.00</td>
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<tr>
<td>234.00 - 264.00</td>
<td>156.00 - 180.00</td>
<td>135.00 - 100.20</td>
<td>184.00 - 203.00</td>
<td>204.00 - 234.00</td>
<td>172.50 - 202.50</td>
<td>193.00 - 223.00</td>
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</tbody>
</table>

Note. LL = 95% Confidence Lower Limit; UL = 95% Confidence Interval Upper Limit; NC = Naturally Cycling; AP = Active Pill Phase; HFI = Hormone Free Interval; MRT = Mental Rotation Task; POMS = Profile of Mood States; CES-D = Center for Epidemiologic Studies Depression (scale); p > .05 not reported.

a Mann-Whitney Test.
b Hodges-Lehmann Median Difference Estimate.
c Values reported based on average performance across two place trials.

Table 7 study 2 comparison of group medians
Table 8
Spearman Nonparametric Correlations Across Groups for Study 1

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<th>3</th>
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<th>7</th>
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<th>11</th>
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</table>

Note. n = 30; * p < .05 level; ** p < .01 level; MRT = Mental Rotation Task; POMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression Scale.

Table 8
Table 9
Spearman Nonparametric Correlations Across Groups for Study 2

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</tbody>
</table>

Note. DS = Dual-Solution; MRT = Mental Rotation Task; PoMS = Profile of Mood State; CES-D = Center for Epidemiologic Studies Depression [scale]; *p < .05 level; **p < .01 level.

a Values reported based on average performance across two place trials.
### ELIGIBILITY QUESTIONS FOR ALL SUBJECTS

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Are you between 18 and 31 years of age?</td>
<td></td>
</tr>
<tr>
<td>2. Are you right-handed?</td>
<td></td>
</tr>
<tr>
<td>3. Do you have a chronic illness that is treated with hormones (e.g., thyroid disease, diabetes, reproductive dysfunction, etc.)?</td>
<td></td>
</tr>
<tr>
<td>4. Do you currently or have you had a reproductive related illness (e.g., ovarian cancer)?</td>
<td></td>
</tr>
<tr>
<td>5. Are you currently taking or in the past month have you taken a neuroleptic (e.g., antidepressants, antipsychotics)?</td>
<td></td>
</tr>
<tr>
<td>6. Is your vision normal or corrected to normal?</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX B

ELIGIBILITY QUESTIONS FOR NATURALLY CYCLING FEMALES

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.</td>
<td>Are you, or were you in the past 3 months, pregnant or breast feeding?</td>
</tr>
<tr>
<td>8.</td>
<td>Have you had a regular period for the past three months?</td>
</tr>
<tr>
<td>9.</td>
<td>Are you currently taking a hormonal contraceptive</td>
</tr>
<tr>
<td>10.</td>
<td>Have you used an oral contraceptive in the past 3 months? Depo-Provera, norplant or a hormonal IUD in the past 12 months?</td>
</tr>
<tr>
<td>11.</td>
<td>Have you used emergency contraception in the past month?</td>
</tr>
<tr>
<td>12.</td>
<td>Have you been seriously ill in the 7 days? been ill (cold, etc.)?</td>
</tr>
<tr>
<td>13.</td>
<td>What was the first day of your last period (when bleeding began)?</td>
</tr>
</tbody>
</table>
APPENDIX C

ELIGIBILITY QUESTIONS FOR
COMBINED ORAL CONTRACEPTIVE FEMALES

<table>
<thead>
<tr>
<th>Question</th>
<th>Eligible contraceptives:</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Are you, or were you in the past 3 months, pregnant or breast feeding?</td>
<td>Allesse, Jolessa, Lutera, Ortho Tri-Cyclen, Seasonique, TriPrevifem</td>
</tr>
<tr>
<td>8. Have you had a regular period for the past three months?</td>
<td>Apri, Kariva, Lybrel, Ortho Tri-Cyclen Lo, Solia, TriSprintec</td>
</tr>
<tr>
<td>9. Which hormonal contraceptive are you currently taking? (must be on list below).</td>
<td>Aviane, Lessina, Mercilon, Orto-Cept, Sprintec, TriVora</td>
</tr>
<tr>
<td>10. Have you taken a hormonal contraceptive NOT on this list in the past three months?</td>
<td>Cesiaphene, Levlen, Mircette, Portia, Sronyx, Velivet</td>
</tr>
<tr>
<td>11. Have you used Depo-Provera, norplant or a hormonal IUD in the past 12 months?</td>
<td>Cesia, Levlen, Mircette, Portia, Sronyx, Velivet</td>
</tr>
<tr>
<td>12. In the past 7 days have you been seriously ill? In the past 30 days have you used emergency contraception?</td>
<td>Cesia, Levlen, Mircette, Portia, Sronyx, Velivet</td>
</tr>
<tr>
<td>13a. Hormone-Free Interval Females: When did the inactive (sugar) pill phase of your current birth control pack begin?</td>
<td>Eligible contraceptives:</td>
</tr>
<tr>
<td>13b. Active Pill Females: How many active pills of your current birth control pack have you taken as of today?</td>
<td>Eligible contraceptives:</td>
</tr>
</tbody>
</table>
REFERENCES


