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Research report

Synesthesia in twins: Incomplete concordance in monozygotes suggests extragenic factors

Hannah G. Bosley [1], David M. Eagleman *

Department of Neuroscience, Baylor College of Medicine, Houston, TX, United States

HIGHLIGHTS

- We conducted the first comparative twin study of colored-sequence synesthesia (CSS).
- We found 73.9% pairwise concordance in monozygotic and 36.4% in dizygotic twins.
- Same-sex dizygotic pairs had 75% concordance; opposite-sex pairs had 14.3%.
- It seems that environmental or epigenetic factors influence expression of CSS.
- Findings suggest a mechanism of CSS development that differs from previous models.

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ABSTRACT

Colored-sequence synesthesia (CSS) is a neurological condition in which sequential stimuli such as letters, numbers, or days of the week trigger simultaneous, involuntary color perception. Although the condition appears to run in families and several studies have sought a genetic link, the genetic contribution to synesthesia remains unclear. We conducted the first comparative twin study of CSS and found that CSS has a pairwise concordance of 73.9% in monozygotic twins, and a pairwise concordance of 36.4% in dizygotic twins. In line with previous studies, our results suggest a heritable element of synesthesia. However, consonant with the findings of previous single-pair case studies, our large sample size verifies that synesthesia is not completely conferred by genetics; if it were, monozygotic twins should have 100% concordance. These findings implicate a genetic mechanism of CSS that may work differently than previously thought: collectively, our data suggest that synesthesia is a heritable condition with incomplete penetrance that is substantially influenced by epigenetic and environmental factors.

1. Introduction

Synesthesia is a neurological condition in which stimuli such as letters or musical notes trigger a simultaneous, involuntary perception in another sensory modality. A prevailing hypothesis is that synesthesia arises from neural "cross-talk" – in other words, activation in one area of the brain elicits activation in another area [1–3].

Anecdotal evidence suggests that synesthesia may be heritable [4], and family linkage studies have supported this possibility [5–7], although none has yet identified a specific genetic mechanism by which synesthesia is transmitted. One genetic linkage analysis of five synesthetic families found evidence of linkage to chromosome 16q in two of the families [7]. Specifically, results from this study pointed to the 16q12.2-23.1 region which contains 343 genes, many of which are expressed in the brain. The authors highlighted six of these as candidate genes that may fit the profile of synesthesia; however, no variants or polymorphisms of these genes emerged in their analysis of affected individuals. Another study determined significant linkage to chromosome 2q24 (HLOD = 3.025, $p = .047$), and also identified suggestive linkage to several other chromosomal loci [5]. These results suggest the possibility that multiple genes independently influence the development of synesthesia.

Although it has been suggested that synesthesia has a single-gene X-linked dominant mode of inheritance [8,9], studies and pedigree analyses have shown this to be unlikely. For example, Asher et al. [5] confirmed male-to-male transmission of synesthesia in two families [5], and Smilek et al. [10] identified a pair of male monozygotic twins in which only one twin experienced synesthesia [10].

* Corresponding author. Tel.: +1 713 798 6224.
E-mail addresses: bosley@berkeley.edu (H.G. Bosley), eagleman@bcm.edu (D.M. Eagleman).
[1] Current address: Department of Psychology, University of California, Berkeley, United States.

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Genetics may not be the only factor at play in the development of synesthesia. Smilek et al. [11] reported a pair of discordant female monozygotic twins; one experienced synesthesia and one did not [11]. The authors hypothesized that the discordance was due to either X chromosome inactivation or an epigenetic event. These findings indicate that genetic inheritance alone may not offer a full explanation of synesthesia.

Our study seeks to understand the genetic contribution to synesthesia by examining both monozygotic and dizygotic twins, recruiting a large cohort of twin pairs for the analysis, and using rigorous phenotyping. We compare the concordance rates of synesthesia in monozygotic and dizygotic twin pairs to estimate the extent to which epigenetic or environmental factors may play a role in the development of synesthesia.

2. Methods

Many varieties of synesthesia have been reported; for this study we focus specifically on colored-sequence synesthesia (CSS) in which sequences such as letters, numbers, days of the week, and months trigger color perception. Our previous analysis of 3194 colored-sequence synesthetes revealed that having synesthetic color associations for one type of sequence (e.g., letters) gives a ~79% likelihood of having color associations for another type of sequence (e.g. numbers), but only chance likelihood of having another form of synesthesia [12], indicating that CSS is a distinct subtype of synesthesia that may result from a distinct mechanism. Motivated by this finding, our prior genetics research has also focused on CSS [7].

For phenotyping, we used the Synesthesia Battery, a standardized online test that distinguishes synesthetes from non-synesthetes [13]. The Synesthesia Battery presents participants with graphemes (A–Z, 0–9), months (January–December) or weekdays (Monday–Sunday). All stimuli are presented in random order three times each. Participants are asked to select the synesthetically associated color for each stimulus from a color palette of 16.7 million colors. The color choices for each grapheme are analyzed for consistency across the three trials within the session (for example, choosing the same color of red each time a participant saw the letter A; for full details, see [13]). Non-synesthetic controls have low consistency in associating the colors to the stimuli, while synesthetes display high consistency in their repeated choices.

From the pool of Synesthesia Battery participants, we contacted 151 individuals who completed the grapheme-color portion of the test and reported having a twin in an optional text field. 38 twin pairs (76 individuals) gave consent to participate in the study. We conducted interviews with interested twin pairs online and over the phone to determine zygosity (via self-report), concordance, and demographic information (including whether the pair was reared together – which was true for all pairs in the sample). All twins reporting synesthesia or uncertainty about having synesthesia were instructed to take the Synesthesia Battery to verify their phenotype.

Recent research [25] indicated that a Synesthesia Battery consistency score of 1.43 is an optimal cutoff score to discriminate synesthetes from non-synesthetes. Thus, participants scoring below a threshold of 1.43 on any of the colored-sequence Battery tests (numbers, letters, weekdays, or months) were categorized as probands. Those who either reported having no experience of CSS or scored above 1.43 on all of the colored-sequence Battery tests were categorized as unaffected. Eagleman et al. [13] originally suggested a more conservative Battery score of 1.0 as the optimal cutoff score; however, only four participants (representing three twin pairs) scored above 1.0 and would have been excluded at the more conservative classification threshold (see Supplementary Tables 1a and 1b for details). Analyses were repeated with and without these three twin pairs to obtain results at both classification thresholds.

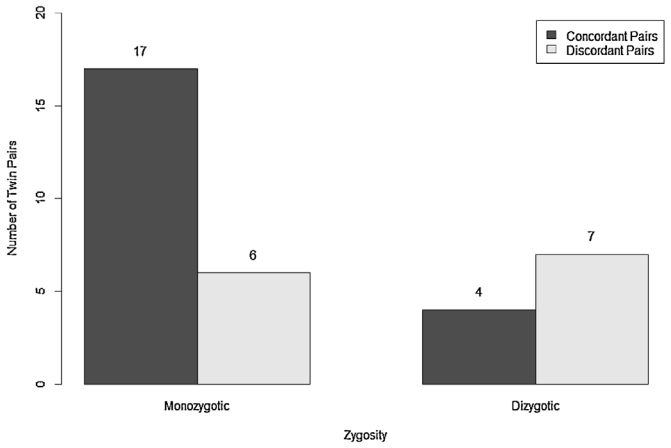

Fig. 1. Monozygotic twins have a greater pairwise concordance for CSS than dizygotic twins. Seventeen of 23 monozygotic twin pairs (73.9%) were concordant for colored sequence synesthesia, while only 4 of 11 (36.4%) dizygotic pairs were concordant.

Supplementary Tables 1a and 1b related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2015.02.024.

After interviews and testing, one twin pair was excluded from the study because neither twin scored below 1.43 on a colored-sequence battery test, classifying both twins as unaffected. Two pairs were excluded due to lost contact, and one pair was excluded because of indeterminate zygosity. Although zygosity was determined through self-report, all pairs included in the analysis either reported reasonable confidence in their zygosity or provided medical evidence (e.g. blood tests, physicians' reports at birth). The final analysis includes 34 twin pairs (68 individuals; based on [25]) and is repeated at the more conservative classification threshold with 31 twin pairs (62 individuals; based on [13]).

3. Results

The study had complete ascertainment as all probands met testing criteria for having CSS. Of the participant twins, 23 pairs were monozygotic (21 female, 2 male) and 11 pairs were dizygotic (3 female, 1 male, 7 sex-discordant). Participant information and test results are detailed in Supplementary Tables 1a and 1b.

The monozygotic group contained 17 concordant twin pairs (pairwise concordance 73.9%) and the dizygotic group had 4 concordant pairs (pairwise concordance 36.4%), as shown in Fig. 1.

Fisher's Exact Test was used to assess the significance of the difference in concordance between the two groups ($p = 0.059$, odds ratio = 4.69). This result approaches but does not reach the .05 threshold of statistical significance; however, we can interpret this finding as suggestive of possible group differences. Repeating the analysis without the three twin pairs who scored above 1.0 on the synesthesia battery, we see a decrease in the trend toward statistical significance ($p = 0.218$, odds ratio = 3.19).

Potential sex differences also emerged. Of the 68 individuals in the study, 56 were probands. The probands in this study had a male:female ratio of 1:6 (8 males, 48 females), while the 12 unaffected individuals had a male:female ratio of 5:7. Fisher's Exact Test indicates this difference is not statistically significant ($p = 0.109$, odds ratio = 0.279). However, when we exclude the three twin pairs who met criteria for inclusion at the 1.43 threshold but not the more conservative 1.0 threshold (see Supplementary Tables 1a

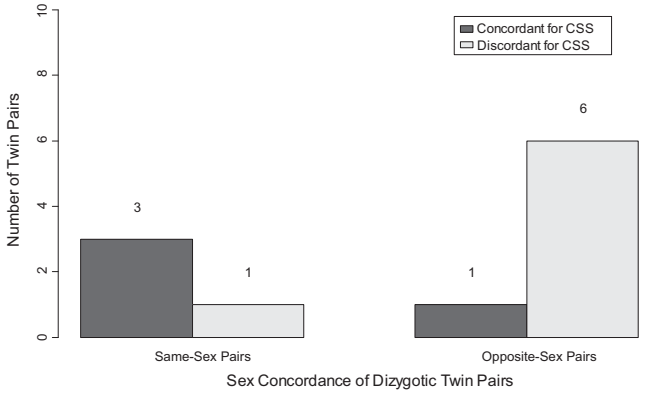

Fig. 2. Dizygotic twin pairs of the same sex have greater concordance of CSS than opposite-sex pairs. Three of four (75%) same-sex dizygotic twin pairs are concordant for CSS while only one of seven (14.28%) opposite-sex pairs are concordant for CSS.

and 1b), the sex difference becomes significant ($p = 0.018$, odds ratio = 0.166).

Looking only at dizygotic twin pairs, it appears that same-sex twins have greater concordance of CSS than opposite-sex pairs: three out of four same-sex dizygotic twin pairs were concordant for CSS (75% pairwise concordance), while only one of seven opposite-sex twin pairs were concordant for CSS (14.28% pairwise concordance; see Fig. 2). These differences fall slightly above the threshold of statistical significance ($p = 0.087$, odds ratio = 12.23); however, we note that the size of our dizygotic sample is small and thus insufficiently powered.

4. Discussion

Although previous genetics work has been inconclusive in identifying a genetic mechanism of synesthesia, this study may partially validate previous proposals that there is a heritable component [5,7,9,14–16]. Crucially, this study also points toward the importance of the role of the environment in the development of synesthesia. Contrary to our predictions, monozygotic twin pairs did not have significantly greater concordance of CSS than dizygotic twin pairs. However, the difference in concordance between the monozygotic and dizygotic groups did closely approach the level of statistical significance at the more liberal classification threshold, so we should be cautious in our interpretation of this finding as ruling out the influence of genetics. Rather, we conclude that while genetics may play a role in the development of CSS, extragenic factors (such as epigenetic events or environmental influences) likely have a significant effect. The presence of six discordant monozygotic twin pairs in this study provides additional support for the role of extragenic factors in the development of synesthesia.

One possibility is that synesthetic associations can be imprinted at a young age [17–20] – for instance, if one twin gravitates toward colored number- or letter-toys as a child, that twin might establish perceptual associations [18,21]. Genetics might confer a predisposition to develop and retain these associations, but synesthesia will not come to realization in all cases. A possible genetic underpinning for this hypothesis is that epigenetic events such as DNA methylation [22] could moderate the expression of the genes underlying CSS differently in different individuals. Fraga et al. [23] showed that epigenetic changes such as DNA methylation are responsible for monozygotic twins' continual phenotypic differentiation throughout the lifespan [23]. While monozygotic twins are generally identical at birth, different life experiences and correspondingly different epigenetic events could lead to one twin developing CSS even though the co-twin does not express the trait. Future work could test this hypothesis by identifying an array of target genes and bisulfite sequencing to detect gene methylation in discordant monozygotic twins.

Additional clues may be available from the sex differences. Our 56 probands with CSS had a male to female ratio of 1:6. Prior studies have found increased prevalence of synesthesia in females – for example, Ward and Simner [16] found a male:female ratio of 1:2 [16] and Tomson et al. [7] showed a ratio of 1:2.7 [7]. Our sample had a substantially greater proportion of females than prior studies have indicated. One possible reason might be that selection bias plays a role in the sex discrepancy of the sample. We only contacted Synesthesia Battery participants who reported having a twin in an optional text field; if male synesthetes were less likely than female synesthetes to reveal information about themselves by mentioning their twin in a text field, they would not have been contacted. Future directions could include polling all of the synesthete participants from the battery regardless of their direct mention of a twin to determine if there are male twin synesthetes who did not get contacted in our initial round of emails.

Interestingly, sex-concordant dizygotic twins appeared to have a higher concordance of CSS than sex-discordant dizygotic twins. Perhaps this provides evidence for an environmental influence on the development of synesthesia. Plausibly, same-sex twins would have more shared experiences and interests during childhood, allowing more opportunities for both twins to imprint synesthetic associations from toys or books. Additionally, this could explain our observation that monozygotic twin pairs had greater concordance for CSS than dizygotic pairs. As monozygotic twins are inherently same-sex pairs, they may be exposed to similar environments and shared experiences as described above.

This could be explored in further studies by examining the variance between each twin's color palette: if twins had imprinted associations from the same sources, their synesthetic colors should have similarities. Due to our limited sample size of dizygotic twin pairs (and the small number of male dizygotic twins in the present sample), it is not yet possible to draw definitive conclusions about whether sex concordance actually predicts concordance of CSS.

An essential limitation of our study is that we were unable to concretely measure environmental influences, as the data was gathered remotely via the Internet (and, further, this was not longitudinal research). Thus, we are unable to draw conclusions about the effect of the environment on the development of CSS from the present study. Future research will explore this relationship further by continuing to recruit dizygotic twins with synesthesia. Nonetheless, the present results support previous suggestions that sex may have an effect on the heritability or development of synesthesia [1,7,16]. The findings of the present study suggest the potential importance of environmental factors for future research paradigms.

It is not clear why we found a disparity in our sample sizes of monozygotic and dizygotic twins. As monozygotic twin births are less common than dizygotic twin births, a greater number of dizygotic twin recruits would have been expected. Our high number of monozygotic relative to dizygotic twin pairs could be due to response bias – e.g., perhaps monozygotic twins are more interested in research involvement than dizygotic twins. However, Hur et al. [24] indicated that observing more monozygotic than dizygotic twin pairs in a volunteer sample is not necessarily the result of bias; dizygotic twinning rates have declined over the past 50 years in Caucasians and birth rates from 1971 to 1984 showed higher rates of monozygotic than sex-concordant dizygotic twin births [24]. In any case, the added statistical power of a larger dizygotic sample size would be beneficial for future studies.

Another limitation of the present study is that we did not conduct genotyping to determine zygosity. Instead, zygosity was determined by self-report, which may be unreliable. While many of the twins were able to cite medical evidence, other twins made the determination based on factors such as appearance. This may, to

some extent, contribute to the higher prevalence of monozygotic twins. Ideally, genotyping will be used in future studies to draw a clearer and more reliable distinction between monozygotic and dizygotic pairs. Future studies should also use genotyping to investigate the genetic or heritable epigenetic components underlying discordance.

In line with previous studies [5,7,9,16], we find suggestive evidence of a heritable element of synesthesia. However, consonant with the findings of Smilek et al. [10,11] our large sample size verifies that synesthesia is not completely conferred by genetics (otherwise monozygotic twins would have had 100% concordance). Collectively, the available evidence to date suggests that a combination of genes, epigenetic events, and environmental influences underlie an individual's development of CSS. Further research is necessary to determine the relative contributions of environmental influences (e.g., imprinting) on synesthesia, as well as the potential impact of other heritable factors such as DNA methylation.

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