



Short communication

## Is digit ratio (2D:4D) a reliable pointer to speech laterality?



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### HIGHLIGHTS

- First study to examine the effects of prenatal testosterone on blood flow during speech.
- Prenatal testosterone as indicated by digit ratio was not related to speech representation.
- There was no association between sex and speech representation.
- The results are inconsistent with androgen theories of cerebral lateralisation.

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### ABSTRACT

The relative length of the second and fourth digits (2D:4D ratio) is sexually dimorphic and a retrospective biomarker of prenatal hormonal exposure. Low ratios indicate higher prenatal testosterone (pT) and lower estrogen exposure, whereas the reverse pattern is associated with high ratios. Elevated levels of pT exposure have long been thought to modulate hemispheric specialisation; subsequently many studies use the 2D:4D ratio as a proxy index for pT to examine the effects of prenatal hormonal exposure on lateralised cognitive abilities. Here we used Transcranial Doppler ultrasonography and digit ratio to investigate whether pT has an influence on speech laterality. We tested 34 right and 14 left handed adults. Our results indicate that speech representation is unrelated to digit characteristics and therefore purportedly pT. We discuss these findings in relation to androgen theories of lateralisation.

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### 1. Introduction

The origin of speech lateralisation in the human brain remains elusive. Androgen accounts of cerebral lateralisation hypothesise that exposure to higher levels of prenatal testosterone (pT) produce neuronal alterations which lead to structural and functional hemispheric asymmetries [19]. There is debate nevertheless, over how these processes may modulate speech laterality, with differing albeit not mutually exclusive perspectives postulating contrasting predictions. According to Geschwind–Behan–Galaburda's (GBG) theory of cerebral lateralisation [9], elevated pT exposure slows the development and cytoarchitecture of the left hemisphere whilst facilitating the generation of compensatory neural mechanisms and functional status of the right hemisphere. Thus it is

postulated that pT reduces the verbal ability of the left hemisphere and increases the likelihood of right hemisphere or bilateral speech representation (for a critique see [6]. In contrast, Callosal theory [24] posits that elevated levels of pT enhance the potential for lateralisation. According to this view, laterality is modulated by the size of the corpus callosum. Low indices of lateralisation are associated with greater callosal integrity and increased functional interhemispheric connectivity. Higher pT exposure is hypothesised to trigger axonal pruning of the corpus callosum which reduces the level of coherence between the hemispheres and increases the potential for typical language dominance.

A widely accepted retrospective measure of pT is the ratio between the lengths of the second and fourth digits (2D:4D ratio). Lower 2D:4D ratios, or in particular, the difference between the ratios of the left and right hands (dR-L) are associated with higher levels of pT exposure and lower levels of prenatal estrogen (pE) exposure; the reverse pattern is associated with high ratios [17]. The link between 2D:4D and prenatal sex steroids has further been demonstrated experimentally. [25] demonstrated that

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chondrocyte proliferation in the fourth digit is sensitive to the balance between pT and pE. The process was shown to be enhanced by relative increases in pT– pE, whereas a relative increase in pE–pT reduced the process. Notably, the modulation of 2D:4D by sex steroids was shown only to occur prenatally and was not influenced by circulating sex hormone levels (see also, [10,22]). A growing body of research has subsequently used digit ratio as a putative biomarker to examine the lateralisation effects of pT on a range of tasks generally associated with the left (e.g., [4]) or right hemispheres (e.g., [5]). These studies however tend to deploy indirect functional measures from which laterality is inferred on the basis of handedness and acknowledged brain-behaviour relations. A limitation of this approach is that handedness does not correlate perfectly to speech laterality. Although it is well known that for most humans the left-hemisphere is dominant for speech and handedness, some right-handers, and many ambilaterals and left-handers have bilateral or right-hemisphere representation [14]. Moreover, handedness is multidimensional and there is no clear consensus on the precise components of handedness that may be related to speech representation. Here we sought to investigate the effects of pT exposure on speech representation by measuring asymmetries in brain activation during speech production directly. In this study, changes in cerebral blood flow velocity (BFV) were unequivocally assessed with a functional Transcranial Doppler ultrasonography (fTCD) system. Functional TCD has high temporal resolution and constitutes a reliable, valid and economical technique for the assessment of language lateralisation. Lateralisation indices (LIs) measured with fTCD correlate highly to those measured with functional MRI [21]. Support for Callosal theory will be evident if an inverse relationship between 2D:4D ratio and strength of typical lateralisation is observed. A positive relationship between these variables would be consistent with the GBG hypothesis.

## 2. Method

### 2.1. Participants

Forty eight adults participated in the study, 24 males (mean = 21.96 years, SD = 7.03) and 24 females (mean = 23.13 years, SD = 8.68). We aimed to test a reasonable group of participants with atypical speech representation, so recruited more left-handers ( $n = 14$ ) than would be expected in a representative sample. No participants had a known neurological disorder nor were any taking medication known to affect the CNS or circulatory system. None had impairments in language or reading. All had normal or corrected to normal vision and gave informed consent prior to participating in the study. All participants were of Caucasian origin apart from one male of African descent. His digit characteristics however were not unusual (within 1 SD) and so his data was retained. Participants were recruited through internal communications at the University of Lincoln and were paid £6.00 for helping with the research. The investigation was approved by the ethics committee of the School of Psychology, University of Lincoln.

### 2.2. Digit ratio

The lengths of the second and fourth fingers of each hand were measured from the ventral proximal crease to the tip with vernier calipers. Right and left 2D:4D ratios were calculated for each hand and also averaged across the hands to derive the mean 2D:4D value. To obtain dR-L, the left 2D:4D ratio was subtracted from the right 2D:4D ratio.

### 2.3. Handedness questionnaire

We used the same questionnaire as that deployed by [8]. In short, respondents indicated their preferred hand (right, left, either) for executing 14 unimanual and 7 bimanual tasks. The questionnaire contains the same primary and secondary activities as those described by [1] and additional activities from [18]. A handedness quotient (HQ) was calculated according to  $[(\text{right} - \text{left}) / (\text{right} + \text{left}) \times 100]$ . Positive values indicated right-handedness, negative values indicated left-handedness. In addition, we classified hand preference according to Annett's [2] criteria for small samples.

### 2.4. Speech laterality

Fluctuations in cerebral BFV were measured with fTCD (DWL Doppler-Box™X; manufacturer, DWL Compumedics Germany GmbH). The system records alterations in BFV within the right and left middle cerebral arteries (MCAs) during language production, using two 2-MHz transducer probes attached to a headset and positioned slightly superior to each zygomatic arch.

The fTCD task involved a word generation paradigm (see [12]). For each trial, a letter appeared on a computer screen and the participant was given 15 s to silently generate as many words as possible that began with the letter. During the following 5 s period they were asked to report the words aloud. A 35 s period of relaxation (baseline) then followed before the start of the next trial. There were 23 trials in total.

A MATLAB (Mathworks Inc., Sherborn, MA, USA) software package called dopOSCCI [3], 2012 was used to compute a laterality index (LI) for each participant.<sup>1</sup> The LI was derived from the maximum peak difference between the right and left side within a 2 s period of interest, when compared to a baseline rest period of 10 s. The activation window was centralized to the time point at which the left-right deviation was greatest within the period of interest (POI). In the present paradigm the POI ranged from 3 to 13 s following presentation of the stimulus letter.

## 3. Results

Speech laterality was determined by the protocol outlined by [13]. According to this criterion laterality is considered to be clear in all cases in which the LI deviates by  $>2$  SE from 0. Left-hemisphere (typical) or right-hemisphere (atypical) speech dominance is indicated by positive or negative indices respectively. Cases with an  $LI < 2$  SE from 0 are categorized as having bilateral speech representation. Using these criteria the following distribution for speech representation was found: typical 85% (mean  $LI = 3.10$ ,  $SD = 1.47$ ), atypical 15% (mean  $LI = -1.78$ ,  $SD = 0.92$ ). The LI for all participants deviated by  $>2$  SE from 0 and therefore none were considered to have bilateral speech representation.

The Kolmogorov–Smirnov test denoted that the 2D:4D ratios deviated from normality [ $D(48) = 0.13$ ,  $p = 0.04$ ], subsequently

<sup>1</sup> DopOSCCI makes a number of computations in order to summarize the fTCD data and advance the validity of measuring hemispheric differences in BFV. First, the numbers of samples were reduced by downsampling the data from ~100Hz to 25Hz. Second, variations in cardiac cycle which may contaminate task-related signals were corrected using a cardiac cycle integration technique. Third, data contaminated by movement or 'drift' were removed prior to normalization. Normalized epochs were subsequently screened and excluded as measurement artifacts if activation values exceeded the acceptable range ( $\pm 40\%$  mean BFV). Fourth, to control for physiological process that can influence BFV (e.g., breathing rate, arousal, cardiac cycle), the mean activation of the baseline period was subtracted from each individual epoch. Deviations in left versus right activity were therefore baseline corrected and reflect relative changes in BFV.

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