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THE 2D:4D RATIO, HANDEDNESS, AND SEX ACROSS THE AGE SPAN

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ABSTRACT

The ratio of the difference between the 2nd and 4th digits of the hand (2D:4D ratio) has been demonstrated to be an indirect indicator of prenatal testosterone levels. Prenatal testosterone has been found to play a role in brain development *in utero*, and thus may influence lateral asymmetries, such as handedness. Consequently, one of the aims of the current study was to examine relationships between the 2D:4D ratio, hand preference, and hand performance with the factors of sex (males and females), handedness (right handers and left handers), and age considered. A total of 104 participants were tested, 90 right handers and 14 left handers (age range = 5-to-90, mean age = 31.93, SD = 20.18, females = 58). Participants completed the Waterloo Handedness Questionnaire (WHQ) as an indicator of hand preference and the Tapley-Bryden Dot Marking (TBDM) task to evaluate hand performance. Right and left 2D:4D ratios were measured for all participants using Vernier calipers, measured to the nearest 0.01mm. Regardless of age and sex, left handers had significantly reduced hand preference strength and trended in having reduced hand performance differences between the hands. Furthermore, although only significant in the 50+ years age group, it appeared as though males tended to have decreased handedness compared to females. No significant relationships nor main effects were found with regards to the 2D:4D ratios measured, though.

Additional testing was conducted including participants with ASD, who have been illustrated to have lower than average 2D:4D ratios, as well as increased hand ambiguity. Relationships were examined between the 2D:4D ratio, hand preference, and hand performance and comparisons were analyzed between neurotypical participants and participants with ASD. A total of 5 participants with ASD were tested, 4 right handers and 1 left hander (age range = 6-to-36, 5 males). With the small sample size, all relationships were found to be insignificant and

were not generalizable. Comparisons did display significant differences in hand performance, where individuals with ASD illustrated greater hand ambiguity.

Overall, the study has demonstrated that sex, handedness, and age influence hand preference and hand performance. However, no relationships were found between handedness and the 2D:4D ratios. Moreover, continuing research on hand ambiguity in individuals with ASD could better the understanding of brain lateralization.

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CHAPTER 1: REVIEW OF LITERATURE

There is an ongoing debate on what determines whether a person will be left handed or right handed. Many argue that handedness has a genetic basis, while others suggest an environmental cause. One of the key factors at play may be fetal testosterone. Fetal testosterone is said to influence the development of handedness as it plays a role in early cerebral lateralization during pregnancy (Cohen-Bendahan, Buitelaar, van Goozen, & Cohen-Kettenis, 2004; Grimshaw, Bryden, & Finegan, 1995; McManus & Bryden, 1991). Fetal testosterone has also been shown to influence the 2D:4D ratio, which is the ratio of the difference in length between the index finger (second digit) and the ring finger (fourth digit) on the same hand (Hönekopp & Watson, 2010; Manning, Scutt, Wilson, & Lewis-Jones, 1998). In addition, the relationship between the amount of existing fetal testosterone during gestation and developmental disorders such as autism spectrum disorder (ASD) has been a growing area of research. A significant difference in the 2D:4D ratio of individuals with ASD compared to those of neurotypical development has been documented, as those with ASD have been illustrated to show a significantly lower ratio, such that the fourth digit is longer than the second digit (de Bruin, Verhij, Wiegman, & Ferdinand, 2006; Manning, Baron-Cohen, Wheelwright, & Sanders, 2001; Teatero & Netley, 2013). Researchers have also noted that individuals with ASD do not exhibit as strong a hand preference compared to neurotypical individuals (Dane & Balci, 2007; Escalante-Mead, Minshew, & Sweeney, 2003; Hauck & Dewey, 2001; McManus, Murray, Doyle, & Baron-Cohen, 1992; Soper et al., 1986). Given these relationships, fetal testosterone could potentially be an indicator of whether or not autistic characteristics may develop in a child. Understanding the 2D:4D ratio and early hand preference could lead to an earlier diagnosis and more prompt treatment that can help these children develop fundamental cognitive, motor, and social skills. For these reasons, understanding the importance of fetal testosterone and cerebral lateralization in the development of an individual is crucial, and research involving traits that can be evaluated to help in this understanding, the 2D:4D ratio and handedness of individuals, will assist in this endeavour. The first section of this document will discuss cerebral lateralization, handedness, and the 2D:4D ratio in a neurotypical population. The second section will then examine cerebral lateralization, handedness, and the 2D:4D ratio specifically in individuals with ASD.

Cerebral Lateralization

During both prenatal and postnatal development the brain undergoes a process called cerebral lateralization. Cerebral lateralization happens as one side of the brain starts to dominate certain functions such as language and emotion and it controls the individual's processes in those areas (Toga & Thompson, 2003). The callosal hypothesis suggests that cerebral lateralization occurs through the pruning of callosal axons in certain regions of the brain, where greater axonal pruning will lead to greater lateralization of functions (Grimshaw, Bryden, & Finegan, 1995). Grimshaw, Bryden, and Finegan (1995) proposed that callosal pruning is partially mediated by fetal testosterone. Geschwind and Galaburda (1985) also proposed that testosterone plays a strong role in cerebral lateralization, such that there is a critical period during fetal growth where it plays a key role on brain development. McManus and Bryden (1991) further developed a model to account for how testosterone affects many neurological outcomes. The model indicates factors that can affect prenatal testosterone levels (e.g., genetic factors, tissue sensitivity, multiple embryos, endocrine environment in pregnancy), which in turn influence brain development, followed by the outcomes that may result. Included among these outcomes are handedness, developmental learning disorders, immune disorders, and neural abnormalities

(McManus & Bryden, 1991). It has also been shown that if there is too much testosterone, growth retardation may occur, shifting the dominance of functions, like language, to the right hemisphere (Grimshaw, Bryden, & Finegan, 1995). Shifting of brain hemisphere dominance has been proposed as the reason there may be greater incidences of left handedness and developmental disorders among males, as there will be greater amounts of fetal testosterone during prenatal development. This idea ties into the sexual differentiation theory, as it states that prenatal testosterone exposure accounts for sex differences in lateralization. Again, testosterone levels may be the reason males are associated with more left handedness and a higher degree of lateralization for cognitive functions (Grimshaw, Bryden, & Finegan, 1995).

Grimshaw, Bryden, and Finegan (1995) conducted a study that examined fetal testosterone levels from the amniotic sac during the 2nd trimester and followed up with the 53 participants (females = 25) at 10 years of age. Females with higher fetal testosterone levels showed a stronger right hand dominance and left hemispheric speech recognition. Males with higher fetal testosterone levels showed stronger right hemisphere specialization for emotion recognition (Grimshaw, Bryden, & Finegan, 1995). Cohen-Bendahan and colleagues (2004) conducted a study to determine any differences in cerebral lateralization between females from opposite sex twin groups (n = 67) and same sex twin groups (n = 53). The researchers predicted that females from opposite sex twin groups would be exposed to higher levels of testosterone prenatally, leading to greater lateralization. A dichotic listening task was used as an indirect method of determining hemispheric specialization for language. The score for the dichotic listening task was calculated based on correct and incorrect responses to stimuli indicating whether a left or right ear advantage was present. A score closer to 0 indicated that the individual was less lateralized for language. The results showed that opposite sex female twins had greater left hemispheric lateralization than those of same sex female twins, thus demonstrating the effects that prenatal testosterone may have on brain asymmetries (Cohen-Bendahan, Buitelaar, van Goozen, & Cohen-Kettenis, 2004). More recently, Chura and her colleagues (2010) examined how fetal testosterone may influence the physical structure of the brain, specifically the corpus callosum in 28 right-handed male participants (mean age = 9.5 years). The researchers found that there was a significant relationship between fetal testosterone and callosal asymmetry, specifically in the isthmus, where fetal testosterone predicted 36% of variance in asymmetry in this particular region. They concluded that these results strongly support the theory in which fetal testosterone plays a role in early brain lateralization (Chura et al., 2010).

Overall, cerebral lateralization plays a fundamental role in the maturation of the brain and without it certain functions such as handedness, language, and emotion may never be fully developed. Future studies should aim to provide a thorough understanding of lateralization and the requisite conditions for this process, such as how fetal testosterone may affect the size and asymmetry of the structure of the brain.

Handedness

What makes a person left handed or right handed is still not fully understood, although factors such as genetics and the environment may play a role. After reviewing previous literature, McManus and Bryden (1992) noted that the number of left-handed children rises when one or more of their parents is left handed. The percentage of left-handed children is highest when both of their parents are left handed. The trait of handedness does not, however, follow the classic Mendelian genetic inheritance pattern. It appears that there is a stronger chance of the child being left handed when the mother is also left handed in comparison to when the father is left handed, suggesting that there may be a sex-linked component to handedness (McManus & Bryden, 1992).

Annett (1999) proposed a model for hand preference, termed the right-shift theory, where two alleles are involved in determining whether an individual will be right or left handed. One allele, RS (right shift) +, coding for right-handedness, is dominant over the other allele, RS-, which is a chance allele for either right or left hand dominance. Therefore, the majority of the population will be right handed. However, if an individual is RS-/RS-, handedness is normally distributed and half of the people will be left handed while the other half will be right handed (Annett, 1999). McManus proposed a similar model in that the allele C (chance) had no control over lateralization, and therefore CC individuals resulted in 50% right-handers and 50% left-handers (McManus & Bryden, 1992). The D (dextral) allele, however, was dominant for right-handedness, so a DD homozygous individual would be 100% right handed. When individuals had a DC allele combination though, it could result in anywhere from 0-50% of them being left-handed, with the remainder being right-handed individuals (McManus & Bryden, 1992). More recent literature by Medland and her colleagues (2009) suggested that approximately 25% of variance in handedness is due to additive genetic effects, while the other 75% is due to non-shared environmental influences. Non-shared environmental influences can include parental treatment, sibling interactions, classroom experiences, and relationships with peers (Plomin, 2011). Based on the previous models mentioned, it has been illustrated that there is a genetic link to handedness, and genes do influence whether an individual will be right or left handed.

In addition, there may be factors consisting of both genetic and environmental components that play a role in determining whether a person will be left or right handed in the prenatal environment. A meta-analysis of 43 articles conducted by Sommer et al. (2008) found that males have a 25% higher prevalence of being non-right handed in comparison to females. Prenatal testosterone, as previously mentioned, affects cerebral lateralization, which can in turn influence handedness (Grimshaw, Bryden, & Finegan, 1995; McManus & Bryden, 1991). Therefore, there may be a link between the amount of prenatal testosterone an individual is exposed to and whether that individual develops left or right handedness. Expanding on this topic, Lust and her colleagues (2011) extracted amniotic fluid from 65 pregnant women and calculated the correlation between prenatal testosterone levels and handedness of the 65 children at a mean age of 6.43 years old. Results showed that higher prenatal testosterone levels were related to a decreased strength of handedness, which was measured through performance on age-appropriate tasks that required active motor action (e.g., drawing, tooth brushing, opening a lid) (Lust et al., 2011). Therefore, the genetics involved in determining the amount of fetal testosterone that will then be present during gestation illustrates one way in which genetics and the environment interact together in determining handedness.

A postnatal environmental aspect that may influence handedness is early childhood practice as cerebral lateralization is still occurring. A longitudinal study examining the early development of handedness in three infants found that a bias towards a certain hand had great variability within the first year of life (Corbetta, Williams, & Snapp-Childs, 2006). Certain locomotor patterns affected these handedness trends, such as hands-and-knees crawling and first independent steps. These novel sensory-motor experiences are key for infants in understanding and developing a preferred hand (Corbetta, Williams, & Snapp-Childs, 2006). It is still under debate as to whether superior hand performance of one hand leads to a preference for that hand, or if hand preference comes first followed by an increase in that hand's performance. However, as an individual continually uses one hand over the other to perform tasks, its overall functioning will increase, naturally leading to that hand being viewed as the preferred hand (Michel, Nelson, Babik, Campbell, & Marcinowski, 2013). Therefore, the developmental process of identifying a dominant hand starts early in life and through experience the different roles for each hand will be shaped.

In order to obtain the most accurate representation of handedness, Brown and her colleagues (2004) analyzed multiple measures in 62 participants aged 18 to 25 years old (left handers = 20) to determine the best predictors of hand preference as well as hand performance. Brown et al. (2004) found that together the Waterloo Handedness Questionnaire with the Wathand Cabinet Test and the score of the grooved pegboard task were the best predictors of handedness (Brown, Roy, Rohr, Snider & Bryden, 2004). Simply completing one task does not provide the best indicator of handedness, and thus, it is important to use both hand preference and hand performance when analyzing handedness.

There are many genetic and environmental components that go into an individual's handedness, and further research still needs to be done to achieve a greater understanding of their roles. The genetic aspects, such as individual genes coding for handedness, genetic and environmental influences, like testosterone levels, and everyday environmental impacts, like hand utilisation, are therefore all important areas for future studies.

The 2D:4D Ratio

The 2D:4D ratio has captured researchers' attention as it has been proposed that this digit ratio can be a good indicator of many factors, such as prenatal testosterone levels, handedness, writing hand, and autistic characteristics. Table 1 below provides clarification of the terminology with regard to the 2D:4D ratio.

Table 1. Sample measurements of the 2D:4D ratio, indicating the direction in which the digit ratio falls. An approximation of testosterone levels is given based on the direction of the digit ratio.

2D Measurement	4D Measurement	2D:4D Ratio	Direction	Testosterone Level
66.46	75.43	0.881	Low ratio	+++
71.44	71.96	0.993	Equal ratio	++
63.73	63.21	1.008	Equal ratio	++
73.27	68.39	1.071	High ratio	+

The 2D:4D ratio is the length of the index finger relative to the length of the ring finger on the same hand, specifically the 2nd digit divided by the 4th digit (2D/4D = 2D:4D ratio). Manning, Scutt, Wilson, and Lewis-Jones (1998) illustrated a significant difference in digit ratios between males (n = 400) and females (n = 400). Vernier callipers were used to measure finger length on the ventral surface of the hand from the basal crease to the top of the digit. Males tended to have a longer 4th digit than 2nd digit, indicating a low ratio, whereas females tended to have similar lengths in the 2nd and 4th digits, resulting in an equal ratio (Manning, Scutt, Wilson, and Lewis-Jones, 1998). Later, a meta-analysis consisting of 110 studies completed by Hönekopp and Watson (2010) provided evidence that the amount of testosterone in fetal development significantly influences the 2D:4D ratio. More specifically, a negative relationship is observed where the more fetal testosterone encountered during gestation, the lower the 2D:4D ratio (Hönekopp & Watson, 2010), providing a potential explanation as to why males' and females' digit ratios differ. Further investigations took place of the physiological aspects behind fetal testosterone and how it could be influencing the 2D:4D ratio. Manning and colleagues (2003) described that the variation in an androgen receptor gene, which plays an important role on fetal growth, determines the sensitivity to fetal testosterone. They found that a high activation of the androgen receptor gene, indicating high sensitivity to testosterone, was correlated to a low digit ratio (Manning, Bundred, Newton, & Flanagan, 2003). Therefore, with high testosterone levels during fetal development, a low 2D:4D ratio will result. Additionally, a study involving mice found that androgen receptor and estrogen receptor activity is higher in digit 4 than in digit 2 (Zheng & Cohn, 2011). As a result, the length of the 4th digit will have sex differences based on the activation and inactivation of these receptors with it having increased growth in male mice and decreased growth in female mice (Zheng & Cohn, 2011). Overall, based on these findings, it appears that there is a strong connection between fetal testosterone levels and the 2D:4D ratio.

Beaton and his colleagues (2011) investigated whether adult testosterone levels were related to the 2D:4D ratio or handedness. The 2D:4D ratio was measured in 38 participants (15 females and 23 males; mean age = 34.61 years) using Vernier callipers. Measures of hand preference (Annett's Hand Preference Questionnaire) and hand performance (Annett's peg-moving task) were completed. A saliva sample was also taken to test for testosterone levels. It was found that there was no relationship between adult testosterone levels and the 2D:4D ratio or handedness. However, significant differences between the 2D:4D ratios for the left and right hands were related to hand preference and hand performance on a peg-moving task, such that the hand with the greater difference in length between the fourth digit and second digit tended to be the preferred hand. The researchers concluded that the 2D:4D ratio was influenced only by prenatal testosterone levels, not adult testosterone levels, and those with high fetal testosterone resulted in a low digit ratio. Another finding was that the 2D:4D ratio is a significant indicator of hand preference (Beaton, Rudling, Kissling, Taurines, & Thome, 2011). Manning and Peters (2009) found similar results in a large scale study investigating the 2D:4D ratio and hand preference for writing in over 250,000 participants. Individuals would be presented as left handed when the left digit ratio was much lower than the right digit ratio. The opposite held true for right-handed individuals, as the right hand 2D:4D ratio was significantly lower than the left

2D:4D ratio, the majority of people were right handed when writing. Therefore, the 2D:4D ratio has been shown to be a significant predictor of handedness, and specifically with respect to the writing hand (Manning & Peters, 2009).

To conclude, Peters, MacKenzie, and Bryden (2002) reviewed the general trends that have been observed when examining the 2D:4D ratio. A main observation was that recently more studies have been showing a lower ratio for males than females (Peters, MacKenzie, & Bryden, 2002). One of the contributions of this difference in 2D:4D ratios is thought to be the levels and sensitivity of the individual to prenatal testosterone during fetal development (Manning, Scutt, Wilson, & Lew-Jones, 1998). Once the 2D:4D ratio is fixed, which may be as early as the first trimester of gestation, it can then be a significant indicator of handedness of an individual (Beaton, Rudling, Kissling, Taurines, & Thome, 2011).

Focus of the Current Study

Research has shown that the 2D:4D ratio is linked to the writing hand, in the sense that the lower of the two ratios is the preferred hand. Furthermore, the 2D:4D ratio is thought to be sex-linked, in that males tend to have lower ratios in general in comparison to females due to prenatal testosterone levels. To date, most studies focus on the correlation between the 2D:4D ratio and hand preference. The current study will analyze both hand preference and hand performance in regards to the 2D:4D ratio, and whether the digit ratio is a clear indicator of both of these measures. Differences between the sexes will also be examined to see how the relationships between the digit ratios, hand preference, and hand performance may vary. Furthermore, age of the participants will be considered to determine whether any differences lie across an age continuum in regard to handedness and the 2D:4D ratio. These findings can help advance the understanding of how fetal testosterone may influence the handedness of a person, through the indirect measure of the 2D:4D ratio. Furthermore, as handedness is one way to observe lateralization of the brain, this study can provide fundamental knowledge on the indirect ways fetal testosterone may influence such an important component and developmental process of the brain.

Autism Spectrum Disorder

Overall, 1 in 68 children are diagnosed with Autism Spectrum Disorder (ASD), and it is three to four times more likely to occur in boys than it is in girls (Autism Society Canada (ASC), 2014; American Psychiatry Association (APA), 2014). Approximately 515,000 people in Canada are living with ASD. As the prevalence of ASD continues to rise, it is key to determine ways in which ASD can be diagnosed as early as possible to allow for intervention programs (ASC, 2014; APA, 2014).

ASD is a neurological disorder that causes developmental disabilities (ASC, 2014; APA, 2014). These disabilities are seen in the form of difficulties with social interaction, problems with communication, both verbal and nonverbal, as well as repetitive behaviours and restricted interests. All autism disorders including autism, Asperger syndrome and pervasive developmental disorder-not otherwise specified (PDD-NOS) were merged into one diagnosis and are newly classified in the DSM5 as ASD. Articles reviewed in the present study that were published before this new classification occurred though, will be discussed based on the author's use of the terminology of ASD, whether it being autism, Asperger syndrome, or PDD-NOS. ASD is classified on a spectrum as every individual diagnosed with ASD is unique and falls somewhere on this continuum depending on the severity of developmental impairments present. However, early detection can lead to early treatment, which has been shown to result in an improvement in learning, communication, and social skills. If the signs and symptoms of

ASD are reduced through the use of early treatment plans, an overall improvement in the quality of life can be seen (ASC, 2014; APA, 2014).

ASD tends to appear within the first 3 years of age; however, it can be diagnosed earlier (ASC, 2014; APA, 2014). Diagnostic criteria consist of deficits in social communication and interaction such as not maintaining eye contact during conversations or showing no interest in peers. Another criterion is restricted and repetitive behaviours or interests, such as the need for a constant routine, or having hyper-reactivity to sensory inputs. Based on these diagnostic criteria, the individual may be placed somewhere along the spectrum depending on the level of severity of the characteristics observed (ASC, 2014; APA, 2014).

The diagnosis of ASD has increased ten-fold in the past 40 years and this increase in prevalence is only partially due to the improved diagnosis (American Psychological Association, 2012; APA, 2014). ASD has been linked to genetic factors, as there are a number of gene changes and gene mutations that are associated with ASD that have been found (APA, 2014). Furthermore, a family with one child with ASD is more likely to have a second child with ASD. However, the genetic component does not explain ASD fully and other factors may play a role. For example, environmental factors, such as advanced age of the mother and father at the time of conception, low birth weight, a deficiency in folic acid before conception, and obesity in the mother (APA, 2014), show a relationship with ASD. Therefore, both genetic and environmental factors appear to play a role in whether an individual will develop ASD and may be contributing to the increase in prevalence.

With the prevalence of ASD rising so rapidly and the noted benefits of an early diagnosis, such as improved cognitive, motor, and social skills, it is crucial to know the signs and symptoms to look for in an individual to allow for the earliest treatment possible.

Cerebral Lateralization in ASD

To further the understanding of cerebral lateralization and its effect on asymmetric processes, Escalante-Mead, Minshew, and Sweeney (2003) examined how brain asymmetry functions in children with high-functioning autism. Participants included 47 individuals with autism with delayed early language development, 22 individuals with autism with typical early language development, and 112 individuals with no current or past history of psychiatric or neurological disorders. Using the Edinburgh Handedness Inventory, they found that children with autism and early language disturbances had a reduced rate of establishing a preferred hand, indicating an atypical cerebral dominance in the brain (Escalante-Mead, Minshew, & Sweeney 2003). Examining cerebral activity in the brain through functional magnetic resonance imaging (fMRI) in males with high-functioning autism (n = 53) and males with neurotypical development (n = 39), Anderson and his colleagues (2011) found abnormalities in interhemispheric functional connectivity, specifically relating to regions that control cognitive and neurological functions, in the males with autism. The fMRIs showed differences in the sensorimotor cortex, frontal insula, and superior parietal lobule between the control participants and the participants with autism, where the interhemispheric correlation was significantly reduced for those with autism. They concluded that cerebral lateralization is not fully developed in individuals with autism (Anderson et al., 2011). Muller and colleagues (1999) found support for this idea in a study examining blood flow in the brain using a positron emission tomography (PET) scan in 5 individuals with high-functioning autism (4 males, 1 female). Overall, there appeared to be a reduction in left-hemisphere language lateralization in the group with autism, which was evidenced through a decrease in blood flow during cerebellar activation and auditory cortex activation. Muller and colleagues (1999) concluded that there was atypical hemispheric language lateralization in the sample of individuals with autism. Furthermore, Vidal et al., (2006) specifically examined the corpus callosum in 24 male individuals with autism (mean age = 10.0 years) and found aberrant connections leading to abnormal cortical activity. Overall, males with autism had a reduced corpus callosum size that may be constraining interhemispheric connectivity, and therefore result in deficits in the integration of information, which is commonly seen in ASD (Vidal et al., 2006).

In an experiment conducted by Hauck and Dewey (2001), hand preference was examined in children with autism (n = 20), children with developmental disorders (no genetic or chromosomal abnormalities) (n = 20), and children with neurotypical development (n = 20) to investigate brain development. The nonspecific developmental delays group was chronologically and mentally age matched to the children with autism, and the typically developing participants were mentally age matched to the children with autism. Comparing all three groups to one another, the group with autism had a much higher rate of ambiguous hand preference, meaning they did not have a preference for one hand, than the other two groups, leading them to believe that it was a result of bilateral brain damage. With bilateral brain damage, the theory proposes that neither hemisphere of the brain becomes lateralized for certain asymmetrical functions. With less cerebral lateralization occurring, this could in turn affect how strongly the individual prefers and performs with one hand over another (Hauck & Dewey, 2001).

Lindell and Hudry completed a systematic review of the literature in 2013 that analyzed cerebral lateralization in individuals with ASD. It was concluded that individuals with ASD show atypical lateralization with a specific reduction in structural asymmetry. This led Lindell and Hudry (2013) to propose a model in which both genetic and environmental factors will cause an altered development of cerebral lateralization, leading to more ambiguous handedness and impaired functions, such as language deficits. The following section will specifically discuss handedness in more detail and examine how it may differ in individuals with ASD compared to those with neurotypical development.

Handedness in ASD

As cerebral asymmetry appears to be reduced in individuals with ASD and cerebral lateralization influences handedness, hand performance, and hand preference have been closely studied in those with ASD. Dane and Balci (2007) examined handedness in autism and there was a clear increased rate of mixed handedness (n = 37; 27 males, 10 females), meaning the number of left-handed individuals was comparable to that of right-handed individuals (Dane & Balci, 2007). In addition, Soper and his colleagues (1986) previously showed that individuals with autism that had lower cognitive functioning, indicating less cerebral lateralization, tended to be more ambiguous in their handedness. Therefore, as the amount of cerebral lateralization is decreased, it would seem plausible to see a reduction in handedness as well (Soper et al., 1986).

Examining both preference and performance is important when studying handedness as an individual may strongly prefer the right hand, however, on motor tasks the two hands may perform equally well. Therefore, to truly understand whether individuals with ASD have different handedness patterns than individuals who are neurotypical, both hand preference and hand performance should be examined. McManus, Murray, Doyle and Baron-Cohen (1992) investigated both the preference and skill of hands in 20 children with autism, all of which were receiving special education and had no previous neurological problems recorded (15 males and 5 females, mean age = 11.1 years). The participants completed thirteen unimanual tasks and a laterality index was calculated to indicate the degree of lateralization and to determine hand preference. The Annett pegboard task was used to assess the hand skill asymmetry. There was a preference for the right hand; however, there was no actual difference in the skill level between the hands. Therefore, they concluded that the preference for a hand developed prior to skill asymmetry (McManus, Murray, Doyle, & Baron-Cohen, 1992).

Overall, one common occurrence in the studies that examined handedness and ASD is that there is a much greater rate of hand ambiguity in comparison to individuals who are neurotypical (Dane & Balci, 2007; Hauck & Dewey, 2001; Lindell & Hudry, 2013; McManus, Murray, Doyle, & Baron-Cohen, 1992; Soper et al., 1986). Also, the rate of left handedness is higher in ASD populations compared to neurotypical populations, being 15-20% compared to 9%, respectively (Hauck & Dewey, 2001). The increased occurrence of mixed handedness is still not fully understood, yet there appears to be a clear link between handedness and the degree of cerebral lateralization that may be occurring. Hauck and Dewey (2001) found that those children who had a stronger preference for one hand over the other had overall higher functioning than those who were ambiguous in handedness. Such evidence suggests that if children with ASD are taught to focus on developing a hand preference early on in life, it may lead to better future functioning, as it could promote further brain lateralization (Hauck & Dewey, 2001). Furthermore, such research suggest that handedness can be seen as an indicator of the levels of cerebral lateralization in an individual, and it has the potential to be used as an external trigger to increase brain asymmetry in people at a young age with ASD.

The 2D:4D Ratio and ASD

It was previously mentioned that prenatal testosterone levels significantly influence the 2D:4D ratio. It was also noted that individuals with ASD appear to have higher testosterone levels during fetal development. Specifically, fetal testosterone levels were positively associated with Childhood Autism Spectrum Test scores and Child Autism Spectrum Quotient scores (Auyeung, Baron-Cohen, Ashwin, Knickmeyer, Taylor, & Hackett, 2009). These findings led to many studies examining how the 2D:4D ratio may differ between individuals with neurotypical functioning and individuals with ASD. A recent meta-analysis conducted by Teatero and Netley (2013) studying the relationship between the 2D:4D ratio and ASD found a distinct relationship between ASD characteristics and the digit ratio. When ASD characteristics were present, the individual tended to have a much lower digit ratio where the 4th digit is longer than the 2nd digit, indicating what is considered a masculine ratio. Seeing as how high fetal testosterone levels are linked to ASD, a lower 2D:4D ratio is expected, as fetal testosterone has a significant influence on the digit ratio. Specifically, higher levels of testosterone lead to a lower 2D:4D ratio. The relationship between the 2D:4D ratio and ASD characteristics seen by Teatero and Netley (2013), however, was more strongly associated with the right hand. Overall, the researchers found that their results supported the extreme male brain theory when discussing ASD, as the 2D:4D ratio indicated a more masculine digit ratio, and thus, higher prenatal testosterone levels (Teatero & Netley, 2013).

Similar results were seen in a study done by Manning, Baron-Cohen, Wheelwright, and Sanders (2001) where they measured the 2D:4D ratio in families with at least one child with ASD and compared all family members to a control population. The children of the families had either autism or Asperger syndrome and were classified as having either a normal or high IQ. All family members had their 2D:4D ratios measured with Vernier callipers, measuring to 0.01mm, from a photocopy of the ventral surface of their hands, which had been proved to be a highly repeatable measure. Overall, 72 children with autism or Asperger syndrome, 34 typically developing siblings, 88 fathers and 88 mothers were tested. All participants were sex matched, and adult participants were age matched as well, to control participants. Relationships between family members, as well as relationships between the children with autism, children with

Asperger syndrome, and children with neurotypical development were examined. Overall, children with autism had a significantly lower 2D:4D ratio in comparison to the control population and to the individuals with Asperger syndrome. The family members of the individuals with autism also had lower digit ratios compared to the normative values. The researchers concluded that the results suggest that a low 2D:4D ratio may be associated with an increased risk of autism, as it is related to high prenatal testosterone levels (Manning, Baron-Cohen, Wheelwright, & Sanders, 2001).

An additional study investigated the 2D:4D ratio in males between the ages of 6 and 14 years with psychiatric disorders were compared to males between the ages of 6 and 13 years from the general population (n = 96) (de Bruin, Verhij, Wiegman, & Ferdinand, 2006). Those with autism or Asperger syndrome were classified into one group (n = 24), individuals with PDD-NOS formed a second group (n=26), individuals with ADHD or oppositional defiant disorder (ODD) classified a third group (n = 68), and the last group was made up of individuals with one or more anxiety disorders (n = 26). The researchers found that the left 2D:4D ratios for those in the autism/Asperger syndrome group were significantly lower than those in the comparison group, and the right 2D:4D ratios for the autism/Asperger syndrome group were significantly lower than the males with anxiety disorders. Again, the results indicated that higher prenatal testosterone levels, indirectly measured by the 2D:4D ratio, may be present and play a role in ASD in males (de Bruin, Verhij, Wiegman, & Ferdinand, 2006).

From past literature, it is apparent that individuals with ASD have a lower 2D:4D ratio, meaning the ring finger is significantly longer than the index finger, in comparison to the general population. Researchers have proposed that fetal testosterone levels play a vital role in determining the 2D:4D feature, and greater levels of fetal testosterone will cause a lower digit ratio (Beaton, Rudling, Kissling, Taurines, & Thome, 2011; Hönekopp & Watson, 2010). Consequently, research investigating fetal testosterone and ASD is vital in understanding whether a link between the two exists.

Focus of the Current Study

Many studies have focused on the relationship between the 2D:4D ratio and individuals with ASD. It appears that those with ASD have a significantly lower 2D:4D ratio, indicating higher levels of prenatal testosterone. Additionally, individuals with ASD have been shown to have an increase in hand ambiguity. Also, the percentage of left-handed individuals is significantly higher in an ASD population compared to individuals of neurotypical development. Part of the reason for this difference in hand preference has been attributed to the effects of cerebral lateralization, as it appears as though individuals with ASD display smaller amounts of cerebral lateralization compared to a control population. However, there is little research to date linking the 2D:4D ratio and hand preference to individuals with ASD. Therefore, as the 2D:4D ratio is a key indicator of prenatal testosterone levels, and handedness is an indirect measurement of cerebral lateralization, the purpose of the current study is to determine whether there is a correlation between these two factors in individuals with ASD compared to individuals of neurotypical development. This may increase the understanding behind the role fetal testosterone can play in determining cerebral lateralization, specifically through the indirect measurement of handedness, in individuals with ASD.

CHAPTER 2: METHODOLOGY

Participants

Participants included both children and adults over the age of five years old. Those under the age of five years would be unable to understand the questions being asked of them so they were excluded from the current study. The following study was reviewed and received ethics clearance through the Research Ethics Board at Wilfrid Laurier University. For participants under the age of 16, the primary guardians of the participants were provided with an information letter and written consent form. The study details were explained to the child participants in written form through email, as well as verbally described by the primary investigator. Verbal consent was obtained from the child participants prior to participation as well. For those participants over the age of 16 years old, the study details were explained directly to them in written form through an email as well as verbally described by the primary investigator. A consent form was provided to those who wished to participate in the study. If the participant wanted to have a trusted person review the consent form before signing, the option was always available to them.

Participants with Neurotypical Development

There were 46 males, 8 left-handers, and 58 females, 6 left-handers, recruited for the current study for a total of 104 participants. The ages ranged from 5-to-90 years old, with a mean age of 31.93 years old. Refer to Table 2 for descriptive statistics of the study participants.

Table 2. Descriptive characteristics of the study participants with the factors of sex and handedness considered.

Sex	Handedness	Average Age	N
Male	Right-handed	33.71	38
	Left-handed	23.25	8
	Total	**31.89**	**46**
Female	Right-handed	32.13	52
	Left-handed	30.5	6
	Total	**31.97**	**58**

Total	Right-handed Left-handed	32.8 26.36	90 14
	Total	**31.93**	**104**

Participants with ASD

Participants with ASD were recruited via recruitment posters, through word of mouth, and through emails by the primary investigator. A total of five participants with ASD took part in the current study. Therefore, due to the small sample size, the results should be interpreted with caution. Comparisons were made between the sample of neurotypically-developing individuals and those individuals with ASD. The individuals with ASD who participated in the study were diagnosed by a physician and then they self-reported if they were over the age of 16 or if they were under the age of 16 their primary caregiver reported on their behalf. Please refer to Table 3 for additional information.

Table 3. Additional information provided for the participants with ASD.

Participant	Additional Information
1	None
2	Smith Magenis Syndrome, physician diagnosed and then reported by primary caregiver
3	ADHD, hearing loss, vision impairments, and mental health issues, self-reported
4	ADHD, speech impairments, reported by primary caregiver
5	Speech/language impairments, reported by primary caregiver

Questionnaires

Background Questionnaire

The participants completed a background questionnaire to provide the researcher with relevant information, particularly for the participants with ASD (see Appendix A). Participants who were unable to understand the questions being asked had their primary guardian help them complete the background questionnaire on their behalf. The background questionnaire was used to gain knowledge on the participant, such as any known behavioural deficits, social deficits, cognitive deficits, motor disabilities, and sensory impairments. Information from the questionnaire was to be used as a reference to see if there were any relationships between the level of cognitive, social, behavioural, motor, or sensory impairments present in the participant and their 2D:4D ratios as well as information pertaining to their handedness. However, due to the small sample size of participants with ASD (n = 5) and all being fairly high-functioning, the background questionnaire was not examined further.

Waterloo Handedness Questionnaire

The Waterloo Handedness Questionnaire (WHQ) has been shown to be a strong indicator of hand preference of an individual (see Appendix A); however, it must be noted that the WHQ is a subjective self-report questionnaire (Bryden & Roy, 2006). In this study, all participants completed the WHQ, which involved a series of 32 questions to determine the degree of handedness of an individual. The questions required the participant to indicate which hand they would use to perform skilled and unskilled unimanual tasks. The WHQ in Appendix A indicates which questions are considered skilled and which questions are considered unskilled (Bryden & Roy, 2006). Culturally irrelevant questions from the WHQ were removed for those participants under the age of 16 to increase clarity and understanding for them. If the participants were unable to complete the WHQ due to their age or they were unable to understand the questions being asked, either the participant's primary guardian assisted them when filling out the WHQ or the primary researcher would simplify the questions and provide the participant with tools when necessary to assist in answering the questions. Participants rated the frequency that they use a certain hand for each unimanual task or activity being asked based on a 5-point scale; right always (RA), right usually (RU), equal (EQ), left usually (LU), and left always (LA). The responses were then given a numerical value of RA +2, RU +1, EQ 0, LU -1, LA -2. These numbers were added up based on the responses given by the participant, or participant's guardian, to calculate a laterality index value. Strong right-handed individuals acquired a high positive score, while strong left-handed individuals acquired a low negative score. Those who tended not to have a strong preferred hand obtained a score around zero. Both relative WHQ scores, which included the direction of hand preference, and absolute WHQ scores, which only indicated strength of hand preference, were used in the analyses.

2D:4D Measurements

The 2D:4D ratio of all participants was calculated as it is a strong indirect indicator of the prenatal testosterone levels that they were exposed to during pregnancy. In this study, both the left hand and right hand's digit ratios of the participants were calculated. The primary investigator, using a digital Vernier calliper calculating to the nearest 0.01mm, took direct measurements of the 2^{nd} digit and 4^{th} digit on the left hand and the right hand. Measurements were taken on the ventral surface of the hand from the basal crease to the apex of the finger. Although a precise measurement can be taken with the Vernier calliper, it should be noted that there is still always the potential for human error when measuring digit lengths.

Calculating the 2D:4D Ratio

Measurements for the finger lengths were obtained and the ratio for both hands was calculated by dividing the length of the 2^{nd} digit by the length of the 4^{th} digit (2D/4D = 2D:4D ratio). Ratios less than one indicated that the 4^{th} digit was longer than the 2^{nd} digit. Ratios greater than one indicated that the 2^{nd} digit was longer than the 4^{th} digit. The farther the ratio was from one, the larger the difference there was between the two finger lengths.

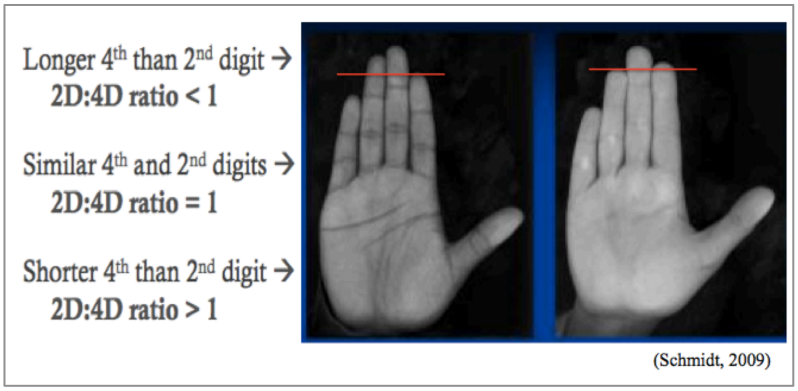

Figure 1. Descriptions and example illustrations of the 2D:4D ratio.

Measurements of Handedness

Tapley-Bryden Dot Marking Task

87 of the 104 neurotypical participants and four of the five participants with ASD completed the Tapley-Bryden Dot Marking task (TBDM task) as a performance-based measurement (see Appendix B). 18 participants had been recruited and tested before the TBDM task had been added to the methodology of the current study. It has been illustrated that the TBDM task is highly correlated with the writing hand of an individual, unlike the pegboard task as illustrated by previous researchers (Tapley & Bryden, 1985). To complete the task, each participant received a piece of paper with four separate trials on it. The individual was provided standardized instructions to make a dot in the circles shown following the pattern on the paper both with speed and accuracy. The first and fourth trials were done with the preferred hand, and the second and third trials were completed with the non-preferred hand. Each trial was 20 seconds in length, which was recorded with a stopwatch. The number of circles that were correctly completed was counted for both the preferred and non-preferred hand trials. The hand difference was then calculated by taking the difference between the total number of circles completed for the right hand trials and the total number of circles completed for the left hand

trials, divided by the total number of circles completed on all four trials ((R-L)/(R+L)). Therefore, a laterality index close to 0 indicated a small performance difference between the hands whereas scores increasing or decreasing away from 0 indicated a person having a larger performance difference between the hands. Positive scores represented a stronger right hand performance, while negative scores represented a stronger left hand performance. Both relative TBDM task scores, which included direction of hand performance, and absolute TBDM task scores, which only indicated strength of hand performance, were used in analysis.

Measurements, Data Preparation, and Hypotheses for the Neurotypical Participants

Data were originally examined for outliers. The only outliers were for the WHQ and TBDM task and they did not appear to skew the overall means. Therefore, no data were removed prior to analyses. Furthermore, the normality of the 2D:4D ratios and hand measurements were examined by inspecting the histograms for a typical normal curve, as well as considering the skewness and kurtosis of the graphs. Overall, all 2D:4D measurements, the absolute WHQ scores, and the absolute TBDM task scores were all determined to be normally distributed. The relative WHQ and relative TBDM task scores did not follow a normal distribution; however, the left handers' scores appeared to be pulling the graphs to the left, which could be causing the negative skew, thus affecting the normality of these two curves.

Analysis of covariance (ANCOVA) tests, with age as the covariate and the factors of handedness and sex, were performed to analyze the 2D:4D ratios, hand preference, and hand performance scores. In addition, analysis of variance (ANOVA) tests, with the factors of handedness, sex, and age as a categorical variable, were performed to analyze the 2D:4D ratios, hand preference, and hand performance scores. When required, pairwise comparisons consisting of independent samples t-tests with a Bonferroni correction were completed following ANOVA tests. Correlational tests (Pearson-correlation) were used to examine relationships between hand preference, hand performance, and 2D:4D ratios. Lastly, independent samples t-tests were performed to compare the neurotypical group to the group of individuals with ASD. All statistical analyses were completed using SPSS Statistics 22.0. A p value of <0.05 was considered significant.

Hand Preference

Hand preference was examined using a laterality index calculated from the WHQ. Sex (male and female), handedness (right handers and left handers), and age were all examined to determine the effects on an individual's hand preference outcome. The relationship between hand preference and age was also examined. Handedness was expected to influence hand preference, such that left handers would not have as strong of a hand preference compared to right handers. A significant effect of age was also hypothesized where hand preference would be lower in children/adolescents and middle to older aged adults compared to young adults.

Hand Performance

Hand performance was examined through a laterality index calculated from the TBDM task. Sex, handedness, and age were all examined to determine the effects on the performance difference between the hands. The relationship between hand performance and age was also examined. Handedness was expected to influence hand performance, where left handers would result in a smaller hand performance difference between the two hands compared to right handers.

Hand Preference and Hand Performance

Correlational tests were run between the WHQ and TBDM task to determine any significant relationship between hand preference and hand performance. A strong positive relationship was hypothesized, where as the WHQ score increased, the TBDM task score would also increase. For example, an individual who strongly prefers their right hand over their left hand would be expected to have a large difference in hand performance scores, where the right hand completed the task much faster than the left hand.

2D:4D Ratio

Analyses were completed to observe the effects sex or handedness may have had on participants' 2D:4D ratios. The right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, which was based on the participant's writing hand, and the difference between the right and left 2D:4D ratios (R-L ratios) were all examined. A comparison of the right hand and left hand digit ratios of participants was completed, specifically studying any differences there may be between the hands' digit ratios among males, females, right-handers, and left-handers. A significant difference between males' and females' digit ratios was expected, where males 4^{th} digit would be longer than the 2^{nd} digit while females would have similar 4^{th} and 2^{nd} digit lengths. Furthermore, it was hypothesized that the individual's preferred hand would have the greater difference in digit lengths.

Hand Preference and the 2D:4D Ratio

Correlational tests were performed between hand preference scores and the 2D:4D ratios. The right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left digit ratios were all examined. The analysis was to determine whether any of the 2D:4D ratios have a strong relationship with hand preference in an individual. A negative relationship was expected between the 2D:4D ratios, specifically the preferred ratio, and hand preference; the lower the 2D:4D ratio, the greater the hand preference score.

Hand Performance and the 2D:4D Ratio

Correlational tests were performed between hand performance scores and the 2D:4D ratios. Again, the right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left digit ratios were all examined. The analysis was to determine whether any of the 2D:4D ratios have a strong relationship with hand performance in an individual. A negative relationship was also hypothesized between the 2D:4D ratios, specifically the preferred ratio, and hand performance; the lower the 2D:4D ratio, the greater the difference in hand performance between the hands.

Measurements, Data Preparation, and Hypotheses for the Participants with ASD

Hand Preference and Hand Performance

Hand preference, indicated by the WHQ, and hand performance, using the TBDM task, were both examined. Correlational tests were performed between the two measures to determine any significant relationship between hand preference and hand performance. A strong positive relationship was hypothesized, where as the WHQ score increased, the TBDM task score would also increase.

2D:4D Ratio

Further analysis was completed to compare the participants' 2D:4D ratios with the hand measurement scores. Hand preference and hand performance were individually correlated with the 2D:4D ratios to examine the strength of the relationships and whether the relationships varied between the two hand measurements. The analysis was to determine whether the 2D:4D ratio could be a reliable predictor of hand preference or hand performance in an individual. A negative relationship was expected between the 2D:4D ratios and hand preference; the lower the

2D:4D ratio, the greater the hand preference score. A negative relationship was also hypothesized between the 2D:4D ratios and hand performance; the lower the 2D:4D ratio, the greater the difference in hand performance between the hands.

Measurements, Data Preparation, and Hypotheses for the Comparison Between the Neurotypical Participants and the Participants with ASD

Comparisons were made between the group of participants with neurotypical development and participants with ASD. Hand preference, hand performance, and the 2D:4D ratios were examined to determine any differences in these outcome measures between the two groups. Participants from the neurotypical group were then matched in the following order for sex, handedness, and age to the participants with ASD. Comparisons between the sex-, handedness-, and age-matched groups were performed, analyzing any differences in the outcome measures of hand preference, hand performance, and the 2D:4D ratios. The participants with ASD were hypothesized to have a decreased hand preference strength and a decreased difference in hand performance scores between the hands in comparison to the group of neurotypical participants. In addition, participants with ASD were expected to have lower 2D:4D ratios than neurotypical participants.

CHAPTER 3: RESULTS

Hand Preference in Neurotypical Participants

** Refer to Appendix D: Table 1, red section for a summary of all results*

Previous literature has demonstrated that hand preference measures may change across the age span (Sivagnanasunderam et al., 2015). In order to take in to consideration any affects age may have had on hand preference scores, analysis of covariance (ANCOVA) tests were performed with age as a covariate. Both relative and absolute WHQ scores were examined (see Table 4 for mean scores).

Table 4. The relative and absolute mean scores of the WHQ for all participants with the factors of sex (males and females) and handedness (right handers and left handers) considered.

Handedness	Sex	N	Relative WHQ	Absolute WHQ
Right-handed	Males	38	41.29	41.29
	Females	8	47.21	47.21
	Total	**46**	**44.71**	**44.71**
Left-handed	Males	52	-2.00	18.50
	Females	6	-12.33	23.67
	Total	**58**	**-6.43**	**20.71**
Total	Males	90	33.76	37.33
	Females	14	41.05	44.78
	Total	**104**	**37.83**	**41.48**

The relative WHQ scores were initially examined using a 2 sex (males and females) by 2 handedness (right handers and left handers) ANCOVA with age as the covariate, to ensure that left handers would result in negative scores while right handers would result in positive scores. No main effects of age or sex were found. A significant main effect of handedness was found, $F_{(1,99)} = 159.23$, $p<0.001$, $\omega = 0.62$, where left handers scored an average of -6.43, while right handers scored an average of 44.71 (see Figure 2). There was also a significant interaction between handedness and sex, $F_{(1,99)} = 4.42$, $p = 0.038$, $\omega = 0.04$, where right-handed males' scores (41.29) were significantly lower than right-handed females' scores (47.21).

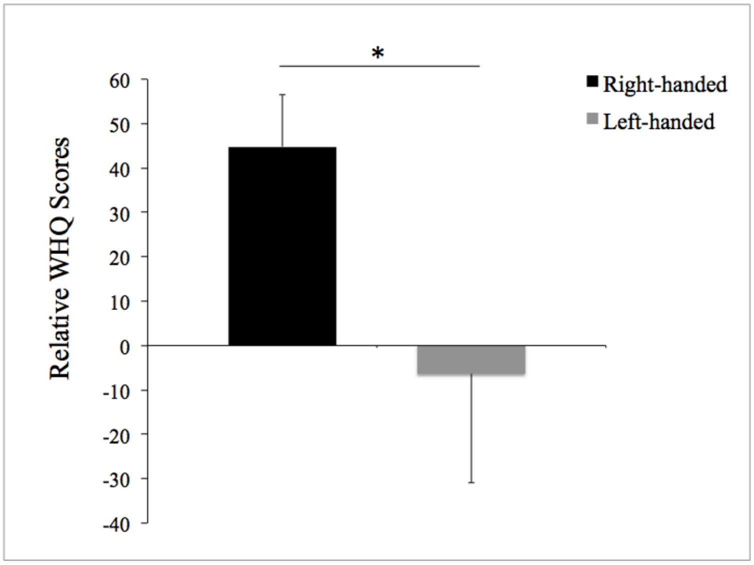

Figure 2. A comparison of the relative WHQ scores between right handers and left handers; right handers scored an average of 44.71, while left handers scored an average of -6.43. $F_{(1,99)} = 159.23$, $p<0.001$. * indicates significance at the 0.05 level.

A second ANCOVA 2 sex (males and females) by 2 handedness (left and right handers) with age as a covariate was performed using the absolute WHQ scores to determine the differences in the strength of hand preference when sex and handedness were considered. No main effects of sex or age were observed. A main effect of handedness was found, $F_{(1,99)} = 43.74$, $p<0.001$, $\omega = 0.31$, where right handers (44.71) and left handers (20.71) absolute WHQ scores were significantly different (see Figure 3). Therefore, left handers did not have as strong of a preference for their preferred hand compared to right handers.

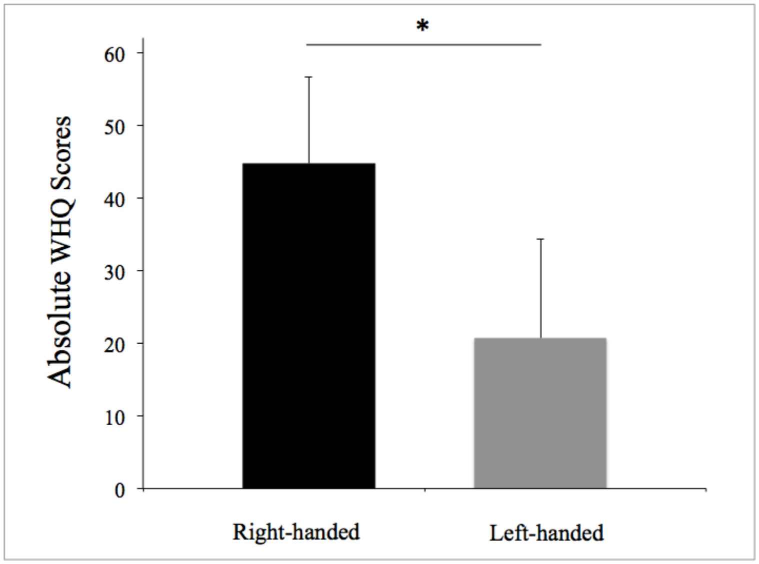

Figure 3. A comparison of the absolute WHQ scores between right handers and left handers; right handers scored an average of 44.71, while left handers scored an average of 20.71. $F_{(1,99)} = 43.74$, $p<0.001$. * indicates significance at the 0.05 level.

To examine if there was a relationship between age and hand preference a correlational test was performed between age and the absolute WHQ scores. No significant relationship was found ($r = 0.11$, $p>0.05$) indicating that hand preference did not seem to vary greatly across the ages (see Figure 1 in Appendix C). The lack of a relationship could explain why there was no main effect of age in the previous ANCOVAs performed that examined the relative and absolute WHQ scores.

Next, age was classified into three categories, which will be used throughout the document, to determine any potential differences in WHQ scores. The children and adolescent age group (5-to-15) was formed with the upper boundary as <18 years old; 18 being considered as the transition year from adolescent to adulthood (Statistics Canada, 2014). The second age group was classified as young adults (20-to-30), which consisted of university students and recent graduates. The lifestyle of the young adults could be influenced by the fact that they have all received a formal education and consequently the results obtained could be affected. Lastly, based on past literature, the middle and older-aged adults were classified as 50+ years old (Brickman et al., 2006; Porac, 1993; Raz, Ghisletta, Rodrigue, Kennedy, & Lindenberger, 2010); the two were classified together due to the small sample of older adults tested. In addition, the older-aged adults were likely a selected sample, as all of them still lived independently and it was expected that their results would be similar to middle-aged adults. Refer to Table 5 for more information.

Table 5. A summary of the characteristics of each age group including the number of participants in each group, the mean age, and the distribution of males and females and left handers and right handers.

Age group (years)	Sample size	Mean age (years)	Males / Females	Right handers / Left handers
5-15	25	8.6	11 / 14	20 / 5
20-30	35	24	14 / 21	31 / 4
50+	27	59.67	12 / 15	25 / 2

A 2 sex by 2 handedness by 3 age group ANOVA was performed to examine the relative WHQ scores. A main effect of handedness was found, $F_{(1,76)} = 100.17$, $p<0.001$, $\omega = 0.57$, where right handers resulted in positive relative WHQ scores (44.86) and left handers resulted in negative relative WHQ scores (-6.00). Additionally, a main effect of age group was found, $F_{(2,76)} = 6.74$, $p = 0.002$, $\omega = 0.15$, where participants 50+ years had significantly greater relative WHQ scores (43.56) compared to both 5-to-15 year olds (35.92) and 20-to-30 year olds (36.26) (see Figure 2 in Appendix C). With respect to interactions, a significant handedness by age group interaction was observed, $F_{(2,76)} = 6.78$, $p = 0.002$, $\omega = 0.15$, where, after examining pairwise comparisons, left-handed participants who were 50+ years (23.00) had greater relative WHQ scores than left-handed 5-to-15 year olds (-8.20) and left-handed 20-to-30 year olds (-17.75) (see Figure 3 in Appendix C). Furthermore, a significant sex, handedness, and age group three-way interaction was found, $F_{(1,76)} = 7.43$, $p = 0.008$, $\omega = 0.09$. After pairwise comparisons were performed, female right handers and left handers significantly differed in their relative WHQ scores in both 5-to-15 year olds (44.92, 7.00, respectively; p = 0.003) and 20-to-30 year olds (45.53, -28.50, respectively; p<0.001) (see Figure 4). Male right handers and left handers significantly differed in the direction of their relative WHQ scores in both 5-to-15 year olds (50.74, -12.00, respectively; p<0.001) and 20-to-30 year olds (39.58, -7.00, respectively; p<0.001). However, in the 50+ years age category, male right handers' (38.50) and left handers' (23.00) scores did not significantly differ in direction from one another (p = 0.096) (see Figure 5). Additionally, a significant difference between male right handers (38.50) and female right handers (49.67) in the 50+ years age category was found (p = 0.024), where females had a stronger hand preference than males (see Figure 6). There was a significant difference between 5-to-15 year-old right-handed males (50.71) and 50+ years' right-handed males (38.50) on relative WHQ scores, where the 5-to-15 year olds had a stronger hand preference than the 50+ years (p=0.040) (see Figure 5). Lastly, a significant difference was observed between left-handed females in the 5-to-15 year olds (7.00) and 20-to-30 year olds (-28.50) on the relative WHQ scores, indicating that children and adolescents prefer their right hand for unimanual tasks even when left-handed in terms of writing unlike left-handed young adults (p=0.017) (see Figure 4). For a list of all mean relative WHQ scores, refer to Table 6.

Table 6. The relative and absolute mean scores for the WHQ by sex (males and females) and handedness (right handers and left handers) within the three age groups.

Handedness	Sex	Age Group	Relative WHQ	Absolute WHQ
Right-handed	**Males**	5-15	50.71	50.71
		20-30	39.58	39.58
		50+	38.50	38.50
		Total	**41.90**	**41.90**
	Females	5-15	44.92	44.92
		20-30	45.53	45.53
		50+	49.67	49.67
		Total	**46.68**	**46.68**
	Total	5-15	46.95	46.95
		20-30	43.23	43.23
		50+	45.20	45.20
		Total	**44.86**	**44.86**

Left-handed	**Males**	5-15 20-30 50+	-12.00 -7.00 23.00	13.50 24.00 23.00
		Total	**-2.00**	**18.50**
	Females	5-15 20-30 50+	7.00 -28.50 --	7.00 28.50 --
		Total	**-16.67**	**21.33**
	Total	5-15 20-30 50+	-8.20 -17.75 23.00	12.20 26.25 23.00
		Total	**-6.00**	**19.27**
Total	**Males**	5-15 20-30 50+	27.91 32.93 35.92	37.18 37.36 35.92
		Total	**32.41**	**36.84**
	Females	5-15 20-30 50+	42.21 38.48 49.67	42.21 43.90 49.67
		Total	**42.88**	**45.16**
	Total	5-15	35.92	40.00
		20-30	36.26	41.29
		50+	43.56	43.56

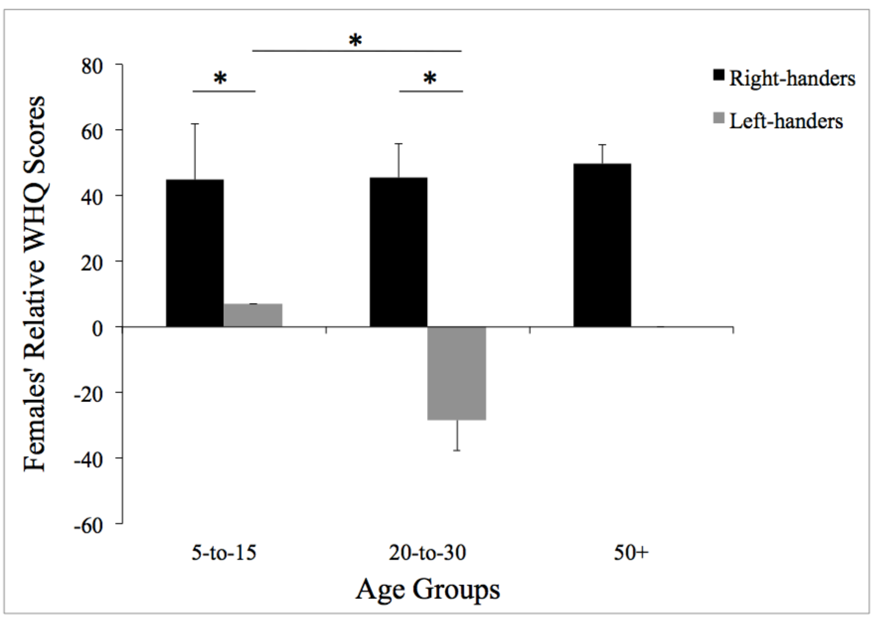

Figure 4. The relative WHQ scores of female participants within the three age groups. 5-to-15 and 20-to-30 year-old right handers and left handers differed in relative WHQ scores. Left-handed females in the 5-to-15 year age group and 20-to-30 year age group differed in relative WHQ scores. * indicates significance at the 0.05 level.

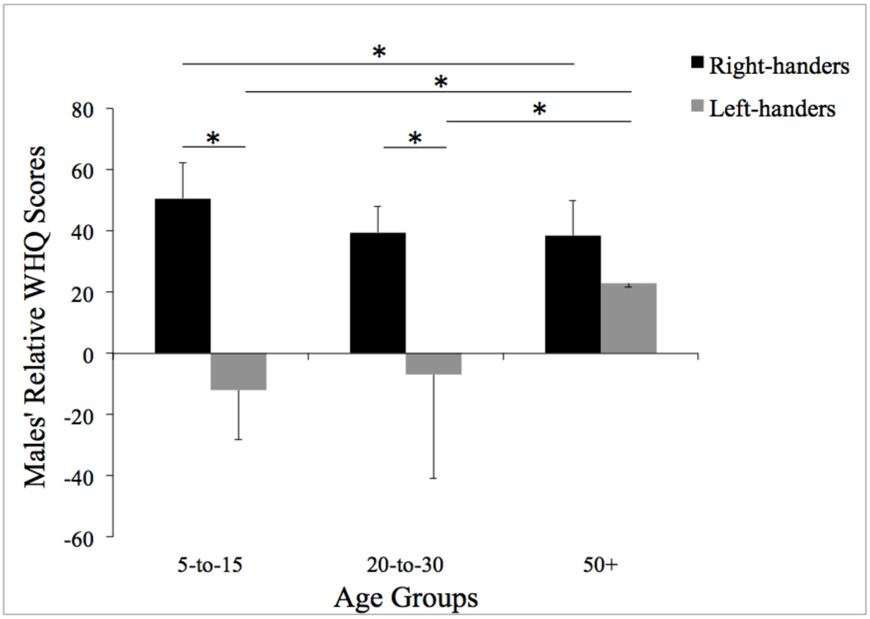

Figure 5. The relative WHQ scores of male participants within the three age groups. 5-to-15 year-old and 20-to-30 year-old right handers and left handers differed in the direction of their relative WHQ scores. Left-handed 5-to-15 year olds and 20-to-30 year olds differed from left-handed 50+ year olds in the direction of their relative WHQ scores. Right-handed 5-to-15 year olds and 50+ year olds differed in relative WHQ scores. * indicates significance at the 0.05 level.

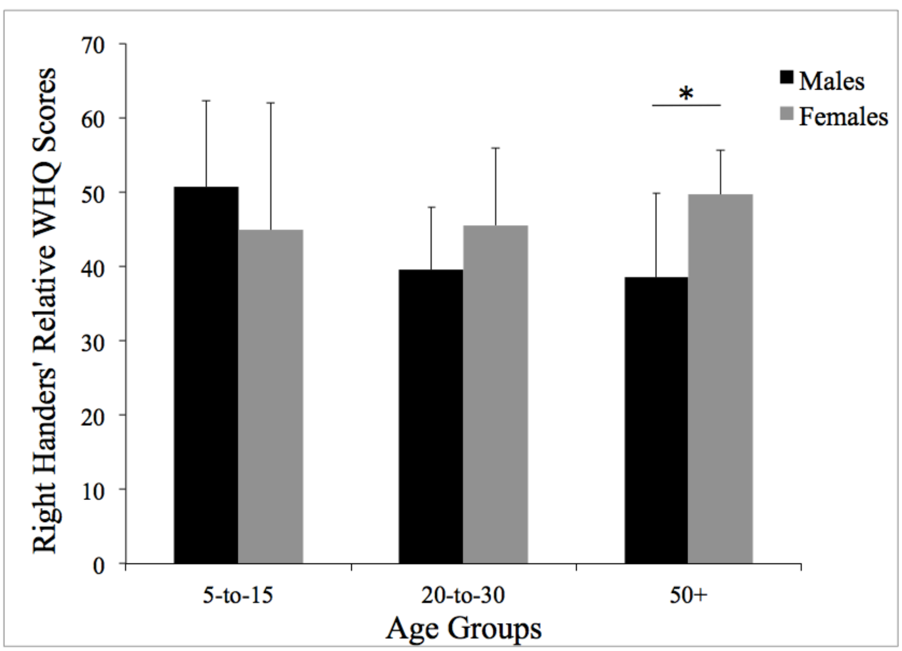

Figure 6. Relative WHQ score comparison of right-handed male and female participants across the three age groups. 50+ year-old males and females differed in relative WHQ scores. * indicates significance at the 0.05 level.

Finally, a 2 sex by 2 handedness by 3 age group ANOVA test was performed to examine the absolute WHQ scores, as differences in the strength of hand preference across the age groups was the main objective. However, no main effect of age group was found. A main effect of handedness was the only significant outcome, $F_{(1,76)} = 32.08$, $p<0.001$, $\omega = 0.30$, where right handers (44.86) had a stronger hand preference than left handers (19.27) on the absolute WHQ scores (see Figure 4 in Appendix C).

Hand Performance in Neurotypical Participants

** Refer to Appendix D: Table 1, green section for a summary of all results*

Previous literature has demonstrated that hand performance measures may change across the age span (Kalisch, Wilimzig, Kleibel, Tegenthoff, & Dinse, 2006). As a result, a 2 sex by 2 handedness ANCOVA was performed with age as a covariate to examine any potential differences in hand performance measurements using the relative TBDM task scores (see Table 7 for all mean values). Here, no main effects of sex or age were found. A main effect of handedness was observed, $F_{(1,82)} = 339.50$, $p<0.001$, $\omega = 0.81$, where right handers resulted in positive TBDM task scores (0.240) and left handers resulted in negative TBDM task scores (-0.197) (see Figure 7).

Table 7. The relative and absolute mean scores of the TBDM task for all participants with the factors of sex (males and females) and handedness (right handers and left handers) considered.

Handedness	Sex	N	Relative TBDM Task	Absolute TBDM Task
Right-handed	Males	34	0.238	0.238
	Females	43	0.241	0.241
	Total	**77**	**0.240**	**0.240**
Left-handed	Males	5	-0.213	0.213
	Females	5	-0.181	0.181
	Total	**10**	**-0.197**	**0.197**
Total	Males	39	0.181	0.235
	Females	48	0.197	0.235
	Total	**87**	**0.190**	**0.235**

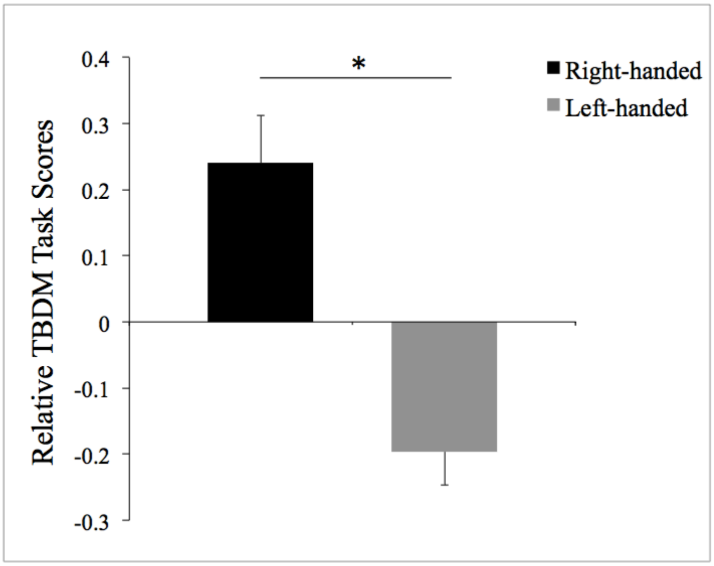

Figure 7. A comparison of the relative TBDM task scores between right handers and left handers; right handers scored an average of 0.240, while left handers scored an average of -0.197. $F_{(1,82)} = 339.50$, $p<0.001$. * indicates significance at the 0.05 level.

Next, the absolute values of the TBDM task were analyzed in a 2 sex by 2 handedness ANCOVA with age as a covariate (see Table 7 for all mean values). There were no main effects of sex or age indicating that hand performance measures appeared to be very similar for males and females across the age span. A non-significant effect of handedness was found, p = 0.058, however one could suggest there is a general trend where right handers (0.240) had a greater hand performance difference between their preferred and non-preferred hand compared to left handers (0.197) (see Figure 8).

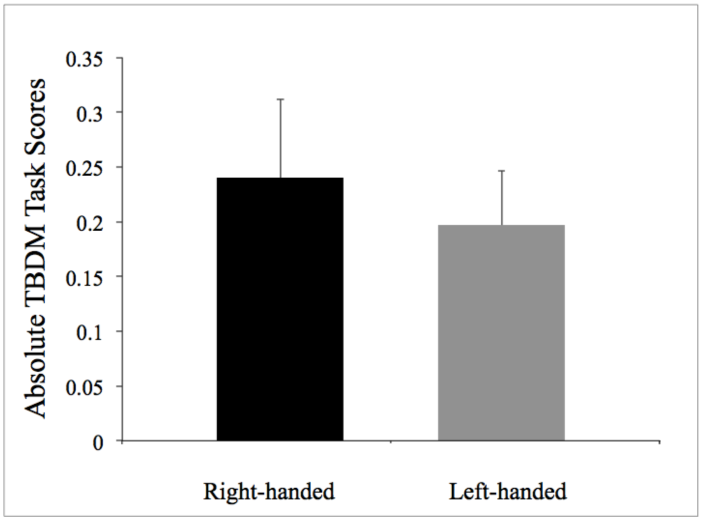

Figure 8. A comparison of the absolute TBDM task scores between right handers and left handers; right handers scored an average of 0.240, while left handers scored an average of 0.197. $F_{(1,82)} = 3.69$, p=0.058.

A correlational test was performed between age and the absolute TBDM task scores to determine any relationships present. However, no significant relationship was found, r = -0.17, p>0.05, indicating that hand performance scores did not seem to vary greatly across the age span (see Figure 5 in Appendix C). The lack of relationship could be a possible explanation as to why there was no main effect of age in the previous ANCOVAs performed when analyzing the relative TBDM task scores.

A 2 sex by 2 handedness by 3 age group ANOVA was performed to analyze the relative TBDM task scores. No main effects of sex or age groups were found. There was a main effect of handedness though, $F_{(1,60)} = 161.20$, $p<0.001$, $\omega = 0.73$, where right handers resulted in positive TBDM task scores (0.240) while left handers resulted in negative TBDM task scores (-0.197). Furthermore, a non-significant interaction between sex and age groups was observed, $F_{(2,60)} = 2.89$, p = 0.063 (see Figure 9). Pairwise comparisons revealed a general trend in which 5-to-15 year-old males' scores (0.127) and females' scores (0.240) as well as 50+ years males' scores (0.130) and females' scores (0.259) were different from one another, where males showed consistently lower relative TBDM task scores. In addition, 20-to-30 year-old females (0.195) appeared to have lower TBDM task scores than both 5-to-15 year-old females (0.240) and females 50 years or older (0.259). Refer to Table 8 for a list of all mean TBDM task scores.

Table 8. The relative and absolute mean scores for the TBDM task by sex (males and females) and handedness (right handers and left handers) within the three age groups.

Handedness	Sex	Age Group	Relative TBDM Task Scores	Absolute TBDM Task Scores
Right-handed	**Males**	5-15	0.258	0.258
		20-30	0.260	0.260
		50+	0.189	0.189
		Total	**0.231**	**0.231**
	Females	5-15	0.240	0.240
		20-30	0.236	0.236
		50+	0.259	0.259
		Total	**0.245**	**0.245**
	Total	5-15	0.247	0.247
		20-30	0.245	0.245
		50+	0.231	0.231
		Total	**0.240**	**0.240**
Left-handed	**Males**	5-15	-0.264	0.264
		20-30	-0.237	0.237
		50+	-0.162	0.162
		Total	**-0.213**	**0.213**
	Females	5-15	--	--
		20-30	-0.196	0.196
		50+	--	--
		Total	**-0.196**	**0.196**
	Total	5-15	-0.264	0.264
		20-30	-0.217	0.217
		50+	-0.162	0.162
		Total	**-0.208**	**0.208**
Total	**Males**	5-15	0.127	0.259
		20-30	0.189	0.257
		50+	0.130	0.184
		Total	**0.157**	**0.228**
	Females	5-15	0.240	0.240
		20-30	0.195	0.232
		50+	0.259	0.259
		Total	**0.223**	**0.243**
	Total	5-15	0.183	0.250
		20-30	0.192	0.242
		50+	0.202	0.226

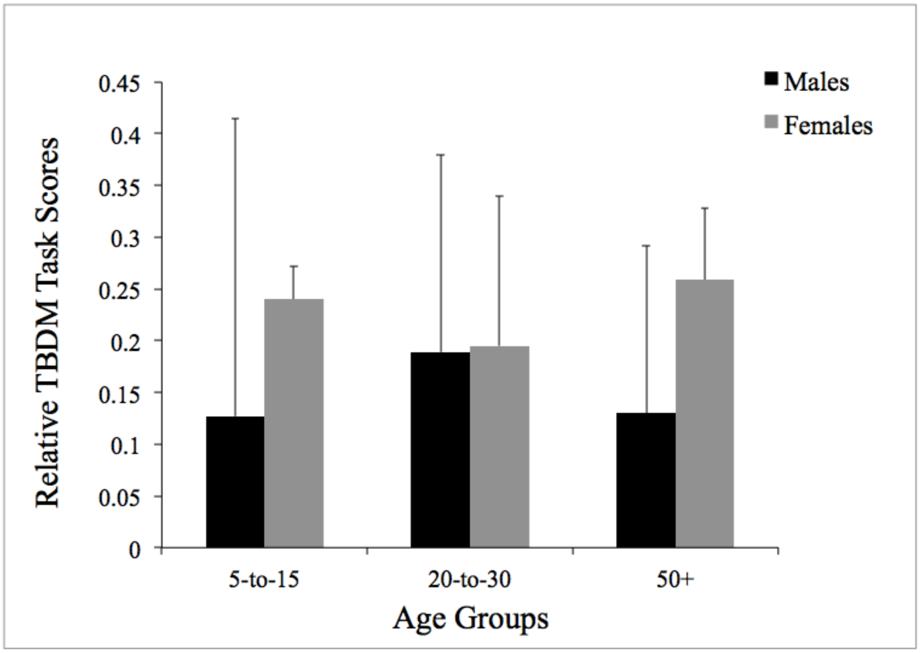

Figure 9. A comparison of the relative TBDM task scores of males and females across the three age groups. A non-significant trend, $F_{(2,60)} = 2.89$, $p = 0.063$, emerged between males and females in the 5-to-15 and 50+ age groups where males had lower TBDM task scores compared to females. Additionally, 20-to-30 year-old females appeared to have lower TBDM task scores compared to 5-to-15 year-old and 50+ year-old females.

Finally, a 2 sex by 2 handedness by 3 age group ANOVA was performed to analyze the absolute TBDM task scores. A non-significant interaction between sex and age group was found, $F_{(2,60)} = 2.89$, $p = 0.063$, and pairwise comparisons indicated a general trend where males (0.184) had smaller differences in hand performance between the hands compared to females (0.259) in the 50+ years age category (see Figure 10). No further significant main effects or interactions were observed.

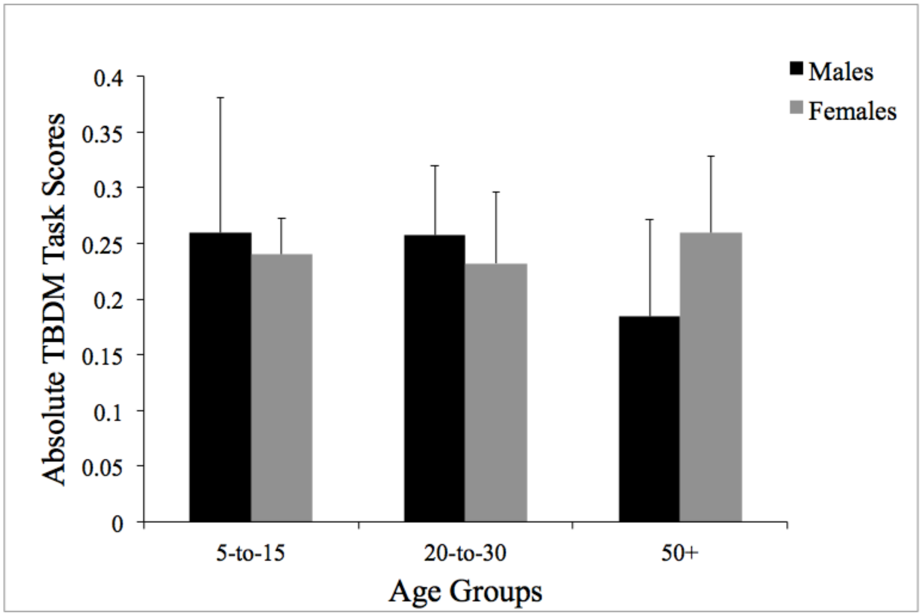

Figure 10. A comparison of the absolute TBDM task scores of males and females across the three age groups. A non-significant trend, $F_{(2,60)} = 2.89$, $p = 0.063$, emerged where males and females in the 50+ age group appeared to differ in their absolute TBDM task scores.

Hand Preference and Hand Performance in Neurotypical Participants

** Refer to Appendix D: Table 1, blue section for a summary of all results*

A strong positive relationship was predicted between the WHQ scores and the TBDM task scores. Therefore, an individual with a very strong hand preference for one hand would also demonstrate a greater difference in hand performance between the hands, specifically favouring the preferred hand. A correlational test was performed between the relative WHQ scores and the relative TBDM task scores to determine the strength of the hand preference and hand performance relationship. A significant Pearson correlation coefficient was found, $r = 0.71$, $p<0.001$, indicating a strong positive relationship between hand preference and hand performance (see Figure 11).

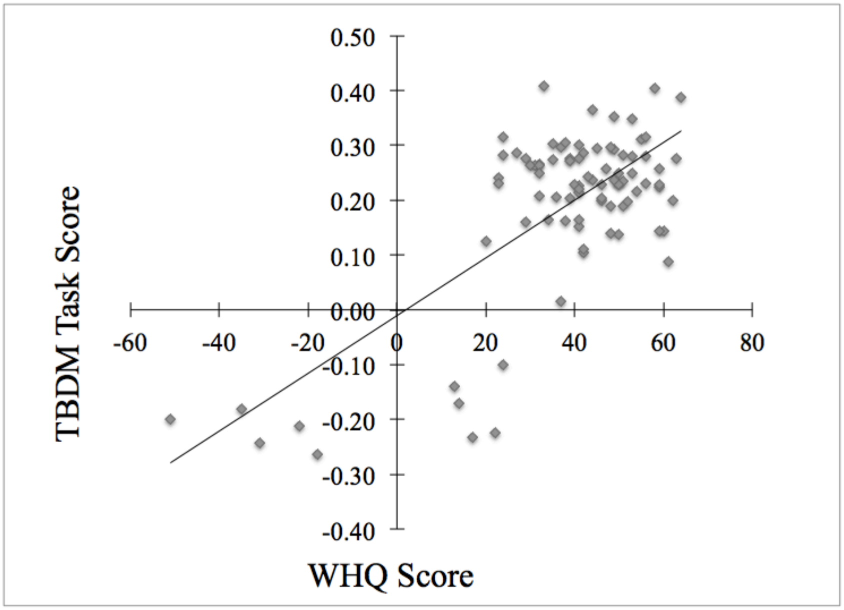

Figure 11. Relationship between hand preference (WHQ scores) and hand performance (TBDM task scores) for right handers and left handers; $r = 0.71$, $p<0.001$.

To determine whether the left handers were influencing the relationship between the two hand measurements though, a correlational test was performed between the absolute values for both the WHQ and TBDM task to examined how the strength of hand preference changes in regard to the strength of hand performance. No significant relationship was observed with a Pearson correlation coefficient of $r = 0.13$, $p>0.05$, thus illustrating that the strength of hand preference and the strength of hand performance are not related.

The 2D:4D Ratio in Neurotypical Participants
** Refer to Appendix D: Table 1, orange section for a summary of all results*

The 2D:4D ratio means of all participants, including the right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left 2D:4D ratios, are displayed in Table 9 below.

Table 9. The average 2D:4D ratio means by sex (males and females) and handedness (right handers and left handers) for all participants.

Handedness	Sex	N	Right 2D:4D Ratio	Left 2D:4D Ratio	Preferred-Hand 2D:4D Ratio	Difference Between the Right and Left 2D:4D Ratios
Right-handed	**Males**	38	0.972	0.980	0.972	-8.142×10^{-3}
	Females	52	0.988	0.995	0.988	-7.380×10^{-3}
	Total	**90**	**0.981**	**0.988**	**0.981**	$\mathbf{-7.702 \times 10^{-3}}$
Left-handed	**Males**	8	0.994	0.987	0.987	6.627×10^{-3}
	Females	6	0.972	0.984	0.984	-1.143×10^{-2}
	Total	**14**	**0.985**	**0.986**	**0.986**	$\mathbf{-1.111 \times 10^{-3}}$
Total	**Males**	46	0.976	0.981	0.974	-5.574×10^{-3}
	Females	58	0.986	0.994	0.987	-7.799×10^{-3}
	Total	**104**	**0.981**	**0.988**	**0.982**	$\mathbf{-6.815 \times 10^{-3}}$

Based on previous literature age should not influence the 2D:4D ratio (Garn, Burdi, Babler, & Stinson, 1975; Manning, Scutt, Wilson, & Lew-Jones, 1998; Trivers, Manning, & Jacobson, 2006). To test this, a 2 sex by 2 handedness ANCOVA with age as a covariate was performed to examine any influence age may have had on the preferred-hand 2D:4D ratio (preferred-hand selected, as it is the digit ratio most commonly used). No main effect of age was found, $p = 0.639$. Correlational tests were also performed between age and the right 2D:4D ratio ($r = -0.098$), left 2D:4D ratio ($r = -0.077$), preferred-hand 2D:4D ratio ($r = -0.060$), and the difference between the right and left digit ratios ($r = -0.024$). No significant relationships were found between age and the 2D:4D ratios. Therefore, the results indicated that age does not have an effect on the 2D:4D ratio.

Four 2 sex by 2 handedness by 3 age group ANOVA tests were performed to examine the right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left 2D:4D ratios (see Table 10 for all mean values). No main effects or interactions were found for any of the outcomes analyzed (see Figure 6 in Appendix C). All of the above ANOVAs were repeated excluding participants that were currently enrolled in a kinesiology program, both at the undergraduate and master's level (see Appendix D: Table 2 for 2D:4D ratio values excluding kinesiology students). However, there were still no main effects of sex, handedness, or age groups found. There was a non-significant interaction between sex and age group when analyzing the difference between the right and left digit ratios, $F_{(2,49)} = 2.93$, $p = 0.063$. A general trend emerged indicating that female 20-to-30 year olds (-3.580×10^{-2}) differed from both female 5-to-15 year olds (1.299×10^{-2}) and 50+ year olds (3.490×10^{-3}).

Table 10. The average 2D:4D ratio means by sex (males and females) and handedness (right handers and left handers) within the three age groups.

Handedness	Sex	Age Group	Right 2D:4D Ratio	Left 2D:4D Ratio	Preferred-Hand 2D:4D Ratio	Difference Between the Right and Left 2D:4D Ratios
Right-handed	**Males**	5-15	0.963	0.984	0.963	-2.008×10^{-2}
		20-30	0.971	0.990	0.971	-1.869×10^{-2}
		50+	0.967	0.973	0.967	-5.245×10^{-3}
		Total	**0.968**	**0.983**	**0.968**	$\mathbf{-1.439 \times 10^{-2}}$
	Females	5-15	0.997	0.986	0.997	1.085×10^{-2}
		20-30	0.982	1.010	0.982	-2.762×10^{-2}
		50+	0.985	0.982	0.985	3.490×10^{-3}
		Total	**0.987**	**0.995**	**0.987**	$\mathbf{-7.048 \times 10^{-3}}$
	Total	5-15	0.985	0.985	0.985	0.026×10^{-3}
		20-30	0.978	1.002	0.978	-2.416×10^{-2}
		50+	0.978	0.978	0.978	-0.004×10^{-3}
		Total	**0.980**	**0.990**	**0.980**	$\mathbf{-9.849 \times 10^{-3}}$
Left-handed	**Males**	5-15	0.991	0.983	0.983	7.167×10^{-3}
		20-30	1.026	1.011	1.011	1.458×10^{-2}
		50+	0.968	0.970	0.970	-2.404×10^{-3}
		Total	**0.994**	**0.987**	**0.987**	$\mathbf{6.627 \times 10^{-3}}$
	Females	5-15	1.010	0.970	0.970	4.079×10^{-2}
		20-30	0.959	0.973	0.973	-1.322×10^{-2}
		50+	--	--	--	--
		Total	**0.976**	**0.972**	**0.972**	$\mathbf{4.786 \times 10^{-3}}$
	Total	5-15	0.995	0.981	0.981	1.389×10^{-2}
		20-30	0.993	0.992	0.992	0.681×10^{-3}
		50+	0.968	0.970	0.970	-2.404×10^{-3}
		Total	**0.989**	**0.983**	**0.983**	$\mathbf{6.125 \times 10^{-3}}$
Total	**Males**	5-15	0.973	0.984	0.971	-1.017×10^{-2}
		20-30	0.979	0.993	0.977	-1.394×10^{-2}
		50+	0.967	0.972	0.968	-4.771×10^{-3}
		Total	**0.974**	**0.984**	**0.972**	$\mathbf{-9.844 \times 10^{-3}}$
	Females	5-15	0.998	0.985	0.995	1.299×10^{-2}
		20-30	0.980	1.006	0.981	-2.624×10^{-2}
		50+	0.985	0.982	0.985	3.490×10^{-3}

		Total	**0.987**	**0.993**	**0.987**	**-6.338×10^{-3}**
	Total	5-15	0.987	0.985	0.985	2.799×10^{-3}
		20-30	0.980	1.001	0.980	-2.132×10^{-2}
		50+	0.977	0.978	0.978	-0.182×10^{-3}

A 2 sex by 2 handedness by 3 age group repeated measures ANOVA test was performed to determine whether there were any differences between an individual's left and right hand digit ratios. No main effects of sex, handedness, or age group on the right and left hand's 2D:4D ratios were found.

Hand Preference and the 2D:4D Ratio in Neurotypical Participants

** Refer to Appendix D: Table 1, purple section for a summary of all results*

Correlational tests were performed to examine the relationships between the 2D:4D ratios and hand preference. The absolute values of the WHQ were used, as the strength of hand preference was the main outcome being analyzed. A Pearson correlation coefficient was calculated for all correlations performed between the absolute WHQ scores and the right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left 2D:4D ratios. No significant relationships were observed between the 2D:4D ratios and the absolute WHQ scores. Table 11 summarizes all Pearson correlation coefficients and the significance levels.

Next, the WHQ was divided into skilled and unskilled unimanual task questions and an additional correlational test was performed with the 2D:4D ratio of participants. The preferred-hand 2D:4D ratio was selected for analysis, as this was the participant's writing hand and therefore the digit ratio most commonly used by each participant. No relationship was found between the preferred-hand 2D:4D ratio and skilled components of the WHQ, $r = -0.008$, $p>0.05$. Right handers and left handers were then separated and correlational tests were performed between their preferred-hand 2D:4D ratios and the skilled WHQ scores. No significant relationships were found (see Table 11). Therefore, the preferred-hand digit ratio did not appear to have any correlation with hand preference strength.

Table 11. Summary of the Pearson correlation coefficients and significance levels for all correlational tests performed between the absolute WHQ scores (including WHQ scores of skilled components only) and the 2D:4D ratios.

	2D:4D Ratio	Pearson Correlation Coefficient	Significance Level
Absolute WHQ	Right 2D:4D Ratio	r = -0.060	p = 0.271
	Left 2D:4D Ratio	r = 0.036	p = 0.357
	Preferred-Hand 2D:4D Ratio	r = -0.011	p = 0.909
	Difference Between the Right and Left 2D:4D Ratios	r = -0.105	p = 0.145
Skilled Components of the Absolute WHQ	Preferred-Hand 2D:4D Ratio	r = -0.008	p = 0.467
	Right-Handers: Preferred-Hand 2D:4D Ratio	r = 0.018	p = 0.434
	Left-Handers: Preferred-Hand 2D:4D Ratio	r = 0.085	p = 0.386

Hand Performance and the 2D:4D Ratio in Neurotypical Participants

** Refer to Appendix D: Table 1, pink section for a summary of all results*

Correlational tests were performed to analyze relationships between hand performance and the 2D:4D ratio. Pearson correlation coefficients were calculated for the correlational tests between the absolute TBDM task scores and the right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left 2D:4D ratios (see Table 12). All relationships were found to be non-significant, thus leading to the conclusion that the 2D:4D ratio does not have any relationship with hand performance.

Table 12. Summary of the Pearson correlation coefficients and significance levels for all correlational tests performed between the absolute TBDM task scores and the 2D:4D ratios.

	2D:4D Ratio	Pearson Correlation Coefficient	Significance Level
Absolute TBDM task	Right 2D:4D Ratio	r = -0.052	p = 0.316
	Left 2D:4D Ratio	r = -0.128	p = 0.120
	Preferred-Hand 2D:4D Ratio	r = -0.078	p = 0.236
	Difference Between the Right and Left 2D:4D Ratios	r = 0.084	p = 0.219

Participants with ASD

Five participants with ASD were examined. Four of the five participants were right-handed and the one participant who was left-handed did not complete the TBDM task; therefore, hand measurements in terms of handedness (right handers vs. left handers) could not be analyzed. Moreover, as all participants with ASD were male, gender could also not be taken into account and analyzed further. Given the low sample, any statistical tests should be interpreted with great caution.

Hand Preference and Hand Performance in Participants with ASD

Table 13 summarizes the scores for the hand measurements tested across all 5 participants, including the overall means of each task. A correlational test was performed between the absolute WHQ scores and absolute TBDM task scores. A non-significant negative relationship was found ($r = -0.527$, $p>0.05$) between the two hand measurements, resulting in an unexpected direction. The non-significant relationship illustrated that as the WHQ scores increased (indicating a stronger hand preference), the TBDM task scores decreased (indicating a weaker hand performance difference between the hands).

Table 13. A comparison of the hand measurement scores for all participants with ASD; both relative and absolute scores are included.

Participant	WHQ	Absolute WHQ	TBDM Task	Absolute TBDM Task
1	30	30	0.12	0.12
2	54	54	0.071	0.071
3	29	29	0.174	0.174
4	-1	1	0.128	0.128
5	-42	42	--	--
Mean Scores	**14.00**	**31.20**	**0.123**	**0.123**

The 2D:4D Ratio in Participants with ASD

Table 14 summarizes the 2D:4D ratios of all five participants with ASD as well as the average ratios. The 2D:4D ratios and hand measurement outcomes were analyzed to see any emerging trends among the two factors. Specifically, correlational tests were performed between the preferred-hand 2D:4D ratio and the absolute WHQ and TBDM task scores. The preferred-hand 2D:4D ratio appeared to decrease as the absolute WHQ scores increased (r = -0.671). A minimum of 9 participants needed to be tested for this relationship to be significant, though. The opposite relationship emerged with regard to hand performance where as the preferred-hand 2D:4D ratio decreased, the TBDM task score also decreased (r = 0.631). An additional six participants, for a minimum of 11 participants, would be needed for this relationship to become significant. Therefore, to determine if these observed relationships hold true on a larger scale and to further the understanding of the 2D:4D ratio and handedness, the collection of data from additional participants with ASD is needed.

Table 14. A comparison of the 2D:4D ratios for all participants with ASD.

Participant	Right 2D:4D Ratio	Left 2D:4D Ratio	Preferred-Hand 2D:4D Ratio	Difference Between the Right and Left 2D:4D Ratios
1	1.035	1.03	1.035	5.77×10^{-3}
2	0.911	0.98	0.911	-6.91×10^{-2}
3	0.995	1.003	0.995	-7.82×10^{-3}
4	1.015	1.026	1.015	-1.18×10^{-2}
5	0.926	0.999	0.999	-7.36×10^{-2}
Mean Scores	**0.976**	**1.008**	**0.991**	$\mathbf{-3.13 \times 10^{-2}}$

Neurotypical Participants vs. Participants with ASD

Comparisons were made between the group of neurotypical participants and the participants with ASD. Independent t-tests were completed for the right and left 2D:4D ratios, preferred-hand 2D:4D ratios, the difference between the right and left digit ratios, absolute WHQ scores, and absolute TBDM task scores. No significant differences were found between the two groups for any of the 2D:4D ratio measures (right ratio, left ratio, preferred-hand ratio, and the difference between the ratios) as well as no difference between the absolute WHQ scores. There was a significant difference found between the TBDM task score of neurotypical participants ($\bar{x}$ = 0.235) and participants with ASD ($\bar{x}$ = 0.123), $t_{(89)}$ = 3.12, p = 0.002, indicating that the difference between the right and left hands' performance on the TBDM task for participants with ASD was smaller compared to neurotypical participants. Examining the absolute WHQ scores further, although not significantly different, individuals with ASD ($\bar{x}$ = 31.20) illustrated a decreased strength of hand preference compared to the neurotypical participants ($\bar{x}$ = 41.48), which matches with the results found for the TBDM task scores.

The participants with ASD were age and sex matched to neurotypical participants and the previous tests were repeated (see Table 15).

Table 15. Summary of the age and sex matched participants' 2D:4D ratios and hand measurement data.

Participant	**Sex**	**Age**	**Absolute WHQ**	**Absolute TBDM Task**	**Right 2D:4D Ratio**	**Left 2D:4D Ratio**	**Preferred-Hand 2D:4D Ratio**	**Difference Between the Right and Left 2D:4D Ratios**
Participants with ASD								
1	Male	15	30	0.12	1.035	1.03	1.035	5.77×10^{-3}
2	Male	8	54	0.071	0.911	0.98	0.911	-6.91×10^{-2}
3	Male	36	29	0.174	0.995	1.003	0.995	-7.82×10^{-3}
4	Male	9	1	0.128	1.015	1.026	1.015	-1.18×10^{-2}
5	Male	6	42	--	0.926	0.999	0.999	-7.36×10^{-2}
Mean Scores	**--**	**14.8**	**31.20**	**0.123**	**0.976**	**1.008**	**0.991**	$\mathbf{-3.13 \times 10^{-2}}$
Participants with Neurotypical Development								
82	Male	18	24	0.316	0.951	0.952	0.951	-8.39×10^{-4}
47	Male	8	58	0.404	0.995	1.012	0.995	-1.68×10^{-2}
29	Male	35	49	0.293	0.998	1.005	0.998	-7.48×10^{-3}
38	Male	10	31	0.264	0.920	0.960	0.920	-3.97×10^{-2}
11	Male	6	1	--	1.048	0.983	0.983	6.51×10^{-2}
Mean Scores	**--**	**15.4**	**32.60**	**0.319**	**0.982**	**0.982**	**0.969**	$\mathbf{4.20 \times 10^{-5}}$

Again, a significant difference between the TBDM task scores of participants with ASD and neurotypical participants was still observed. However, the difference between the two groups was increased as participants with ASD obtained a mean score of 0.123, while the neurotypical participants had a mean score of 0.319; $t_{(6)} = 5.34$, $p = 0.002$ (see Figure 12). No significant differences were found between the two groups in terms of absolute WHQ scores and all 2D:4D ratio measurements analyzed (right 2D:4D ratio, left 2D:4D ratio, preferred-hand 2D:4D ratio, and the difference between the right and left digit ratios).

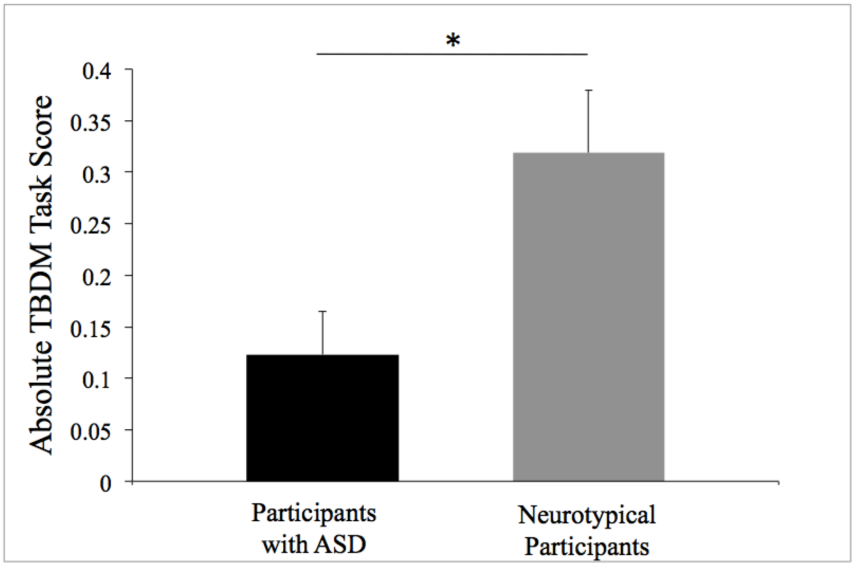

Figure 12. A comparison of the TBDM task scores between the participants with ASD (0.123) and neurotypical participants (0.319); $t_{(6)} = 5.34$, $p = 0.002$. * indicates significance at the 0.05 level.

CHAPTER 4: DISCUSSION

The current studied examined factors influencing the 2D:4D ratio including, hand preference, and hand performance. Comparisons of the three outcome measures between participants with neurotypical development and participants with ASD were investigated. It was hypothesized that handedness would influence both hand preference and hand performance, sex and handedness differences would be observed in the 2D:4D ratios, negative relationships would result between the 2D:4D ratios and hand preference and performance, individuals with ASD would have a decreased handedness, and individuals with ASD would have lower digit ratios than the neurotypical population.

Hand Preference in Neurotypical Participants

Differences in the strength of hand preference have been illustrated between right handers and left handers (Porac & Coren, 1981; Steenhuis & Bryden, 1999). The WHQ scores in the present study differed in strength as a function of handedness, where right handers had significantly stronger WHQ scores than left handers, indicating greater lateralization of right handers. Numerous studies (Bryden, Roy, & Spence, 2007; Mamolo, Roy, Rohr, & Bryden, 2006; Michel, Nelson, Babik, Campbell, & Marcinowski, 2013; Steenhuis & Bryden, 1999; Steenhuis & Bryden, 1989) have shown similar results, perhaps due to the fact left handers must utilize the non-preferred hand (Porac & Coren, 1981) or due to increased amounts of bilateral cerebral activation in left handers, suggesting a reduction in the dominance of one hemisphere of the brain (Pujol, Deus, Losilla, & Capdevila, 1999).

To date, there are mixed results on whether hand preference fluctuates with age (Bryden & Roy, 2006; Dittmar, 2002; Fleminger, Dalton, & Standage, 1977; Sivagnanasunderam et al., 2015). Participants were categorized into three age groups for the present study (children and adolescents ages 5-to-15, young adults ages 20-to-30, and middle to older-aged adults 50+ years). The current study found no significant effect of age on hand preference nor was there a significant linear relationship between age and hand preference. Age-related changes in hand preference may not start occurring until >65 years old (Dittmar, 2001) and the current study only tested five participants >65 years old. Furthermore, children tend to be more variable in hand preference under the age of 6 (Bryden & Roy, 2006; Carlier, Doyen, & Lamard, 2006), of which only 3 participants were tested. Thus, the current study might not be able to detect age-related changes in hand preference.

An interaction between age group and handedness was revealed, indicating that left-handed 50+ year olds had positive WHQ scores that did not differ from right-handed 50+ year olds. Although these participants were left handed when writing, they still scored as having a right-hand preference. Studies conducted by Hugdahl et al. (1993) and Porac (1996) suggest that the middle- to older-aged adult cohorts report a higher incidence of hand preference switch attempts compared to younger generations, which may account for these findings. Likewise, everyday tasks are easier to accomplish left handed nowadays than in previous generations (Hawkyard, Dempsey, & Arthur-Kelly, 2014; Hugdahl, Satz, Mitrushina, & Miller, 1993; Porac, 1996) providing a plausible reason for why left hand writers in the two younger age groups preferred their left hand across a range of tasks on the WHQ.

While no main effect of sex was found, hand preference and sex did interact, where right-handed males demonstrated a weaker hand preference compared to right-handed females. A similar, but non-significant trend was seen in left handers ages 20-to-30. Such findings are consistent with previous work. For example, Sommer et al. (2008) found that males have a higher prevalence of being non-right handed and they are more ambiguous in their hand preference. Furthermore, Lust et al. (2011) found that higher prenatal testosterone levels were related to weaker handedness, providing a potential explanation as to why males from the current study may have trended towards a decreased strength of hand preference. Consequently, research is needed to explain any relationships between fetal testosterone and the handedness of an individual.

Overall, after examining sex, handedness, and age, it was concluded that all three factors play a role in shaping hand preference. Left handers showed weaker hand preference in comparison to right handers, and males also have weaker hand preference compared to females. All three factors appear to interact at some level, suggesting that the developmental trajectory of hand preference for males, females, right handers, and left handers follow a unique path.

Hand Performance in Neurotypical Participants

Handedness, sex, and age affected hand performance in the current study. As would be expected, the direction of hand performance depended upon handedness, where individuals performed best with their preferred hand (Tapley & Bryden, 1985). Although non-significant, the preferred hand of right handers trended to outperform the preferred hand of left handers when completing a task. The fact that left handers must adapt to their surroundings and perform many tasks with their right hand (Mamolo, Roy, Rohr, & Bryden, 2006; Porae & Coren, 1981; Steenhuis & Bryden, 1999), may help account for these findings. Consequently, when left handers complete a task, a smaller difference between the two hands may result. Right handers, however, do not undergo the same practice with the non-preferred hand (Porae & Coren, 1981). As such, a greater difference in performance skill between the two hands is expected. It can thus be argued that left handers not only have a decreased hand preference but they also appear to perform better with their non-preferred hand, supporting previous findings by Steenhuis and Bryden (1999).

There was no effect of age on hand performance in the present study. Based on past research, the influence of age can be considered task dependent (Sivagnanasunderam et al., 2015). Furthermore, similar to the reasoning with hand preference, the current study may not have detected age-related changes in hand performance due to the small sample sizes and variability in the <6 year-old and >65 year-old groups (Bryden & Roy, 2006; Carlier, Doyen, & Lamard, 2006; Dittmar, 2001). However, there are mixed results in the literature on how hand performance may change with age (Bryden & Roy, 2006; Kalisch et al., 2006; Przybyla et al., 2011; Sivagnanasunderam et al., 2015), and the current study is not able to provide a definite answer.

In addition, sex also did not have a main effect on hand performance, however, when age and sex were considered together, there was a non-significant trend where 50+ year-old males had smaller hand performance differences than 50+ year-old females ($p = 0.063$). Dittmar (2011) found similar findings where females started to have a stronger hand performance with their preferred hand while aging. The study did also show that males were becoming more asymmetrical in hand performance as they aged except it was not to the same extent as it was for the females. Overall, based on the current study's findings, 50+ year-old women had significantly greater hand performance asymmetries in comparison to 50+ year-old men.

In conclusion, hand performance does not appear to be affected by sex, handedness, and age when analyzed separately, at least in the current study. Overall, the findings provide evidence of the importance for considering handedness, sex, and age together when investigating hand performance, as the interactions may be what effects an individual's handedness.

Hand Preference and Hand Performance in Neurotypical Participants

An individual with a strong hand preference would be expected to perform better with their preferred hand over the non-preferred hand. However, this does not always appear to be the case. In the current study, relationships were significant only for the relative scores. Absolute scores, however, showed no relationship. Therefore, based on the results, one cannot assume that someone with a very strong hand preference will also have a large difference in hand performance. As a result, the present study further supports Brown et al. (2004) by stressing the need for multiple measures when studying the handedness of individuals, as one measure is not necessarily a reliable indicator.

2D:4D Ratio in Neurotypical Participants

Studies have demonstrated that sex and handedness play a role on the 2D:4D ratio. Specifically, males have longer 4^{th} digits than 2^{nd} digits, while females have similar 4^{th} and 2^{nd} digit lengths (Honekopp & Watson, 2010; Manning, Scutt, Wilson, & Lewis-Jones, 1998). In addition, the preferred hand of an individual tends to have the greater difference between the two digits in comparison to the non-preferred hand (Beaton, Rudling, Kissling, Taurines, & Thome, 2011; Manning & Peters, 2009). One of the aims of the current study was to determine if these observed trends hold true when investigating a large age range. Age was not found to influence the 2D:4D ratio, thus supporting previous research (Garn, Burdi, Babler, & Stinson, 1975; Manning, Scutt, Wilson, & Lew-Jones, 1998; Trivers, Manning, & Jacobson, 2006).

In addition, no significant influence of sex was found on the 2D:4D ratio. Manning and colleagues' (1998) study that found males' ratios significantly lower than females' ratios had 800 participants (females = 400). Since the digit ratio is such a small scale measurement, the current study (n = 104) likely had too small a sample size to observe an effect of sex. Considering the sample included participants from a Kinesiology program, which likely included a high proportion of athletes and athleticism tends to be related to low 2D:4D ratios (Moffit & Swanik, 2010), the current study removed this subsample. However, there were still no significant differences between males and females. In conclusion, the current study was believed to have had too small of a sample size to illustrate a sex difference on the 2D:4D ratios.

Finally, the role of handedness on the 2D:4D ratio was examined. Manning and Peters (2009) found that individuals who have a right digit ratio that is significantly lower than the left digit ratio are typically right-handed for writing. The reverse held true for left-handers (Manning & Peters, 2009). In addition, Beaton and colleagues (2011) found that an individual's hand with the greater difference in length between the 2nd and 4th digits was most often their preferred hand. Similar results were expected of the current study, however, no significant effect of handedness was found. Thus, the difference in digit ratios did not indicate the direction of one's handedness. Investigating sample size numbers again though, the study conducted by Manning and Peters (2009) had 255,116 individuals participate. Therefore, in order to reveal a significant effect of handedness on digit ratio difference between the hands, a large sample size may be needed. In conclusion, the 2D:4D ratio cannot be an indicator of handedness of an individual by examining the differences between a person's right and left hand digit ratios.

Hand Preference, Hand Performance, and the 2D:4D Ratio in Neurotypical Participants

Research to date has shown that higher fetal testosterone levels are indicative of increased amounts of cerebral lateralization, and one way this has been illustrated is through an increase in the strength of handedness of an individual (Grimshaw, Bryden, & Finegan, 1995; McManus & Bryden, 1991). Fetal testosterone has also been linked to the 2D:4D ratio, where there is a negative relationship between the amount of fetal testosterone and the 2D:4D ratio

(Beaton, Rudling, Kissling, Taurines, & Thome, 2011; Honekopp & Watson, 2010; Manning, Scutt, Wilson, & Lewis-Jones, 1998). Taking both of these findings into consideration, the current study hypothesized that as the strength of handedness increased, in terms of both preference and performance, the 2D:4D ratio would decrease. To date, no study has examined the direct link between handedness and the 2D:4D ratio with both variables being measured on a continuous scale. The correlational tests performed between hand preference and the 2D:4D ratios and hand performance and the 2D:4D ratios did not, however, show any relationships.

Additional correlational tests were performed between the skilled components of the WHQ (Steenhuis & Bryden, 1989) and the 2D:4D ratios. Here it was thought that the digit ratio might have a stronger relationship to only skilled tasks, as skilled tasks may expose hand preference to a greater degree. As a result, extreme right handers and extreme left handers would become more visible and relationships may be easier to see between the 2D:4D ratios. However, again no significant relationships were found.

Therefore, the current study demonstrated that the degree to which the 2D:4D ratio changes across a population is not related to the degree hand preference or hand performance changes across the same population. These results then question whether fetal testosterone, having been linked to both cerebral lateralization and the 2D:4D ratio, acts in a completely separate manner on both of these factors.

Hand Preference and Hand Performance in ASD

Participants with ASD were expected to have similar trends as those participants with neurotypical development when analyzing the hand preference and hand performance relationship. As the strength of the preference for the preferred hand increases, hand performance was also expected to increase, resulting in a greater difference in hand performance skill between the two hands. However, in the five participants with ASD tested, the opposite trend appeared. Hand preference scores increased, while hand performance scores decreased. McManus and his colleagues' (1992) findings could help explain the unexpected trend, as the participants with ASD had a preference for the right hand, but there was no actual skill difference between the hands. Such results indicate an increase in hand ambiguity (McManus, Murray, Doyle, & Baron-Cohen, 1992), which could explain the unusual relationship between hand preference and hand performance found in the current study.

The 2D:4D Ratio, Hand Preference, and Hand Performance in ASD

The 2D:4D ratio, hand preference, and hand performance were examined in individuals with ASD. It should be noted that research to date has not investigated the change of the 2D:4D ratio with respect to the change of hand preference or hand performance in participants with ASD. As higher fetal testosterone levels have been linked to ASD, a lower 2D:4D ratio was expected (Teatero & Netley, 2013). In addition, individuals with ASD appear to have weaker handedness, and thus more hand ambiguity (Dane & Balci, 2007; Hauck & Dewey, 2001; Lindell & Hudry, 2013; McManus, Murray, Doyle, & Baron-Cohen, 1992; Soper et al., 1986). As a result, the relationship between fetal testosterone levels, indirectly measured by the 2D:4D ratio, and handedness in individuals with ASD was of interest to the current study.

As lower 2D:4D ratios were expected to be associated with stronger hand preference and hand performance scores in the participants with neurotypical development, similar findings were predicted to occur in individuals with ASD. The observed relationships must be interpreted with caution, as only five participants with ASD were tested. The positive relationship between the 2D:4D ratio and hand preference was expected, indicating potentially higher fetal testosterone related to greater strengths of hand preference. Interestingly, the opposite relationship was found for hand performance, indicating potentially higher fetal testosterone related to a decrease in the difference between the hands for performance. Again, such a finding could be due to the increased hand performance ambiguity among those individuals with ASD (Dane & Balci, 2007; Hauck & Dewey, 2001; Lindell & Hudry, 2013; McManus, Murray, Doyle, & Baron-Cohen, 1992; Soper et al., 1986).

Neurotypical Participants Compared to Participants with ASD

Comparisons between participants with ASD and those with neurotypical development indicated no differences in the 2D:4D ratios. It was hypothesized that those with ASD would have significantly lower 2D:4D ratios than neurotypical individuals, however, this was not found. It is plausible that these results were a function of the low sample size. For example, Teatero and Netley (2013) found digit differences when comparing ASD (N=352) to control participants (N=353). Furthermore, Manning and colleagues (2001) included 72 children with ASD to compare to both siblings and parents, all of which were then sex and/or age matched to control participants. Clearly, more participants were needed in the current study.

Hand preference and hand performance were also compared between the participants with ASD and neurotypical participants. It was expected that those with ASD would have decreased strengths of hand preference and reduced differences in the performance between the hands. No significant differences were found for hand preference; however, overall individuals with ASD did have lower scores than the neurotypical group, indicating weaker hand preference. No differences were found when participants were age and sex matched. All participants with ASD tested were relatively high functioning, and Soper and colleagues (1986) did find that hand ambiguity was more common in individuals with ASD that were lower functioning. Thus, hand preference differences could be more apparent if participants with ASD that ranged along the ASD spectrum were tested.

Hand performance was significantly different between participants with ASD and neurotypical participants, as found by McManus and colleagues (1995). Such results could be attributed to abnormal cerebral lateralization, specifically a reduction in brain asymmetries (Dane & Balci, 2009; Lindell & Hudry, 2013) in individuals with ASD, resulting in weaker handedness.

Overall, participants with ASD did not have lower 2D:4D ratios; although, they did have reduced hand performance differences between the hands in comparison to neurotypical participants. Furthermore, no relationships were found between the 2D:4D ratios and hand preference and hand performance.

Limitations

There was a lack of an adequate sample size in order to determine differences in the 2D:4D ratios between males and females, preferred and non-preferred hands, and neurotypical participants and participants with ASD. Further testing would need to be completed to observe any such differences. In addition, the sample of participants collected, with the young adults being university students and the older adults being selectively sampled, was not as varied as a random sample would have been. As such, this is a limitation to the study as the reduced variability could have influenced the hand measurements tested.

Hand preference questionnaires, specifically the WHQ used in the current study, can be inaccurate with regards to children due to verbal comprehension (Bryden, Roy, & Spence, 2007), and recall that the majority of the children and adolescent participants did not complete the hand performance task. As such, the reflected handedness of the children and adolescents could be inaccurate. For future studies, the collection of data should involve an addition hand preference measure, such as the WatHand Cabinet Task, as well as a hand performance task to gain a truer understanding of the handedness of a child.

Future Directions

Future studies could test the levels of fetal testosterone during pregnancy and follow up with the children testing both handedness and 2D:4D ratios before the age of 6, when handedness is quite varied (Bryden & Roy, 2006; Carlier, Doyen, & Lamard, 2006), and in young adulthood, when handedness is more developed (Bryden & Roy, 2006; Kalisch et al., 2006; Przybyla et al., 2011). Age-related analyses and correlations with fetal testosterone could be examined for both handedness and digit ratios, providing evidence for possible relationships between the outcomes analyzed. Unfortunately, due to the longitudinal design though, it would be many years before these data could be analyzed.

The current study gathered indirect information on potential ways fetal testosterone and cerebral lateralization may be linked. However, the study's results illustrated no existing relationships between digit ratios and handedness. As such, the exploration of fetal testosterone and cerebral lateralization must be examined directly. A starting point would be to manipulate fetal testosterone levels in animal models and record the resulting cerebral lateralization. Although results would not be directly transferrable to humans, it would help gain a better understanding of the importance of fetal testosterone on brain development. Taking this further, critical periods of fetal testosterone's impact on brain development could be noted.

Gaining a more conclusive understanding about hand ambiguity in individuals with ASD, with fetal testosterone as a potential explanation, could provide insight into differences between the brain of neurotypical individuals and individuals with ASD. As Hauck and Dewey (2001) suggested, the early promotion for children with ASD to focus on one hand could have an effect on brain lateralization. If better future functioning results from this, research must then be done to investigate whether this could be a plausible intervention for children with ASD.

Conclusions

The current study found that handedness, sex, and age were all important factors when examining a neurotypical individual's hand preference and hand performance. Furthermore, the results supported previous research by concluding that those with ASD do have reduced handedness, particularly in the form of hand performance (Lindell & Hudry, 2013; McManus, Murray, Doyle, & Baron-Cohen, 1992). Unfortunately, as no strong relationships could be observed between the 2D:4D ratios and hand preference and hand performance, it could not be concluded that fetal testosterone may have an influence on the strength of handedness of an individual.

Researchers are trying to find ways to diagnose ASD earlier, and thus provide earlier interventions. It is important, however, to fully understand the neurotypical population before any studies can be undertaken in individuals with ASD. This study has contributed to the body of knowledge and now, knowing more about the relationships, or lack thereof, between the 2D:4D ratio and handedness in a neurotypical population, future research can move in a direction that will be more helpful in understanding ASD.

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Appendix A: Questionnaires

Background Questionnaire: Parent/Guardian **Date: ________________**

Please complete the following questions by either checking the box with the most appropriate answer or filling in the blank space provided.

Questions about you:

1. Please indicate your age: ________

2. What is your relationship with the child?

____

3. What is your gender?

☐ Male ☐ Female

Questions about your family member:

1. What is your family member's gender? ☐ Male ☐ Female

2. What is your family member's date of birth (dd/mm/yyyy):

____

3. Has your family member ever experienced a hand or finger injury? If so, please explain below:

____
____
____

4. Does your family member have any of the following conditions? Check all that apply.

- ☐ Autism Spectrum Disorder
- ☐ Attention Deficit/Hyperactivity Disorder
- ☐ Down Syndrome
- ☐ Learning Disability
- ☐ Deafness/Hearing Loss
- ☐ Speech/Language Impairment
- ☐ Vision Impairment
- ☐ Other? Please specify: ____

Continued on reverse.

5. Does your family member exhibit any of the following signs and/or symptoms? Check all that apply. If selected, please rate severity on a scale of 1 to 10 (1 being minimal deficits, 5 being moderate, and 10 being severe).

□ Social/Communication deficits
1 2 3 4 5 6 7 8 9 10

□ Behavioural deficits
1 2 3 4 5 6 7 8 9 10

□ Cognitive/learning delays
1 2 3 4 5 6 7 8 9 10

□ Physical/motor disabilities
1 2 3 4 5 6 7 8 9 10

□ Sensory impairments
1 2 3 4 5 6 7 8 9 10

Thank you for taking the time to complete this background questionnaire!

Background Questionnaire: Participant **Date: ___________________**

Please complete the following questions by either checking the box with the most appropriate answer or filling in the blank space provided.

Questions about you:

1. What is your gender? ☐ Male ☐ Female

2. What is your date of birth (dd/mm/yyyy):

____

3. Have you ever experienced a hand or finger injury? If so, please explain below:

____
____
____

4. Do you have any of the following conditions? Check all that apply.

- □ Autism Spectrum Disorder
- □ Attention Deficit/Hyperactivity Disorder
- □ Down Syndrome
- □ Learning Disability
- □ Deafness/Hearing Loss
- □ Speech/Language Impairment
- □ Vision Impairment
- □ Other? Please specify: ____

5. Do you exhibit any of the following signs and/or symptoms? Check all that apply. If selected, please rate severity on a scale of 1 to 10 (1 being minimal deficits, 5 being moderate, and 10 being severe).

□ Social/Communication deficits
1 2 3 4 5 6 7 8 9 10

□ Behavioural deficits
1 2 3 4 5 6 7 8 9 10

□ Cognitive/learning delays
1 2 3 4 5 6 7 8 9 10

□ Physical/motor disabilities
1 2 3 4 5 6 7 8 9 10

□ Sensory impairments
1 2 3 4 5 6 7 8 9 10

Thank you for taking the time to complete this background questionnaire!

WATERLOO HANDEDNESS QUESTIONNAIRE

<u>Instructions</u>

Answer each of the following questions as best you can. If you <u>always</u> use one hand to perform the described activity, circle **RA** or **LA** (for right always or left always). If you <u>usually</u> use one hand circle **RU** or **LU** (for right usually or left usually), as appropriate. If you use both hands equally often, circle **EQ**.

Do not simply circle one answer for all questions, but imagine yourself performing each activity in turn, then mark the appropriate answer. If necessary, stop and pantomime the activity.

1. Which hand do you use for writing? *Skilled*

   LA LU EQ RU RA

2. In which hand would you hold a heavy object? *Classified as neither*

   LA LU EQ RU RA

3. With which hand would you unscrew a tight jar lid? *Unskilled*

   LA LU EQ RU RA

4. In which hand do you hold your toothbrush? *Skilled*

   LA LU EQ RU RA

5. With which hand would you pick up a penny off a desk? *Unskilled*

   LA LU EQ RU RA

6. In which hand would you hold a match to strike it? *Skilled*

   LA LU EQ RU RA

7. With which hand do you throw a baseball? *Skilled*

   LA LU EQ RU RA

8. With which hand would you pet a cat or a dog? *Unskilled*

   LA LU EQ RU RA

9. Which hand would you use to pick up a nut or a washer? *Unskilled*

   LA LU EQ RU RA

10. Which hand do you consider the strongest? *Unskilled*

    LA LU EQ RU RA

11. Over which shoulder would you swing an axe? *Classified as neither*

    LA LU EQ RU RA

12. With which hand would you pick up a comb? *Unskilled*

    LA LU EQ RU RA

13. With which hand do you wind a stopwatch? *Skilled*

    LA LU EQ RU RA

14. With which hand would you pick up a bat? *Unskilled*

    LA LU EQ RU RA

15. With which hand would you pick up a piece of paper off a desk? *Unskilled*

    LA LU EQ RU RA

16. With which hand do you use a pair of tweezers? *Skilled*

    LA LU EQ RU RA

17. With which hand would you throw a spear? *Skilled*

    LA LU EQ RU RA

18. With which hand would you hold a cloth when dusting furniture? *Unskilled*

    LA LU EQ RU RA

19. With which hand do you flip a coin? *Skilled*

    LA LU EQ RU RA

20. In which hand would you hold a knife to cut bread? *Skilled*

    LA LU EQ RU RA

21. With which hand do you use the eraser on the end of a pencil? *Skilled*

    LA LU EQ RU RA

22. With which hand would you pick up a toothbrush? *Unskilled*

    LA LU EQ RU RA

23. With which hand would you hold a needle when sewing? *Skilled*

    LA LU EQ RU RA

24. On which shoulder do you rest a baseball bat when batting? *Classified as neither*

    LA LU EQ RU RA

25. In which hand would you carry a briefcase full of books? *Classified as neither*

    LA LU EQ RU RA

26. In which hand would you pick up a jar? *Unskilled*

    LA LU EQ RU RA

27. With which hand do you hold a comb when combing your hair? *Skilled*

    LA LU EQ RU RA

28. With which hand would you pick up a pen? *Unskilled*

    LA LU EQ RU RA

29. Which hand do you use to manipulate implements such as tools? *Skilled*

    LA LU EQ RU RA

30. Which hand would you use to put a nut washer on a bolt? *Skilled*

    LA LU EQ RU RA

31. With which hand would you pick up a baseball? *Unskilled*

LA	LU	EQ	RU	RA

32. Which hand is the most adept at picking up small objects? *Unskilled*

LA	LU	EQ	RU	RA

33. Is there any reason (ie. injury) why you do not use the hand you prefer to use for any of the above activities?

YES NO (Circle one)

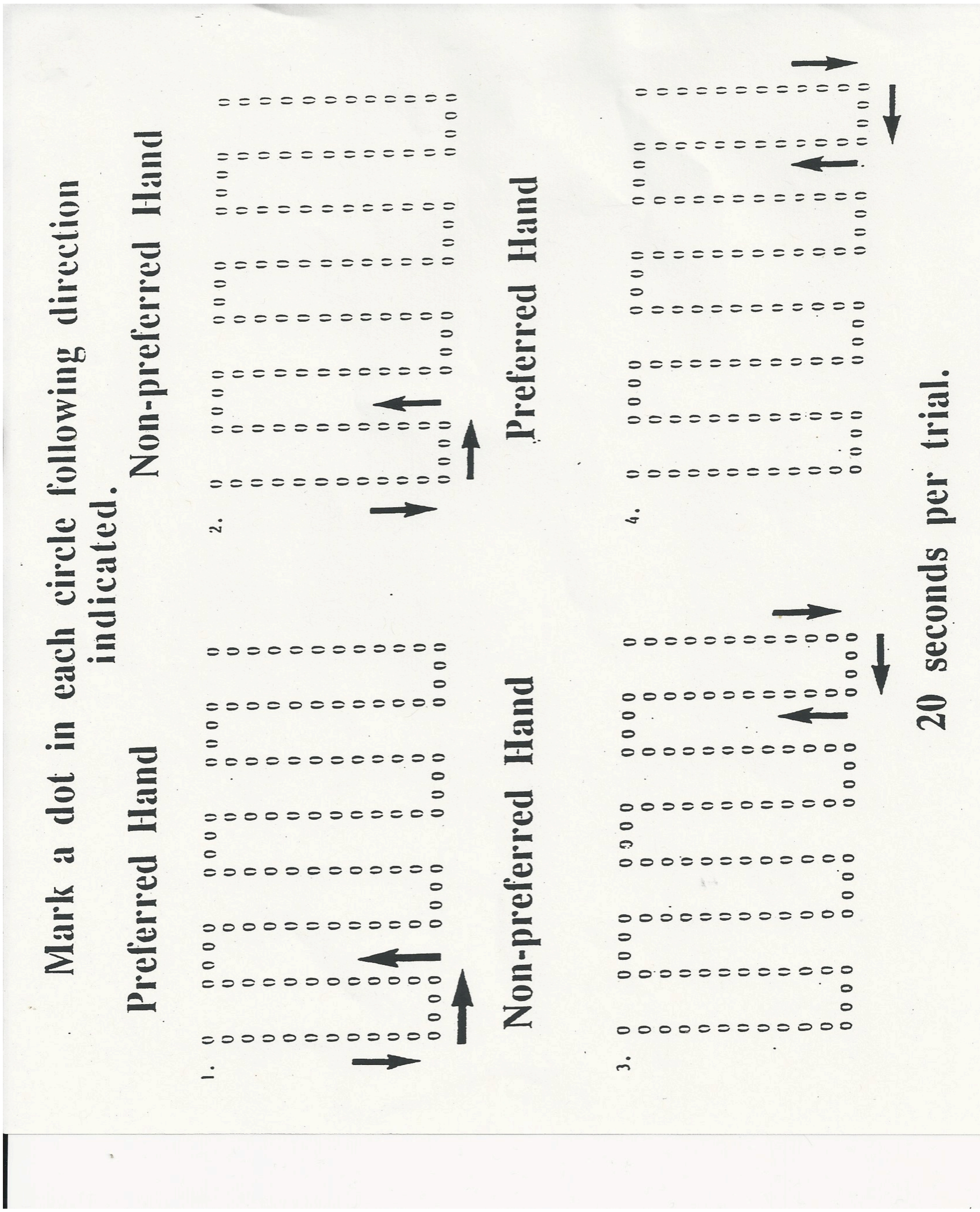
If yes, please explain why you do not use your preferred hand and which activities are affected.

34. Have you ever been given special training or encouragement to use a particular hand for certain activities?

YES NO (Circle one)

If yes, please explain the special training and which activities are affected.

Appendix B: Methodology – Figures



(Tapley & Bryden, 1985)

Figure 1. The Tapley-Bryden Dot Marking Task. The participant must place a dot in the circles shown following the given pattern on the sheet. The task consists of 4 trials in total; the 1st and 4th trials are to be completed with the writing hand and the 2nd and 3rd trials are to be completed with the non-writing hand. Twenty seconds are allotted for each trial.

Appendix C: Results – Figures

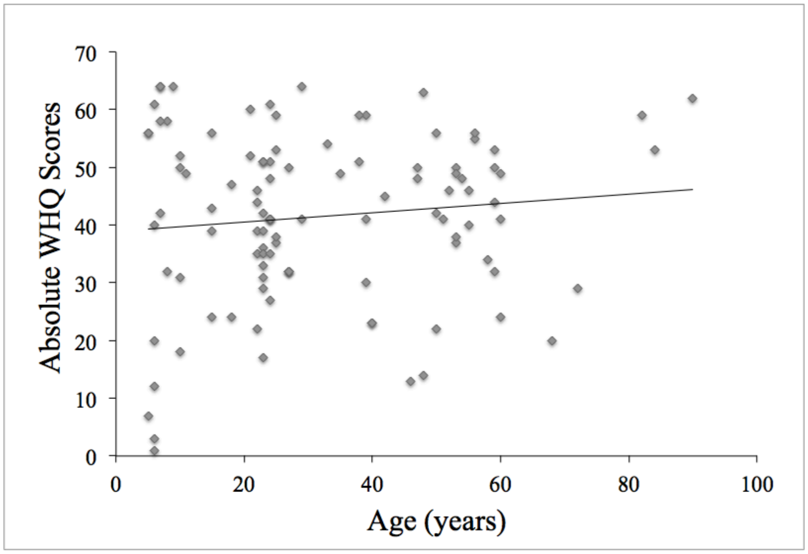


Figure 1. The correlation between absolute WHQ scores and age, r = 0.11, p>0.05, indicating no significant relationship among hand preference and age.

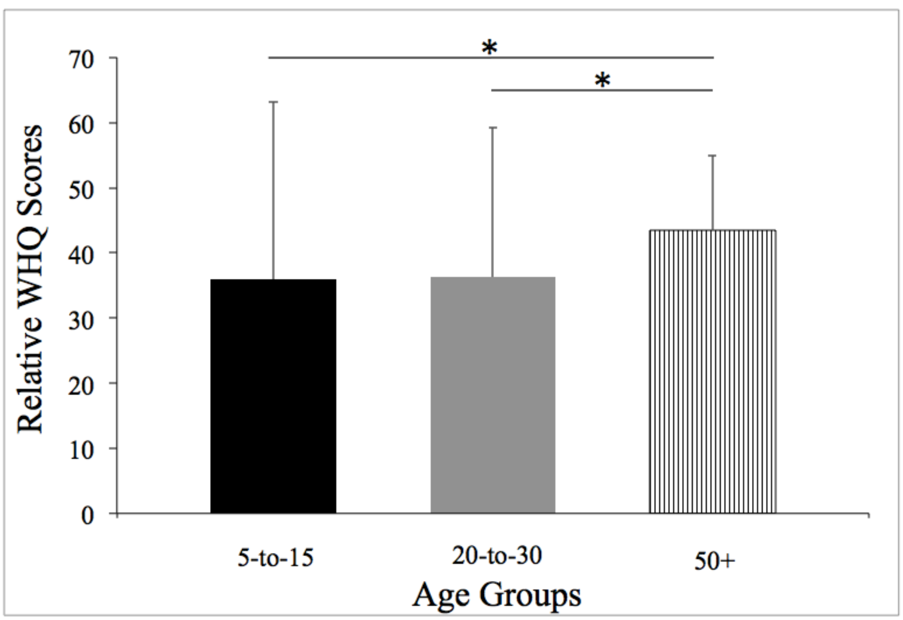


Figure 2. A comparison of the relative WHQ scores across the three age groups. 5-to-15 year olds averaged 35.92, 20-to-30 year olds averaged 36.26, and 50+ years averaged 43.56; $F_{(2,76)} = 6.74$, p = 0.002. * indicates significance at the 0.05 level.

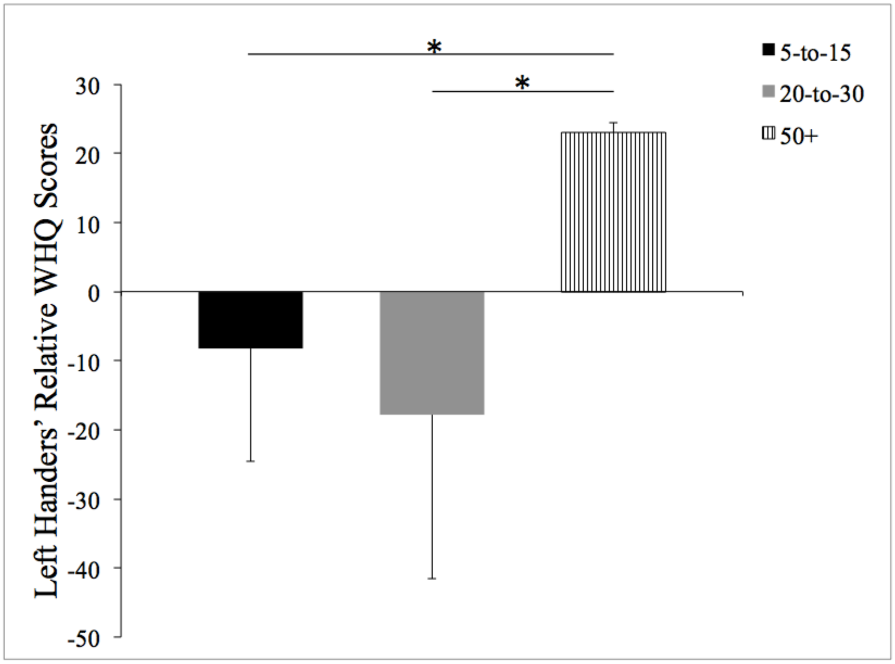


Figure 3. A comparison of the relative WHQ scores of left handers across the three age groups. 5-to-15 year olds averaged -8.20, 20-to-30 year olds averaged -17.75, and 50+ years averaged 23.00; $F_{(2,76)} = 6.78$, $p = 0.002$. * indicates significance at the 0.05 level.

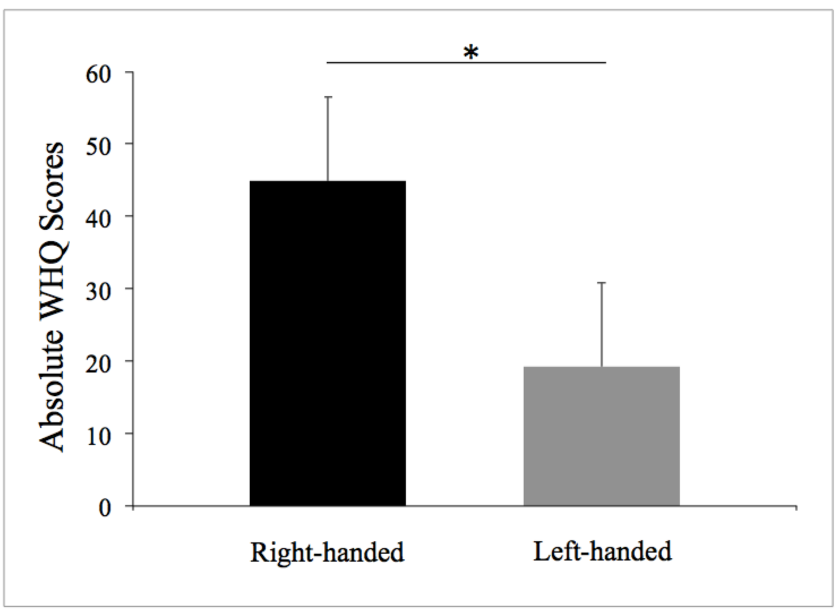


Figure 4. A comparison of the average absolute WHQ scores between right handers and left handers among the three age groups; right handers scored an average of 44.86, while left handers scored an average of 19.27. $F_{(1,76)} = 32.08$, $p<0.001$. * indicates significance at the 0.05 level.

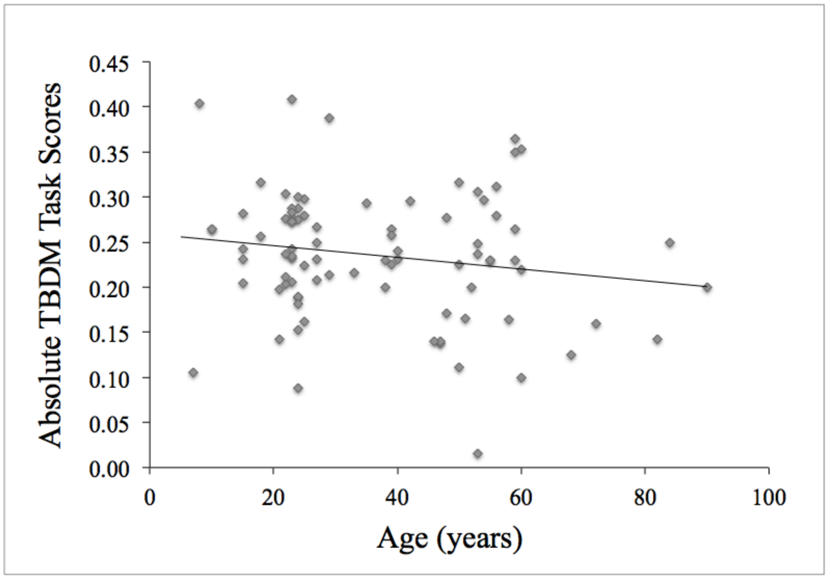


Figure 5. The correlation between the absolute TBDM task scores and age, r = -0.17, p>0.05, indicating no significant relationship among hand performance and age.

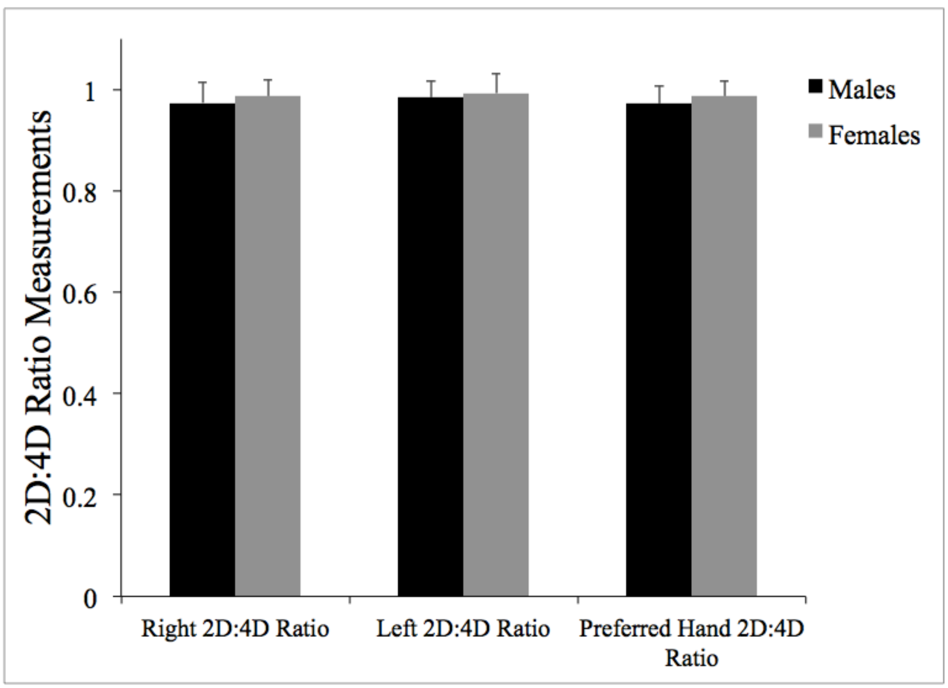


Figure 6. Comparing the right 2D:4D ratio, left 2D:4D ratio, and preferred-hand 2D:4D ratio of males and females. No significant differences were found across the sexes for any of the outcome measures.

Appendix D: Results – Tables

Table 1. A summary of the results for each section analyzing neurotypical participants.

Test	Results	Meaning
Hand Preference		
2 sex x 2 handedness ANCOVA with age covariate → relative WHQ	Main effect of handedness: $F_{(1,99)} = 159.23$, $p<0.001$	Left handers had negative relative WHQ scores (-6.43) while right handers had positive relative WHQ scores (44.71)
	Interaction between sex & handedness: $F_{(1,99)} = 4.42$, $p = 0.038$	Right-handed males' relative WHQ scores (41.29) were significantly lower than right-handed females' relative WHQ scores (47.29)
2 sex x 2 handedness ANCOVA with age covariate → absolute WHQ	Main effect of handedness: $F_{(1,99)} = 43.74$, $p<0.001$	Right handers had higher absolute WHQ scores (44.71) compared to left handers absolute WHQ scores (20.71)
Correlation → absolute WHQ vs. age	Pearson correlation: $r = 0.112$, $p = 0.130$	No relationship between age and hand preference
2 sex x 2 handedness x 3 age group ANOVA → relative WHQ	Main effect of handedness: $F_{(1,76)} = 100.17$, $p<0.001$	Right handers had positive relative WHQ scores (44.86) while left handers had negative relative WHQ scores (-6.00)
	Main effect of age group: $F_{(2,76)} = 6.74$, $p = 0.002$	50+ year olds (43.56) had greater relative WHQ scores than 5-15 year olds (35.92) (p=0.012) 50+ year olds (43.56) had greater relative WHQ scores than 20-30 year olds (36.26) (p<0.001)
	Interaction between handedness & age group: $F_{(2,76)} = 6.78$, $p = 0.002$	Left-handed 50+ year olds (23.00) significantly differed from left-handed 5-15 year olds (-8.20) on relative WHQ scores (p=0.020) Left-handed 50+ year olds (23.00) significantly differed from left-handed 20-30 (-17.75) year olds on relative WHQ scores (p<0.001)
	Interaction between sex, handedness, & age group: $F_{(1,76)} = 7.43$, $p = 0.008$	Right handers (44.92) and left handers (7.00) who were female and 5-15 years old differed on relative WHQ scores (p=0.003) Right handers (45.53) and left handers (-28.50) who were female and 20-30 years old differed on relative WHQ scores (p<0.001) Right handers (50.74) and left handers (-12.00) who were male and 5-15 years old differed on relative WHQ scores (p<0.001) Right handers (39.58) and left handers (-7.00) who were male and 20-30 years old differed on relative WHQ scores (p<0.001) Males (38.50) and females (49.67) who were right-handed and 50+ differed on relative WHQ

		scores (p=0.024) 5-15 year olds (50.71) and 50+ year olds (38.50) who were male and right-handed differed on relative WHQ scores (p=0.040) 5-15 year olds (50.71) and 20-30 year olds (39.58) who were male and right-handed differed on relative WHQ scores (p=0.052), non-significant 5-15 year olds (-12.00) and 50+ year olds (23.00) who were male and left-handed differed on relative WHQ scores (p=0.001) 20-30 year olds (-7.00) and 50+ year olds (23.00) who were male and left-handed differed on relative WHQ scores (p=0.014) 5-15 (7.00) and 20-30 (-28.50) year olds who were left-handed females differed on relative WHQ scores (p=0.017)
2 sex x 2 handedness x 3 age group ANOVA → absolute WHQ	Main effect of handedness: $F_{(1,76)} = 32.08, p<0.001$	Right handers (44.86) had significantly greater absolute WHQ scores than left handers (19.27)
Hand Performance		
2 sex x 2 handedness ANCOVA with age covariate → relative TBDM	Main effect of handedness: $F_{(1,82)} = 339.50, p<0.001$	Right handers (0.240) had positive relative TBDM task scores and left handers (-0.197) had negative relative TBDM task scores
2 sex x 2 handedness ANCOVA with age covariate → absolute TBDM	Non-significant effect of handedness: $F_{(1,82)} = 3.69, p = 0.058$	General trend: right handers (0.240) appeared to have a greater difference in hand performance between the hands compared to left handers (0.197)
Correlation → absolute TBDM task vs. age	Pearson correlation: $r = -0.170, p = 0.115$	No relationship between TBDM task and age, slight decrease of TBDM scores as age increases (more equal)
2 sex x 2 handedness x 3 age group ANOVA → relative TBDM	Main effect of handedness: $F_{(1,60)} = 161.20, p<0.001$ Non-significant interaction between sex & age group: $F_{(2,60)} = 2.89, p = 0.063$	Right handers (0.240) had positive relative TBDM task scores while left handers (-0.197) had negative relative TBDM task scores Difference between males (0.127) and females (0.240) who are 5-15 years old on relative TBDM task scores (p<0.001) Difference between males (0.130) and females (0.259) who are 50+ years old on relative TBDM task scores (p<0.001) 5-15 (0.240) and 20-30 (0.195) year olds who are female differed on relative TBDM task scores (p<0.001) 20-30 (0.195) and 50+ (0.259) year olds who are female differed on relative TBDM task scores (p<0.001)

2 sex x 2 handedness x 3 age group ANOVA → absolute TBDM	Non-significant interaction between sex & age group: $F_{(2,60)} = 2.89$, $p = 0.063$	Males (0.184) and females (0.259) who are 50+ years old differed on their absolute TBDM task scores ($p = 0.019$)
Hand Preference & Hand Performance		
Correlation → relative WHQ vs. relative TBDM task	Significant correlation: $r = 0.712$, $p < 0.001$	As WHQ scores increased, TBDM task scores appeared to increase *influenced by left-handers
Correlation → absolute WHQ vs. absolute TBDM task	No significant correlation: $r = 0.125$, $p = 0.125$	No relationship seen
2D:4D Ratio		
2 sex x 2 handedness ANCOVA with age covariate → preferred-hand 2D:4D ratio	No main effects or interactions	Sex, handedness, and age play no role on the preferred-hand 2D:4D ratio
2 sex x 2 handedness x 3 age group ANOVA → right 2D:4D ratio	No main effects or interactions	Sex, handedness, and age groups play no role on the right hand 2D:4D ratio
2 sex x 2 handedness x 3 age group ANOVA → left 2D:4D ratio	No main effects of interactions	Sex, handedness, and age groups play no role on the left hand 2D:4D ratio
2 sex x 2 handedness x 3 age group ANOVA → preferred-hand 2D:4D ratio	No main effects or interactions	Sex, handedness, and age groups play no role on the preferred-hand 2D:4D ratio
2 sex x 2 handedness x 3 age group ANOVA → difference between the right and left 2D:4D ratios	No main effects or interactions	Sex, handedness, and age groups play no role on the difference between the right and left 2D:4D ratios
* All above tests repeated excluding kinesiology students at both the undergraduate and master's levels: no significant main effects or interactions found		
2 sex x 2 handedness x 3 age group ANOVA → difference between the right and left 2D:4D ratios * ***excluding kinesiology students***	Non-significant interaction between sex and age group: $F_{(2,49)} = 2.93$, $p = 0.063$	20-to-30 year-old females (-3.580×10^{-2}) differed from 5-to-15 year-old females (1.299×10^{-2}) and 50+ year-old females (3.490×10^{-3})
2 sex x 2 handedness x 3 age group repeated measures ANOVA → right ratio vs. left ratio	No main effects or interactions	No differences between a participant's right and left digit ratios considering sex, handedness, and age group
Hand Preference and the 2D:4D Ratio		
Correlation → absolute WHQ vs. right 2D:4D ratio	No significant correlation: $r = -0.060$, $p = 0.271$	No relationship between absolute WHQ scores and right 2D:4D ratios
Correlation → absolute WHQ vs. left 2D:4D ratio	No significant correlation: $r = 0.036$, $p = 0.357$	No relationship between absolute WHQ scores and left 2D:4D ratios
Correlation → absolute WHQ vs. preferred-hand 2D:4D ratio	No significant correlation: $r = -0.011$, $p = 0.909$	No relationship between absolute WHQ scores and preferred-hand 2D:4D ratios
Correlation → absolute WHQ vs.	No significant correlation: $r = -0.105$, $p = 0.145$	No relationship between absolute WHQ scores and the difference between the right and left digit

difference between the right and left digit ratios		ratios
Correlation → skilled components of the absolute WHQ vs. preferred-hand 2D:4D ratio	No significant correlation: r = -0.008, p = 0.467	No relationship between absolute WHQ scores of skilled components only and preferred-hand 2D:4D ratios
Correlation → skilled components of the absolute WHQ vs. preferred-hand 2D:4D ratio (right-handers only)	No significant correlation: r = 0.018, p = 0.434	No relationship between right handers' absolute WHQ scores of skilled components only and preferred-hand 2D:4D ratios
Correlation → skilled components of the absolute WHQ vs. preferred-hand 2D:4D ratio (left-handers only)	No significant correlation: r = 0.085, p = 0.386	No relationship between left handers' absolute WHQ scores of skilled components only and preferred-hand 2D:4D ratios
Hand Performance and the 2D:4D Ratio		
Correlation → absolute TBDM task vs. right 2D:4D ratio	No significant correlation: r = -0.052, p = 0.316	No relationship between absolute TBDM task scores and right 2D:4D ratios
Correlation → absolute TBDM task vs. left 2D:4D ratio	No significant correlation: r = -0.128, p = 0.120	No relationship between absolute TBDM task scores and left 2D:4D ratios
Correlation → absolute TBDM task vs. preferred-hand 2D:4D ratio	No significant correlation: r = -0.078, p = 0.236	No relationship between absolute TBDM task scores and preferred-hand 2D:4D ratios
Correlation → absolute TBDM task vs. difference between the right and left digit ratios	No significant correlation: r = 0.084, p = 0.219	No relationship between absolute TBDM task scores and difference between the right and left digit ratios

Table 2. The average 2D:4D ratio means by sex (males and females) and handedness (right handers and left handers) within the three age groups, excluding participants enrolled in a kinesiology program.

Handedness	Sex	Age Group	Right 2D:4D Ratio	Left 2D:4D Ratio	Preferred-Hand 2D:4D Ratio	Difference Between the Right and Left 2D:4D Ratios
Right-handed	**Males**	5-15	0.963	0.984	0.963	-2.008×10^{-2}
		20-30	0.965	0.949	0.965	1.615×10^{-2}
		50+	0.967	0.973	0.967	-5.245×10^{-3}
		Total	**0.966**	**0.976**	**0.966**	$\mathbf{-9.825 \times 10^{-3}}$
	Females	5-15	0.997	0.986	0.997	1.085×10^{-2}
		20-30	0.981	1.017	0.981	-3.580×10^{-2}
		50+	0.985	0.982	0.985	3.490×10^{-3}
		Total	**0.989**	**0.989**	**0.989**	$\mathbf{-0.438 \times 10^{-3}}$
	Total	5-15	0.985	0.985	0.985	0.026×10^{-3}
		20-30	0.978	1.005	0.978	-2.714×10^{-2}
		50+	0.978	0.978	0.978	-0.004×10^{-3}
		Total	**0.981**	**0.984**	**0.981**	$\mathbf{-3.184 \times 10^{-3}}$

Left-handed	**Males**	5-15	0.991	0.983	0.983	7.167×10^{-3}
		20-30	0.979	1.010	1.010	-3.109×10^{-2}
		50+	0.968	0.970	0.970	-2.404×10^{-3}
		Total	**0.982**	**0.983**	**0.983**	$\mathbf{-1.033 \times 10^{-3}}$
	Females	5-15	1.010	0.970	0.970	4.079×10^{-2}
		20-30	--	--	--	--
		50+	--	--	--	--
		Total	**1.010**	**0.970**	**0.970**	$\mathbf{4.079 \times 10^{-2}}$
	Total	5-15	0.995	0.981	0.981	1.389×10^{-2}
		20-30	0.979	1.010	1.010	-3.109×10^{-2}
		50+	0.968	0.970	0.970	-2.404×10^{-3}
		Total	**0.986**	**0.982**	**0.982**	$\mathbf{-4.194 \times 10^{-3}}$
Total	**Males**	5-15	0.973	0.984	0.971	-1.017×10^{-2}
		20-30	0.972	0.979	0.987	-7.471×10^{-3}
		50+	0.967	0.972	0.968	-4.771×10^{-3}
		Total	**0.970**	**0.978**	**0.971**	$\mathbf{-7.363 \times 10^{-3}}$
	Females	5-15	0.998	0.985	0.995	1.299×10^{-2}
		20-30	0.981	1.017	0.981	-3.580×10^{-2}
		50+	0.985	0.982	0.985	3.490×10^{-3}
		Total	**0.990**	**0.988**	**0.989**	$\mathbf{1.624 \times 10^{-3}}$
	Total	5-15	0.987	0.985	0.985	2.799×10^{-3}
		20-30	0.978	1.006	0.983	-2.770×10^{-2}
		50+	0.977	0.978	0.978	-0.182×10^{-3}