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Automatic Letter-Colour Associations in Non-Synaesthetes and their Relation to Grapheme-Colour Synaesthesia

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Abstract

Although grapheme-colour synaesthesia is a well-characterized phenomenon in which achromatic letters and/or digits involuntarily trigger specific colour sensations, its underlying mechanisms remain unresolved. Models diverge on a central question: whether triggered sensations reflect (i) an overdeveloped capacity in normal cross-modal processing (i.e., sharing characteristics with the general population), or rather (ii) qualitatively deviant processing (i.e., unique to a few individuals). We here address this question on several fronts: first, with adult synaesthesia-trainees and second with congenital grapheme-colour synaesthetes. In Chapter 3, we investigate whether synaesthesia-like (automatic) letter-colour associations may be learned by non-synaesthetes into adulthood. To this end, we developed a learning paradigm that aimed to implicitly train such associations while keeping participants naïve as to the end-goal of the experiments (i.e., the formation of letter-colour associations), thus mimicking the learning conditions of acquired grapheme-colour synaesthesia (Hancock, 2006; Witthoft & Winawer, 2006). In two experiments, we found evidence for significant binding of colours to letters by non-synaesthetes. These learned associations showed synaesthesia-like characteristics despite an absence of conscious, colour concurrents, correlating with individual performance on synaesthetic Stroop-tasks (experiment 1), and modulated by the colour-opponency effect (experiment 2) (Nikolic, Lichti, & Singer, 2007), suggesting formation on a perceptual (rather than conceptual) level. In Chapter 4, we probed the nature of these learned, synaesthesia-like associations by investigating the brain areas involved in their formation. Using transcranial Direct Current Stimulation to interfere with two distinct brain regions, we found an enhancement of letter-colour learning in adult trainees following dlPFC-stimulation, suggesting a role for the prefrontal cortex in the release of binding processes. In Chapter 5, we attempt to integrate our results from synaesthesia-learners with the neural mechanisms of grapheme-colour synaesthesia, as assessed in six congenital synaesthetes using novel techniques in magnetoencephalography. While our results may not support the existence of a "synaesthesia continuum," we propose that they still relate to synaesthesia in a meaningful way.

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Dedication

For my parents, my brother, and Giorgos.

*And for **you**, for taking the time to read this.*

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Author's Declaration

I certify that this doctoral dissertation is my original work and that all references to the work of others have been clearly identified and fully attributed.

Chapter 1: Introduction

Synaesthesia: Defined

'Synaesthesia' originates from the Greek words *syn*, meaning "union," and *aisthises*, meaning "of the senses," literally expressing a "joining together" of two senses in a singular experience. It is characterised by a paradoxical perception in which stimulation in one sensory modality automatically, involuntarily, and systematically elicits a conscious perception either in an additional sensory modality or in a different aspect of the same modality. Synaesthetes may thus "see music," "hear colours," or "taste shapes," while they simultaneously hear music, see colours, or taste flavours the way non-synaesthetes would if the corresponding two senses were stimulated concurrently.

Defining Characteristics and Phenomenology

Central to the definition of synaesthesia and differentiating it from seemingly comparable phenomena like illusions and hallucinations, is that synaesthesia must always be *elicited* by a stimulus. Furthermore, the induced synaesthetic percept always exists in conjunction with, and never overrides, the inducing stimulus, i.e., taste-shape synaesthetes continue to taste flavours in addition to feeling tactile shapes. It is automatic, highly consistent, and specific (Baron-Cohen, Wyke, & Binnie, 1987), in addition to often being quite vivid (Grossenbacher & Lovelace, 2001). However, most synaesthetes do not generally confuse their induced experiences with actual components of the external world (Rich & Mattingley, 2002). In addition to the observable characteristics of synaesthesia, it has been proposed that its underlying causes also be considered. However, there is still much debate regarding what these entail (see subsection, "Underlying Neural Mechanisms"), whether there are multiple causal pathways, and whether developmental (congenital) and acquired (for example, following sensory loss) synaesthesia share the same neural bases.

With respect to test-retest reliability, consistency tends to be between 80%-100% in synaesthetes, compared to 30%-50% in controls (Walsh, 1999). It should be noted that although consistency (of associations) is commonly

accepted as a marker of synaesthesia (but see Simner (2012)), it alone does not warrant diagnosis and is never used without an additional first-person report of the phenomenon. In fact, consistency alone does not lead to the same physiological manifestations (i.e., brain activity, startle response) as real synaesthesia (Elias, Saucier, Hardie, & Sarty, 2003; Meier & Rothen, 2009; Zeki & Marini, 1998).

The eliciting stimulus is termed 'inducer' and the resulting percept the 'concurrent' – and the particular type of synaesthesia is always referred to in the corresponding inducer-concurrent pair, so that, for example, 'touch-colour' denotes the form of synaesthesia in which tactile sensations induce coloured percepts (Grossenbacher & Lovelace, 2001). Although inducers can be representational (i.e., linguistic), concurrents normally comprise simple perceptual features like colour (Grossenbacher & Lovelace, 2001).

The most common types of inducers are linguistic (letters, digits, words), and the most common types of concurrents are visual (colours, textures, spatial forms). Among the most common types of synaesthesia are grapheme-colour, day-colour, and mirror-touch synaesthesia, the former of which has a prevalence rate of about 1.4% (Shapley & Hawken, 2011; Simner et al., 2006). In grapheme-colour synaesthesia, digits, letters, and/or words induce colour perceptions (Cohen Kadosh & Henik, 2007; Rich & Mattingley, 2002; Simner et al., 2006).

Unidirectional versus Bidirectional

The experience of synaesthesia is typically unidirectional (Rich & Mattingley, 2002), but multiple studies have proposed that synaesthesia is actually implicitly bi-directional, given that behaviour has been shown to be influenced by "reverse" synaesthetic associations (i.e., concurrents acting as inducers) (Brang, Edwards, Ramachandran, & Coulson, 2008; Cohen Kadosh, Cohen Kadosh, & Henik, 2007; Cohen Kadosh & Henik, 2006; Cohen Kadosh et al., 2005; Gebuis, Nijboer, & van der Smagt, 2009; Johnson, Jepma, & de Jong, 2007; Knoch, Gianotti, Mohr, & Brugger, 2005; Meier & Rothen, 2007; Rothen, Nyffeler, von Wartburg, Muri, & Meier, 2010; Ward & Sagiv, 2007; Weiss, Kalckert, & Fink, 2009). Additionally, one study using TMS supports the mediation of both unidirectional and bidirectional effects by the same brain

areas (parieto-occipital areas) (Rothen et al., 2010). Interestingly, the question of whether synaesthesia has an implicit, bi-directional component is directly related to the question of whether the "consciousness" of concurrents is, or should be, a defining characteristic of the phenomenon. Does synaesthesia necessitate a conscious concurrent, or does it only sometimes (or often times) elicit one? The point of view claiming that synaesthesia exists along a continuum (Marks, 1987; Martino & Marks, 2000) would suggest that the consciousness (or vividness) of the induced concurrent merely separates "strong" synaesthesia from weaker forms of the phenomenon.

Low versus High, and Projectors versus Associators

The majority of synaesthesia studies to date have explored grapheme-colour, since it is one of the most prevalent variants. Thus, much of the terminology characterizing synaesthesia derives from it. Whether or not the corresponding classifications/ distinctions (see below) apply to all other types of synaesthesia is not entirely clear.

While the reality of the synaesthetic experience is now widely accepted, its phenomenological aspects are poorly understood. How closely is the synaesthetic experience of colours equivalent to real colour perception? Additionally, what do synaesthetes mean when they say that they see "coloured" achromatic (or differently coloured) graphemes? Synaesthetic percepts can be elicited perceptually (for example, by seeing a printed digit) and/ or conceptually (for example, by merely thinking about a specific digit, or by seeing a conceptual representation of that digit in the form of Roman numerals or clusters of dots) (Grossenbacher & Lovelace, 2001). This distinction is sometimes referred to as "higher" and "lower" synaesthesia (Ramachandran & Hubbard, 2001b). Furthermore, it is generally accepted that there exist two subtypes of synaesthetes: (1) projectors, who experience their perceptions in a field of view external to their bodies, either as a transient mist, a transparent coloured overlay, or as saturating the printed letter; and (2) associators, who report mental imagery in their "mind's eye" (Dixon, Smilek, & Merikle, 2004). Projectors typically experience coloured graphemes simultaneously in their veridical and synaesthetic colours, but these experiences neither mix nor occlude each other (Kim, Blake, & Palmeri, 2006; Palmeri, Blake, Marois,

Flanery, & Whetsell, 2002). Recently, however, much doubt has been cast on this latter distinction (see Eagleman (2012)) primarily because self-report often depends on the phrasing of the questions asked, and also tends to be inconsistent (Edquist, Rich, Brinkman, & Mattingley, 2006) or else too bimodal when contrasted with synaesthetes' actual self-assessments (Rouw & Scholte, 2007), see Appendix). There is high variability in the questionnaires administered to synaesthetes, and often the phrasing used in these conveys ambiguity to synaesthetes. There have been attempts to rectify these discrepancies by proposing illustrative, in addition to descriptive, measures to depict synaesthetic experiences (Skelton, Ludwig, & Mohr, 2009); these aim to avoid textual ambiguities by accompanying verbal descriptions with clear illustrations. Similarly, Rothen and colleagues (2013) have recently designed a questionnaire, based on a large-scale study, that aims to capture the heterogeneity of grapheme-colour synaesthesia and provide test-retest reliability. Unfortunately, none of these questionnaires are yet widely or uniformly used among synaesthesia researchers and thus classifications into synaesthetic subtypes remains, to some extent, unreliable.

Nevertheless, there is evidence to suggest that the projector-associator distinction, or some variation of it, may correlate with both behavioural as well as neurobiological characteristics in synaesthetes. First, the distinction is predictive of individual differences in performance on the synaesthetic Stroop task (M. J. Dixon et al., 2004); and second, neuroimaging has supported the existence of disparate neural mechanisms for each subtype (see subsection, "Underlying Neural Mechanisms" for more information).

Characteristics Linked to Synaesthesia

Synaesthesia is associated with several positive cognitive enhancements, including superior memory, though not all aspects of memory (Rothen & Meier, 2010a; Simner, Mayo, & Spiller, 2009; Smilek, Dixon, Cudahy, & Merikle, 2002; Yaro & Ward, 2007), heightened visual imagery (Barnett & Newell, 2008), and elevated performance on perceptual tests/ heightened perception in the "synaesthetic" sense (Banissy, Walsh, & Ward, 2009; Barnett, Foxe, et al., 2008; Ramachandran & Hubbard, 2001a). Additionally, it is linked to other

characteristics like schizotipy (Banissy et al., 2012), out-of-body experiences (Terhune, 2009), and *mitempfindung* (Burrack, Knoch, & Brugger, 2006).

There are also claims that synaesthetes tend to be creative (Rich, Bradshaw, & Mattingley, 2005; Ward, Thompson-Lake, Ely, & Kaminski, 2008), artistic (Rothen & Meier, 2010b), and highly emotional individuals; that they are mostly left-handed; and that they suffer from left-right confusion (Rich et al., 2005), poor arithmetical reasoning (Ward, Sagiv, & Butterworth, 2009), and/or deficient topographical cognition (Baron-Cohen, Burt, Smithlaittan, Harrison, & Bolton, 1996; Rich & Mattingley, 2002). These claims, however, have not been backed by systematic investigations and thus are contested.

Establishing Objective Measures and Consistency

Synaesthesia is highly idiosyncratic, resulting in inter-individual variability among synaesthetes of the same type, so that for example middle C may induce a shade of red for one synaesthetes but a shade of green for another. There is, however, some evidence pointing to non-random associations between inducer and concurrent pairings, resulting in inter-individual agreement, for example of high frequency graphemes paired to high frequency colour names (Simner et al., 2005).

Even though synaesthesia seems highly idiosyncratic, intra-individual variation of grapheme-colour pairs is low, making synaesthetic percepts highly consistent over time. In psychophysics and cognitive neuroscience, this forms the basis of objective identification of most types of synaesthesia, as well as of most methods of investigation into synaesthesia. In tests of consistency, inducer-concurrent pairings are analysed for stability over time, while synaesthetes are unaware that a re-test will be administered. Among the types of synaesthesia currently confirmed through tests of consistency are grapheme-colour (Baron-Cohen et al., 1987; Walsh, 1999), time-space synaesthesia (Smilek, Callejas, Dixon, & Merikle, 2007), and sound-colour synaesthesia (Ward, Huckstep, & Tsakanikos, 2006).

Stroop Test as a Marker of Synaesthesia

Aside from consistency, the most robust measure of synaesthesia is a modified version of the Stroop test heretofore referred to as the modified-Stroop, or synaesthetic-Stroop test (M. J. Dixon, Smilek, Cudahy, & Merikle, 2000; Walsh, 1999). Here, inducers replace colour names (as presented in the original Stroop); for example, for grapheme-colour synaesthetes, single coloured graphemes are presented and participants are asked to name their print colour as fast as possible, which they have been shown to be slower to name when they appear in a print colour incongruent to synaesthetes' induced synaesthetic colour, and faster when the print colour matches the colour concurrent. This interference effect has been found for several types of synaesthesia, including grapheme-colour (Walsh, 1999), music-taste (Beeli, Esslen, & Jancke, 2005), music-colour (Ward et al., 2006), mirror-touch (Banissy & Ward, 2007), and spatial forms of synaesthesia (Sagiv, Simner, Collins, Butterworth, & Ward, 2006). Importantly, Stroop interference demonstrates that synaesthesia is automatic and (under normal attentional circumstances), obligatory. However, the Stroop test cannot distinguish between perceptual and conceptual associations, as interference can result from overlearned associations, such as in trained controls who just know rather than perceive their associations (Colizoli, Murre, & Rouw, 2012; Elias et al., 2003; Meier & Rothen, 2009) and who claim to experience no phenomenological indications of synaesthesia (i.e., a first-person "synaesthetic" experience). This is true even for long-term trainees, as in the control participants included in Elias et al. (2003), who were experts in cross-stitching for approximately 8 years prior to the study and thus held strong semantic associations between numbers and colours.

Nonetheless, the modified-Stroop task does give some insight into the synaesthetic experience, as it has been shown that there are systematic differences in Stroop interference between projector and associator grapheme-colour synaesthetes (M. J. Dixon et al., 2004), such that projectors show greater interference in both colour naming (169 msec vs. 106 msec, classical modified-Stroop task) and photism naming (60 msec vs. 34 msec, the same task but ignoring print colours and instead naming induced colour concurrents). These patterns suggest that photisms are more automatically induced in projectors than associators, possibly because, being externally projected, they are also

more difficult to ignore. Whether these differences represent categorical or rather continuous (i.e., along a spectrum) differences is debated; nevertheless, it points to the fact that synaesthesia subtypes, i.e., differences in first-person report, can be corroborated by third-person objective measures and additionally may reflect differences in underlying mechanisms.

Synaesthesia: Prevalence and Acquisition

In the adult general population, the prevalence of synaesthesia was initially estimated to be about 1 in 2,000, and even higher in infants/children, arguably being a feature of normal development that disappears with normal neural pruning following birth (Baron-Cohen et al., 1996; Rich et al., 2005). Additionally, it was claimed to be about five times more common in females than in males (Baron-Cohen et al., 1996; Rich et al., 2005). These estimates, however, were based on responses to newspaper adverts and thus likely were skewed by (i) number of respondents relative to reportability, as well as (ii) a higher number of female respondents. Synaesthetes usually report more than one (and often several) forms of synaesthesia, and they normally manifest surprise upon learning that others do not share their same perceptual experiences, thus often naïvely failing to report their synaesthesia (Grossenbacher & Lovelace, 2001). More recent studies screening large populations in addition to using objective measures of synaesthesia have reported prevalence rates of ~4% and a female to male ratio of 1:1 (Simner et al., 2006; Ward & Simner, 2005).

Among the most common types of synaesthesia are day-colour (2.8%, (Simner et al., 2006)), mirror-touch (1.6%) and grapheme-colour (1.4%, (Shapley & Hawken, 2011)) synaesthesia, as well as types of synaesthesia relating to spatial forms (2.2%, (Brang, Teuscher, Ramachandran, & Coulson, 2010; Sagiv et al., 2006).

Underlying Neural Mechanisms

There are several accounts describing the neural mechanisms of synaesthesia. While they all seek to explain deviant cross-talk between brain areas, they approach the topic from two fundamentally different standpoints,

disagreeing on whether the brain areas representing the synaesthetic inducer and concurrent are functionally or anatomically connected. The first theory is based on functional anomalies, positing altered inhibitory interactions (i.e., a release from inhibition) or recurrent processing between brain areas consisting of entirely normal neural connections (Grossenbacher & Lovelace, 2001; Hubbard & Ramachandran, 2005; Smilek, Dixon, Cudahy, & Merikle, 2001). These two variations both propose abnormal disinhibited feedback, either flowing back from a multisensory nexus (i.e., long-range) or else from one relevant brain area to the other (i.e., aberrant re-entrant processing). The second theory is based on structural anomalies, arguing for the existence of deviant brain architecture (i.e., increased connectivity) between relevant brain areas, for example due to excess anatomical connections or to a failure of pruning following birth (Ramachandran & Hubbard, 2001a). However, recently this theory of local cross-activation has been revised to reflect both new models of grapheme recognition (i.e., as a process of hierarchical feature analysis, for reviews see (Dehaene, Cohen, Sigman, & Vinckier, 2005; Vinckier et al., 2007)) as well as evidence for parietal cortex involvement in synaesthetic associations. This updated theory, referred to as the cascaded cross-tuning model (Hubbard, Brang, & Ramachandran, 2011), is primarily founded on principles of Cross-Activation but also acknowledges a (normal, i.e., not unique) role for top-down influences from the parietal cortex (i.e., in the “hyperbinding” of grapheme and colour features).

Most studies investigating the neural substrates of synaesthesia have focused on grapheme-colour, the most common type; however, these studies are inconclusive. The bulk of studies have taken a neuroimaging approach, but evidence has been conflicting; on one hand, several studies support the hypothesis that synaesthesia is governed by excess connectivity giving rise to local cross-activation between early visual areas (Hubbard, Arman, Ramachandran, & Boynton, 2005; Ramachandran & Hubbard, 2001a, 2001b; Rouw & Scholte, 2007; Sperling, Prvulovic, Linden, Singer, & Stirn, 2006), while on the other hand, several other studies have failed to find activation of early visual areas and/or reveal involvement of higher processing areas, thus supporting the alternate hypothesis that synaesthesia is governed by inhibitory interactions mediated by entirely normal neural connections (Elias et al., 2003;

Hupe, Bordier, & Dojat, 2012; Rich et al., 2005; Weiss, Zilles, & Fink, 2005). In one of these most recent studies, Hupe and colleagues (2012) not only failed to find involvement of area V4, but also highlighted severe methodological flaws in many of the studies mentioned above, and implying that the corresponding results may be statistically unreliable. Importantly, neuroimaging is considered a weak test between models of synaesthesia, as the low temporal resolution of fMRI makes both theories plausible *even* given activation in early visual areas.

There have also been studies employing DTI (diffusion tensor imaging) (Rouw & Scholte, 2007) or VBM (voxel-based morphometry) (Jancke, Beeli, Eulig, & Hanggi, 2009; Weiss & Fink, 2009) showing structural connectivity differences between brain areas in grapheme-colour synaesthetes, but not controls. Of particular interest, Rouw and Scholte (2007) showed, not only greater anisotropic diffusion in grapheme-colour synaesthetes as compared to non-synaesthetic controls, but also differential white matter connectivity *between projector and associator subtypes*, manifested as greater connectivity in inferior temporal cortex near the fusiform gyrus in projectors as compared to associators. This has led to the idea that different neural mechanisms may underlie projector and associator subtypes, in this way accounting for individual differences in synaesthesia (see also van Leeuwen (2010)). Whether the structural differences observed in synaesthetes reflect causal properties of synaesthesia or are rather epiphenomena of repeated, synaesthetic associations (i.e., changes in white matter resulting from training-induced plasticity effects) remains unresolved (see Rouw, Scholte, and Colizoli (2011) for a review).

While electrophysiological approaches may provide the best method for disentangling the two main models of grapheme-colour synaesthesia, there have been a few EEG studies and these have primarily addressed modulations of synaesthetic congruency (i.e., in congruently versus incongruently coloured graphemes) in the context of semantic priming (Brang et al., 2008; Brang, Kanai, Ramachandran, & Coulson, 2011). Thus, despite modulations of early ERP components (such as the N1 and P2 components), it is not clear how these may relate to the neural mechanisms underlying the induced, synaesthetic percept. Additionally, these studies could not accurately localise the underlying neural generators of the electrophysiological components due to volume conduction

limitations typically characteristic of EEG data. There has only been one MEG study to date (Brang, Hubbard, Coulson, Huang, & Ramachandran, 2010), which provides evidence that neural activity in area V4 is significantly more active in projector grapheme-colour synaesthetes than in controls between 111-130 ms after grapheme onset. Additionally, this activity reached significance only 5 ms after that of the grapheme processing area, posterior temporal grapheme area (PTGA). However, it should be noted that the results obtained by Brang and colleagues (2010) rely almost entirely on methodologically, very challenging techniques, including retinotopic mapping of area V4 in the MEG, which has not yet proven robust (i.e., no published MEG studies to date using this method). In fact, retinotopic mapping is typically obtained from high-resolution fMRI and then used to spatially constrain the source estimates from electrophysiologically-derived data (Hagler et al., 2009; Wibral, Bledowski, Kohler, Singer, & Muckli, 2009).

Genetic versus Developmental: An Interaction?

Synaesthesia is common among biological relatives and is thus hypothesized to result from a genetic predisposition; in fact, its frequency among first-degree relatives of synaesthetes exceeds 40% (Barnett & Newell, 2008; Baron-Cohen et al., 1996; Ward & Simner, 2005). It has been proposed that synaesthesia may be acquired through transmission of an X-linked autosomal dominant gene (Baron-Cohen et al., 1996; Rich & Mattingley, 2002), in great part because there appears to exist a predominance of synaesthesia in females; however, the male:female ratio varies across studies and the bias has not been supported by genetic data. Recent studies conducting whole-genome linkage analyses (Asher et al., 2009; Tomson et al., 2011), in addition to other previous studies (Barnett, Finucane, et al., 2008; Ward & Simner, 2005), have pointed to alternate modes of inheritance and even reveal common genetic markers for clusters of synaesthesia.

While most cases are, in fact, congenital, there are also cases of developed synaesthesia following sensory deafferentation (Armel & Ramachandran, 1999), acquired blindness (Armel & Ramachandran, 1999; Steven & Blakemore, 2004), and ingestion of hallucinogenic substances (though the latter's relationship to synaesthesia is debated) (Grossenbacher & Lovelace,

2001). It has also been proposed that synaesthesia is a *learned* phenomenon, or at least experience-dependent. Evidence in favour of this hypothesis came initially from a study indicating that the induced colours of a grapheme-colour synaesthete (of projector subtype) were learned from a set of refrigerator magnets in childhood and later transferred from English to Cyrillic in a systematic way (Witthoft & Winawer, 2006). Similar studies describing the cases of acquired grapheme-colour synaesthesia (Hancock, 2006; Witthoft & Winawer, 2013) also document individuals who developed their particular synaesthetic associations following repeated exposure to the same pairings during childhood (i.e., refrigerator magnets, jigsaw puzzle). It should be noted, however, that the learned and the genetic accounts of synaesthesia are not mutually exclusive, as a genetic predisposition to synaesthesia may still require environmental triggers to provoke development into "full blown," phenotypic synaesthesia.

Synaesthesia: Unique versus Universal

Related to the question of whether synaesthesia is genetic, developmental, or an interaction between the two, is the question of its *universality*. This point can be addressed on two complementary fronts. First, it has recently been questioned whether synaesthetic associations are truly arbitrary (i.e., random inducer-concurrent mappings), or whether there are recurrent patterns reflecting shared mappings across synaesthetes (Brang, Rouw, Ramachandran, & Coulson, 2011; Eagleman, 2010; Rich et al., 2005; Simner et al., 2005). Similarly to many normal (i.e., non-synaesthetic) cross-modal associations (see Spence (2011) for a review), these mappings may be acquired from exposure to regularities or statistically frequent pairings in the environment. While such "learned probabilities" cannot explain the idiosyncrasies of synaesthesia (i.e., making it nonreducible to previous exposure), they imply the presence of common mechanisms across synaesthetes, or at least some susceptibility to environmental input. Interestingly, there is also evidence that synaesthetes and non-synaesthetes use the same heuristics for cross-modal matching, e.g., of graphemes or sounds to colours, or of spatial sequences to inherent spatial mappings of non-synaesthetes (Cohen Kadosh et al., 2007; Cohen Kadosh & Henik, 2007; Eagleman, 2009; Rich et al., 2005; Simner et al., 2005; Ward et al., 2006). Similarly, other findings show that synaesthetic correspondences can influence multisensory perception in the

general population, even if detrimental to task performance (Bien, Ten Oever, Goebel, & Sack, 2012; Eagleman, 2012; Simner, 2012). Together, these studies suggest that synaesthesia and normal cross-modal integration are closely related and even fall along a spectrum (Eagleman, 2012; Martino & Marks, 2000; Simner, 2010), indicating that synaesthesia-training may be possible.

Trained Synaesthesia

The recent debates regarding the development of grapheme-colour synaesthesia, as well as its relationship to normal cross-modal integration in non-synaesthetes, has sparked an interest in whether synaesthesia can be trained in the adult general population. The underlying idea is that with training, automatic, perceptual, and arbitrary associations may be acquired by adult non-synaesthetes, eventually crossing the threshold of awareness and manifesting as conscious concurrents similar to those of associator grapheme-colour synaesthetes. However, there have only been three synaesthesia-training studies to date (Cohen Kadosh, Henik, Catena, Walsh, & Fuentes, 2009; Colizoli et al., 2012; Meier & Rothen, 2009). These, along with the studies presented in this thesis, will be explored in an attempt to assess their relationship to canonical grapheme-colour synaesthesia.

Human Colour Processing

As this thesis sets out to investigate and further understand the relationship between colour and form in trained non-synaesthetes, as well as the induced colour concurrents of grapheme-colour synaesthetes, a brief account of colour perception is considered. While the specific mechanisms of colour processing are beyond the scope of this thesis, the hierarchy of colour processing is discussed, with a slight emphasis on the possible role of V4 as a colour centre.

Despite extensive research on colour processing, there is disagreement regarding how colour perception works, and recent models have challenged Zeki's classically accepted scheme of colour processing (Zeki & Marini, 1998), which comprises three main stages. According to Zeki's model, wavelength information is initially processed in V1 and V2, after which colour constancy occurs in "colour area" V4, followed by the association of colour with form, likely in inferior temporal cortex (IT). However, more recent studies have

challenged Zeki's view and redefined the roles for each of these cortical areas in the hierarchy of colour processing.

Humans visibly perceive the spectrum of light between the wavelengths ~400-700 nm. Essentially, colour vision is possible through the processing and comparison of signals from three types of cone photoreceptors: short (S), medium (M), and long (L) cones, maximally sensitive to ~430 nm (corresponding to blue), ~530 nm (corresponding to green), and ~560 nm (corresponding to red), respectively (Solomon & Lennie, 2007). Hence derives the "trichromacy" of human colour vision.

The visual pathway begins in the retina, where light entering the eye passes through multiple layers (including the different photoreceptors as well as different types of specialized cells, like bipolar, horizontal, amacrine, and ganglion cells) before projecting to the lateral geniculate nucleus (LGN) via the optic nerve. The LGN is organized into six layers, each reflecting the type of ganglion cell that provides input to that particular layer(Solomon & Lennie, 2007). Here, the four more dorsal layers are termed the parvocellular (P) layers, and the two more ventral layers the magnocellular (M) layers. Additionally, the koniocellular (K) layers are found ventral to both of these. Each layer-type responds to signals from different combinations of photoreceptors, giving rise to three opponent channels (see Conway (2009) for a review): (1) P-cells oppose signals from L- and M-cones (L vs. M), and thus are important for red-green colour vision (in addition to spatial vision) (Solomon & Lennie, 2007); (2) K-cells oppose signals from L- and M-, and S-cones (L+M vs. S) and are important for blue-yellow colour vision; and (3) M-cells respond to signals from L- and M-cones (L+M), making them sensitive only to achromatic stimuli (light vs. dark).

The axons of the LGN project differentially to layer 4 of primary visual cortex, V1, where neurons differ in terms of receptive field properties, and where there exist two types of cells: colour-luminance cells (most abundant), and colour-preferring cells (rare, account for ~10% cell population) (Solomon & Lennie, 2007). Colour-luminance cells are sensitive to colour *contrast* rather than to spatially uniform modulations of colour (Shapley & Hawken, 2002). This indicates that the perception of colour contrast (including colour constancy) may begin as early as V1, rather than in extrastriate visual cortex as predicted in the classically accepted theories of colour processing. Colour constancy refers to the

visual system's ability to perceive colour even under varying illumination conditions, and more accurately reflects how colour is perceived by humans.

In contrast to Zeki's classical view of primary visual cortex and "colour area" V4, more recent studies have implicated these areas in broader roles, including (1) V1 and V2 in the processing of hue and luminance, in addition to wavelength, and (2) V4 in the perception and learning of form, selective attention to form and other attributes, and memory (see Walsh (1999), for a review). In the competing views, awareness of colour is attributed to IT. Thus, although V4 is still referred to as a "colour centre," it is important to consider its role in the analysis and synthesis of visual form (see Shapley and Hawken (2011) for a review). This is particularly relevant to the synaesthesia community, since much of the focus of neuroimaging studies has been on V4 and the implications of its role as a "colour centre" especially in the context of synaesthetic concurrents.

Chapter 2: Methods and Techniques

transcranial Direct Current Stimulation

Although the use of uncontrolled electrical stimulation dates back to early history (Kellaway, 1946), it was not until the invention of the electric battery in the 18th century that it begun its development into a controlled, systematic technique (Zago, Ferrucci, Fregni, & Priori, 2008). Transcranial direct current stimulation (tDCS) is a non-invasive, neuromodulatory technique that induces neuronal, as well as behavioural, changes via the application of a low-amplitude electric current to the head. It is a quickly-growing technique, primarily due to its low cost, simple application, well-tolerated effects, and recent success as a therapeutic (substitutive or additional) treatment for psychiatric disorders (for example, depression, obsessions, bipolar disorders, post-traumatic stress disorder), neurological diseases (for example, Parkinson's disease, tinnitus, epilepsy), rehabilitation (of aphasia or hand function following stroke), pain syndromes (for example, migraine, neuropathies, or lower-back pain), and internal visceral diseases (cancer) (Nitsche et al., 2008; Wagner, Valero-Cabre, & Pascual-Leone, 2007).

The tDCS apparatus merely consists of a DC source attached to scalp electrodes, which are typically made of conductive rubber plates and placed inside saline-soaked sponges; these are placed on the head and deliver a pre-defined, constant current for which the voltage is constantly adjusted by a potentiometer. Although the electric current that successfully passes through the various tissue layers of the head and eventually reaches the brain does *not* typically elicit an action potential, it modifies the transmembrane neuronal potential in a polarity-dependent way and thus modulates the spontaneous firing rate of neurons as well as their responsiveness to afferent synaptic input (Bikson et al., 2004; Bindman, Lippold, & Redfearn, 1964b; Nitsche et al., 2008; Priori, Hallett, & Rothwell, 2009), affecting neuronal excitability. The anode is presumed to increase cortical excitability, and the cathode to decrease it (Nitsche & Paulus, 2000). Furthermore, given its long-lasting after-effects, tDCS also modifies the synaptic microenvironment in multiple ways, ranging from processes similar to long-term potentiation (LTP) to prolonged neurochemical changes (see Brunoni et al. (2012) for a review). There is also recent evidence

that tDCS may exhibit connectivity-driven effects on remote cortical areas (Boros, Poreisz, Munchau, Paulus, & Nitsche, 2008; Villamar, Santos Portilla, Fregni, & Zafonte, 2012). Consequently, tDCS may induce controlled changes in neuropsychologic activity and behaviour.

In conventional tDCS, a low-amplitude, constant current is delivered to the head via the scalp electrodes, which normally have a surface area of 25-35 cm^2 (Wagner et al., 2007). However, a more focal version of tDCS has recently been developed, called high-definition tDCS (HD-tDCS), which employs variable multi-electrode ring-configurations with smaller electrode sizes (< 12 mm diameter): for example, a 4x1-ring containing one "active" (anode) disc electrode and four "return" (cathode) disc electrodes, each having a radius of 4 mm (Datta et al., 2009; Minhas et al., 2010). In conventional tDCS, the current applied to the head typically ranges from 0.5-2 mA and lasts anywhere from seconds to minutes.

The areas affected by stimulation are presumed to lie broadly underneath the scalp electrodes, as well as in the interconnected neural networks (Villamar et al., 2012). However, there is evidence that the peak magnitude of the induced electric field lies not directly underneath the scalp electrodes, but rather at an intermediate area between the anode and cathode (Datta et al., 2009). Thus, conventional tDCS has limited focal capacity, as neighbouring anatomical areas are also affected. Several studies have addressed the various parameters of tDCS stimulation that may contribute to its focality, including inter-electrode distance (Moliadze, Antal, & Paulus, 2010) and sponge size (Nitsche et al., 2007). It is presumed that HD-tDCS is more focal than conventional tDCS (Datta et al., 2009); but given how novel it is, this point remains to be confirmed by future studies. In the 4x1-ring configuration, the active (centre) electrode defines the polarity of stimulation (anodal vs. cathodal), and the radii of the return electrodes confine the area modulated by the applied current (Datta et al., 2009). There is some evidence that the after-effects of HD-tDCS may outlast those of conventional tDCS (Kuo et al., 2013). (See Villamar et al. (2013) for a short review of the 4x1-ring configuration.) Despite the nonfocality of tDCS, electrode placement is critical and changing the

electrode sites can change, or even eliminate, the desired effects (Antal, Kincses, Nitsche, & Paulus, 2003; Boggio et al., 2008; Fregni et al., 2005).

Of course, the current densities in the brain are very different from those measured at the scalp surface, as the current must pass through several surfaces before reaching the brain, including the skin, skull, and cerebrospinal fluid (CSF). In addition, current distributions must take into account true head anatomy, tissue properties, and electrode properties (Wagner et al., 2007). For example, as the current passes through the scalp surface, "shunting" occurs (a flow of current along the scalp surface), an effect that is considerably larger for smaller electrode sizes (Wagner et al., 2007). The current that crosses into the skull, which is the most highly resistant of the aforementioned surfaces, is significantly attenuated before reaching the highly conductive CSF.

Much of what we know about the current densities, current distributions, and DC effects on cortical neurons comes from measurements using various electrophysiological recording techniques in either animal studies (Bikson et al., 2004; Bindman, Lippold, & Redfearn, 1964a; Fritsch et al., 2010; Liebetanz, Fregni, et al., 2006; Liebetanz, Klinker, et al., 2006; Purpura & McMurtry, 1965; Rush & Driscoll, 1968) or human patients (for example, during pre-surgical evaluation for epilepsy) (Dymond, Coger, & Serafetinides, 1975), pharmacologic studies combined with tDCS (see Brunoni et al. (2012) for a review), or computational models of brain current flow – though all of these are still scarce, remain difficult to test/are ethically inaccessible for testing, and/or require further empirical (re-)confirmation.

Recent computational modelling of current flow in the brain has challenged common electrode-placement assumptions, for example the "AeCi" (polarity-specific) effects of tDCS. This point has recently been investigated in a meta-analytical review that suggests that while the polarity-specific effects of tDCS may hold for the motor domain, it may not for the cognitive domain (Jacobson, Koslowsky, & Lavidor, 2012). Rather, anodal stimulation is likely to have excitatory effects, while cathodal stimulation rarely causes inhibition, possibly due to compensatory processes by other brain networks. This may be possible, given that the reverse has also been reported (mental costs of cognitive enhancement by tDCS, see Iuculano and Cohen Kadosh (2013)).

Additionally, the electrode montage used (placement and size) may significantly modulate the actual current flow in the brain (Bikson, Datta, Rahman, & Scaturro, 2010).

Magnetoencephalography

In the 1960s, the possibility of recording the brain's magnetic fields emerged with the first induction-coil magnetometer, a single-channel instrument which had 2 million turns of copper wire wound around a ferrite core and required an electric reference (Hari & Salmelin, 2012). By the early 1990s, with the invention of the Superconducting QUantum Interference Devices (SQUIDs), this had already evolved into a whole-scalp 122-sensor MEG system. In comparison with EEG, MEG allowed much better localisation of the underlying neural generators of the recorded signal. In EEG, the electric potential is measured on the scalp, and thus it is subject to distortion and smearing due to the low electrical conductivity of the skull. On the other hand, the electric currents that give rise to the magnetic fields measured by the MEG are confined to the intracranial space, and their magnetic fields pass through the head unperturbed. The magnetic fields outside the head are in the hundreds of femto (10-15) Tesla, about 100 million times smaller than Earth's geomagnetic field. The generators of both EEG and MEG signals are synchronous postsynaptic (intracellular) currents in the pyramidal neurons of the cerebral cortex (Hari, 1990). MEG is thus most sensitive to superficial cortical currents tangential to the skull, in the walls of cortical fissures, whereas EEG also picks up signals from deep and radial sources.

Modern MEG systems contain more than 300 SQUID sensors maintained at extremely low temperatures (~4 K) in liquid helium, and they are housed in magnetically shielded rooms. The SQUIDs receive their input from different kinds of flux transformers: magnetometers, axial gradiometers, or planar gradiometers. They all have different sensitivity profiles but are all situated as close as possible to the participant's head. Importantly, a single source can produce correlated signals on several sensors, even 10 cm apart (Hari & Salmelin, 2012).

The analysis of MEG data are typically performed either in the sensor or source space. In the sensor space, data acquired from the MEG sensors is directly analysed in time, frequency or time-frequency domains. Normally, it is first analysed and explored on this level, where the most common type of analysis is the derivation of Event Related Fields (ERFs), in which data are split in trials locked to a specific event (i.e., the stimulus onset) and then averaged at each time point (i.e., across trials). Importantly, this type of analysis highlights brain activity triggered by a specific event in a temporally consistent way. In the source space, the analysis is performed on a model of the cortical sheet or of the brain volume, onto which the MEG sensor data are projected. In general, this type of analysis is more complex than sensor-level analysis, as it requires (1) the derivation of a model of the subject's brain (normally acquired from a structural MRI scan) as well as (2) the derivation of projection vectors through which the MEG sensor data are projected inside the brain model. This latter step (i.e., the derivation of these projection maps) is termed the "inverse solution." A variety of methods exist for the computation of this "inverse solution", the most appropriate of which normally varies, as it depends on the particular scientific question at hand and/or on the characteristics of the data.

Aside from the sensor and source spaces, which are defined in Euclidean space, there are also other mathematically defined and interpreted spaces that have recently been incorporated into MEG analysis, with the aim of un-mixing and dissociating the superimposed magnetic fields of different neural sources and highlighting activity from even subtle neural sources. The most commonly used of these spaces are Principal Components and Independent Components (Vigario, Sarela, Jousmaki, Hamalainen, & Oja, 2000). Principal Component Analysis (PCA) transforms the highly correlated MEG data (i.e., due to field spread) into a set of components termed Principal Components, which are linearly uncorrelated (Makeig, Jung, Bell, Ghahremani, & Sejnowski, 1997). Independent Component Analysis (ICA) decomposes the MEG data into a set of components termed Independent Components, which are not only linearly uncorrelated but also statistically independent (Makeig et al., 1997). In the following subsections, some basic principles pertaining to MEG analysis are outlined and described.

The Forward Model

In MEG, the forward problem refers to the calculation of the magnetic field at specific locations outside the head, produced by a given current distribution inside the brain (Hamalainen, Hari, Ilmoniemi, Knuutila, & Lounasmaa, 1993). The forward problem makes several assumptions regarding the brain. First, it assumes that the brain is a closed volume with finite conductivity and permeability. Second, it assumes that there are two types of currents inside the brain: passive and primary, where the former refers to currents resulting from the macroscopic electric field and the latter to all other currents. Thus, passive currents flow everywhere in the brain, while primary currents are considered to be generated by neuronal activity (i.e., in the vicinity of neurons) (Hamalainen et al 2003). The solution to the forward problem is provided by a model of the resulting magnetic field, as produced by the combination of these (passive and primary) currents within the brain and measured at specific locations outside the head.

In the forward problem, the brain is represented by a finite number of brain locations. In turn, the current in each of these brain locations is represented by a single current dipole (Hamalainen et al., 1993). The relationship between each of these current dipoles and their (generated) magnetic field values (i.e., as measured at the MEG sensor locations) are described by a linear transformation termed the Leadfield (Λ). In simple terms, it could be said that the Leadfield describes what the MEG sensor data would look like, as generated by a single current dipole in the brain (i.e., providing a "map" from a single location in the brain to the MEG sensors). The estimation of these leadfields highly depends on the brain conductor model employed. Various such models have been tested in MEG analysis, such as single sphere, multiple spheres (Hamalainen et al., 1993) and single shell conductor (Nolte, 2003). Of these, the latter is considered the most realistic brain conductor model.

The Inverse Problem

The inverse problem is described as ill-posed because a given magnetic field outside the head has an infinite number of electrical current distributions that could have created it. The various methods for source localization make

different assumptions about how the brain works, and thus certain methods are better suited to certain kinds of brain responses.

Dipole Fitting

In Dipole Fitting, the assumption is that only one (or a handful) of brain areas is strongly time-locked to an external stimulus. This technique locates the equivalent current dipoles (ECDs) in the head by estimating certain parameters (i.e., the location, direction, and strength of current flow in a point-like source, as a function of time) in a way that best "matches" the observed (i.e., measured) magnetic field signal. This simple dipole model can be thought of as an infinitesimal concentration of directed current flow, which is essentially moved around the cortex until the magnetic field that it generates most closely "matches" the observed magnetic field (i.e., the measured signal). "Matching" is generally based on the widely-used least-squares (LS) technique, which attempts to minimize the (square of the) difference between the model predictions and the actual observations (i.e., measured signal). In Multi-Dipole Modelling, many ECDs are brought together and the strength (i.e., amplitude) of each one is varied in order to best account for the observed magnetic field (i.e., the measured signal) over the time interval of interest.

One important weakness of Dipole Fitting is that the more dipoles that are incorporated into the model, the more unstable they become. However, due to other issues beyond the scope of this thesis, including noise contamination, most research studies take a more conservative approach, using fewer dipole sources (typically less than 5). In general, Dipole Fitting works best for brain functions that average well, like sensory and motor processes, but not for higher cognitive functions. Averaging time-locked evoked responses over many trials attenuates the "noise" by drowning-out the signal produced by non-time-locked responses (and thus increasing signal-to-noise ratio). It has primarily been used to show basic somatotopy (Meunier et al., 2003; Baumgarter et al., 1991; Okada et al., 1984), primary auditory (Zimmerman, Reite & Zimmerman, 1981) and visual responses (Lehmann, Darcy & Skrandies, 1982). Importantly, Dipole Fitting is often criticised for some of its "subjective" aspects, like knowing the number of sources in advance as well as choosing the subset of MEG sensors to be included in the localization procedure.

Minimum Norm Based Approaches

Instead of modelling the measured magnetic field by using just a small number of discrete dipole sources, the Minimum Norm based approach simultaneously estimates the current *distribution* within a set of pre-defined sources (i.e., the brain volume as modelled by a 3D grid containing thousands of locations). As this solution is derived for *all sources* simultaneously, it is dependent on the number and location of pre-defined [potential] sources. In contrast to Dipole Fitting, which estimates a few focal current dipoles, Minimum Norm algorithms thus result in a current *distribution* over a large number of sources (i.e., the entire cortical sheet). Typically, the number of pre-defined [potential] sources exceeds the number of MEG sensors, resulting in an underdetermined inverse solution problem. As there are infinite solutions to this undetermined problem, a number of constraints are needed in order to derive a single solution. In Minimum Norm based approaches, the constraint used is the minimization of current required to produce the observed magnetic field (i.e., the measured signal). This minimization can be applied with respect to the L1- or L2-norms of the current. The main disadvantage of this method results from this very constraint, because it tends to bias solutions to superficial sources of the brain, where less power is required to produce the observed signal as compared to deeper structures. (See Appendix for a detailed, mathematical and theoretical description of the Minimum Norm method.)

Beamforming

Beamforming mainly differs from the Minimum Norm approach in that the contribution of each source location in the brain is estimated independently of all other source locations, rather than solving for all source locations simultaneously. These algorithms are extensively used for source localization because they are adaptive to the actual dataset, through the use of the data covariance matrix (in the solution). They are also good at localizing distributed, rather than point-like, sources. Additionally, the inverse solution is computed independently for each brain source, thus removing the dependence of the solution to the number of brain areas considered (as potential sources). One of the main disadvantages of Beamforming algorithms is their dependence on the (inversion of) the data covariance matrix. This means that for highly collinear

sensor time-series, the covariance matrix is rank deficient and thus cannot be inverted.

Independent Component Analysis

While Independent Component Analysis (ICA) has proven to be an efficient tool for artefact rejection from MEG data, it has only recently been applied to the analysis of analysed brain signals Here, it is used to decompose the event-related activity with the aim of extracting its dominant patterns (on a single-subject level).

Broadly, ICA seeks to separate statistically independent sources that have been mixed in the combined signal (i.e., MEG measurements) (http://sccn.ucsd.edu/~scott/tutorial/icafaq.html). It is generally assumed that the co-varying field measurements of a single component (i.e., the signal contained by a single component, which is a fraction of the entire signal) reflect single processes (either focal or distributed), or networks within the brain. ICA can thus be used as a tool for separating statistically independent brain responses to external stimuli (for example, event-related fields).

ICA assumes that the signal measured by the MEG sensors is a superposition of the magnetic fields from many individual current dipoles inside the brain. The purpose of ICA is thus to decompose the recorded signal into these individual components. In general, ICA algorithms use two criteria to perform this separation. The first criterion is that the mutual information between these components should be minimum (non-correlated linearly or non-linearly). The second criterion is that these components should be maximally non-Gaussian. This latter criterion comes from the Central Limit Theorem, which states that the superposition of many independent random variables produces a variable with Gaussian distribution. MEG measurements, which have a Gaussian distribution, can be considered as the superposition of the magnetic fields of many individual non-Gaussian sources. Under this assumption, ICA tries to identify such sources with non-Gaussian distributions.

Typically, a single component can capture either a single dipole emanating from a focal cortical area or more complex, distributed dipole fields.

The main advantage offered by this type of decomposition is that it isolates such components from the rest of the brain signal and background noise so that they can be subsequently investigated in a "cleaner" fashion.

Most ICA algorithms make no use of any information regarding the spatial location of MEG sensors; rather, the identified patterns depend solely on the statistical characteristics of the *time series* of each of the sensors. In contrast to Principal Component Analysis, which is always derived through the singular value decomposition (i.e., is always the same no matter how many times it is recomputed), ICA is usually computed by recursive numerical algorithms, which try to minimize mutual information and maximize non-Gaussianity; the identified independent components (ICs) differ from run to run. *Thus, it is difficult to compare components extracted from different decompositions.*

Localising individual Independent Components in the brain is an area of active research in the field of neuroscience methods. For each Independent Component (IC), the corresponding covariance matrix at the sensor level has rank 1, meaning that all sensor time-series are co-linear, as they are weighted versions of the same IC. In such cases, inverse solution methods that use the data covariance matrix ,i.e. Beamformers, are not suitable because such covariance matrices cannot be inverted. Rather, the main classes of inverse solutions for such problems are dipole fitting and minimum norm. In order to perform dipole fitting, the brain sources must be very focal, approximated by a point, and the number of sources known. However, brain activity can involve non-focal distributed sources, which are difficult to approximate with a given number of dipoles. For such cases, the use of Minimum Norm methods offers more flexible inverse solutions.

Minimum Norm solutions for single ICA components have already been used (de Pasquale et al., 2010; Mantini et al., 2011). In these approaches, the Minimum Norm regularization parameter has been chosen for each IC differently, although the details of these procedures are not described in the corresponding publications.

Chapter 3: Formation of automatic letter-colour associations in non-synaesthetes through likelihood manipulation of letter-colour pairings

Introduction

Synaesthesia is characterised by paradoxical perception in which stimulation in one sensory modality automatically, involuntarily, and systematically elicits a conscious perception either in an additional sensory modality, or in a different aspect of the same modality. One of the most common types, along with day-colour and mirror-touch synaesthesia (Shapley & Hawken, 2011), is grapheme-colour synaesthesia, with a prevalence rate of about 1.4% (Simner et al., 2006). In this type of synaesthesia, orthographic forms of digits, letters, and/or words induce colour perceptions (Cohen Kadosh & Henik, 2007; Rich & Mattingley, 2002; Simner et al., 2006). Although synaesthesia is highly idiosyncratic, intra-individual variation of grapheme-colour pairs is low, making individual synaesthetic percepts highly consistent over time. In psychophysics and cognitive neuroscience, this forms the basis of objective identification of synaesthesia, as well as of most methods of investigation into this phenomenon.

Although grapheme-colour synaesthesia is well-documented, its underlying neural mechanisms remain unknown, and a number of questions linger regarding its manifestation across the general population. It has been shown that synaesthesia is far more common than previously assumed (Rich et al., 2005; Simner et al., 2005), and that synaesthetes and non-synaesthetes use the same heuristics for cross-modal matching, e.g., of graphemes or sounds to colours (Cohen Kadosh et al., 2007; Simner et al., 2005; Ward et al., 2006). In addition, grapheme-colour synaesthesia has proven difficult to capture due to variability in the phenomenological experience, manifested across synaesthetes as graded effects on perception measured through various cognitive tasks, including digit search and modified-Stroop tasks (M.J. Dixon, Smilek, Duffy, & Merikle, 2006; Simner, 2012). From all this derives the hypothesis that synaesthesia may recruit mechanisms of normal cross-modal perception, albeit in an exaggerated form. If synaesthesia represents an overdeveloped capacity in cross-modal processing that we all possess, synaesthesia-like behaviour should

also be expressed in the general population, rather than being unique to a few individuals (Cohen Kadosh & Henik, 2007; Hubbard et al., 2005; Mann, Korzenko, Carriere, & Dixon, 2009). This view has been supported by several recent findings (Bien et al., 2012; Eagleman, 2012; Martino & Marks, 2000; Simner, 2012). Related to this is the question of whether synaesthesia has a learned or a genetic basis. If synaesthesia arises from common rather than unique cross-modal mechanisms, then it is possible that synaesthesia may be learned to some degree by non-synaesthetes into adulthood.

Evidence for *developed* synaesthesia following sensory deafferentation (Armel & Ramachandran, 1999; Steven & Blakemore, 2004), late blindness (Armel & Ramachandran, 1999; Steven & Blakemore, 2004), and intake of hallucinogens (Grossenbacher & Lovelace, 2001) indeed indicates that aspects of synaesthesia can be learned, or at least are *experience-dependent*. This is further supported by case-studies into synaesthesia. Some grapheme-colour synaesthetes seem to have acquired grapheme-colour associations in childhood through repeated exposure to grapheme-colour pairings, e.g., in the form of refrigerator magnets (Witthoft & Winawer, 2006) or a jigsaw puzzle (Hancock, 2006). Additionally, there is evidence for non-random, structured biases in synaesthetic grapheme-colour experiences across individuals, indicating that environmental factors influence grapheme-colour associations (Simner et al., 2005). Besides this evidence for the experience-dependence of synaesthesia, there is also support for a genetic basis. Studies highlighting the frequency of synaesthesia among biological relatives (Ward & Simner, 2005), as well as family linkage analyses (Asher et al., 2009; Tomson et al., 2011), reveal common genetic markers for clusters of synaesthesia. Thus, it seems likely that the phenomenon arises from an interaction between environmental influences and a genetic predisposition.

One key to better understand synaesthesia is therefore to study the extent to which adult non-synaesthetes may acquire synaesthesia-like associations, for instance via brief cross-modal associative learning. This is likely to provide information on a number of outstanding points, including the learning account of synaesthesia, and on whether synaesthesia-like associations are present in the general population, or unique to a few individuals. Two recent

attempts to train adult non-synaesthetes with specific grapheme-colour associations using brief training paradigms (<=7 days) were successful (Cohen Kadosh et al., 2009; Meier & Rothen, 2009). The first study (Cohen Kadosh et al., 2009) used post-hypnotic suggestion to train digit-colour associations in four highly hypnotically susceptible non-synaesthetes. The second study (Meier & Rothen, 2009) trained a group of non-synaesthetes in letter-colour pairings over the course of seven days using a reinforcement task. Both studies made explicit that specific colour-grapheme pairings had to be learned. Cohen Kadosh et al. (2009) corroborated synaesthetic induction by both objective (digit search on coloured background) and subjective measures (phenomenological reports). Meier & Rothen (2009) found behavioural evidence (Stroop interference) but neither physiological (skin conductance) nor perceptual evidence (phenomenological experience) for induction of synaesthesia-like grapheme-colour binding.

In contrast to the above studies, we here adopted a training paradigm which mimics the natural conditions under which some synaesthetes seem to have learned their grapheme-colour pairings (Witthoft & Winawer, 2006; Hancock, 2006). Our paradigm involved frequent exposure to specific grapheme-colour pairings which were, in turn, task-irrelevant, and thus not learned intentionally. Specifically, we aimed to consolidate specific letter-colour associations in adult non-synaesthetes using a visual letter search paradigm combined with statistical learning (adapted from Fecteau, Korjoukov, and Roelfsema (2009)). Participants were instructed to search an array of six coloured letters for one of three predefined target letters, whilst we manipulated the likelihood of specific target letter-colour associations within the search array: two of the three target letters appeared more often in one colour each (biased colours) leading to frequent exposure to two specific letter-colour pairings, while the third target letter was presented in all colours equally. Importantly, the nature of the training paradigm allowed us to continuously quantify during exposure the interaction between letters and colours (i.e. whether letter-colour binding may have occurred). This was accomplished by comparing search performance when letters appeared in their congruent biased colour (frequent pairing) as compared to when presented in their incongruent colour (i.e., biased to another target letter and thus an

infrequent pairing). With no binding, search performance should be independent of letter-colour pairings, as any target letter and any biased colour appeared with equal likelihood over trials. Binding between specific letters and colours, on the other hand, is expected to manifest as disproportionately improved search performance (faster reaction times) for target letters in their congruent biased colours (match between associated and real colour of the target letter), and/or disproportionately impaired search performance (slower reaction times) for target letters in their incongruent colours, i.e. biased to another target letter (mismatch between associated and real colour of the target letter).

We first examined whether the above search task (with likelihood manipulation of grapheme-colour pairings) leads to binding of colours to graphemes in non-synaesthetes, as indexed by interference of incongruent pairings with task performance (relative to congruent pairings). Because attention to features plays an important role in synaesthesia (e.g. (Mattingley, Payne, & Rich, 2006; Walsh, 1999)), we sought to manipulate depths of processing of the task-subordinate feature (colour). To this end, we informed one group of participants that two colours would be more often associated with the two target letters and identified these colours (colour-bias aware), while not informing the other group (colour-bias unaware). In addition, we manipulated the duration of training. In two experiments, we show that colours can be bound to letters in non-synaesthetes on a short time scale (as measured by letter-colour interference during search), but without evoking conscious colour-concurrents as is present in synaesthesia.

We then assessed to what extent these learned letter-colour bindings in non-synaesthetes relate to synaesthetic grapheme-colour associations (are synaesthesia-like) by testing for the following synaesthesia-characteristics: In experiment 1, we correlated our letter-colour binding measure derived from search performance with a common objective measure of synaesthesia, namely the modified-Stroop test assessed at the end of the search task (M. J. Dixon et al., 2004; Mills, Boteler, & Larcombe, 2003; Ward, Li, Salih, & Sagiv, 2007). In addition, we compared the strength of Stroop-interference in non-synaesthetes with synaesthetic Stroop-interference in three confirmed synaesthetes. In experiment 2, we tested whether letter-colour interference between the

associated and real colour of search targets is strongest when these colours are opponent colours, in analogy to findings in synaesthesia (Nikolic et al., 2007). Dependence of letter-colour interference on the relative position of the chosen colours in colour space (colour-opponency vs. non-opponency) would suggest formation of these associations at a perceptual rather than conceptual level, because depending on low-level (colour) features of the stimuli. Our results reveal that, although learning did not induce conscious (additional) colour experiences in non-synaesthetes, the learned letter-colour associations were synaesthetic-like, because correlating with synaesthesia Stroop-interference and showing a colour-opponency effect.

Materials and Methods

All experiments were conducted in accordance with the ethical guidelines established by the Declaration of Helsinki, 1994, and were approved by the local ethical committee of the College of Science and Engineering, University of Glasgow. All participants gave written informed consent prior to inclusion in the study. All participants had normal or corrected-to-normal vision, including self-reported normal colour vision.

Experiments 1 and 2: Search task with likelihood manipulation of letter-colour pairings

In both experiments (experiments 1 and 2), participants performed the same visual search task in which search targets were pre-defined letters. Over trials, certain target letters were more often associated with a given colour (to promote statistical associative learning through repeated exposure). Figure 1 illustrates the search display.

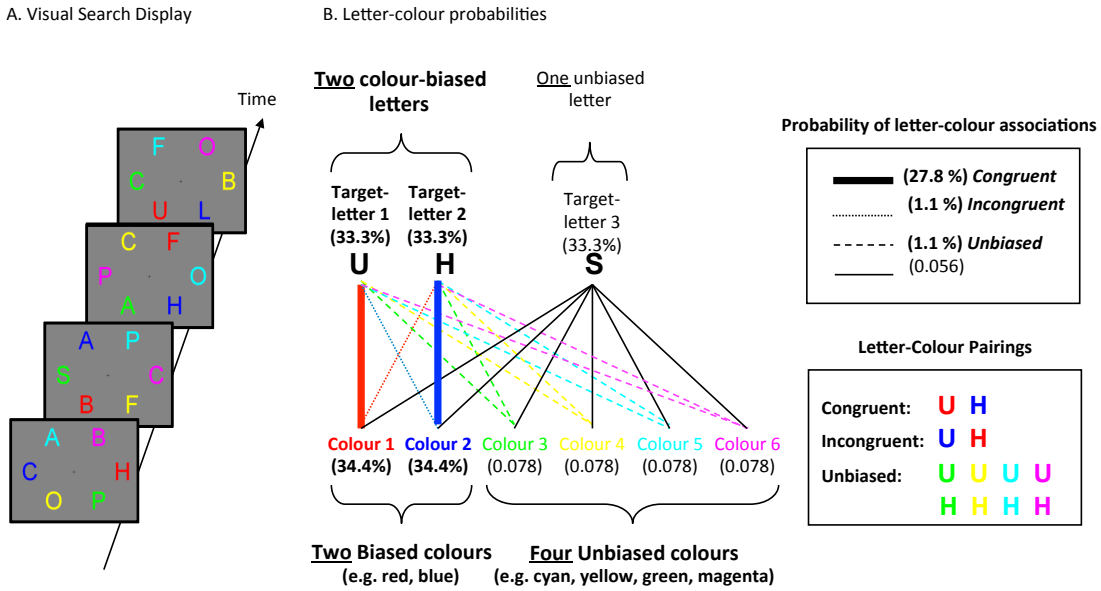

Figure 1. Visual search task and stimuli used in experiments 1 and 2. A. Visual search task, during which non-synaesthetes were more frequently exposed to specific grapheme-colour associations. The task was to detect one target letter (among five distracters). Colour was not a target dimension. **B.** Letter-colour probabilities. Of three possible target-letters, two (target-letters 1&2) most often appeared in one colour each (biased colours 1&2). Congruent pairings refer to colour-biased targets appearing in biased colours (letter 1-colour 1, letter 2-colour 2). All other combinations were far less frequent, including incongruent pairings (letter 1-colour 2, letter 2-colour 1).

Each trial began with the presentation of a fixation cross for 800 ms, after which the search array appeared. Every search array was composed of six letters; one letter was the target, which was randomly selected from a pre-defined set of three target letters (U, H, and S), and the remaining five letters were distracters, randomly selected from a set of seven potential distracter letters (A, F, B, L, C, O, or P). Each letter subtended 2° in the vertical dimension and 1.5° in the horizontal dimension. Every letter in the search array was printed in a unique colour against a medium grey background. The six colours used were red, green, blue, yellow, cyan, and magenta, in their corresponding maximal RGB values. The stimuli consistently appeared in the same six locations and were centred 5° from the central fixation cross. On any given trial, any target or distracter letter could appear in any colour, though not necessarily at chance frequencies (see below). Moreover, no correlation existed either between letter and location, or between colour and location – any letter and colour could appear at any location. The search array remained on the screen until participants generated a key-press or 6000 ms had elapsed.

The task was to indicate whether the target letter appeared to the left or right of the central fixation cross. Responses were given with the index and middle finger of the right hand, by pressing the 'b'- and 'n'-keys, respectively. The participants were instructed to respond as quickly and accurately as possible. Note that the manual response was dissociated from the identity of the target letter, in order to avoid introducing response biases for any letter (which could influence subsequent grapheme-colour association testing in the modified-Stroop tests, where colours are the targets but letters are obligatorily present).

Statistical learning was accomplished by manipulating the likelihood that a particular target letter would appear in a particular colour (Figure 1B). Only a single target letter appeared in each trial; thus, the likelihood of seeing each target letter was 33.3 % (p=0.333, 1 out of 3). Two of the three target letters (i.e., U and H, see targets 1 and 2 in Figure 1B) were chosen to appear more often in a particular colour (colour-biased letters; for biased colours, see colours 1 and 2 in Figure 1B). The frequency with which each of these two letters appeared in their respective colours was 83.3% (5 out of 6), and the frequency with which they appeared in either of the 5 remaining colours (randomly chosen) was 16.7% (1 out of 6). Thus, the likelihood of a trial to feature a particular colour-biased target (i.e., U or H) in its biased colour (congruent condition: target1-colour1 or target2-colour2) was 27.8% (p=0.278, 1/3 * 5/6). Since two targets were colour-biased, the likelihood of observing any colour-biased target letter in its biased colour was 55.6%. Conversely, the likelihood of a trial to feature a colour-biased target in the opposite biased colour (incongruent condition: target1-colour2 or target2-colour1) was 1.1% (p=0.011, 1/3 * 1/6 * 1/5). The remaining target letter (i.e., S) was not colour-biased (unbiased letter, see target 3 in Figure 1B), i.e. it appeared in every colour with equal likelihood (Figure 1B). The colours that were biased were chosen randomly for each participant.

In brief, these manipulations led to two grapheme-colour pairings of particular interest: (1) frequent pairings of a colour-biased letter with its respective colour (target1-colour1, target2-colour2), for which letters and colours should become "congruent" over time if repeated exposure indeed leads to grapheme-colour binding; (2) pairings of a colour-biased letter with the

opposite biased colour (target 1-colour 2, target 2-colour 1), which were much more infrequent (but not altogether absent) and therefore allowed tracking of performance under the "incongruent" condition (again, if grapheme-colour binding were to occur). It is important to note that the likelihood of these grapheme-colour pairings ranged from frequent (congruent pairings: 0.556) to infrequent (incongruent pairings: 0.022); but when considered on their own, any of these targets or colours occurred with equally high likelihood across trials (target-letter 1 or 2: probability= 0.666 (2*0.333), colour 1 or 2: probability= 0.688 (2*0.344); Figure 1B).

Experiment 1

Aims

The main aims were twofold: (1) to study the extent to which letter-colour bindings can be induced in non-synaesthetes using the visual search task detailed above, and (2) to relate the strength of learned letter-colour associations (2.1) to the participants' performance in synaesthetic-Stroop tasks administered following the search task, and (2.2) to synaesthetic-Stroop performance of confirmed synaesthetes. As a secondary aim, we (3) explored whether strength of letter-colour binding may depend on attention to stimulus features during search by manipulating depth of processing of the task-subordinate feature (colour) in two groups of non- synaesthetes.

Participants

Twenty-eight university students without synaesthesia participated in this experiment (age range: 18-37, m/f=11/17, right/left-handed=24/4). One participant performed below-chance during the search task and was thus discarded from the analysis. Additionally, three grapheme-colour synaesthetes were invited to participate in a modified-Stroop task only (also students, age range: 19-29, f=3) in order to compare the strength of letter-colour binding in non-synaesthetes to synaesthetes.

In the non-synaesthetes, synaesthesia was ruled out based on screening for grapheme-colour synaesthesia using a questionnaire adapted from (Shapley & Hawken, 2011) (see Appendix for a copy of questionnaire). None of the 28

participants claimed to experience the phenomenon (as tested by the questionnaire). To confirm synaesthesia in the three control synaesthetes (recruited separately), a consistency test of grapheme-colour synaesthesia was used, adapted from the Texsyn Toolbox (Eagleman, Kagan, Nelson, Sagaram, & Sarma, 2007), a freeware synaesthesia battery for Matlab. Both the non-synaesthetes and synaesthetes were kept naïve as to the purposes of the experiment (which was why synaesthesia questionnaires were given to non-synaesthetes only at the end of the experiment).

Experimental Procedure (Non-synaesthetes)

Participants performed the task detailed above, searching for specific target letters (U, H, or S; see subsection, "Experiments 1 and 2: Search task with likelihood manipulation of letter-colour pairings," and Figure 1) appearing in distinct colours. The task was performed over 12 blocks, each consisting of 135 trials (leading to a total of 1620 trials). The colours to which target letters were paired (i.e., the biased colours) were randomly assigned for each participant from a pre-defined selection. This random procedure yielded the following distribution of biased colours: yellow-green in 8 participants, red-blue in 8 participants, and cyan-magenta in 12 participants. All colour pairs therefore were non-opponent and served as the biased feature in at least one participant. To manipulate the depth of colour processing in the search task, participants were divided into two groups: colour-bias aware and unaware (n=14 vs. n=13). The colour-bias unaware group was not informed of any statistical manipulations, whereas the aware group was told, as well as shown, before each experimental block of the biased colours and their associations to the two targets. To allow consolidation overnight, the experiment was split into 2 sessions performed over 2 consecutive days (2 days x 6 blocks). Each session took approximately 45 minutes to complete. Participants were encouraged to take breaks between blocks.

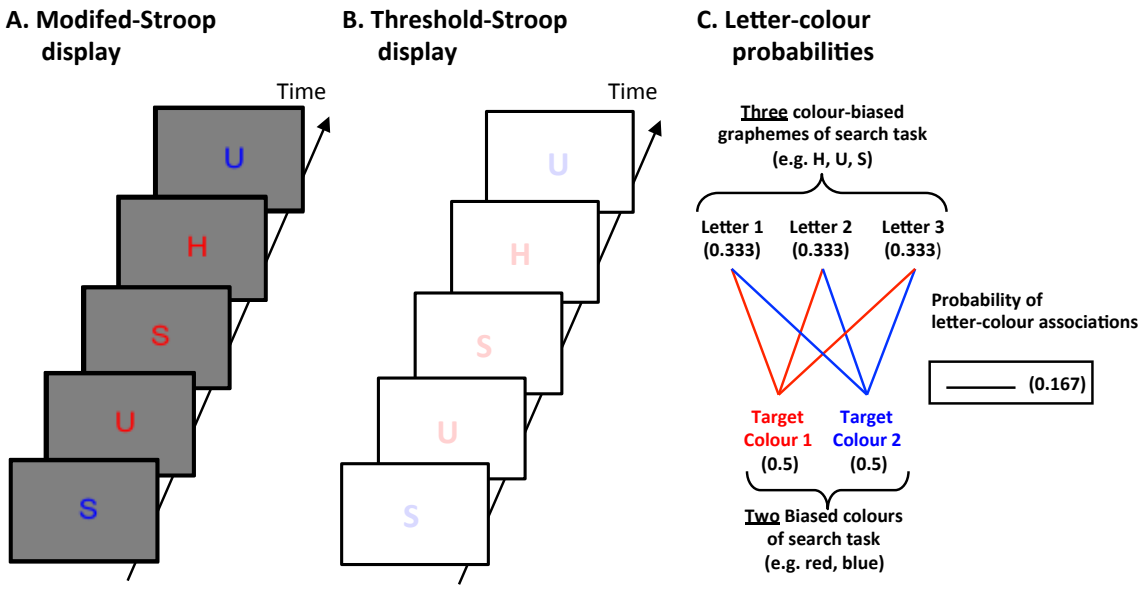

Figure 2. Task and stimuli in modified Stroop-tests (experiment 1). After repeated exposure to specific grapheme-colour associations, participants were presented with **A.** the modified-Stroop and **B.** a threshold-Stroop task. The single letters of these modified Stroop tests consisted of target letters 1-3 of the search tasks and were presented in biased colours only (colours 1&2, equal probability). The task was to identify the colour (letter was task irrelevant). The modified-Stroop and threshold-Stroop were identical, except that in the latter, colours were very faint and set against a white background. **C.** Probabilities of letter-colour pairings

After each completed session (of 6 blocks), participants performed 2 versions of the synaesthetic Stroop task, commonly used as a marker of synaesthesia (M. J. Dixon et al., 2004; Ward et al., 2007). Figure 2 illustrates these two tasks. In the first modified-Stroop task (Fig 2A), each trial began with presentation of a central fixation cross over a medium grey background. A single letter, always a former search target letter (i.e., U, H, or S), then appeared in the centre of the screen. This letter was shown in black ink for the first 800 ms before changing to one of two possible colours for 200 ms (50% probability). The two colours were the same biased colours previously presented in the search task. For example, if 'U' had been presented mostly in blue and 'H' mostly in red, then these were the two colours used in the modified-Stroop task. The task was to indicate the print colour of the single letter. The second modified-Stroop task (Fig 2B), henceforth referred to as threshold-Stroop task, was identical to the former with the exception that the colours were faint, i.e., in the direction of threshold values for colour perception. We implemented this variant with the rationale that if a synaesthetic experience in non-synaesthetes were induced, this experience may be weak, i.e., not strong enough to interfere with the saturated colours in the first Stroop task. Here, the central fixation cross was

presented over a white background for 800 ms before changing directly to a coloured letter for 200 ms. No black letter preceded the coloured letter in order to avoid afterimages created by the former and thus interfering with perception of the latter. In both tasks, participants had to indicate the print colour of the single letter as quickly and accurately as possible using pre-defined keys with their right index and middle fingers (again, the keys 'b' and 'n' were used). In both tasks (Fig 2C), each of the three single letters was presented an equal number of times (33.3%), and appeared half of the time in one colour and half in the other colour. Thus, each of the two colour-biased target letters (i.e., U and H) appeared in their congruent colour in 16.7% of all trials and in their incongruent colour also in 16.7% of trials. Similarly, the non-biased target letter (i.e., S) also appeared in one or the other colour in 16.7% of all trials respectively. There were a total of 150 trials, which took about 5 minutes to complete.

At the very end of testing on day 2, participants were debriefed and a questionnaire testing for the synaesthetic experience was given. E-prime software was used to control stimulus presentation and data collection during search and modified-Stroop-tasks.

Experimental Procedure (Control synaesthetes)

To evaluate the strength of potentially induced grapheme-colour associations in trained non-synaesthetes, we asked three confirmed grapheme-colour synaesthetes to perform the same version of the modified-Stroop task. For each synaesthete, we chose two synaesthetically induced colours that matched, as closely as possible, the colours used for trained non-synaesthetes. The corresponding letters (plus one letter evoking no colour) were presented half of the time in one colour and the other half in the other colour (analogous to the modified-Stroop tasks for the trained non-synaesthetes).

Grapheme-colour Synaesthesia Consistency Task (Control synaesthetes)

To test and confirm grapheme-colour synaesthesia in the three synaesthetes, we used the computerized protocol by (Eagleman et al., 2007) (also providing normative data). Each trial began with presentation of a

colourless grapheme (black on a medium grey background), together with a colour palette. Participants then selected, by mouse navigation within the palette, the colour that most closely matched their synaesthetic experience to the simultaneously presented grapheme (or a "no colour" option if they lacked a colour experience). Participants were instructed to take their time, and to be as accurate as possible. Upon selection of a colour, the corresponding RGB value was automatically recorded, and the next trial began. In total, there were 150 trials, corresponding to the full set of graphemes, A-Z and 0-9, repeated three times each in randomized order. Matlab 2007b (The MathWorks, Inc.) and an adapted version of the Texsyn Toolbox (Eagleman et al., 2007) were used to control stimulus presentation, as well as data collection.

After a minimum delay of three weeks and without prior knowledge, all three participants were re-tested in the exact same task. All individual grapheme-colour pairings were then tested for consistency per synaesthete across sessions based on the formula established by (Eagleman et al., 2007): the total distance between colours selected for *each grapheme* is calculated in normalised RGB colour space. These colour distances are then averaged across *all graphemes* within, and subsequently across sessions to yield a Consistency Score. All three grapheme-colour synaesthetes fell within the normative synaesthesia range provided by (Eagleman et al., 2007), i.e. exhibiting Consistency Scores below 1 (0.59, 0.67, and 0.73).

Data Analysis

Experiment 1, Aim 1: Testing for letter-colour binding during search performance in non-synaesthetes

Only colour-biased targets were analysed (congruent, incongruent and unbiased conditions), in order to test for evidence of letter-colour binding, by comparing search performance between congruent and incongruent letter-colour pairs (letter1-colour1/letter2-colour2 vs. letter1-colour2/letter2-colour1), also in relation to these same letters displayed in unbiased colours (letter1-unbiased colour/letter2-unbiased colour). To this end, reaction times to these target letters were subjected to an overall 2 x 2 x 6 x 3 mixed-design ANOVA with the between-subject factor Training Group (colour-bias unaware vs. colour-bias aware) and the within-subject factors Day (day 1 vs. day 2), Experimental Block

(blocks 1-6), and Congruency (congruent, incongruent, unbiased letter-colour pairs). Main effects and interactions were further probed with simple effects where appropriate.

Experiment 1, Aim 2.1: Relating letter-colour Binding Index (derived from search performance) to individual Stroop interference in the synaesthetic Stroop tasks (non-synaesthetes)

In order to relate letter-colour binding to Stroop interference across participants, we first estimated per participant the strength of letter-colour binding (letter-colour binding index) and Stroop interference (Stroop-interference index). The binding index was calculated based on search performance at the end of testing (in blocks 6 and 12, hence closest in time to Stroop testing) according to formula 1, below. We first normalised per participant mean reaction times of both congruent and incongruent pairings to the unbiased condition ($RT_{(congruent)}$ divided by $RT_{(unbiased\ colour)}$, and $RT_{(incongruent)}$ divided by $RT_{(unbiased\ colour)}$), before taking the difference between congruent from incongruent conditions. Normalising to the unbiased condition weighs reaction time differences between congruent and incongruent conditions (i.e., the index of binding) according to the general speed of target detection, i.e. without the associated colour advantage/disadvantage (same letters/unbiased colours).

(1) Grapheme-colour binding index =
Normalised $RT_{(incongruent)}$ – Normalised $RT_{(congruent)}$

The Stroop interference index was calculated according to formula 2, below.

(2) Stroop-interference index = $RT_{(incongruent)}$ minus $RT_{(congruent)}$

We then related these indices in two ways. First, in order to be able to test for statistical differences between training groups, we performed 3 x 2 factorial ANOVAs on Stroop-interference (dependent variable), with the between-subject factors Binding Strength (3 bins) and Training group (unaware vs. aware). One ANOVA was performed per Stroop variant and day of testing. Effects of bin were further explored by planned polynomial linear contrasts (across Binding bins) to test for linear relationships. Second, we followed up these results by planned correlations between binding index and Stroop-interference across all participants (Pearson and Spearman).

Experiment 1, Aim 2.2: Comparing the strength of letter-colour interference in non-synaesthetes with synaesthetic Stroop interference in synaesthesia

Descriptive statistics was used to compare performance between non-synaesthetes and three confirmed grapheme-colour synaesthetes.

Experiment 2

Aims

The main aims were twofold: (1) to reproduce the findings of experiment 1 (significant letter-colour associations in non-synaesthetes by means of the same likelihood manipulation task), and (2) to further explore the relation of the learned letter-colour associations to synaesthesia by testing whether letter-colour interference depends on the position of the chosen colours (associated and real) in colour space (opponent versus non-opponent), in analogy to synaesthesia (Nikolic et al., 2007). As a secondary aim, we sought to (3) determine whether longer training (in the search task) would strengthen letter-colour binding.

Participants

Twenty-two university students without synaesthesia participated in this experiment (age range: 18-27, m/f=4/18, right/left-handed=20/2). As in experiment 1, synaesthesia was ruled out in all participants via a questionnaire adapted from (Shapley & Hawken, 2011) (see Appendix for a copy of questionnaire). All participants were kept naïve as to the purposes of the experiment (which was why synaesthesia questionnaires were given to participants only at the end of the experiment).

Experimental Procedure

Participants performed the task detailed under 2.1, searching for specific target letters (U, H, or S) appearing in distinct colours. The task was performed over 18 blocks, each consisting of 135 trials (leading to a total of 2430 trials). Participants were divided into two groups: Opponent and Non-Opponent Colour group (n=11 vs. 11). The random selection procedure that we implemented to select biased colours for each participant yielded the following distribution: in

the Opponent Colour group, red-green in 6 participants and blue-yellow in 5 participants; and in the Non-Opponent Colour group, red-blue in 5 participants, yellow-green in 3 participants, and cyan-magenta in 3 participants. Before each experimental block, both groups were told, as well as shown, that two target letters (U and H) would usually appear in particular colours (colour-aware instructions of experiment 1). To allow consolidation overnight, the experiment was split into 3 sessions (3 days x 6 blocks) performed within a maximum period of 5 consecutive days (one working week). Each session took approximately 45 minutes to complete. Participants were encouraged to take breaks between blocks. At the very end of testing on day 3, participants were debriefed and a questionnaire was given testing for synaesthetic experiences.

Data Analysis

Experiment 2, Aim 1: Testing for letter-colour binding during search performance in non-synaesthetes

Again, only colour-biased targets were analysed (in analogy to experiment 1). Reaction times to these target letters were subjected to an overall 2 x 3 x 6 x 3 mixed-design ANOVA with the between-subject factor Group (Opponent vs. Non-opponent Colour) and the within-subject factors Day (day 1-3), Experimental Block (blocks 1-6), and Congruency (congruent, incongruent, unbiased letter-colour pairs). Main effects and interactions were further probed with simple effects where appropriate.

Experiment 2, Aim 2: Relating letter-colour binding in non-synaesthetes (derived from search performance) to synaesthesia-like letter-colour associations: the colour-opponency effect

Here, we examined the effects of colour opponency on letter-colour interference. Evidence for letter-colour binding (incongruent-congruent effects on search performance) was explored as a function of Group (i.e., interactions of Congruency x Group) using the above overall 2 x 3 x 6 x 3 ANOVA.

Results

Experiment 1

Experiment 1: Evidence for rapid letter-colour binding in non-synaesthetes with repeated exposure to letter-colour pairs during search performance

Figure 3 illustrates search performance in experiment 1 over time as a function of the three colour-biased target conditions, i.e., colour-biased targets appearing (i) in their most frequent colour (congruent pairings), (ii) in the colour associated with the other biased target (incongruent pairings) and (iii) in an unbiased colour. The main comparison of interest is between congruent and incongruent pairings, because significant differences between these two conditions indexes binding between colours and letters.

With time, and as participants learn to detect target letters, search time should decrease progressively. In addition, because the two biased colours were more likely to appear than any other colour (by a factor of 2, see Fig 1B), search time should be faster for the two biased colours than for the unbiased colours (RT advantage for biased colour). Importantly, if targets and biased colours were processed independently (unbound), the RT advantage for biased colours should be independent of specific letter-colour pairings (congruent vs. incongruent), i.e. should occur to an equal extent irrespective of whether a target is shown in its respective colour or in the other biased colour (i.e., that of the other target letter). Conversely, if binding between target letters and specific colours occurred, one would expect target letters and colours to interact as a function of target-colour pairings (congruent vs. incongruent). That is, if binding takes place, target detection should be enhanced for congruent pairings and/or slowed for incongruent pairings.

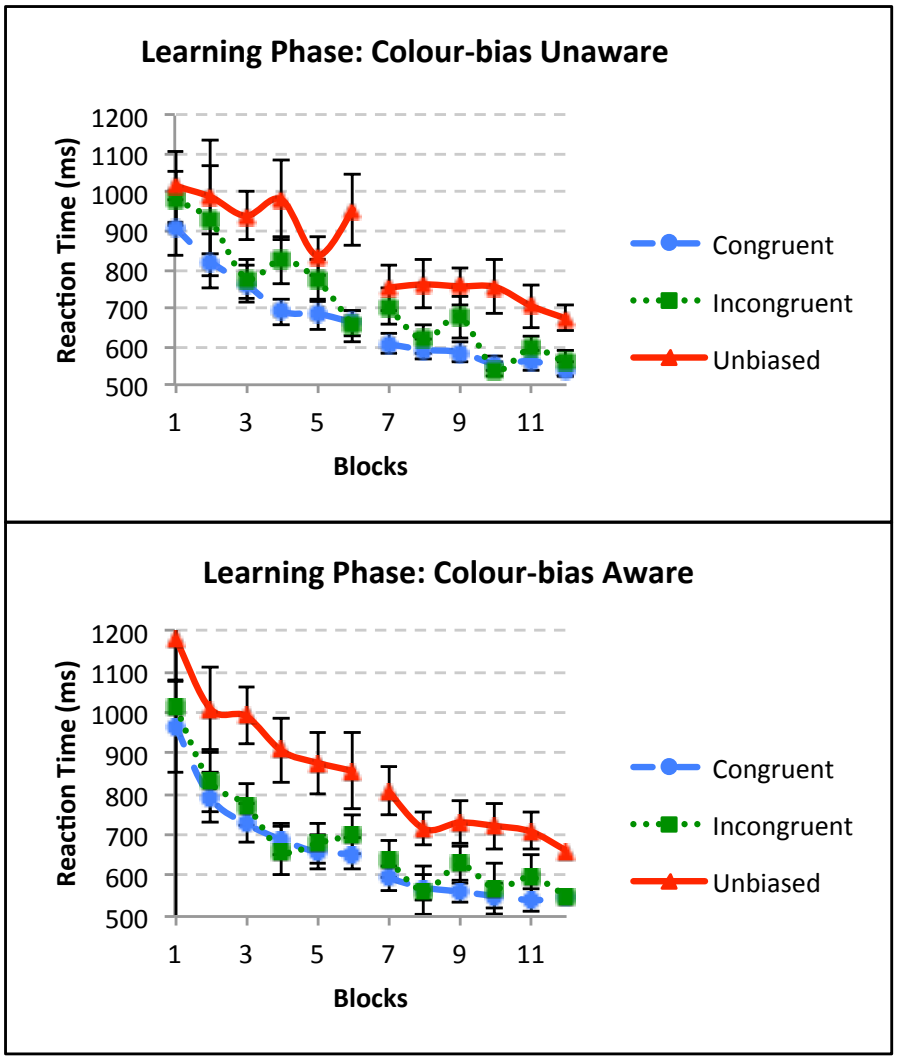

Figure 3. Search performance in Experiment 1. Search time for colour-biased targets (correctly detected) as a function of time (12 experimental blocks over 2 days), letter-colour pairings (dashed line= congruent, dotted line= incongruent, solid line= unbiased) and group (colour-bias unaware vs. aware). There was a progressive decrease in search time over blocks and days, and a difference in search time for targets displayed in congruent vs. incongruent colours (dashed vs. dotted lines), the latter denoting significant letter-colour binding in non-synaesthetes. Error bars denote standard error.

Search performance improved progressively over time (Figure 3, main effects of Experimental Block: $F(5,125)=19.0$, $p<0.00001$, and of Day: $F(1,25)=84.2$, $p<0.00001$), depending on day of testing (interaction Day x Block: $F(5,125)=6.3$, $p=0.00003$). Performance-improvement over blocks was more pronounced during the first day of training (simple effect of Block: $F(5,125)=13.8$, $p<0.00001$) than during the second day ($F(5,125)=8.3$, $p<0.0001$). Crucially, we found an effect of Congruency (congruent vs. incongruent vs. unbiased target-colour pairs) on search time (Figure 3, main effect $F(2,50)=46.5$, $p<0.00001$). Target detection was significantly faster when colour-biased targets appeared in their congruent versus incongruent colours (Fig. 3, dashed vs.

dotted line, 658.84 ± 134.55 ms and 700.48 ± 149.97 ms, simple effect: $F(1,25)=13.2$, $p=0.0013$). This indicates that grapheme-colour binding may indeed have occurred, and that letter and colour detection were not purely additive.

In addition, target detection in the above two conditions (biased colours) was significantly faster than target detection in the unbiased condition (Fig. 3, solid line: 845.56 ± 216.44 ms) (simple effects: congruent vs. unbiased colour: $F(1,25)=73.0$, $p<0.00001$; incongruent vs. unbiased colour: $F(1,25)=29.9$, $p=0.00001$). This shows that on top of the interaction with associated letters, biased colours have a general facilitative effect on search time, as expected. No other main effect nor interaction was significant (all $F<1$ .99, all $p>0.15$). The absence of any interaction with a factor of time (i.e. day or block) is notable, suggesting that letter-colour binding occurred on a short time scale.

Experiment 1: The strength of letter-colour binding derived from search performance relates to Stroop interference in a modified synaesthetic-Stroop task

To explore the extent to which the individual binding indices may relate to individual Stroop interference, and whether this may also depend on type of training (i.e., unaware vs. aware), we analysed Stroop interference (see formula 2 above, subsection “Experiment 1, Aim 2.1”) as a function of binding strength (percentile split of participants into 3 bins) and of training group (unaware vs. aware) (see Figure 4). These data were analysed using 3 x 2 (3 bins of Binding Strength x 2 Training groups) factorial ANOVAs (i.e., one ANOVA per Stroop variant and day of testing), followed up by planned polynomial linear contrasts and correlation analyses.

We found significant changes in threshold-Stroop interference across binding bins depending on Training group (interaction of Binding Strength x Training group; trend for day 1: $F(2,21)=2.9$, $p=0.076$: significant for day 2: $F(2,21)=3.7$, $p=0.042$; no main effects neither for Binding Strength nor Training group). Post-hoc polynomial linear contrasts showed that threshold-Stroop interference linearly increased with estimated Binding Strength (bins) in the colour-aware group (Figure 4, lower panels, black line; day 1: $F(1,11)=4.46$, $p=0.058$; day 2: $F(1,11)=13.6$, $p=0.0035$). No such relationship was found in the

colour-bias unaware group (Figure 4, lower panels, grey line; day 1: $F(1,10)=0.17$, $p=0.69$; day 2: $F(1,10)=1.85$, $p=0.20$). For the classical-Stroop variant (modified Stroop task, Figure 4, upper panels), the 3 x 2 ANOVAs did not yield any significant results, neither for day 1 nor day 2 of testing. In summary, threshold-Stroop interference progressively increased with binding strength (bins 1-3), but only in the colour-bias aware group (black lines, lower panels).

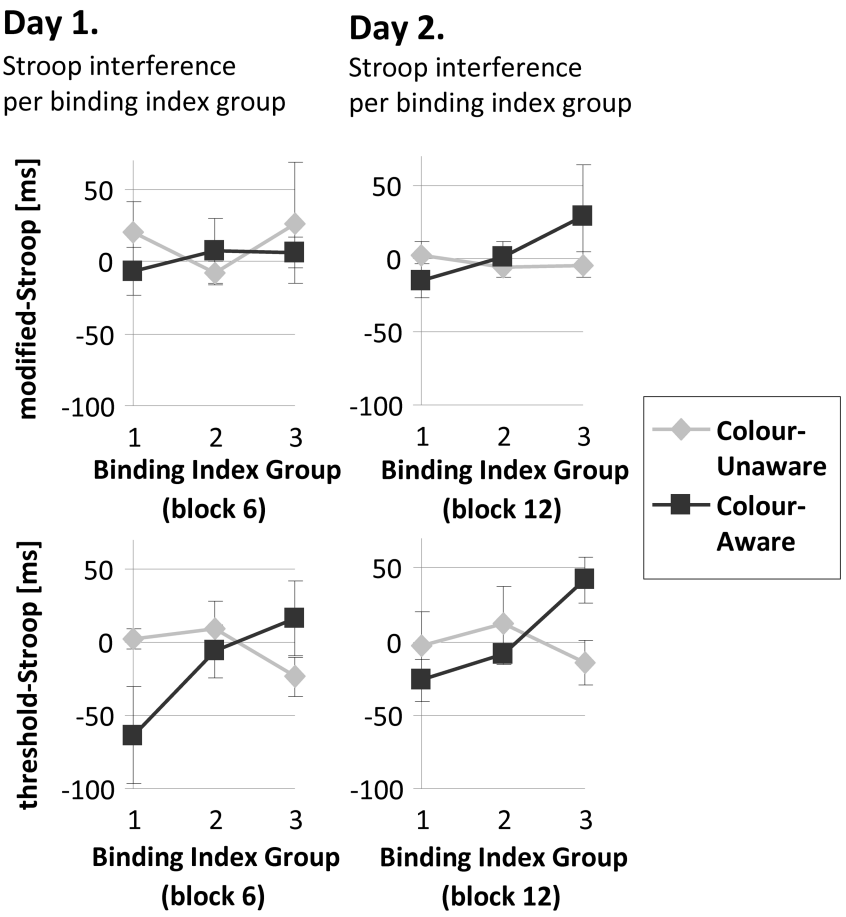

Figure 4. Relationship between strength of letter-colour binding (bins 1-3, x-axis) and synaesthetic Stroop-interference test (y-axis). Data are split according to day of training (day 1 vs. 2), Stroop variant (modified vs. threshold) and group (unaware vs. aware). Note the linear relationship between binding strength and threshold Stroop-interference (lower panels) for the colour-bias aware group (black line) regardless of day, suggesting that the strength of learned letter-colour associations co-varies with a synaesthesia-measure.

To further corroborate the relationship between binding index and threshold-Stroop interference described above, we correlated individual binding strength with Stroop-interference across individuals (Figure 5). In the Colour-Aware group, we found significant Pearson correlations between binding strength and threshold-Stroop performance for day 1 (Figure 5, lower left panel, $r=0.55$, $p=0.043$) and day 2 (Figure 5, lower right panel, $r=0.77$, $p=0.001$). This

remained significant when outliers were eliminated (Pearson Correlations: day1: r=0.55, p=0.053; day2: r=0.58, p=0.039), as well as with non-parametric testing (Spearman Rank: day 1: R=0.57, p=0.034; day2: R=0.77, p=0.0012). Threshold-Stroop interference linearly increased with binding strength (positive correlation). Conversely (and confirming the above analysis across bins), there were no correlations between binding indices and classical-Stroop interference (Figure 5, upper panels). Also in line with the previous analysis across bins, there were no significant correlations for either Stroop variant for the colour-bias unaware group (data not shown).

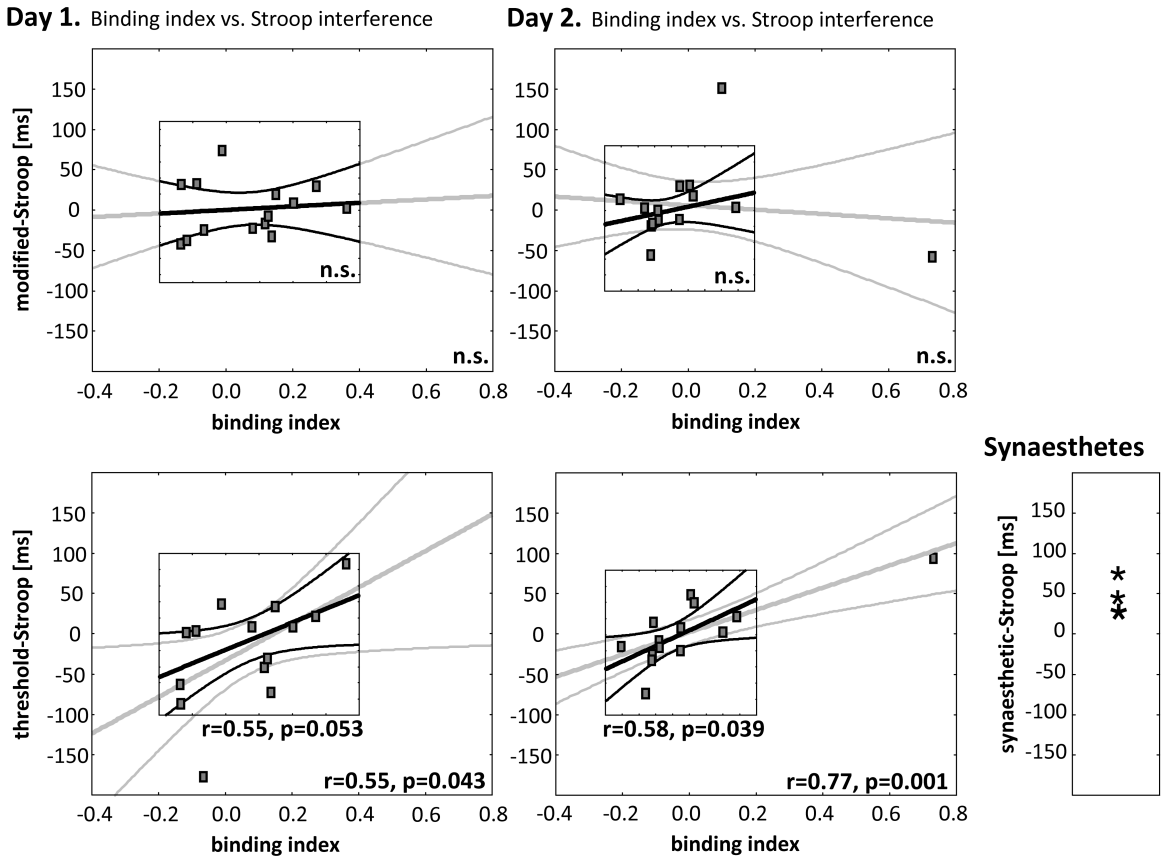

Figure 5. Correlation between individual binding index (x-axis) and individual synesthetic Stroop-interference (y-axis). Same data as in Fig 4. Data are split according to day of training (day 1 vs. 2) and Stroop variant (modified vs. threshold) as in Fig. 4 but shown for each participant. Only data of the colour-bias aware group are represented (non-significant for the colour-bias unaware group). Note the linear relationship between the two variables for the threshold-Stroop test (lower panels), which is present both with (large panel) and without outliers (smaller insets). Synaesthetic Stroop interference values of three synaesthetes are shown in the lower right panel (stars), for comparison with the non-synaesthetes (rectangles).

Experiment 1: Comparing the strength of letter-colour interference in non-synaesthetes with synaesthetic Stroop interference in Synaesthesia

For comparison with the non-synaesthetes, we assessed synaesthetic interference in 3 confirmed synaesthetes, using the same task (modified-Stroop task) and difference measure than for the non-synaesthetes ($RT_{(incongruent)}$ minus $RT_{(congruent)}$). The results showed interference in all 3 synaesthetes (positive Stroop-interference values of 34.8 ms, 46.3 ms, and 71.4 ms, respectively), which were situated in the upper range of the threshold-Stroop interference scores for non-synaesthetes (see Figure 5, panel inset to the lower right).

Experiment 1: Induced letter-colour binding in non-synaesthetes was not associated with colour-concurrents to letters

None of the non-synaesthetes reported seeing colours from letters, neither spontaneously during the course of the experiment, nor at the end of learning as assessed by the synaesthesia-questionnaire and by debriefing. There was, therefore, no conscious (additional) colour experience induced by learning.

In line with this, our data did not reveal any overall Stroop effects. Table 1 illustrates Stroop performance (time for colour identification) as a function of congruent versus incongruent letter-colour pairings, across Days of testing (day 1 vs. day 2) per Stroop-variant (modified-Stroop vs. threshold-Stroop) and Training group (colour-bias unaware vs. colour-bias aware). Using an overall 2 x 2 x 2 x 2 mixed-design ANOVA with the within-subject factors Congruency, Day and Stroop-Variant, and the between-subject factor Training Group, no synaesthetic Stroop-effects were found (i.e., no main effect of Congruency: $F(1,25)=0.01$, $p=0.92$, nor any interactions with Congruency: all $F<2.06$, $p>0.16$). The only significant finding of the ANOVA was a main effect of Stroop-Variant ($F(1,25)=9.5$, $p=0.005$) due to slower performance in the threshold Stroop than in the modified Stroop task (Table 1), suggesting that the former task was more difficult (as intended by design).

Performance in modified synaesthetic Stroop tasks (ms ± SEM) per Stroop variants, Day of training and Training Group.

	Colour-unaware group.				Colour-aware group			
	Day 1		Day 2		Day 1		Day 2	
	Modif. Stroop	Thresh. Stroop	Modif. Stroop	Thresh. Stroop	Modif. Stroop	Thresh. Stroop	Modif. Stroop	Thresh. Stroop
Congruent	470 ± 18	512 ± 26	443 ± 19	472 ± 23	500 ± 33	521 ± 41	462 ± 40	495 ± 37
Incongruent	485 ± 31	507 ± 28	440 ± 19	470 ± 29	501 ± 35	502 ± 31	468 ± 36	498 ± 42

Note: Stroop interference is reflected in RT differences between incongruent and congruent conditions (Incongruent RT–Congruent RT).

Table 1

Experiment 2

Experiment 2: Further evidence for letter-colour binding in non-synaesthetes with repeated exposure to letter-colour pairs during search performance

Figure 6 illustrates search performance in experiment 2 over time as a function of the three colour-biased target conditions, i.e., colour-biased targets appearing (i) in their most frequent colour (congruent pairings), (ii) in the colour associated with the other biased target (incongruent pairings) and (iii) in an unbiased colour. Again, the main comparison of interest is between congruent and incongruent letter-colour pairs.

Search performance improved progressively over time (Figure 6, main effects of Experimental Block: $F(5,100)=11.0$, $p<0.00001$, and of Day: $F(2,40)=109.8$, $p<0.00001$), depending on day of testing (interaction Day x Block: $F(10,200)=8.9$, $p<0.00001$). Performance-improvement over blocks was more pronounced during the first day of training (simple effect of Block: $F(5,100)=13.4$, $p<0.00001$) than during the second and third days (both n.s.). Crucially, we again found a main effect of Congruency (congruent vs. incongruent vs. unbiased target-colour pairs) on search time (Figure 6, main effect $F(2,40)=96.2$, $p<0.00001$). Target detection was significantly faster when colour-biased targets appeared in their congruent versus incongruent colours (Fig. 6, dashed vs. solid line, 599.9±16.4ms vs. 626.2±19.9ms, simple effect: $F(1, 20)=17.4$, $p=0.00048$), indicating binding of colours to letters.

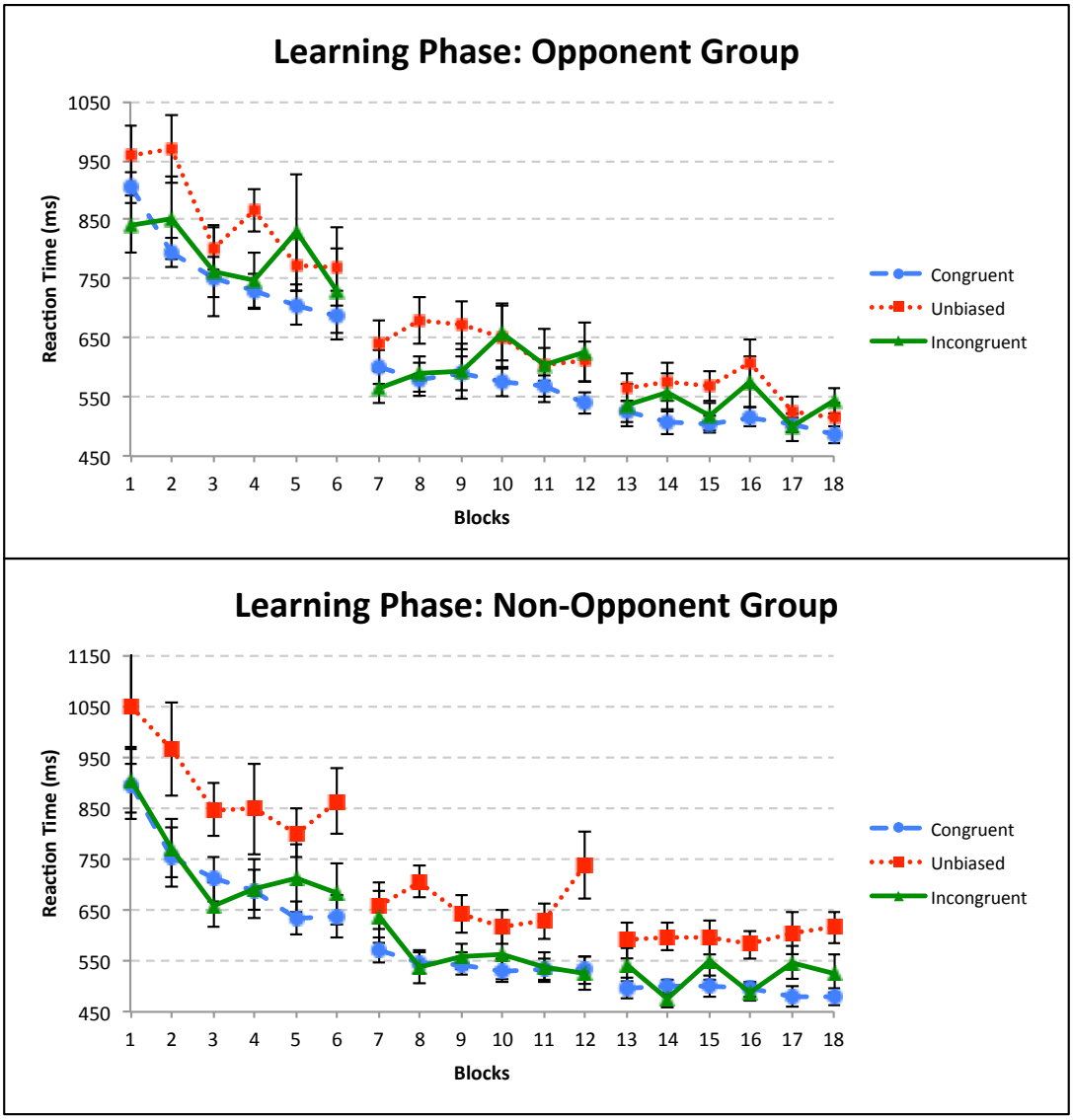

Figure 6. Search performance in Experiment 2. Search time for colour-biased targets (correctly detected) as a function of time (18 experimental blocks over 3 days), letter-colour pairings (dashed line= congruent, solid line= incongruent, dotted line= unbiased) and group (Opponent colour vs. Non-opponent colour). There was a progressive decrease in search time over blocks and days, and a difference in search time for targets displayed in congruent vs. incongruent colours (dashed vs. solid lines), which depended on Group. The opponent-colour group showed stronger letter-colour interference (difference between dashed vs. solid lines) replicating the colour-opponency effect found in synaesthetes (Nikolić et al., 2007). Error bars denote standard error.

In addition to this difference between congruent and incongruent conditions, we found target detection in both these conditions (biased colours) to be faster than target detection in the unbiased condition (702.8±23.9ms) (simple effects: congruent vs. unbiased: $F(1, 20)=142.23$, $p<0.000001$, incongruent vs. unbiased: $F(1, 20)=91.6$, $p<0.000001$), reproducing the general facilitative effect of biased colours on search time of experiment 1. Finally, the effect of Congruency depended on Group (interaction Congruency x Group:

F(2,40)=13.3, p=0.00004), Day of testing (interaction Congruency x Day: F(4,80)=3.5, p=0.011), and also Block (interaction Congruency x Block: F(10,200)=1.9, p=0.039). There was also a three-way interaction of Congruency x Block x Group (F(10,200)=2.0, p=0.035), explored below.

Experiment 2: Relating letter-colour binding in non-synaesthetes during search performance to synaesthesia-like letter-colour associations: the colour-opponency effect

The three-way interaction of Congruency x Block x Group (F(10,200)=2.0, p=0.035) reveals that letter-colour interference evolved differently across blocks for each group, i.e. depends on colour-opponency (Figure 6). For the Opponent Colour Group, congruency depended on block (simple effect: F(10,100)=2.2, p=0.029). Performance evolved such that: (i) in the 1st block (collapsed across days), the congruent and incongruent conditions differed significantly from the unbiased condition (simple effects: F(1,10)=5.9, p=0.036 and F(1,10)=15.6, p=0.002, respectively), with no difference between congruent and incongruent conditions; but (ii) by the 6th block (collapsed across days), the congruent condition became significantly faster than both the incongruent and unbiased conditions (simple effects: F(1,10=6.0, p=0.033; and F(1,10)=12.5, p=0.0053, respectively). Notably, the latter two conditions (incongruent vs. unbiased) ceased to differ by the 6th block (simple effect: F(1,10)=0.004, p=0.95) due to a slowing in the incongruent condition to similar levels as the unbiased condition. That is, letter-colour interference in the Opponent Colour Group is so strong that it abolishes the general facilitative effect of biased colours (Figure 6, upper panel, Opponent Group, 6th blocks per day= blocks no 6, 12, 18). In contrast, for the Non-Opponent Colour Group, congruency did not depend on block. That is, performance in the congruent condition (584.8 ms ± 34.5) was consistently faster than in the incongruent condition (606.0 ms ± 33.7) across blocks (simple effect: F(1,10)=10.8, p=0.0083), and performance in both these conditions were significantly faster than in the unbiased condition (719.2 ms ± 43.1) (simple effects: F(1,10)=103.3, p=0.000001 and F(1,10)=94.5, p=0.000002, respectively).

These results indicate that while both Opponent and Non-Opponent groups show evidence for grapheme-colour binding, the Opponent Group shows significantly stronger letter-colour interference on target detection than the Non-opponent group. Note that this group difference cannot be due to a

difference in strength of grapheme-colour binding, because this should be identical across groups, given that both groups performed the same task (identical in all aspects except the chosen colour pairs). Rather, the difference in strength of interference between associated (learned) colour and real colour across colour-opponent groups is best explained by the learned associations being represented in visual (colour-opponent) space (i.e. at a perceptual rather than conceptual level).

Discussion

We here studied the formation of automatic letter-colour associations in adult non-synaesthetes via a learning paradigm that mimics the natural conditions in which grapheme-colour synaesthetes may learn their associations (Hancock, 2006; Witthoft & Winawer, 2006). Our paradigm involves the search for target letters paired with specific colours (likelihood manipulation); and allows inferring the strength of letter-colour associations by tracking interference with search performance when letters are displayed in congruent (associated) colours versus in incongruent colours (associated with another target). Across two experiments, we find that non-synaesthetes display significant learning of specific letter-colour associations (letter-colour binding), with these associations showing synaesthesia-like characteristics: Experiment 1 showed that the strength of letter-colour binding is linearly related to a classical synaesthetic-Stroop measure. Experiment 2 showed that letter-colour interference during letter search is significantly stronger when the learned (associated) and the real colours of the search target are opponent colours (as compared to when they are unrelated, non-opponent colours). In other words, the strength of interference depended on colour-opponency, indicating that letter-colour associations have been formed on a perceptual (rather than conceptual) level. From this also follows that the learned associations did not result from cognitive strategies, and are therefore likely specific to letter-colour associations. However, while showing synaesthesia-like characteristics, the learned associations did not lead to conscious (additional) colour experiences. In brief, we here present evidence for synaesthesia-like grapheme-colour binding in non-synaesthetes, in line with learning accounts of synaesthesia as well as models assuming common mechanisms underlying automatic crossmodal associations in synaesthetes and non-synaesthetes.

Our first main goal across both experiments was to investigate whether automatic letter-colour associations could be induced using a search task, in which some of the targets (which were letters) appeared more often in certain colours. We call these the biased colours. Because attention to features plays an important role in synaesthesia (Mattingley et al., 2006; Walsh, 1999), we also sought to manipulate the depth of processing of the task-subordinate feature (colour) by manipulating the level of knowledge that participants received regarding the association between colours and letters (colour-bias aware vs. colour-bias unaware groups in experiment 1; all participants were made aware of the colour-bias in experiment 2). Additionally, we manipulated duration of training (2 vs. 3 days in experiment 1 vs. 2). *Importantly, all participants of both experiments remained uninformed of the actual end-goal of the experiment:* to learn the letter-colour pairings. We thus examined the automatic formation of letter-colour associations through likelihood manipulation of letter-colour pairings when these were per se task-irrelevant. We assessed whether associations were formed based on search performance differences between the three target conditions (congruent vs. incongruent vs. unbiased, see Figures 3 and 6).

If formation of automatic letter-colour associations occurred, then one would expect target letters and biased colours to interact as a function of congruency (congruent vs. incongruent vs. unbiased conditions), such that "correct" (i.e., congruent) letter-colour pairings (associated colour is congruent with real colour) facilitate search performance most. Conversely, "incorrect" (i.e. incongruent) letter-colour pairings should slow down search performance. It is important to note here that one would expect biased colours in general to aid target search more than unbiased colours, since these are most often paired with the biased targets. In other words, colour does carry information that is relevant for letter search performance in our paradigm. In our data, this is reflected in the search advantage for targets in biased colours (congruent and incongruent) as compared to the same targets shown in unbiased colours (Figure 3 and 6; irrespective of group). Crucially, however, using colour information per se would not favour target search in the congruent relative to the incongruent condition, but rather would benefit search of both colour-biased targets equally. In contrast, we found a significant difference in search performance *between*

congruent and incongruent letter-colour pairings in both experiments, showing that biased colours facilitate/impair target detection depending on the specific letter-colour pairing. Notably, these letter-colour associations were formed rapidly, occurring early into training (experiments 1 and 2) and not differing between training over two versus three days (experiment 1 versus 2). We therefore conclude that automatic letter-colour associations have been formed, and accordingly, that the paradigm we adopted may be used to study grapheme-colour binding in non-synaesthetes.

The second main goal of our study was to probe to what extent these learned associations may relate to grapheme-colour synaesthesia (are synaesthesia-like). First, we tested whether the learned associations would correlate with common measures of grapheme-colour synaesthesia, such as those inferred from a modified synaesthetic Stroop task (M.J. Dixon et al., 2006). Grapheme-colour synaesthetes are quicker to name the print colour of a presented grapheme when the grapheme-colour combination matches their innate colour association, than when it does not (synaesthetic-Stroop effect), presumably because of interference between the synaesthetically-induced colours and real (i.e., print) colours. Our data revealed a significant correlation between the strength of letter-colour binding during search and Stroop interference. Stroop interference increased progressively with enhanced binding indices across participants, indicating that the strength of letter-colour binding co-varies with a synaesthesia-measure. This relation was restricted to Stroop interference derived from the presumably more sensitive threshold task. In addition, this relation was restricted to participants who were aware of the colour-bias, which may indicate that depth of attention drawn to visual features may not only be important for the expression of the synaesthetic experience in synaesthetes (Mattingley et al., 2006; Walsh, 1999), but also for the formation of new letter-colour associations in non-synaesthetes.

As a further probe into synaesthesia-characteristics, we tested to what extent the learned associations are subject to the colour-opponency effect, present in synaesthesia (Nikolic et al., 2007). This effect reported in synaesthetes consists of stronger letter-colour interference (slowing in incongruent conditions), when the synaesthetically induced (associated) colour

of a grapheme is colour-opponent to its real colour, as compared to when it is non-opponent. Our results show that the colour-opponency effect also applies to the letter-colour associations learned by our non-synaesthetes. From this follows that much of our results are explained by automatic (obligatory) letter-colour associations at a perceptual rather than conceptual level. In analogy to the effects of colour-opponency in synaesthesia (Nikolic et al., 2007), this suggests involvement of early stages of visual processing in letter-colour interference also in our group of non-synaesthetes. Visual areas V4/V8 are candidate structures where such interference could occur, because these areas have been associated with both synaesthetic associations (Hubbard et al., 2005; Hubbard & Ramachandran, 2005; Sperling et al., 2006; Zeki & Marini, 1998) and colour-opponent receptive fields (Chichilnisky & Wandell, 1999; Hubel & Livingstone, 1987; Hurvich & Jameson, 1957; Zeki, 1980), and because in grapheme-colour synaesthetes, activity in these areas is influenced by the distance between competing real and induced colours in colour space (Laeng, Hugdahl, & Specht, 2011). In addition, it seems most plausible that interference effects are triggered via top-down processes initiated by the learned associations (i.e., of colours and letters), more likely implicating later stages of visual colour processing rather than earlier ones. Note that although our participants could have used a cognitive strategy to learn these associations, our data reveal that they did not do so, as in the latter case learning outcome should have been independent of colour opponency. Note also that we designed our paradigm to prevent participants from adopting a cognitive strategy, by likelihood manipulation of colour-letter pairings and avoiding explicit instruction to learn associations. Overall, the presence of a colour-opponency effect in trained non-synaesthetes lends further support to the view that synaesthesia may partially be based on normal mechanisms of cross-modal interactions (Bien et al., 2012; Eagleman, 2012; Martino & Marks, 2000; Simner, 2012).

In contrast to previous studies on the learning of synaesthesia-like grapheme-colour associations “late” in life by adult non-synaesthetes (Cohen Kadosh et al., 2009), our paradigm did not evoke a synaesthetic-Stroop effect at the group level (such as reported in (Colizoli et al., 2012; Meier & Rothen, 2009)), or conscious concurrent colour percepts from the presentation of letters (reported in Cohen Kadosh et al., 2009). Hence, while our findings are in line with synaesthesia-like letter-colour binding in non-synaesthetes, they differ from

previous reports and also synaesthesia in the sense that measures of synaesthesia did not show on the group level and no synaesthetic experience was induced. It may be speculated that, in our participants, (1) the learned associations were qualitatively similar to synaesthesia, just differing in strength (and therefore not leading to *conscious* concurrent colour perceptions); or (2) alternatively, that the learning outcome displayed qualitative differences to synaesthesia. Our data collectively favour the latter scenario. In the case of (1), one would expect the "best" learners of the non-synaesthete group to exhibit "synaesthetic Stroop interference." This was, however, clearly not the case. Although the strongest letter-colour binders (of our participants without synaesthesia) displayed Stroop interference scores in a similar range as actual grapheme-colour synaesthetes, whom we recruited and tested separately for comparison (see Figure 5, compare rightmost data point in correlation plots with synaesthetes plot), this comparison was based on threshold-Stroop interference in non-synaesthetes, versus saturated Stroop interference in synaesthetes. This, together with the absence of any evoked colour-concurrents in non-synaesthetes, speaks against scenario 1, and in favour of scenario 2, i.e. for qualitative differences. This latter scenario accords with the low prevalence rate of synaesthesia (Simner et al., 2006) and the fact that most grapheme-colour synaesthetes are congenital (or learned very early in development), suggesting the existence of a critical period of development for synaesthesia, and/or other factors such as genetic and/or environmental predisposition (Asher et al., 2009; Simner et al., 2005; Tomson et al., 2011; Ward & Simner, 2005).

How can our relatively weak effects be reconciled with previous findings of synaesthetic Stroop-effects at the group level (Meier & Rothen, 2009; see also Colizoli et al., 2012), or even induced colour concurrents by Cohen Kadosh et al. (2009)? One explanation may be duration of training. In comparison to Meier & Rothen (2009), who trained their participants over seven days, we only trained for a maximum of three days. Although possibly playing a role, we believe that duration of training cannot fully explain the discrepancy, as we did find letter-colour binding effects very early after the first day of learning, and not much change with further days of training. In addition, Cohen Kadosh et al. (2009) found robust effects with only one session of training (albeit under hypnosis). We speculate that one dimension that could explain some of these discrepant results

is the training paradigm employed. We emphasized implicit aspects of associative learning (avoiding explicit instructions as to the ultimate goal of the experiment, i.e., the learning of letter-colour associations, see also Colizoli et al., 2012) while both Cohen Kadosh et al. (2009) and Meier & Rothen (2009) did not. It is possible that using explicit instructions may have engaged learning of letter-colour associations on a more conceptual (than perceptual) level, leading to overall stronger Stroop interference scores. This would be reminiscent of theories of sequence learning in the motor domain, according to which several forms of learning may lead to the same behavioural outcome (e.g., performance improvement), albeit by recruiting distinct processes and non-overlapping brain areas (e.g., Clegg, Digirolamo, and Keele (1998)). For instance, it has been suggested that implicit motor learning may rely more on primary (motor) areas, whereas explicit motor learning engages more prefrontal areas (Clegg et al., 1998). We therefore speculate that similar distinctions (i.e. whether associations have been learned implicitly or explicitly) may prove important for understanding the acquisition of synaesthesia-like automatic crossmodal associations in the adult population in future research.

As a final note, the correlation between letter-colour binding and Stroop in our data may suggest that there may be meaningful variability in the predisposition to learn these associations. It would be of interest in future research to explore to what extent this covaries with characteristics previously linked to the synaesthetic experience, such as schizotipy (Banissy et al., 2012), enhanced visual imagery (Barnett & Newell, 2008), superior memory (Yaro & Ward, 2007), out-of-body experiences (Terhune, 2009), and *mitempfindung* (Burrack et al., 2006). It is possible that any number of these manifestations may explain the individual differences observed in our study, and account for the observed variability in the learning of synaesthesia-like letter-colour associations.

In conclusion, using a learning paradigm which mimics the acquisition of grapheme-colour associations in individuals with confirmed acquired synaesthesia, the present study reveals the formation of synaesthesia-like grapheme colour associations in the general adult population, although not leading to conscious (additional) colour percepts from letters. Together with

other previous studies (e.g. Cohen Kadosh et al., 2009), this lends further support to the idea that grapheme-colour synaesthesia may recruit mechanisms of normal neuronal letter/colour representations.

Chapter 4: Brain regions involved in the formation of synaesthesia-like letter-colour associations by non-synaesthetes: a tDCS study

Introduction

Synaesthesia provides a unique way of investigating cross-modal sensory processing, as well as brain (re)organization for cross-modal integration (Mulvenna & Walsh, 2006). In the last decade, many studies have suggested that synaesthesia is an exaggerated form of normal, cross-modal processing (see Sagiv & Robertson, 2005 for a review), and several groups have argued the case for 'weak synaesthesia' (Eagleman, 2012; Martino & Marks, 2000; Simner, 2012). Synaesthesia research has thus recently re-focused its attention to whether adult non-synaesthetes can acquire synaesthesia-like associations: arbitrary, letter-colour pairings that exhibit automaticity, are absolute, of a perceptual nature, and show behavioural interference as observed in real synaesthetes.

Currently, models of grapheme-colour synaesthesia diverge on a central question: whether triggered sensations reflect a variant of normal or rather unique brain organization (i.e., Disinhibited Feedback vs. Cross-Activation models, respectively). The former (i.e., Disinhibited Feedback model, see (Armel & Ramachandran, 1999; Grossenbacher & Lovelace, 2001)) predicts the involvement of normal, multisensory processing areas common to synaesthetes and non-synaesthetes alike, indicating that synaesthesia-learning by adult non-synaesthetes may be possible. Contrarily, the latter (i.e., Cross-Activation model, see Ramachandran and Hubbard (2001a)) stipulates qualitatively unique brain connectivity in grapheme-colour synaesthetes, implying that individuals lacking certain structural elements (i.e., non-synaesthetes) cannot acquire synaesthetic associations following short training periods.

In line with the first model, there is a growing pool of evidence that synaesthesia may normally recruit mechanisms of normal cross-modal perception, but in an exaggerated form, thus leading to automatic, conscious percepts in the associated modality. First, synaesthetes and non-synaesthetes use the same heuristics for cross-modal matching, e.g., of graphemes or sounds to colours (Cohen Kadosh & Henik, 2007; Rich et al., 2005; Simner et al., 2005;

Spector & Maurer, 2008; Ward et al., 2006); even grapheme-colour associations across synaesthetes (Brang, Rouw, et al., 2011; Rich et al., 2005) and non-synaesthetes are non-random (Simner et al., 2005), and spatial sequence synaesthesia reflects the inherent spatial mappings of non-synaesthetes (Eagleman, 2009). Similarly, other findings show that synaesthetic correspondences can influence multisensory perception in the general population, even if detrimental to task performance (Bien et al., 2012; Eagleman, 2012; Simner, 2012). Together, these studies suggest that synaesthesia and normal cross-modal integration are closely related and even fall along a spectrum (Eagleman, 2012; Martino & Marks, 2000; Simner, 2012), indicating that synaesthesia-training may indeed be possible. In line with this view, there is increasing evidence that the environment, alongside genetic (Asher et al., 2009) and structural (Rouw & Scholte, 2007; Weiss & Fink, 2009) factors, may play a key role in the development of synaesthesia; for example, there are studies describing the cases of *acquired* grapheme-colour synaesthetes (Hancock, 2006; Witthoft & Winawer, 2013), who developed their particular synaesthetic associations following repeated exposure to the same pairings during childhood (i.e., refrigerator magnets, jigsaw puzzle).

Several recent studies employing either implicit or explicit short-term training paradigms have reported synaesthesia-like behaviour in the adult general population (Cohen Kadosh et al., 2009; Colizoli et al., 2012; Kusnir & Thut, 2012; Meier & Rothen, 2009), as measured objectively using either a synaesthesia modified-Stroop task or a grapheme search task. However, none of these studies (but one, see Cohen Kadosh et al. (2009)) has successfully induced the phenomenological experience fundamentally characteristic of synaesthesia: an elicited, conscious percept in the un-stimulated modality. Thus, it is not clear in what way these trained letter-colour associations relate to real synaesthesia (see (Deroy & Spence, 2013); Spence (2011)).

Using an implicit training task employing statistical learning of letter-colour pairings in naive participants, we found evidence that newly formed associations may indeed be synaesthesia-like because: (1) their strength not only correlated with interference on the synaesthetic-Stroop task, but more importantly, (2) they showed the colour-opponency effect, normally present in

synaesthetes but not controls (Nikolic et al., 2007) and only generated by perceptual (and not semantic) associations. The colour-opponency effect manifests as increased synaesthetic-Stroop interference when the real colour of a letter is opponent to the synaesthetic (or associated) colour, consistent with the involvement of early visual/colour areas. This is indicative that binding of colours to letters has occurred on a perceptual level, despite the absence of evoked, conscious colour concurrents.

One way of further probing the nature of these learned, synaesthesia-like associations by adult trainees is by investigating the brain areas involved in the formation of these. Thus, we here investigate the brain areas involved in the formation of these following the same visual letter search task we previously used (see Kusnir and Thut (2012), or Chapter 3)). Importantly, this task aims to re-create the learning conditions in which real synaesthetes may have acquired their associations (Hancock, 2006; Witthoft & Winawer, 2013): learning through repeated exposure. Thus, participants perform a coloured visual search task in which colours are actually task-irrelevant, while the frequency of specific letter-colour pairings are manipulated to promote statistical learning. We used bilateral transcranial direct current stimulation (tDCS) to interfere with two brain areas hypothesized to be involved in synaesthesia-like learning, or binding of colours to letters: dorsolateral prefrontal cortex (dlPFC) and posterior parietal cortex (PPC).

These sites were chosen based on several recent studies. First, Cohen Kadosh and colleagues (2009) induced grapheme-colour synaesthesia through post-hypnotic suggestion and subsequently proposed that the observed, induced synaesthetic associations may have resulted from changes in cortical inhibition mediated by frontal cortex, to and between brain regions normally involved in letter-colour binding (such as occipitotemporal and parietal areas). This would suggest that the formation of synaesthesia-equivalent associations in non-synaesthetes is suppressed by frontal cortex under normal conditions, consistent with both the inhibitory role of frontal cortex in cognitive control tasks (Miller & Cohen, 2001) and the disinhibited feedback model of grapheme-colour synaesthesia (Armel & Ramachandran, 1999; Grossenbacher & Lovelace, 2001). Second, it is likely that distinct brain areas of a learning network may

reciprocally interact to support either the formation or suppression of synaesthesia-like automatic associations in non-synaesthetes. While the network mediating these processes is unknown, one recent study (Iuculano & Cohen Kadosh, 2013) reveals reciprocal involvement of the PPC and dlPFC in distinct aspects of learning in a numerical conception task. We thus hypothesize that these two areas may also be differentially implicated in the learning of synaesthesia-like letter-colour associations by adult non-synaesthetes. Together with evidence that PPC plays a causal role in synaesthetic letter-colour binding (Esterman, Verstynen, Ivry, & Robertson, 2006; Rothen et al., 2010), we speculated that dlPFC may show a reciprocal function, i.e., suppressing these associations under normal conditions, in line with the engagement of PPC versus dlPFC in numerical learning (Iuculano & Cohen Kadosh, 2013).

We here tested these predictions by studying the effects of transcranial direct current stimulation (tDCS) over PPC and dlPFC on the formation of synaesthesia-like letter-colour associations, as induced by the implicit letter-colour association task we previously employed (Kusnir & Thut, 2012). Importantly, this task allows continuous quantification of the interaction between letters and colours during exposure, by comparing search performance of congruent (frequent) pairings to incongruent (infrequent) pairings. Letter-colour binding manifests as disproportionately improved search performance for congruent targets, and/or disproportionately impaired search performance for incongruent targets.

We tested two identical groups of participants: one group used non-opponent colour pairs for association learning (non-opponent group), and another used opponent colour pairs for association learning (opponent group). In each group (non-opponent, opponent), we applied tDCS to either PPC or dlPFC in two groups of participants while they performed the letter search task described above (i.e., leading to letter-colour binding through likelihood manipulations of letter-colour associations). A third control group (control groups 1 and 2) performed the same task with identical stimuli but no tDCS (i.e., control group 1 used non-opponent colour pairs for association learning, while control group 2 used opponent colours). In total, this experimental setup resulted in two major groups (Non-opponent, Opponent); within each, there were two stimulation

groups (PPC-Non-opponent and dlPFC-Non-opponent/ PPC-Opponent and dlPFC-Opponent), and a control group (Non-opponent, Opponent).

We find that modulation of dlPFC by tDCS significantly enhances binding of colours to letters by adult non-synaesthetes, to levels in the order of the colour-opponency effect despite the use of non-opponent colour pairs.

Materials and Methods

All experiments were conducted in accordance with the ethical guidelines established by the Declaration of Helsinki, 1994, and were approved by the local ethical committee of the School of Psychology, University of Glasgow. All participants gave their written informed consent prior to inclusion in the study. All participants had normal or corrected-to-normal vision, including self-reported normal colour vision.

Aims

The main aims of this experiment were: (1) to re-confirm, in our Control groups, the colour-opponency effect previously observed in adult trainees performing the same search task (see Kusnir & Thut, 2012, or Chapter 3), and (2) more importantly, to investigate the role of dlPFC and PPC in the learning of synaesthesia-like letter colour associations in adult non-synaesthetes.

Participants

Fifty-nine university students without synaesthesia participated in this experiment (age range: 18-53, m/f=22/37, right/left-handed=58/1; n=10 for PPC Non-Opponent; n=9 for PPC Opponent; n=7 for dlPFC Opponent; n=11 for all other groups). Synaesthesia was ruled out based on screening for grapheme-colour synaesthesia using a questionnaire adapted from Banissy *et al*. (2009) (see Appendix for a copy of questionnaire). All participants were kept naïve as to the purposes of the experiment (which was why synaesthesia questionnaires were given to participants upon completion of the experiment).

Letter Search Task

In the present experiment, we divided participants into six groups: two groups received dlPFC stimulation (Opponent, Non-Opponent), two others PPC stimulation (Opponent, Non-Opponent), and two served as control groups (no tDCS stimulation; Opponent, Non-Opponent). Before each experimental block, all groups were told, as well as shown, the two biased colours and their association to the two targets.

The experimental paradigm was identical in all aspects to the tasks employed in our previous behavioural study (see Chapter 3, "Materials and Methods"), except for the number of blocks tested: 4 versus 6 blocks of testing per day in this experiment, as compared to the previous experiments.

To allow consolidation overnight, the experiment was split into 2 sessions performed over 2 consecutive days. Each session consisted of 4 blocks, each 135 trials. Participants therefore contributed a total of 1080 trials each (divided into 8 blocks), and sessions took approximately 45 minutes in total (including experimental set up, mounting of electrodes and task time, the latter around 20 min). Participants were encouraged to take breaks between blocks.

Trascranial Electrical Stimulation (TES) Protocol

We used transcranial direct current stimulation (tDCS), a neuromodulatory technique that alters overall neural excitability by delivering low electric current to the area of scalp lying directly beneath the electrodes, and thus modifying the resting membrane potentials of the underlying neurons by either hyperpolarizing them (via cathodal stimulation) or partially depolarizing them (via anodal stimulation) (see Wagner et al., 2007, for a review). The sites of stimulation were identified using the International 10-20 system for EEG electrode placement. dlPFC stimulation electrodes were placed over F3 and F4, and PPC stimulation electrodes over P3 and P4. Anodal stimulation was consistently applied to the right hemisphere (F4 or P4), and cathodal to the left hemisphere (F3 or P3). The electrodes consisted of square conductive rubber plates (3 cm^2) each placed inside a saline-soaked sponge; they were mounted directly on the participant's scalp and subsequently held in place using an

adjustable elastic head band. A weak current (1 mA) was applied using a NeuroConn Eldith DC-Stimulator Plus. Stimulation started with the task and lasted for 20 minutes (1200 seconds), with a fade in/fade out of 30 seconds each. The duration was designed to last through all four blocks of the visual search task, and thus the electrodes were always kept in place until task completion. Some participants reported a slight tingling sensation at the onset, or during, stimulation, and no participants reported any discomforts or adverse effects.

Data Analysis

Only colour-biased targets, and blocks 1 and 4 were analysed. While block 1 served as baseline (with no learning expected, and not enough time for tDCS to develop its effects), block 4 served to assess the strength of letter-colour binding following learning under maximum stimulation time (since at the end of each day, both learning and tDCS effects were expected to manifest maximally) with and without concurrent tDCS (stimulation and control groups). Reaction times to target letters were subjected to an overall 6 x 2 x 2 x 3 mixed-design ANOVA with the between-subject factors Group (Opponent, Non-Opponent) and tDCS Stimulation (Control, PPC, and dlPFC); and the within-subject factors Day (days 1 and 2), Experimental Block (blocks 1 and 4), and Type of Letter-Colour Pairing (congruent, incongruent, and unbiased letter-colour pairs). Main effects and interactions were further probed with simple effects where appropriate.

Results

Search Performance

Figure 7 illustrates search performance at the beginning and end of training (i.e., baseline versus end of learning) per day of testing, site of tDCS stimulation, and group, as a function of the three colour-biased target conditions, i.e., colour-biased targets appearing (i) in their most frequent colour (congruent pairings), (ii) in the colour of the other biased target (incongruent pairings) and (iii) in an unbiased colour.

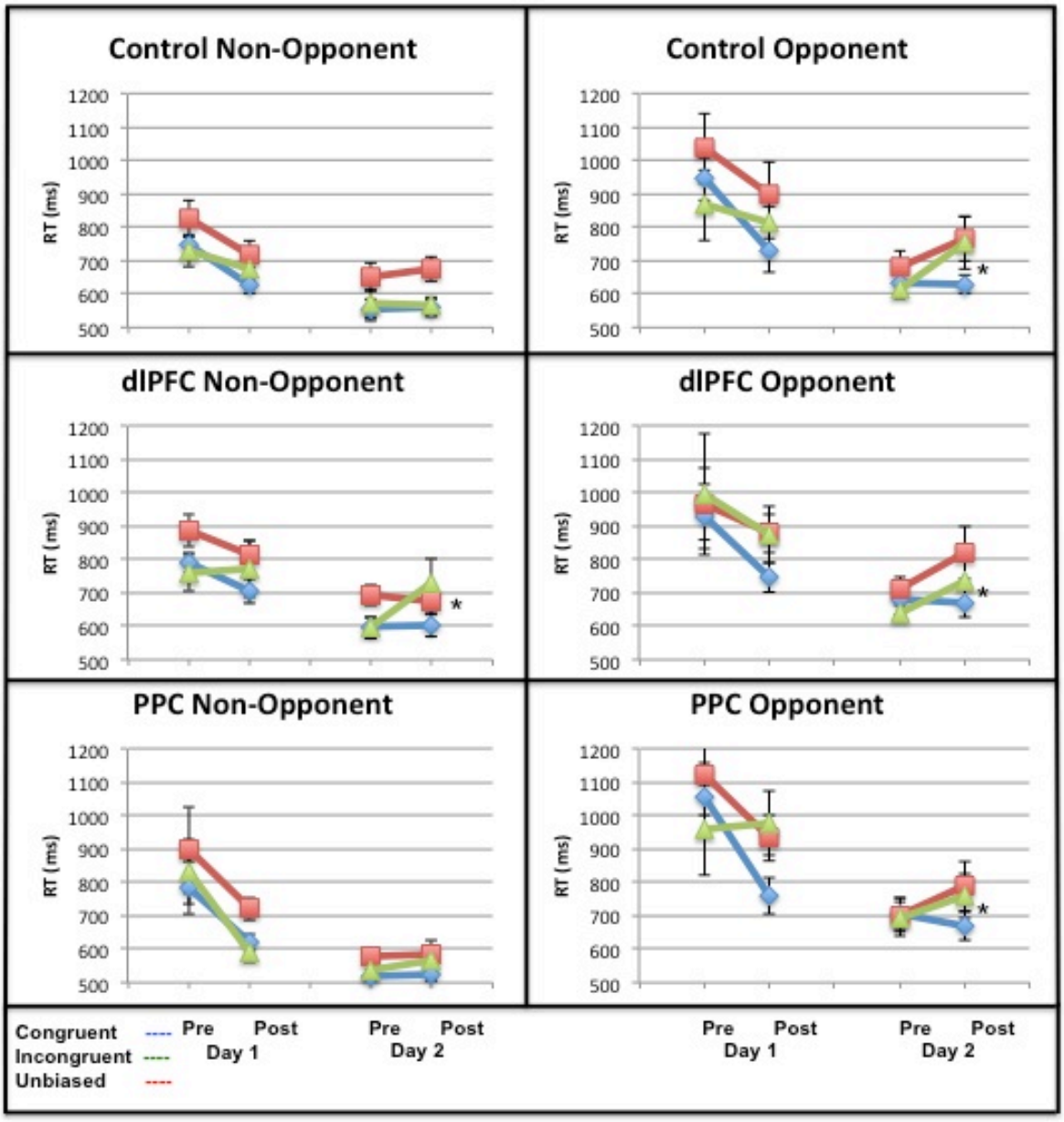

Figure 7. Search performance in Blocks 1 and 4 of each Day in the three training groups, per stimulation site and group (Opponent vs. Non-Opponent). Asterisks show groups in which letter-colour interference manifested as a function of congruent-incongruent differences. Error bars denote standard error.

Overall, participants engaged in the search task, manifesting improvement over the first day of training, and then a plateauing or slowing during the second day of training. Search performance improved from baseline to end of learning (manifested as faster reaction times in block 4 vs. block 1) and from days 1 to 2, as participants improved at target detection (Figure 7, main effects of Block: $F(1,53)=9.07$, $p=0.00397$, and main effect of Day: $F(1,53)=146.08$, $p<0.000001$), with an interaction between the two factors ($F(1,53)=34.48$, $p<0.000001$). On Day 1, participants became faster at the end of

learning compared to baseline (simple effect of Block on Day 1: $F(1,53)=23.81$, $p=0.00001$; Blocks 1 vs. 4, mean RT: 895.72±111.84ms vs. 770.09±96.86ms, collapsed across all groups); while on Day 2 a slight slowing of target detection was observed from baseline to end of learning (simple effect of Block on Day 2: $F(1,53)=11.42$, $p=0.00137$; Blocks 1 vs. 4, mean RT: 631.08±59.04ms vs. 670.99±80.38ms; collapsed across all groups). Additionally, the Opponent group improved slightly more across days than the Non-Opponent group (interaction Day x Opponency: $F(1,53)=4.28$, $p=0.0434$; simple effect of Day, Opponent group: $F(1,24)=73.98$, $p<0.000001$, Day 1 vs. 2, mean RT: 916.16±56.43ms vs. 703.16±26.14ms ; Non-Opponent group: $F(1,29)=68.93$, $p<0.000001$, Day 1 vs. 2, mean RT: 749.65±50.58ms vs. 598.92±52.76ms).

There was also an overall difference between Opponency groups, such that the Non-Opponent group generally performed faster than the Opponent group (main effect of Opponency: $F(1,53)=12.22$, $p=0.00096$, all stimulation sites and conditions collapsed, per group; Non-Opponent group vs. Opponent group, 683.67 ± 60.12 vs. 791.32 ± 57.89).

Letter-colour binding following learning

Binding of letters to colours should manifest as significantly slowed search performance when targets are presented in their incongruent versus congruent colours. Contrarily, no differences between the congruent and incongruent conditions would indicate that colours and letters remain unbound. Because binding should occur as a result of training, the congruency effect should depend on experimental Block (i.e., baseline vs. end of learning). As expected, and reproducing previous results (see Chapter 3, and Kusnir and Thut, 2012), we here find that the effects of Type of Letter-Colour Pairings (including the congruent vs. incongruent contrast) depend on experimental Block (interaction Blocks x Pairing: $F(2,106)=12.11$, $p=0.00002$), suggesting that binding may have occurred with training (see simple tests, below). Crucially, this interaction depended both on Opponency (colour-opponent vs. colour non-opponent) and Site of Stimulation (dlPFC-tDCS vs. PPC-tDCS vs. no-tDCS) (interaction Block x Pairing x Opponency x Stimulation: $F(4,106)=2.75$, $p=0.03212$), suggesting that binding may have been influenced by both Opponency and the type of Stimulation. Since there was no 5-way interaction, Day x Block x Pairing x Opponency x Stimulation

(F(4,106)=1.22, p=0.30779), we collapsed data across Days when probing simple effects of Types of Letter-Colour Pairings (congruent, incongruent, unbiased) across Blocks (baseline vs. end of learning), according to Opponency (Opponent vs. Non-Opponent) in each Stimulation Site group (control, PPC, dlPFC). We thus compare learning between Opponent and Non-Opponent groups in each tDCS stimulation site separately (i.e., interaction Blocks x Pairing x Opponency in each tDCS stimulation group).

Control Group

Results reveal that while in the Non-Opponent group, no letter-colour binding has occurred as a result of training (possibly due to insufficient duration of training, e.g. compare with Chapter 3, and see Kusnir and Thut (2012), where binding was significant with six blocks of training); in contrast, in the Opponent group letter-colour binding may indeed have occurred by the end of learning (as compared to baseline), manifested as letter-colour interference by incongruent pairings. The stronger interference effects observed in the Opponent vs. Non-Opponent group replicate our previous findings (Chapter 3, and see Kusnir and Thut, 2012), implicating colour sensitive areas and further supporting formation of letter-colour associations at a perceptual level.

In general in the Control groups (Opponent, Non-Opponent), the letter-colour pairings (congruent, incongruent, unbiased) developed differently from beginning to end of learning according to Opponency (interaction Block x Pairing x Opponency: F(2,40)=3.65, p=0.03507). In the Opponent group, search performance at the end of learning differed from baseline (simple effects: interaction Block x Pairing: F(2,20)=12.77, p=0.00027); while in the Non-Opponent group, no such interaction was observed.

Simple tests, used to further probe this interaction, revealed that letter-colour binding indeed may have occurred in the Opponent group. This was reflected mainly as a non-significant difference in search performance between incongruent and congruent letter-colour pairings *at baseline* (t(11)=1.41,p=0.190), but with increased search time for incongruent letter-colour pairings as compared to congruent (i.e., a significant slowing of the incongruent condition) *by the end of learning* (t(11)=2.36, p=0.0397).

Additionally, this difference was complemented by a slowing down of the incongruent condition at the end of learning to levels comparable to that of the unbiased condition; this manifested as significantly faster search performance for the incongruent condition than the unbiased in the baseline block (incongruent vs. unbiased: $t(11)=2.80$, $p=0.0186$), with no difference between the two conditions by the end of learning (incongruent vs. unbiased: $t(11)=0.86$, $p=0.407$) (see Figure 7).

PPC Group

Results indicate that PPC-tDCS has no effects on the learning of letter-colour associations by adult non-synaesthetes, as compared to the Control groups. Similarly to the Control groups, the letter-colour pairings developed differently from baseline to end of learning according to Opponency (interaction Block x Pairing x Opponency: $F(2,34)=4.98$, $p=0.01264$). In the Opponent group, search performance differed at the end of learning compared to baseline (simple effects: interaction Block x Pairing: $F(2,16)=5.84$, $p=0.01248$); but not in the Non-Opponent group (simple effects: interaction Block x Pairing). These results suggest that binding may have occurred as a result of training only in the Opponent group. A series of subsequent simple tests confirmed this, reflected mainly as a non-significant difference in search performance between incongruent and congruent letter-colour pairings *at baseline* ($t(9)=0.838$, $p=0.426$), but with increased search time for incongruent letter-colour pairings as compared to congruent (i.e., a significant slowing of the incongruent condition) *by the end of learning* ($t(9)=6.38$, $p=0.000214$, see Figure 7).

dlPFC Group

In contrast to the Control and PPC-tDCS groups, the dlPFC-tDCS group exhibited no differences in the evolution of search performance from beginning to end of learning according to Opponency (interaction Block x Pairing x Opponency: $F(2,32)=2.02$, $p=0.14871$). For this reason, Opponency groups were collapsed in all subsequent analyses, when probing for simple effects of Block x Pairings.

Crucially, *simple tests revealed significant letter-colour binding across Opponency groups*, despite (1) no other Non-Opponent groups (Control, PPC) showing binding as a result of training, and (2) the Non-Opponent dlPFC group performing the task under identical conditions as the other Non-Opponent groups (Control, PPC) but exhibiting interference effects on the order of the colour-opponency effect.

Across both dlPFC groups, performance mirrored Control- and PPC-Opponent groups, such that the incongruent letter-colour pairings did not differ from the congruent at baseline ($t(18)=0.175$, $p=0.863$) but again manifested a significant slowing by the end of learning ($t(18)=3.31$, $p=0.00415$), to levels comparable to that of the unbiased condition (baseline, incongruent vs. unbiased: $t(18)=2.403$, $p=0.027983$; compared to end of learning, incongruent vs. unbiased: $t(18)=0.3988$, $p=0.695$).

In summary, by the end of training, both dlPFC groups displayed differences in search performance between targets appearing in their congruent versus incongruent colours, despite both colour conditions comprising biased colours (which were equally likely to appear across trials); and hence suggesting that letter-colour binding has occurred by the end of training in both Opponent and Non-Opponent groups. In line with these results, target letters appearing in incongruent colours may have hindered search performance, slowing down target detection to levels comparable to that of the unbiased condition by the end of learning.

Importantly, dlPFC-tDCS to the Non-Opponent group enhanced letter-colour interference significantly to levels comparable to that of the Opponent group (despite the use of non-opponent colour pairs), suggesting that dlPFC-stimulation may facilitate the binding of colours to letters via the release of top-down mechanisms directly through stimulation of frontal cortex or indirectly through downstream network effects.

Discussion

We here applied bilateral tDCS over one of two brain areas (dlPFC, PPC) in different groups of adult non-synaesthetes while they performed an implicit

training task leading to synaesthesia-like automatic letter-colour associations (task previously used, see Chapter 3, and Kusnir and Thut (2012)). Our aims were to investigate the brain regions underlying the formation of these associations, while also manipulating the positions of the learned colour pairs in colour space (i.e., opponent versus non-opponent), since the use of the opponent colours normally exacerbates interference of incongruent pairings (Kusnir & Thut, 2012; Nikolic et al., 2007). The training paradigm, which employs likelihood manipulation to implicitly train specific letter-colour pairings, (1) mimics the learning conditions in which grapheme-colour synaesthetes may have learned their associations (Hancock, 2006; Witthoft & Winawer, 2013) and (2) allows for inference of binding between letters and colours by tracking interference of congruent (frequent letter-colour pairings) and incongruent (infrequent letter-colour pairings) conditions during search performance. While previous studies have suggested that the learning of automatic letter-colour associations by non-synaesthetes may be synaesthesia-like (Cohen Kadosh et al., 2009; Colizoli et al., 2012; Kusnir & Thut, 2012; Meier & Rothen, 2009) and even exemplify cases of 'weak synaesthesia' along a synaesthesia continuum (see Eagleman (2012)), we here show for that the network of brain areas involved in this synaesthesia-like learning by adult non-synaesthetes may differ from that implicated in real grapheme-colour synaesthesia. Nevertheless, we suggest that learned, synaesthesia-like associations may still relate to (real) synaesthesia in a meaningful way.

In this study, we interfered with one of two brain areas in two groups of participants (Opponent, Non-Opponent) while they performed the training task. Each of these two groups consisted of three sub-groups: a dlPFC-stimulation group, a PPC-stimulation group, and a Control group. This resulted in six total groups: 1) the dlPFC-Opponent, PPC-Opponent, and Control-Opponent performed the task using opponent colour pairs; while 2) the dlPFC-Non-Opponent, PPC-Non-Opponent, and Control-Non-Opponent performed the task using non-opponent colour pairs.

Our results revealed two main effects: first, a re-confirmation of the colour-opponency effect, here present in our Control groups (Opponent vs. Non-Opponent) and previously observed in a different set of adult trainees who

performed the same task but with longer training (see Chapter 3, and Kusnir and Thut (2012)); and more importantly, a substantial enhancement of letter-colour interference following dlPFC-stimulation *to the Non-Opponent* colour manipulation group (dlPFC-Non-Opponent), in the order of the colour-opponency effect despite the use of non-opponent colour pairs.

First, these findings re-confirm that letter-colour binding in adult trainees is subject to the colour-opponency effect, thus likely occurring on a perceptual level despite the lack of accompanying colour concurrents (see Figure 7, Control-Opponent versus Control-Non-Opponent groups). The colour-opponency effect, also present in grapheme-colour synaesthetes (Nikolic et al., 2007), results in increased interference (i.e., increased reaction times) when incongruently coloured letters are presented in colours *opponent* to the induced/ newly learned colours. Interference is reflected as a slowing down of performance in the incongruent condition relative to the congruent. Since colour-opponent receptive fields are characteristic of neurons in early visual processing areas, an exacerbation of interference by opponent colour pairs indicates the engagement of colour-opponent neurons and thus binding between paired features on a perceptual (rather than conceptual) level. Although participants underwent only four blocks of training per day (as opposed to six in our previous study, see Chapter 3, and (Kusnir & Thut, 2012)), and no letter-colour interference was observed in the Non-Opponent group, the colour-opponency effect still led to letter-colour binding in the Opponent control group, demonstrating its robustness.

Second, and crucially, our results provide evidence for the network of brain areas involved in the formation of automatic, perceptual letter-colour associations in adult non-synaesthetes, different from those that may be involved in real grapheme-colour synaesthesia. While it is the parietal cortex that has repeatedly been implicated in models of synaesthesia (Esterman et al., 2006; Grossenbacher & Lovelace, 2001; Muggleton, Tsakanikos, Walsh, & Ward, 2007; Rothen et al., 2010), our results reveal a key role for the dlPFC in the substantial enhancement of letter-colour binding between congruent pairings. This manifested as a considerable increase of letter-colour interference following dlPFC-stimulation to the Non-Opponent group, even though no

interference was observed in the corresponding Control group (compare dlPFC-Non-Opponent to Control-Non-Opponent). The changes induced by dlPFC-stimulation were thus significant, not only because they produced interference even when none was observed in the corresponding Control group, but also because the induced interference between learned and real colours was considerable, i.e., in the order of the colour-opponency effect despite the use of non-opponent colour pairs.

These enhanced interference effects (i.e., a slowing of the incongruent condition relative to the congruent) may be interpreted as a release of binding (or binding-related pathways) following dlPFC-stimulation. This interpretation would be in line with Cohen Kadosh and colleagues (2009), who proposed that the induced synaesthetic associations observed in their study following post-hypnotic suggestion could have resulted from changes in cortical inhibition between brain regions involved in letter-colour binding (such as occipitotemporal and parietal areas), mediated by frontal cortex. Accordingly, the prefrontal cortex receives connections from various sensory cortices, including the parietal lobe (Hagmann et al., 2008), possessing an integrative role (Jones & Powell, 1970) that may involve emphasis of task-relevant information (Miller & Cohen, 2001). In this way, dlPFC may act as a "gating mechanism" of relevant sensory information. As such, this brain area is subject to modulation following brain stimulation (Hannula et al., 2010). In the context of our study, dlPFC may normally inhibit the binding of colours to letters in cross-modal convergence zones of the posterior brain (i.e., in parietal and/or extrastriate ventral stream areas) that may play a key role in the formation of synaesthesia-like letter-colour associations. In support of this, it has been shown that anodal tDCS stimulation of dlPFC may result in fronto-parietal network effects (Keeser et al., 2011). In addition, dlPFC may also itself represent meaningful cross-modal associations (Fuster, Bodner, & Kroger, 2000).

In contrast to the Non-Opponent dlPFC stimulation group, the Opponent dlPFC stimulation group manifested no changes to letter-colour interference relative to their Control group (Control-Opponent). Both Opponency groups exhibited letter-colour binding, with the congruent condition becoming significantly faster than the incongruent condition by the end of learning,

relative to baseline. Because the use of colour-opponent pairs already produces strong interference, it is possible that participants were already performing at ceiling and thus were not affected by dlPFC-stimulation.

One possible caveat to this interpretation is that there are also studies showing an important role for the prefrontal cortex as a cognitive control mechanism in situations involving *incongruence* between sensory experiences and thus demanding conflict resolution (for example, in Stroop-paradigms). In line with this type of role for the prefrontal cortex, there is evidence that frontal regions are elicited in grapheme-colour synaesthesia specifically during Stroop-interference (Terhune, Cardena, & Lindgren, 2010; Weiss & Fink, 2009). In our study, binding of colours to letters is measured in terms of interference effects (i.e., as a slowing of the incongruent condition relative to the congruent), making the two effects (binding and interference) not easily dissociable. Thus, it is possible that dlPFC-stimulation in our study may have merely exacerbated interference by incongruent letter-colour pairings without affecting binding mechanisms, per se. In other words, dlPFC-stimulation may have merely increased letter-colour interference (manifested as a slowing of the incongruent condition relative to the congruent) by affecting conflict resolution processes, without affecting binding processes. Despite this possibility, we believe it to be unlikely, given (1) the lack of interference effects in the corresponding Control group (Control-Non-Opponent group) in the first place, suggesting that the newly observed interference effects reflect newly formed associations; and (2) the absence of modulation of interference effects in the dlPFC-Opponent group (compare Control-Opponent to dlPFC-Opponent groups).

In contrast to grapheme-colour synaesthetes (Esterman et al., 2006; Muggleton et al., 2007; Rothen et al., 2010), we found no modulation of letter-colour interference following PPC-stimulation in either Opponent or Non-Opponent groups. Interference effects remained unchanged relative to their respective Control groups. While these results may suggest that the mechanisms driving grapheme-colour binding in real synaesthesia may differ from the mechanisms driving letter-colour learning in non-synaesthetes, we propose that they may still relate to (real) synaesthesia in a meaningful way. It is unlikely that the prefrontal cortex plays a key role in grapheme-colour synaesthesia, as it

is the parietal cortex that is repeatedly implicated in both models of grapheme-colour synaesthesia (Grossenbacher & Lovelace, 2001) and in interference of synaesthetic Stroop effects (Esterman et al., 2006; Muggleton et al., 2007; Rothen et al., 2010). Yet, even if parietal cortex is confirmed as a key structure in synaesthesia, it may still be reconcilable with our findings of dlPFC involvement in the *learning* of synaesthesia-like letter-colour associations by adult trainees. We speculate that while parietal cortex may be important for the *expression* of synaesthesia (once acquired), dlPFC may be important for its acquisition. This is conceivable given that virtually all grapheme-colour synaesthetes have acquired their associations in childhood (i.e., they are congenital); consistent with the late maturation of frontal cortex during development (Sowell, Thompson, Holmes, Jernigan, & Toga, 1999; Sowell, Thompson, Tessner, & Toga, 2001), late dlPFC development may promote development of grapheme-colour synaesthesia in children during a critical period of development, similarly to how dlPFC-stimulation in adult trainees may support learning of automatic, letter-colour associations.

Whether PPC and dlPFC could then show a double-dissociation in terms of expression versus acquisition is an open question. Despite strong similarities between learned letter-colour associations and synaesthetic associations (i.e., they are automatic, arbitrary, of a perceptual nature, and show behavioural interference), there are important differences in phenomenology between the two groups (i.e., the presence vs. absence of conscious colour concurrents). Thus, it is also possible that any dissociations between the two brain areas would reflect this discrepancy, rather than differences related to the acquisition and expression of binding, per se.

Chapter 5: Underlying mechanisms of grapheme-colour synaesthesia and relationship to letter-colour association learners

Introduction

Although grapheme-colour synaesthesia, in which graphemes are systematically experienced in particular colours despite their veridical print colours, is one of the most prominent forms of synaesthesia, its neural mechanisms continue to stir a debate within the synaesthesia research community. While it has been highly characterized on both behavioural and psychophysical levels, studies addressing the underlying brain areas have yielded conflicting and often ambiguous results (see (Hupe et al., 2012)).

Two main models describing the underlying neural mechanisms of grapheme-colour synaesthesia have been proposed, and recently a third "hybrid" model merging aspects of both has been introduced (Hubbard et al., 2011). The two main models diverge on a central question: whether triggered synaesthetic sensations reflect (a) a variant of normal brain organisation, at the extreme of the normal spectrum, or (b) qualitatively deviant brain connectivity. The first model, termed "Disinhibited Feedback" (Grossenbacher & Lovelace, 2001), posits the existence of disinhibitory mechanisms in higher cortical areas of the visual processing hierarchy (such as cross-modal convergence zones in the parietal or temporal lobes), resulting in feedback signals to early visual areas (such as area V4). Importantly, Disinhibited Feedback predicts that graphemes are processed in their entirety before then propagating through multiple stages of processing and finally converging in a higher cortical area, such as a multisensory nexus like the superior temporal sulcus (STS). Only then can information flow "back" (in the form of feedback) to earlier visual areas, such as area V4.

In contrast, the "Cross-Activation" model, inspired from the observed neuroplasticity in phantom limb patients (Ramachandran & Hubbard, 2001b), posits structural hyperconnectivity between the brain areas involved in both grapheme and colour processing (i.e., posterior fusiform areas involved in grapheme processing, and adjacent area V4 in the fusiform gyrus and lingual

sulcus). This abnormal, excess neuronal wiring may be attributed to genetically triggered events resulting in decreased pruning between the two (adjacent) brain areas during development (Ramachandran & Hubbard, 2001a), resulting in structural hyperconnectivity and thus leading to a "cross-activation" of colours upon grapheme processing. Importantly, Cross-Activation predicts relatively *early* involvement of area V4 in the initial, feedforward sweep of activity. In fact, graphemes need not be processed in their entirety before their corresponding neural signals are transmitted to area V4, as even their component features (i.e., line segments, curves, etc.) may result in the partial activation of several graphemes (i.e., those containing some or all of these component features) before unique grapheme recognition occurs (see (Dehaene et al., 2005; Vinckier et al., 2007) for a reviews of hierarchical letter processing). . One key, important difference between the two main models of grapheme-colour synaesthesia is thus the predicted time course of early (extrastriate) visual areas: in contrast to Disinhibited Feedback, Cross-Activation predicts early activation during the initial, feedforward sweep of activity.

The bulk of studies investigating the neural mechanisms of grapheme-colour synaesthesia have taken a neuroimaging approach (Elias et al., 2003; Hubbard et al., 2005; Hupe et al., 2012; Rich et al., 2006; Rouw & Scholte, 2010; Sperling et al., 2006; Weiss et al., 2005; Zeki & Marini, 1998) (for a review, see Rouw et al. (2011)) and centre their debate on whether synaesthetically triggered sensations generate activation of area V4, or rather other areas of visual or neo- cortex. However, the imprecise temporal information of fMRI actually makes both models of grapheme-colour synaesthesia plausible given activity in area V4. Only electrophysiological approaches, capable of scrutinizing neural activity on a millisecond timescale, are capable of disentangling the two models. Nonetheless, there have only been a handful of these, and only one has employed MEG (Brang, Hubbard, et al., 2010) rather than EEG (Beeli et al., 2005; Brang et al., 2008; Brang, Kanai, et al., 2011; Jancke et al., 2009; Sagiv & Ward, 2006; van Leeuwen et al., 2010; Volberg, Karmann, Birkner, & Greenlee, 2013). In addition, most of these studies have explored other aspects of synaesthesia, such as semantic congruency effects (in memory or in conceptual processes), general early sensory processing, or functional network connectivity.

Only the MEG study (Brang, Hubbard, et al., 2010) has examined the triggered synaesthetic percept itself, i.e., via the (passive) presentation of achromatic, inducing and non-inducing graphemes. Here, Brang and colleagues (2010) demonstrated nearly simultaneous activity of area V4 (as pre-defined using retinotopic mapping in the MEG) and grapheme areas (also pre-defined in the MEG) in response to achromatic, synaesthesia-inducing graphemes. Importantly, this activity peaked between 111-130 ms after the presentation of graphemes, only a 5-ms delay following the onset of grapheme areas. Although these results support a quick, direct cross-activation between the involved brain areas (i.e., Cross-Activation) only three grapheme-colour synaesthetes, who were strong projector sub-types, were reported. Thus, it is unclear whether these results may generalise to the majority of grapheme-colour synaesthetes, who are typically associator sub-types.

We have here conducted an MEG study on associator grapheme-colour synaesthetes using Independent Component Analysis (ICA) to decompose the acquired signal and identify the dominant patterns using a single-subject approach. ICA is a data-driven, blind source separation method that extracts statistically independent sources that have been mixed in the combined EEG/ MEG signal (Makeig, Debener, Onton, & Delorme, 2004; Makeig et al., 1997). It makes no prior assumptions about the spatial locations of the combined signal. ICA has recently been introduced into the analysis of MEG data (Brookes et al., 2012; Brookes et al., 2011; Capilla, Belin, & Gross, 2013; Spadone, de Pasquale, Mantini, & Della Penna, 2012; Vigario et al., 2000). Since the neural mechanisms and the cortical areas underlying the triggered, synaesthetic percept are still very much under debate, and since additionally no “synaesthetic” event-related components have been defined, ICA is an ideal method for extracting the patterns consistently present across trials in each grapheme-colour synaesthete, including the “weaker” patterns that may not necessarily manifest in the event-related averages of the raw, sensor time-series.

In addition to disentangling the two current models of grapheme-colour synaesthesia, information regarding the underlying mechanisms of (real) synaesthesia would help clarify the relationship of synaesthesia-like, learned associations in adult trainees and (real) synaesthetic associations. Since the

Disinhibited Feedback model posits normal neural circuitry in grapheme-colour synaesthetes, it is also in line with theories of common cross-modal integration across synaesthetes and non-synaesthetes alike, of a "synaesthesia continuum," and of synaesthesia-learning in adult trainees. In contrast, the Cross-Activation model posits structural abnormalities in grapheme-colour synaesthetes, suggesting that the phenomenon is indeed unique, expressed only in a small percentage of the general population, and allows no prospects of synaesthesia-learning following only a short training period.

In this study, we show an absence of early, evoked activity in early (extrastriate) visual areas of associator grapheme-colour synaesthetes (in response to synaesthesia-inducing graphemes). Rather, we show evoked activity peaking approximately 190 ms following grapheme presentation in grapheme-colour synaesthetes but not matched controls, and exhibiting an occipito-parietal topology localised consistently to the inferior occipital gyrus and overlapping with Brodmann area 19. This is, to the best of our knowledge, only the second MEG study to date investigating grapheme-colour synaesthesia, and the first to provide evidence for the Disinhibited Feedback model.

Materials and Methods

All experiments were conducted in accordance with the ethical guidelines established by the Declaration of Helsinki, 1994, and were approved by the local ethical committee of the School of Psychology, University of Glasgow. All participants gave their written informed consent prior to inclusion in the study. All participants had normal or corrected-to-normal vision, including self-reported normal colour vision.

Participants

Six grapheme-colour synaesthetes (age range: 19-34, m/f=0/6, right/left-handed=6/0), and six controls (age range: 21-35, m/f=1/5, right/left-handed=6/0) matched on age, handedness and educational level participated in this experiment. Developmental synaesthesia was established by means of two questionnaires: in the first, participants rated *statements* describing aspects of their synaesthetic experience and provided accompanying written explanations

of these (adapted from Banissy et al. (2009)); while in the second, they rated visual *illustrations* portraying their synaesthetic experience (adapted from Skelton et al. (2009)) and also provided short accompanying statements describing additional aspects of their synaesthetic experience (see Appendix for a copy of both questionnaires). Based on these questionnaires, all six synaesthetes were classified as associators, or at least as more "associator-like" than "projector-like." In addition, we tested and confirmed grapheme-colour synaesthesia in all six synaesthetes by means of Consistency Tests (see below). At the end of the study, all controls were also screened for synaesthesia using the same written questionnaire administered to synaesthetes (described above) (adapted from Banissy et al., 2009).

Consistency Test

To test and confirm grapheme-colour synaesthesia in all six synaesthetes, we used a computerized protocol adapted from Eagleman et al. (2007) (also providing normative data). Each trial began with presentation of a colourless grapheme (black on a medium grey background), together with a colour palette consisting of more than sixty-five thousand colours. Participants then selected the colour that most closely matched their synaesthetic percept of the presented grapheme (or a "no colour" option if they lacked a colour experience for that grapheme). Participants were instructed to take their time and to be as precise as possible. Upon selection of a colour, the corresponding RGB value was automatically recorded and the next trial began. In total, there were 150 trials, corresponding to the full set of graphemes A-Z, digits from 0-9, and fourteen non-letter symbols (see Figure 8), repeated three times each in randomised order. Matlab 2007b (The MathWorks, Inc.) and an adapted version of the Texsyn Toolbox (Eagleman et al., 2007) were used to control both stimulus presentation and data collection.

After a minimum delay of three weeks and without prior knowledge, all six participants were re-tested in the exact same task. All individual grapheme-colour pairings were then tested for consistency, per synaesthete and across sessions based on the formula established by Eagleman et al. (2007): for each of the fifty graphemes, the total distance between the selected colours (i.e., three total colours) was calculated in normalised RGB colour space. Then, the colour

distances were averaged within sessions (i.e., average of fifty colour distances for the first session, and average of fifty colour distances for the second) and subsequently across sessions to yield a Consistency Score. All six grapheme-colour synaesthetes fell within the normative synaesthesia range provided by Eagleman et al. (2007), i.e., exhibiting Consistency Scores below 1 (range of Scores across sessions: 0.55-0.88).

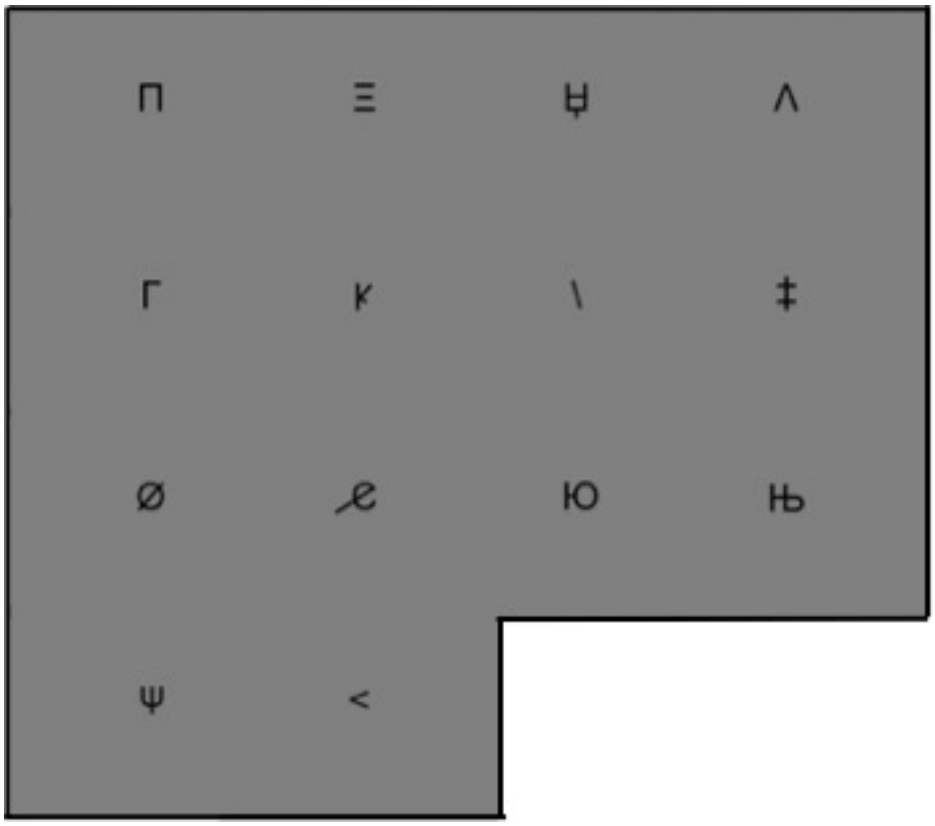

Figure 8. Non-letter symbols presented to grapheme-colour synaesthetes in Consistency Task. These were manually created using similar component features as letters.

Psychophysics of the Synaesthesia-Inducing Stimuli

Following the Consistency Test, all synaesthetes (but not controls) were asked to complete a computer task aimed at: 1) defining the duration of stimulus presentation for the subsequent MEG task, and 2) acquiring psychophysical measures of the inducing and non-inducing stimuli. First, seven colour-inducing letters and seven non-inducing, non-letter symbols were chosen *for each synaesthete* based on individual responses in the (previously administered) Consistency Test. Then, each of the seven selected colour-inducing letters was paired to one of the selected non-inducing symbols, resulting in seven pairs of inducing/non-inducing graphemes. A static sequence

of morph graphemes was then created for each of these seven pairs, such that each complete "morph set" consisted of a colour-inducing letter, a non-inducing symbol, and three "intermediate" morph graphemes representing step-wise transformations between the inducing letter and the non-inducing symbol. The intermediate morph graphemes were created such that they physically resembled a "blend" of their preceding and subsequent graphemes. (See Figure 9) This led to a total of thirty-five graphemes (7 total morph sets x 5 graphemes per morph set). All graphemes were created manually using Microsoft Office Power Point, and were achromatic set against a medium grey background.

The second aim of this task was to correlate the morph sets with the actual experience of synaesthesia, i.e., to confirm that the assigned "morph levels" (ranging from the inducing letter gradually to the non-inducing symbol) correlated with each synaesthete's subjective, triggered colour experience (i.e., strongest synaesthetic experience in response to Morph Level 1, becoming gradually weaker across Morph Levels 2-4 and absent in response to Morph Level 5).

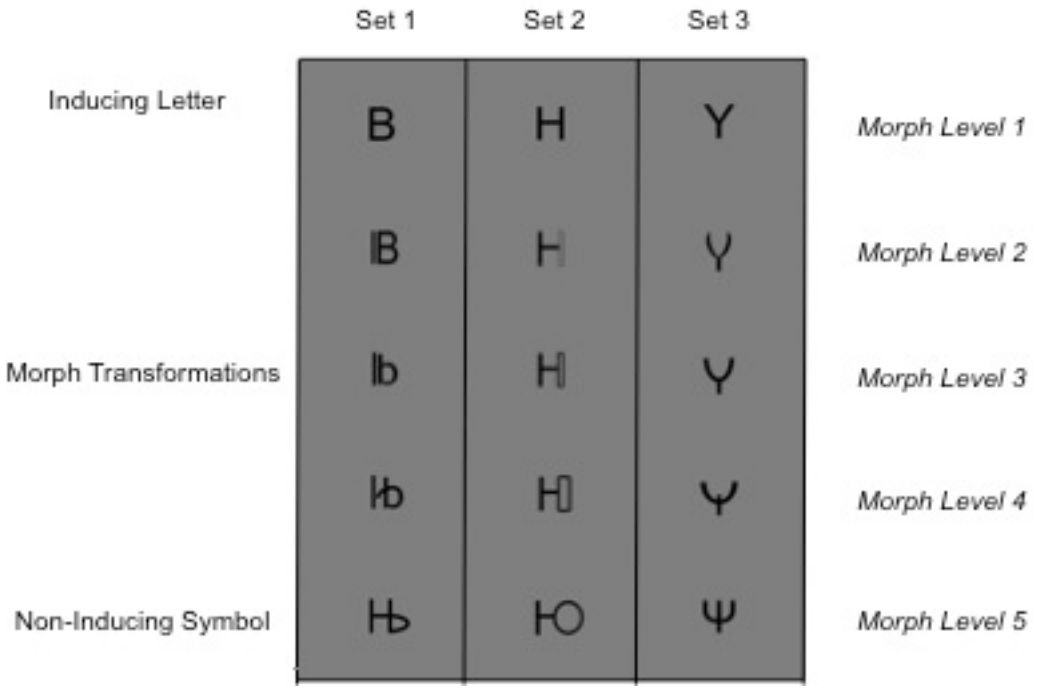

Figure 9. Morph Sets. Seven, static sequences of morph graphemes were created for each grapheme-colour synaesthete, such that each complete "morph set" consisted of a colour-inducing letter, a non-inducing symbol, and three "intermediate" morph graphemes representing step-wise transformations between the inducing letter and the non-inducing symbol. The intermediate morph graphemes were created such that they physically resembled a "blend" of their preceding and subsequent graphemes.

Participants were instructed to focus their attention to the centre of the screen. Each trial began with the presentation of a medium grey screen prompting the participant to press the "spacebar" key as soon as he/she was ready for the next trial. Upon pressing the "spacebar" key, the stimulus appeared against a medium grey background. The stimulus appeared in black and was always one of the thirty-five pre-selected graphemes (i.e., from that particular participant's seven morph sets). The set of stimuli (i.e., the thirty-five pre-selected graphemes) was thus tailored to, and different for, each individual synaesthetic participant. The stimulus remained on the screen for a pre-defined stimulus duration time (either 50 ms, 200 ms, or 1 s). The stimuli were presented in randomized order an equal number of times (fifteen times each), one third of the time in each of the three pre-defined stimulus duration times (i.e., five times for 50 ms, five times for 200 ms, and five times for 1s, also in randomized order). (See Figure 10) This resulted in a total of 525 trials.

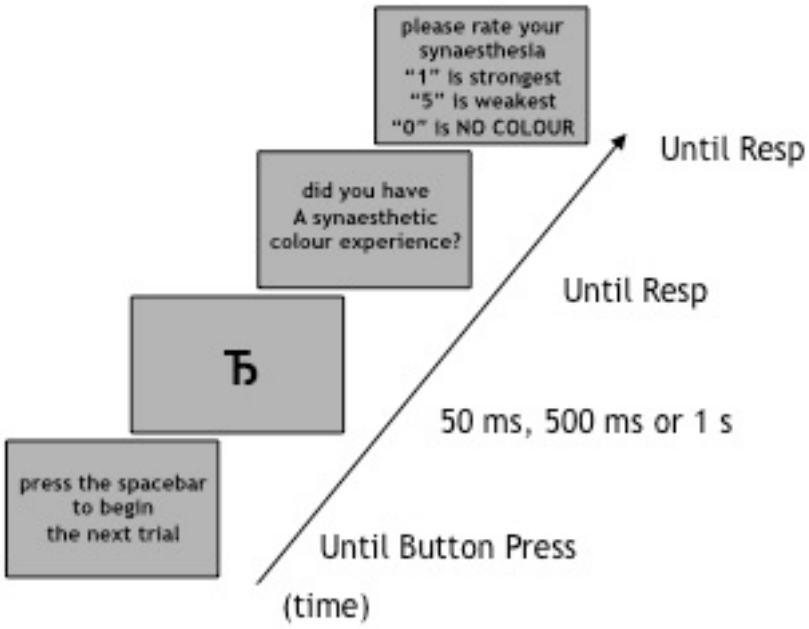

Figure 10. Task used in Psychophysical Testing of MEG Stimuli.

The task was two-fold. First, synaesthetic participants were prompted to indicate "yes" or "no" to whether the presented stimulus induced a synaesthetic colour experience, via the pre-defined keys 'k' and 'l'; and second, they were prompted to rate, from 1 to 5, how strong their synaesthetic experience was via the pre-defined keys 's' to 'h' (though the keys were marked with their corresponding numbers so as to avoid confusion). Synaesthetic participants were

instructed to respond with a '0' (pre-defined key 'a') if they had *not* experienced a synaesthetic colour, and to rate the strength of their synaesthetic colour experiences from 1 to 5 if they had answered "yes" to the previous question, "1" being the strongest and "5" being the weakest. Synaesthetes were encouraged to use all five button presses and were reminded via on-screen instructions of which keys represented the strongest and weakest responses. Both questions, presented separately, remained on the screen until response. Synaesthetic participants were encouraged to take breaks every 20 minutes, as the task lasted between 60-90 minutes, depending on individual pace.

MEG Task

The paradigm administered to participants in the MEG suite was a passive viewing task coupled to a grapheme-comparison task, partially designed to maintain participants' attention to the presented graphemes. In each trial, synaesthetes and controls viewed two successive achromatic graphemes, drawn from the pool of pre-selected graphemes (i.e., morph sets) tailored and assigned to each synaesthetic participant. The presented graphemes could thus be colour-inducing letters, morphs, or non-inducing symbols (i.e., any grapheme within a morph set). Participants were instructed to compare the two presented graphemes on a scale from 1 to 5, where '1' was "very similar" and '5' "very different," and the numbers '2,' '3,' and '4' progressively dissimilar. Participants were encouraged to use all five button presses.

Each trial began with the presentation of a fixation cross set against a medium grey background. After a delay of 1.5 s, the first grapheme appeared on the screen and remained for 50 ms, after which a blank, medium grey screen remained on the display for 2 s. Then, a second (different) grapheme was presented on the screen against a medium grey background and remained there until response. Upon response, a medium grey screen was again presented for 1 s before the next trial began (i.e., signalled by the presentation of a fixation cross). (Figure 11)

Stimuli were presented through a DLP projector (PT-D7700E-K, Panasonic) placed outside the shielded room onto a screen situated 1.90 m away from the participants via an in-room mirror. All stimuli (achromatic) were presented using

Psychtoolbox (Brainard, 1977) on a medium grey background. The fixation cross was presented in the centre of the screen, as were graphemes. Each grapheme was presented a total of twelve times, and was paired three times with each of the other four graphemes in its morph set (separated by a blank screen, as described above). This led to a total of 420 trials, divided into six blocks lasting 6-8 minutes each.

The experiment started with the presentation of instructions. Participants were given instructions to maintain a steady gaze at the centre of the screen, and to blink immediately upon response. They were given unlimited time to rest between runs. On average, the total duration of the task was ~1 h.

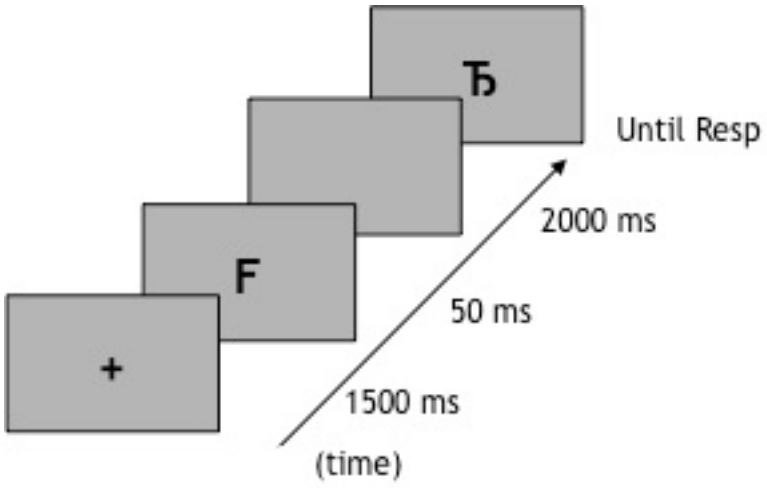

Figure 11. Task administered to grapheme-colour synaesthetes and controls in MEG. Participants were instructed to attend to all stimuli and rate the similarity between presented graphemes following presentation of the second one.

MEG Recording

Brain activity was recorded with a 248-magnetometers whole-head MEG system (MAGNES® 3600 WH, 4-D Neuroimaging) confined in a magnetically shielded room. MEG signal was acquired at a 1017 Hz sampling rate.

Before starting the recording session, 5 coils were positioned on the participant's head, which was localized at the beginning and end of each run. These coils, together with 3 fiducial points and the subject's head shape, were

digitized using a Polhemus system. During the recording session, subjects were seated in a reclining chair and supported their head against the back and top of the magnetometer. Participants were asked to remain as still as possible and were continuously monitored by video camera. They were also instructed to minimize blinking during the presentation of visual stimuli, and instead to synchronize their blinks with the blank grey screen that followed their response.

MEG Analysis

The analysis of the MEG signal was performed using the FieldTrip software package (Oostenveld, Fries, Maris, & Schoffelen, 2011) (see http://fieldtrip.fcdonders.nl/) and in-house Matlab code. It was performed in four main steps: 1) preprocessing aimed at removing artifactual activity; 2) an Independent Component Analysis (ICA) aimed at extracting the dominant patterns of brain activity; followed by 3) a Cluster-Level Analysis on the resulting event related fields (derived from single ICs) evoked by the inducing (versus non-inducing) visual stimuli; and finally 4) source-level analysis aimed at projecting single ICs into source space, and thus identifying the neural generators underlying differences between (inducing vs. non-inducing) conditions.

Preprocessing

The preprocessing of the MEG signal was carried out as follows. First, the signal was epoched in trials of 3 s length (1 s pre-stimulus) time-locked to stimulus onset. We then removed the DC offset and linear trends in the signal to centre the signal around zero. To standardize the whole-signal preprocessing and facilitate any potential source analysis, a common set of MEG sensors (n=8) manifesting low correlation with immediate neighbours (signifying increased levels of hardware noise) were removed from the MEG data set. These MEG sensors were manually selected by computing the correlation between individual channels and their first and second order neighbours over the entire signal length (with bad trials removed, i.e., trials manifesting a variance three z-scores above the average variance, per channel). Then, trials contaminated with SQUID jumps were discarded from further analysis, and the remaining MEG signal was de-noised relative to the MEG reference sensors, as implemented in the

"ft_denoise_pca" function in FieldTrip. Finally, trials with large signal variance were removed from the MEG data set prior to implementing Independent Component Analysis to isolate and reject both eye blinks and cardiac components from the MEG signal ("fastica" algorithm implemented in FieldTrip, after a dimensionality reduction to 20 components).

Independent Component Analysis (ICA) for Analysis of Evoked Signals

In the case of comparing two experimental conditions, as is done here, performing ICA to each of the conditions separately could lead to the undesired situation in which the decomposition of a component was not performed in exactly the same numerical way for both conditions. In such a case, it becomes difficult both to identify and compare components underlying a brain process present in both conditions, but dominant in only one. For these reasons, we performed ICA on the entire data set before isolating our conditions of interest.

Specifically in this study, ICA has been employed to isolate components present in both conditions of interest, on a single-subject level. All components are then compared across the conditions of interest (Inducing vs. Non-Inducing Graphemes), in order to identify components dominated by one condition versus the other. Thus, following preprocessing of the raw data, the "cleaned" data were downsampled to 250 Hz and subjected to an ICA ("runica;" FieldTrip/EEGLAB, http://sccn.ucsd.edu/eeglab/) in a time window between -0.3 s and 1.2 s. This algorithm first performs a PCA-based dimensionality reduction to 40 components, and then performs ICA on these 40 components. A timelocked analysis was then performed on the output components of the ICA (of which the data structure is [components x time], rather than [channels x time]), in order to average each condition of interest separately (Inducing vs. Non-Inducing).

Nonparametric Cluster-Based Permutation Analysis (ICA Space)

The resulting data from the ICA were filtered between 1-30 Hz, since only event related averages were of interest.

We then applied a nonparametric cluster-based permutation analysis (Maris & Oostenveld, 2007), as implemented in FieldTrip, to the resulting data

from the ICA in order to identify clusters of time in which the two conditions of interest (Inducing vs. Non-Inducing) exhibited significant differences. This test controls the family wise error rate (FWER) in the context of multiple comparisons. In this type of analysis, an independent *t*-test between the two conditions of interest is performed *on each time sample of the evoked response*; then, all time samples whose resulting *t*-values exceed a certain, pre-defined threshold (in this case, *t*-values corresponding to a *p*-value of 0.025, as corresponds for a two-tailed test) are *clustered* based on temporal adjacency. Each cluster is then assigned a [cluster-based] statistic equal to the sum of its individual *t*-values (i.e., those *t*-values corresponding to the individual time samples included in each particular cluster). These cluster-based statistics are calculated with each permutation and are used to build a distribution, from which is calculated the Monte Carlo *p*-value: this is the *p*-value used to determine whether the observed [cluster-based] statistic (i.e., that of the original data set) could have been obtained by chance. The permutation distribution can thus be thought of as a histogram of the *t*-values derived from the random re-shuffling of all trials into two partitions (original data split in two to represent the two tested conditions), for which the clustering procedure is repeated each time. Thus, if the original *t*-value falls above 95% of the *t*-values obtained from the randomized (re-shuffled) data, then the null hypothesis is rejected with a Monte-Carlo *p*-value < 0.05. Here, a reference distribution of cluster-level *t* statistics was created from 1000 randomizations.

First, Stimulus-Evoked Activity in Raw Data (Sensor Space)

The aim of identifying the first, stimulus-evoked activity in visual cortex was mainly to identify the time course of the first, feed-forward sweep of activity following grapheme onset. To identify the first, stimulus-evoked visual activity in occipital areas (following grapheme presentation) in all participants, we computed the planar gradient magnitudes for individual participants, derived from the raw, sensor time-series. The preprocessed MEG data were timelocked in each condition of interest, separately. Then, the resulting averaged data (in each condition) were converted to synthetic planar gradients considering both first- and second-order neighbours (maximum distance of 7.4 cm) and using the "sincos" approach implemented in FieldTrip. We chose to use the planar gradient representation because it is stronger over the underlying brain sources

(Hamalainen et al., 1993), in contrast to the magnetic field representation, which is stronger at the poles of the magnetic dipole. The use of planar gradient makes the topology of the results in the sensor level more spatially interpretable with respect to the underlying brain sources.

Source Level Analysis

MEG-Magnetic Resonance Image Co-Registration

T1-weighted structural magnetic resonance images (MRIs) of each participant were co-registered to the MEG coordinate system by a semi-automatic procedure that provided the best fit between the participant's scalp surface, extracted from his/her anatomical MRI, and the digitized head shape from the MEG. To obtain a first approximate alignment between MEG and MRI coordinates, we manually located the three digitized fiducial points (nasion, left and right pre-auricular points) in each individual's MRI.

Head and Forward Models

The brain was segmented and the cortex extracted from each MRI using the segmentation routine implemented in FieldTrip/SPM8 (http://www.fil.ion.ucl.ac.uk/spm). We then constructed a semi-realistic single shell head model (Nolte, 2003) based on each individual's brain. We subsequently adapted a standard grid of 6 mm resolution derived from the Montreal Neurological Institute (MNI) brain to each participant's brain volume. This was achieved by normalizing the individual MRIs to the standard MNI brain through a linear affine transformation (FieldTrip/SPM8). The inverse of the resulting transformation matrix was applied to the MNI-standard grid to transform it into each participant's brain space. Finally, we computed and normalized the lead fields corresponding to the 2 tangential orientations for each voxel.

Inverse Solution (Source Space)

The main aim of the inverse solution, as used here, is to project single ICA activity into source space. This kind of analysis has been already been applied on resting state MEG data (de Pasquale et al., 2010; Mantini et al., 2011). In these

approaches, the regularization parameter has been chosen for each IC differently, but details (of the regularization computation) are not described in the corresponding publications. In this work, a similar methodology has been employed, using weighted-Minimum Norm Least Squares Estimation (wMNLS) for the derivation of the inverse solution, with a different regularization parameter for each IC.

The wMNLS source reconstruction was performed in Fieldtrip (Oostenveld et al., 2011), which employs the algorithm implemented in (Lin et al., 2004). (The theoretical formulation for this algorithm is described in the Appendix, see "Minimum Norm: Theory." For details regarding the specifics of the computation performed here, see the Appendix, "Minimum Norm: Practice.") Importantly, we followed a novel approach for the computation of the regularization parameter for each IC. This procedure was applied to each of the ICs for which a significant, statistical difference was found between the compared conditions (i.e., via the non-parametric cluster-level permutation analysis), both for synaesthetes and controls. The source localization was performed and plotted on a 3-dimensional, template grid with 6mm resolution, warped to individual subjects' brain volumes.

Results

Due to the temporal delay between the stimulus-PC and the projector inside the MEG suite, a delay of ~30 ms must be considered in all analyses (and all figures).

Non-parametric Cluster-Level Permutation Analysis on ICs

Only ICs exhibiting temporal clusters corresponding to a significant difference between the two conditions (i.e., Inducing vs. Non-Inducing, all $p<0.05$) were analysed. In general, Synaesthetes exhibited more significant ICs than Controls. Additionally and in contrast to Controls, all Synaesthetes exhibited at least one significant IC. (Figure 12, Figure 13)

In order to identify the time window of maximal, temporal overlap across participants' significant clusters, all ICs exhibiting significant differences

between conditions were clustered together independently for each group (Synaesthetes, Controls). Most ICs clustered around the same time window for both groups, peaking approximately around 190 ms. Figure 14 shows a histogram, for each group (Synaesthetes, Controls), with the frequency of significant clusters (within ICs) for the analysed time window, in bins of 20 ms.

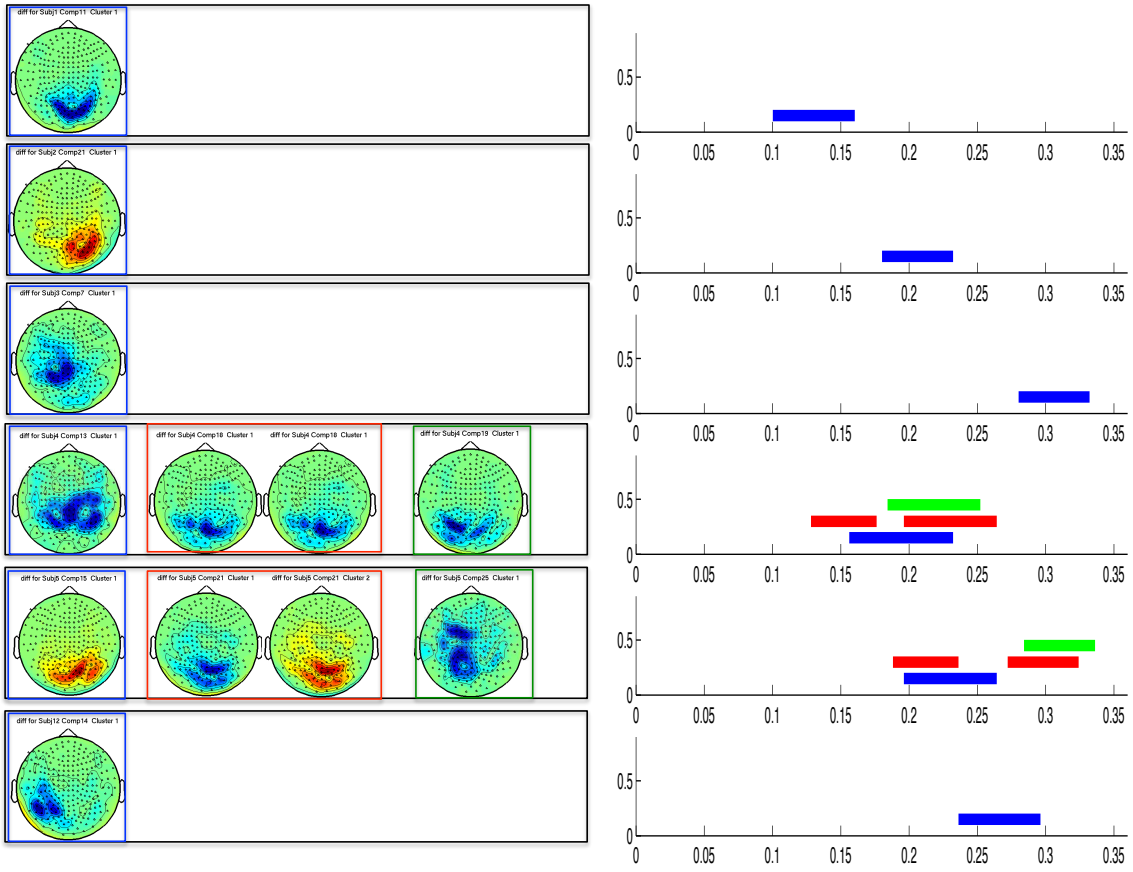

Figure 12. Synaesthetes. Represented, are the ICs (topographies, time) exhibiting clusters with significant differences between conditions (Inducing, Non-Inducing) in Synaesthetic participants. On the left can be seen the topographies of significant ICs, for all six participants. On the right are time bars showing the time period in which significant differences between conditions were found by a non-parametric cluster-level permutation analysis. Coloured boxes and coloured time bars denote a correspondence between ICs and time windows. Stacked time bars denote different ICs, while horizontally aligned time bars (of the same colour) denote >1 significant cluster for one IC.

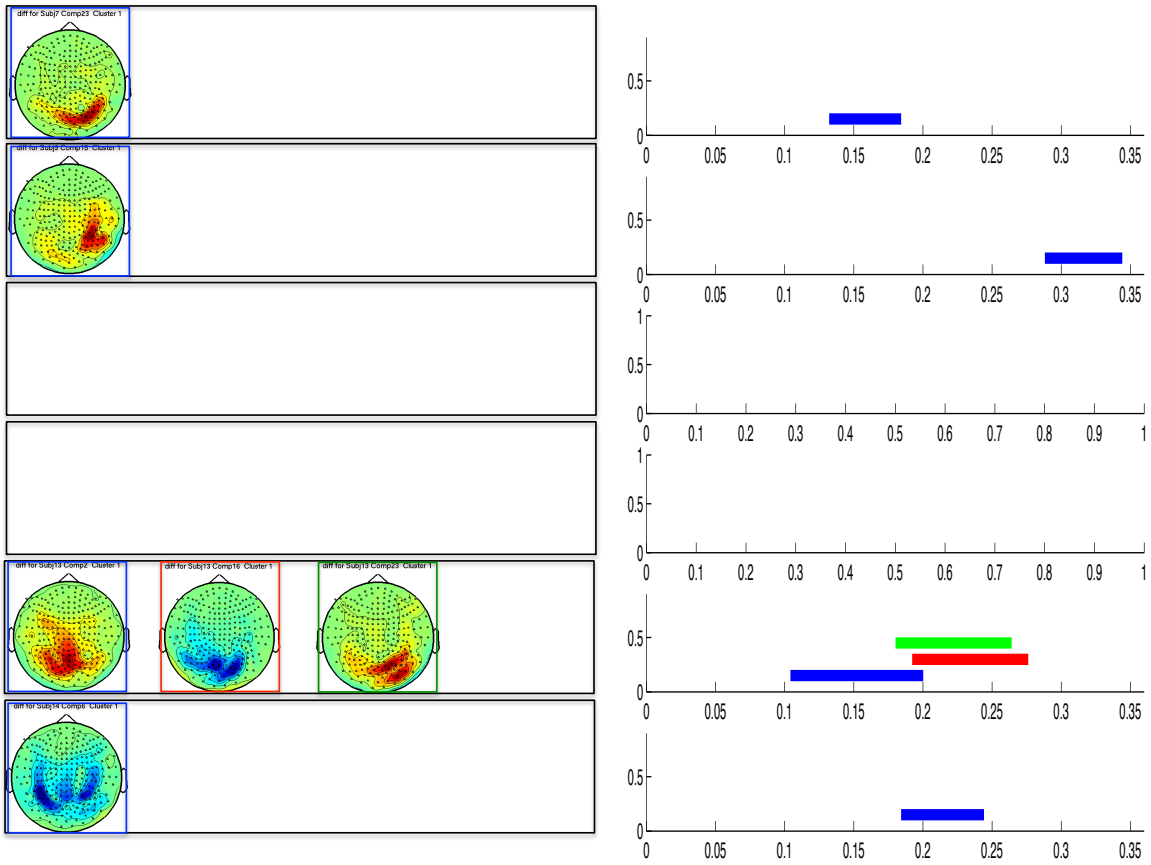

Figure 13. Controls. As Figure 12, but in Control participants.

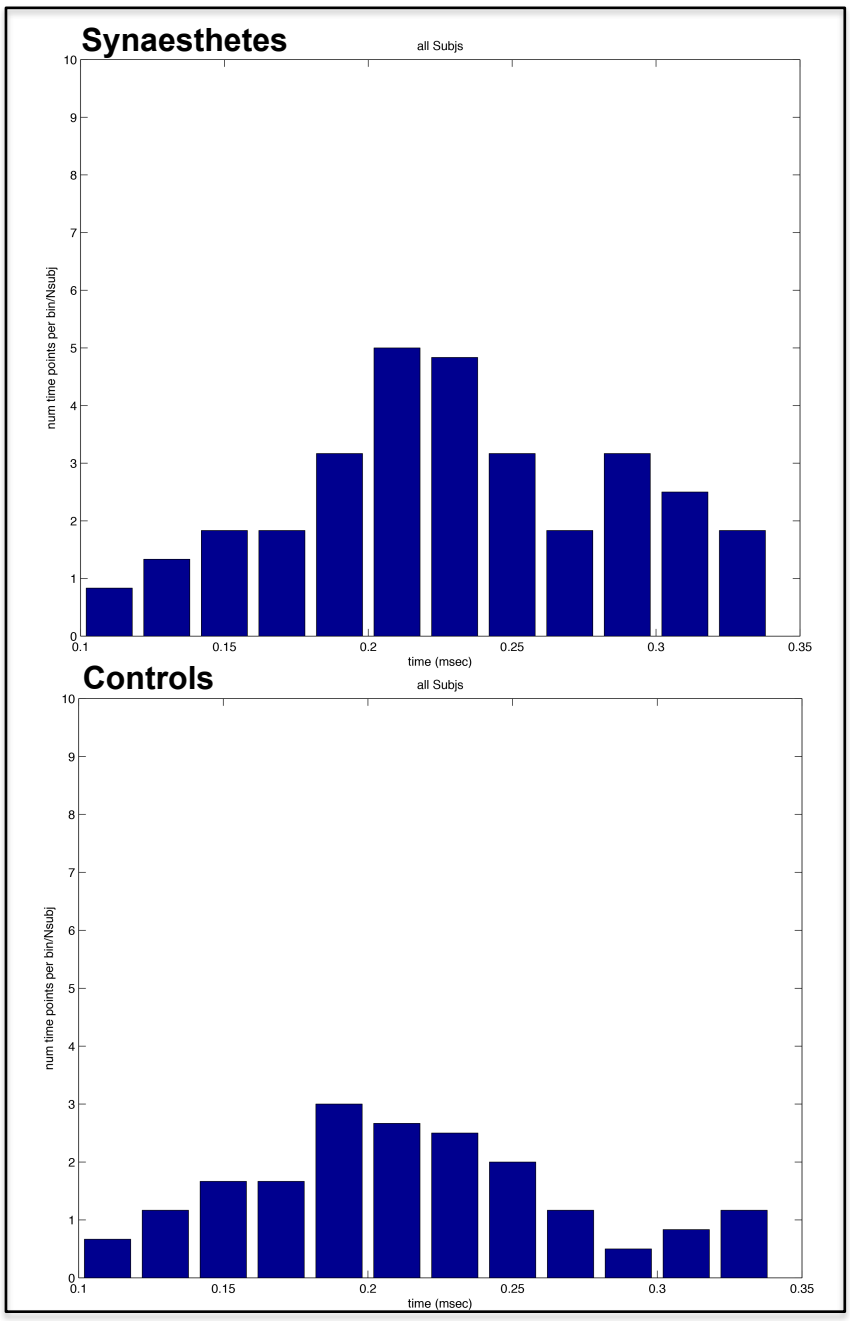

Figure 14. Histogram, for each group (Synaesthetes, Controls), showing the frequency of significant clusters (within ICs) for the entire analysed time window,(70-330 ms) in bins of 20 ms.

Since the maximum temporal overlap across participants in both groups centred approximately at 190 ms, the grand average of all ICs containing significant clusters within the time window 130-230 ms was calculated individually for each group, in order to identify the average brain activity and topography in this (highly significant) time period. Given the difficulties of comparing ICs *between* participants, a grand average of all relevant ICs (i.e. containing significant clusters in the given time window) was one strategy for

identifying similarities or differences across individual participants of each group. In order to calculate the grand averages, individual ICs falling within the pre-selected time window (130-230 ms) were first projected back to sensor space on a single-subject level, after which planar gradient magnitudes were computed before averaging across individual participants of each group. (Figure 15)

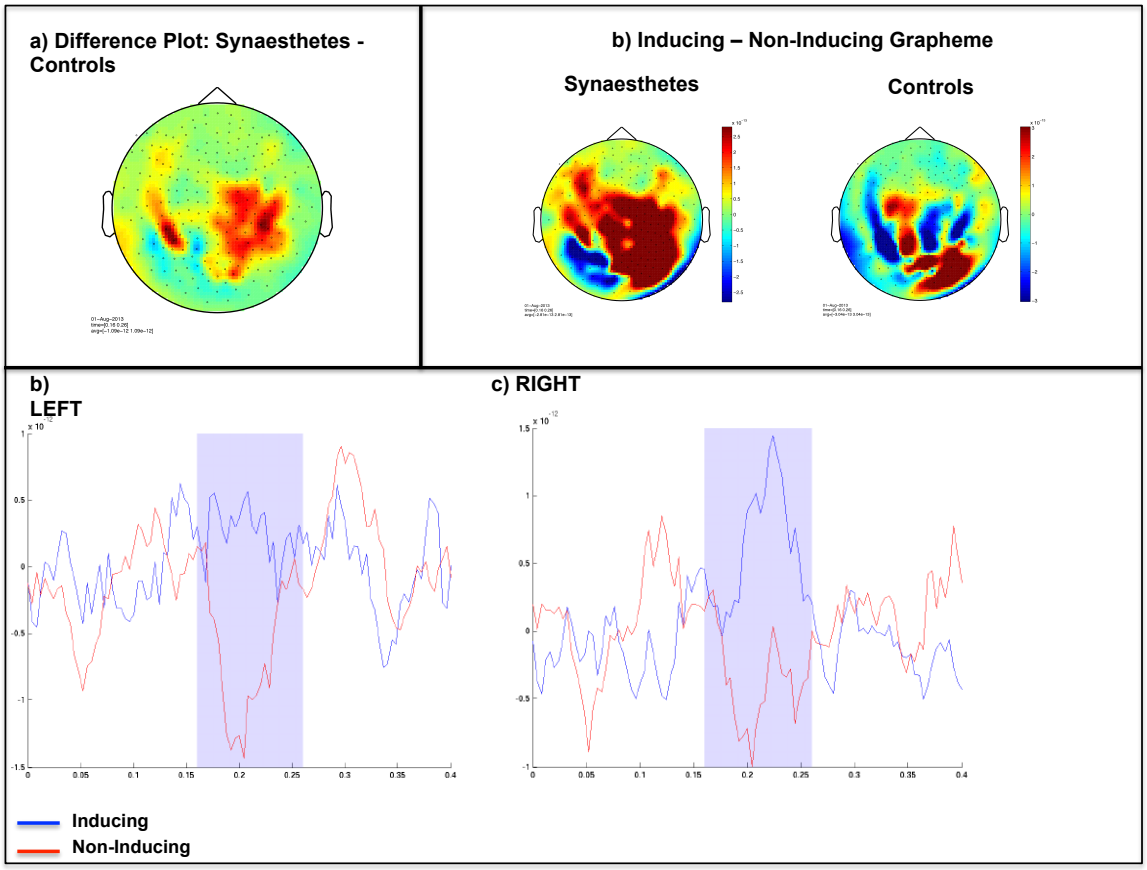

Figure 15. Contrast Plot showing the topography (a) and signal differences (c,d) between Synaesthetes and Controls. For each participant, any ICs exhibiting significant differences between conditions (Inducing vs. Non-Inducing) within the pre-selected time window (130-230 ms) were projected back to Sensor Space and converted to Planar Gradient representations. Then, the data were averaged per group (Synaesthetes, Controls). The difference topography (a) shows the contrast between the groups (Synaesthetes minus Controls), revealing activity in occipito-parietal areas, possibly reflecting an increase of activity in the Inducing condition and/or a suppression in the Non-Inducing condition (b,c). The shaded areas represent the Time Window plotted in the topography (a), (derived from maximum temporal overlap across individual participants' significant ICs).

First, Stimulus-Evoked Visual Activity

The first, stimulus-evoked activity over occipital sensors was calculated independently for each group, as a reference for the time course of visual

activity. The first visual-evoked response occurred just after approximately 100 ms in both groups (see Figure 16).

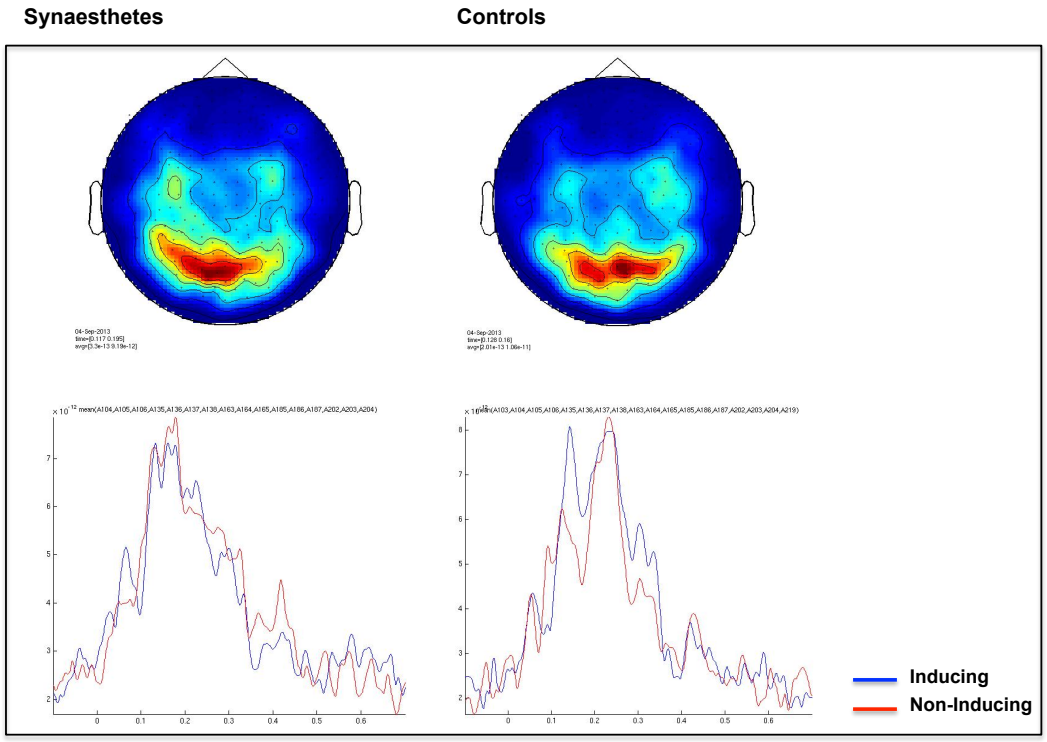

Figure 16. First, visual-evoked activity in Synaesthetes and Controls. Planar gradient representation of activity over occipital sensors, averaged across participants of each group.

Source Level

The wMNLS source reconstruction results, calculated for individual participants, yielded consistent localization across Synaesthetes to visual extrastriate cortex, overlapping with Brodmann area 19 or areas of the Cuneus in the occipital lobe. Synaesthetes are shown in Figure 17. In contrast, source reconstructions across Controls showed no consistency, localizing instead to different areas of the parietal or temporal cortices (only two participants), and to early visual cortex in a third participant.

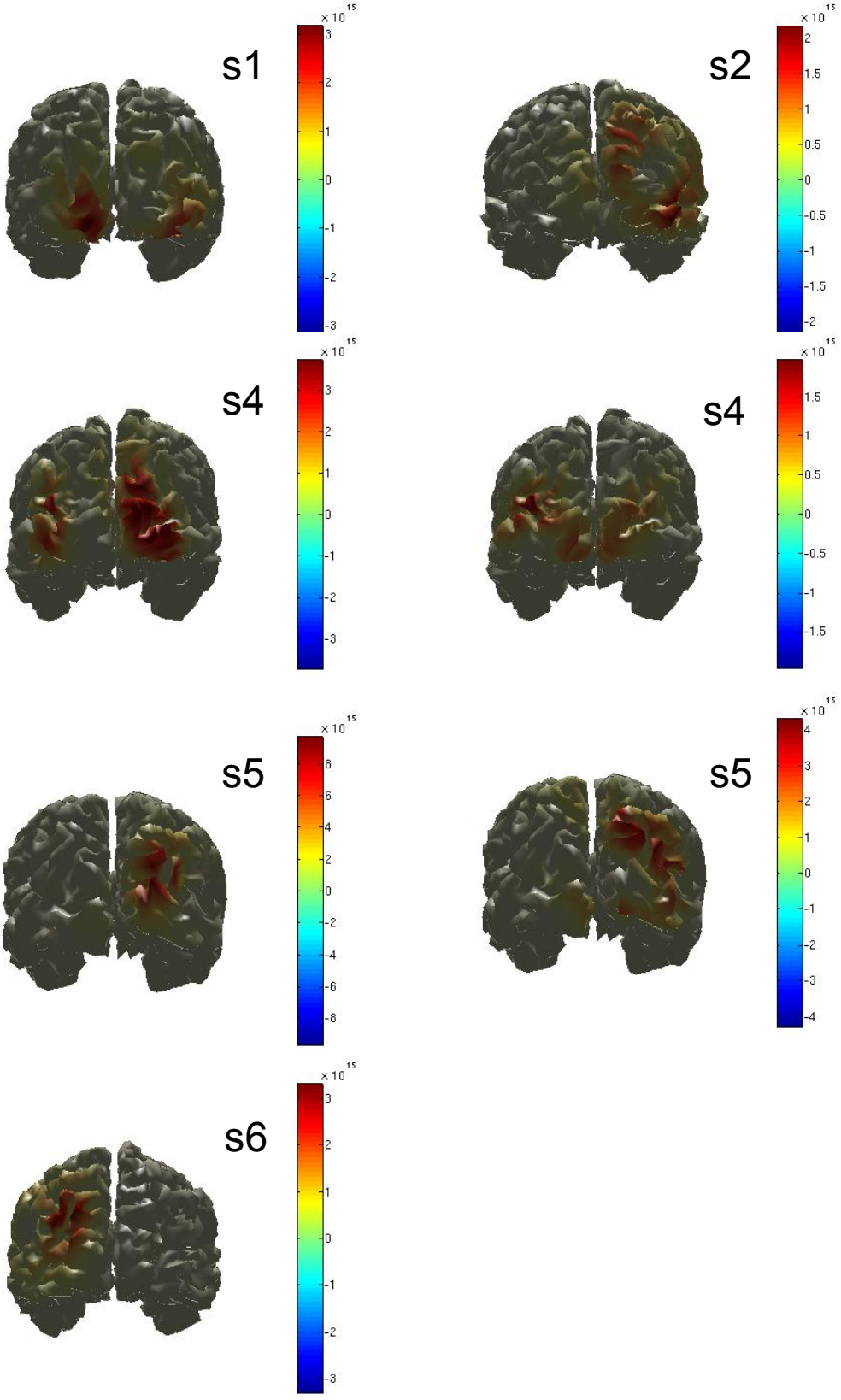

Figure 17. wMNLS source reconstructions in individual participants. All Synaesthetes except one (s3) exhibited ICs with significant differences between conditions. The inverse solutions of individual participants yielded differently lateralized, extrastriate visual cortex. In contrast, the only three Controls exhibiting ICs with significant differences in the analysed time window showed divergent source reconstructions (data not shown).

Discussion

We here provide evidence that grapheme-colour synaesthesia may be governed by neural events occurring *after* the initial, feed-forward sweep of activity in the visual processing stream, peaking between 180-210 ms, after grapheme processing is likely complete (Rey, Dufau, Massol, & Grainger, 2009). These neural events exhibit an occipito-parietal topography and localise consistently across individual grapheme-colour synaesthetes to extrastriate visual cortex in the occipital lobe. Due to the relatively late timing of these neural events, our results more likely support a Disinhibited Feedback model of grapheme-colour synaesthesia, or re-entrant processing from brain areas further down the visual processing hierarchy (Grossenbacher & Lovelace, 2001; Smilek et al., 2001).

Given the current debate regarding the underlying neural mechanisms of grapheme-colour synaesthesia, we here used Independent Component Analysis (ICA) for the blind extraction of the dominant patterns present in individual participants' MEG signal, as they viewed Inducing and Non-Inducing graphemes in the form of letters or pseudoletters. ICA is an ideal tool for identifying patterns consistently present across trials, including subtle patterns that may not be evident in the event-related averages of the raw, sensor time-series. A non-parametric cluster-level analysis was applied to the resulting Independent Components (ICs) of individual participants (i.e., on a single-subject level). This analysis allowed identification of any ICs composed primarily of one condition versus the other (i.e., ICs showing significant differences between Inducing vs. Non-Inducing graphemes) and thus representing patterns of brain activity unique to one condition versus the other. Note that the number of ICs exhibiting significant differences between conditions in the Synaesthetic group nearly doubled that of the Control group (Figures 12 & 13). We then identified the maximum *temporal* overlap across individual participants (independently for each group) in which significant ICs exhibited significant differences between conditions (Figure 14). Both groups showed a maximal ICs (exhibiting significant differences between conditions) in approximately the same time window, i.e., centring around 190 ms.

There have only been a handful of electrophysiological studies (only one MEG) examining the underlying mechanisms of the induced, synaesthetic percept in grapheme-colour synaesthetes. While there is evidence of early processing differences between synaesthetes and controls beginning as early as 100-150 ms after viewing graphemes (Brang et al., 2008; Brang, Kanai, et al., 2011) or hearing words (Beeli et al., 2005), these studies primarily addressed semantic modulation of synaesthesia-inducing graphemes. In addition, they could not accurately localise the underlying electrophysiological components due to volume conduction limitations characteristic of EEG data. Similarly to these studies, the only MEG study to date (also investigating grapheme-colour synaesthesia, see Brang, Hubbard, et al. (2010)) shows early (i.e., beginning around 110 ms) activity in area V4 in response to synaesthesia-inducing graphemes in synaesthetes (versus controls), only ~5 ms after the onset of activity in adjacent grapheme processing areas. This short latency (~5 ms) between activity in grapheme processing areas and colour areas may only be supported by direct, anatomical connections between the two areas, as predicted by the Cross-Activation model. In contrast to Brang et al. (2010a), our results indicate relatively late differences between Inducing and Non-Inducing graphemes (around ~190 ms). Importantly, this time corresponds to activity occurring *after* the first sweep of stimulus-evoked activity, which peaked in all participants (both Synaesthetes and Controls) approximately around 100 ms (Figure 14). This suggests that differences between Inducing and Non-Inducing graphemes likely occur after grapheme processing is complete (>145 ms following grapheme presentation) (Rey et al., 2009), contrary to the predictions of the Cross-Activation model.

Rather, these “late” differences more adequately support a feedback model of synaesthesia, such as the Disinhibited Feedback (Grossenbacher & Lovelace, 2001) or Re-entrant Processing (Smilek et al., 2001) models. Contrary to Cross-Activation, these predict that graphemes (1) are first processed in their entirety and (2) propagate through multiple stages of visual processing before arriving in the more anterior extrastriate cortex, PIT areas, or multisensory areas of the parietal cortex, where synaesthetic processing would begin to differ from normal perception: in synaesthetes, feedback connections from PIT areas or parietal cortex projecting back to earlier visual areas (such as area V4) would

relay back information pertaining to the meaning of the processed grapheme, leading to the induced, synaesthetic percept. In line with these models, a difference topography (i.e., average of Synaesthetes minus average of Controls) implicated occipital-parietal areas (Figure 15, upper panels) in the pre-selected time window (centring around 190 ms) and revealed a general suppression in the Non-Inducing condition, with an enhancement of activity in the Inducing condition (Figure 15, lower panels). Together, the timing, topography, and neural signals as revealed in the corresponding time-series are consistent with Disinhibited Feedback/ Re-entrant Processing models of grapheme-colour synaesthesia, wherein a "release" of feedback signals may manifest as a suppression of activity in response to Non-Inducing graphemes, or as an increase of activity in response to Inducing graphemes.

In addition, we verified the average topographies of synaesthetes and controls by localising the significant ICs of *each individual participant* using a Minimum Norm approach. Importantly, the source reconstructions of Synaesthetes consistently localised to the extrastriate cortex of the occipital lobe (Figure 16). While lateralisation differed between Synaesthetes, previous neuroimaging studies have reported similar findings (see (Gray et al., 2006; Zeki & Marini, 1998)). We speculate that the differences observed between synaesthetic participants may reflect individual differences in the induced, synaesthetic percept, i.e., variability of the synaesthetic experience. Contrary to Synaesthetes, the few Controls who also displayed significant differences between the two conditions showed no consistency in their inverse solutions, possibly reflecting task-specific strategies given that they encountered the presented graphemes for the first time on the day of MEG testing (contrary to Synaesthetes, who were already familiar with the stimuli, see Materials and Methods). Moreover, we did not necessarily expect to observe early visual differences between conditions (Inducing vs. Non-Inducing) based solely on grapheme recognition, given (1) the equivalence between our letters and pseudoletters in terms of low-level visual complexity, and (2) current theories of grapheme recognition as a process of hierarchical feature analysis, together with (3) the lack of letter-centred or language-centred task demands (note that 80% of presented graphemes were pseudoletters, or morphs) (Dehaene et al., 2005; Hubbard et al., 2005; Mitra & Coch, 2009) (but see Rey et al., 2009).

Nonetheless, we cannot rule out that the observed differences between conditions observed in Synaesthetes (but not Controls) reflect visual processing differences unique to grapheme-colour synaesthesia but not specifically related to the induced, conscious colour concurrent. Since our paradigm did not directly address the "consciousness" or phenomenological experience of the synaesthetic concurrent, these are here not dissociable from differences between the Inducing vs. Non-Inducing conditions.

How can we reconcile our results with previous evidence for parietal involvement in the synaesthetic, induced percept, and its role in Disinhibited Feedback models of grapheme-colour synaesthesia? There is increasing evidence showing the importance of parietal cortex in synaesthesia, particularly in IPS (intraparietal sulcus) regions (van Leeuwen et al., 2010; Weiss et al., 2005; Zeki & Marini, 1998). In addition, several TMS studies have unveiled a causal role for parietal areas in the synaesthetic concurrent (Esterman et al., 2006; Muggleton et al., 2007; Rothen et al., 2010). There are also anatomical studies showing increased coherence (FA) in the white matter of IPS (Rouw & Scholte, 2007), and recently there have been studies investigating functional connectivity in synaesthesia (Jancke & Langer, 2011; Specht & Laeng, 2011), demonstrating important hubs in parietal areas (and also in the corresponding early sensory areas, such as fusiform gyrus). Thus, parietal cortex seems to play a crucial (essential) role in the induced, synaesthetic percept, possibly in hyperbinding of visual features elicited in earlier visual areas, or in feedback to earlier visual areas.

First, we have here examined only stimulus-evoked activity; contrarily, oscillatory activity may govern neural communication through synchrony, with even distant neurons transiently linking into larger-scale neural assemblies (Fries, 2005), thus allowing for more efficient communication. Thus, it is possible that other brain areas subserving multisensory integration, possibly in parietal cortex, may be involved in the binding or in the perceptual awareness of the induced, synaesthetic percept. In addition, our stimuli were presented for a short duration (50 ms), as compared to the longer stimulus duration times used in previous electrophysiological studies (>500 ms). Given this and also the nature of our task, which required participants to actively engage in the form-related

features (geometric shape) of the stimuli, it is possible that the induced, synaesthetic percept was partially extinguished, or weak, in some (or all) participants. In fact, two synaesthetic participants reported weaker induced percepts at such short stimulus durations during the behavioural task administered prior to the MEG task. It should be noted, too, that two Synaesthetes did manifest parietal involvement in the difference between Inducing and Non-Inducing graphemes, as in both of these participants one of their significant ICs localised to the superior parietal lobe.

With respect to relating grapheme-colour synaesthesia to synaesthesia-learners, these models implicate functional (as opposed to structural) differences between synaesthetes and controls, and thus they are also consistent with models proposing common mechanisms of cross-modal integration across the two groups, and also imply that synaesthesia-learning may indeed be possible. In fact, the timing observed here is consistent with previous behavioural (RT) as well as electrophysiological (ERP) studies demonstrating the timing of feature selection and binding processes in non-synaesthetes: the selection and processing of attended conjunctions of features becomes evident between 150-260 ms (Schoenfeld et al., 2003), with the integration of irrelevant features occurring even later between 230-250 ms.

Chapter 6: General Discussion

Integrative Summary

We have here studied the formation of synaesthesia-like, arbitrary letter-colour associations in adult non-synaesthetes via an implicit, statistical learning paradigm, first in a behavioural setting and subsequently using brain stimulation over prefrontal or posterior parietal areas. We can now compare the nature of the resulting, trained associations in non-synaesthetes to the natural letter-colour associations of congenital synaesthetes, drawing on information from the literature as well as from our own assessment of grapheme-colour synaesthesia using MEG.

Our paradigm differed from previous studies on synaesthesia-training in non-synaesthetes in three important ways. First, learning of associations occurred implicitly rather than explicitly and under conscious control (see also Colizoli et al. (2012)). Our learning paradigm aimed to mimic the natural conditions in which some grapheme-colour synaesthetes may have learned their associations (Hancock, 2006; Witthoft & Winawer, 2006, 2013). Adult non-synaesthetes searched for targets that were frequently paired with specific colours, but they were instructed to make spatial judgments; in fact, they were unaware that they were partaking in the statistical learning of specific letter-colour associations and that the real end-goal of the task was to learn these pairings. Second, our paradigm provided a measure of learning over time, as search performance could be tracked across blocks in the evolution of both congruently and incongruently coloured letters. Third, our paradigm allowed dynamic quantification of the strength of letter-colour associations over time, manifested as a contrast between the congruent and incongruent conditions across blocks and presumably reflecting interference of (incongruent) colours on letter search due to binding of (congruent) colours to letters. Although participants were expected to generally improve search performance to biased targets over time (i.e., across blocks and over days),the main experimental questions here were whether participants could learn specific, arbitrary letter-colour associations; whether these pairings would be robust enough to interfere with target detection when the (wrongly coloured) incongruent targets were

displayed; and whether they were indeed synaesthesia-like, or quantitatively and qualitatively similar to synaesthetic associations.

Across the first two experiments, we determined that adult non-synaesthetes displayed significant learning of specific letter-colour associations, showing synaesthesia-like characteristics: first, the strength of letter-colour binding (as evidenced by the contrast between congruent and incongruent conditions, i.e., colour interference) was linearly related to synaesthetic-Stroop interference; and second, learning most likely occurred on a perceptual (rather than conceptual) level, as the strength of letter-colour interference during search performance depended on the relative positions of the (biased) colours in colour space. Interference was significantly stronger when the learned (associated) and real colours of the targets were opponent, rather than non-opponent. In summary, results from the first two experiments suggested that the learned letter-colour associations did not result from cognitive strategies, but rather from automatic, involuntary, and perceptual processes, potentially similar to those of congenital grapheme-colour synaesthetes.

Importantly, however, the learned associations did not lead to conscious colour experiences and thus, despite behavioural similarities, adult trainees differed phenomenologically from real synaesthetes. We suggested that these differences exhibited qualitative (rather than quantitative) differences from real synaesthesia, also in accordance with the low prevalence rate of synaesthesia (Simner et al., 2006) and the fact that most grapheme-colour synaesthetes are congenital (or learned their associations very early in development), indicating a critical period of development for synaesthesia, and/or the involvement of other factors such as genetic and/or environmental predisposition (Asher et al., 2009; Tomson et al., 2011 (Simner et al., 2005; Ward & Simner, 2005)). Rather than suggesting a “synaesthesia continuum” (see Eagleman (2012)), the individual learning differences observed here were interpreted as reflecting meaningful variability across adult non-synaesthetes in the predisposition, or propensity, to learn arbitrary crossmodal associations. However, these were considered “synaesthesia-like” because they exhibited similar principles as those of synaesthetes (i.e., in both groups, pairings are automatic, involuntary, arbitrary, absolute, and of a perceptual nature).

The third experiment indirectly addressed the relationship between adult trainees and real synaesthetes by investigating the brain areas involved in the formation of automatic, synaesthesia-like letter-colour associations by adult non-synaesthetes. Knowledge of the brain areas involved could help elucidate whether learned associations depend on the same neural mechanisms as those underlying (real) synaesthetic associations (versus altogether different ones). Given the recent evidence pointing to common mechanisms of crossmodal interactions in synaesthetes and non-synaesthetes (Bien et al., 2012; Cohen Kadosh, Cohen Kadosh, & Henik, 2008; Martino & Marks, 2000; Parise & Spence, 2009), we administered bilateral tDCS stimulation over either prefrontal (dlPFC) or posterior parietal (PPC) brain areas while adult trainees underwent the same learning task as discussed above (i.e., leading to the formation of automatic letter-colour associations). We showed that the network of brain areas involved in the learning of these associations by adult trainees may altogether differ from those implicated in real grapheme-colour synaesthesia. On one hand, this may be taken to suggest that the learned associations are not synaesthesia-like at all (in contrast to recent findings, see (Cohen Kadosh et al., 2009; Colizoli et al., 2012; Kusnir & Thut, 2012; Meier & Rothen, 2009) and do not exemplify cases of 'weak synaesthesia' along a synaesthesia continuum (in contrast to Eagleman (2012). However, dlPFC involvement observed in our study may be reconciled with what we know about PPC involvement in grapheme-colour synaesthesia. Below, I will first cover the role of dlPFC in the learning of synaesthesia-like associations, and then the apparent contradiction to PPC involvement in synaesthesia research.

Rather than revealing a role for the parietal cortex, as suggested by theories of a "synaesthesia continuum" (Eagleman, 2012) and previous studies of grapheme-colour synaesthesia (Esterman et al., 2006; Muggleton et al., 2007; Rothen et al., 2010), our results implicated dlPFC in the enhancement of letter-colour interference (presumably reflecting increased letter-colour binding). In other words, our results suggest that dlPFC-stimulation helped the binding of colours to letters (i.e., the formation of letter-colour associations), a divergence from what would be expected given the "synaesthesia continuum" hypothesis (namely, that instead PPC-stimulation would have interfered with letter-colour binding). The considerable slowing observed in the incongruent condition (i.e.,

relative to the congruent condition) following dlPFC-stimulation may be interpreted as a release of binding (or binding-related pathways), normally suppressed by dlPFC function. This would be in line with the proposal by Cohen Kadosh et al. (2009) that the induced synaesthesia-like associations observed in their study (following post-hypnotic suggestion) were also mediated by frontal cortex, resulting in changes to cortical inhibition between brain regions involved in letter-colour binding (such as parietal and occipitotemporal areas). As to the network interactions underlying such effects, we can only speculate. It is long known that the prefrontal cortex exhibits an "integrative" role, as it receives connections from various sensory cortices (Jones & Powell, 1970). One aspect of this role likely involves inhibition and/or emphasis of task-relevant information (Miller & Cohen, 2001). In this way, dlPFC may act as a "gatekeeper" of sensory information. In the context of this study, we speculate that dlPFC normally inhibits binding of letters to colours in cross-modal convergence zones of the posterior brain (possibly parietal cortex and/or extrastriate, ventral stream areas) that play a key role in letter-colour associations. In support of this hypothesis, it has previously been shown that anodal tDCS stimulation of dlPFC may result in fronto-parietal network effects (Keeser et al., 2011) (presumably via projections between dlPFC and the parietal lobe (Hagmann et al., 2008).

While the results of our first two and also third experiments may not support the existence of a "synaesthesia continuum," could they nevertheless relate to synaesthesia in a meaningful way? It is unlikely that the prefrontal cortex plays a key role in grapheme-colour synaesthesia (but see (Terhune, 2009; Weiss & Fink, 2009)), as it is the parietal cortex that has repeatedly been implicated in models of synaesthesia stipulating disinhibited feedback (Grossenbacher & Lovelace, 2001) from higher, multisensory association areas in parietal cortex to sensory cortices. However, this theory may require further testing, as to the best of our knowledge, there is yet no study looking at the effects of prefrontal transcranial stimulation on synaesthetic experience in grapheme-colour (or any other type of) synaesthetes. Yet, even if parietal cortex is confirmed as a key structure in synaesthesia, it may still be reconcilable with our findings of dlPFC involvement in the learning of synaesthesia-like associations in adult trainees. For example, it is conceivable that parietal cortex is important for the expression of synaesthesia (once

acquired), while dlPFC (dys)function may be important for its acquisition. In other words, the two observations (PPC involvement in synaesthesia, dlPFC involvement in the learning of synaesthesia-like associations) may simply be incomparable because reflecting different processes (i.e., expression versus development of letter-colour associations).

The question then arises of whether dlPFC may play a role in the acquisition of synaesthesia in (real) synaesthetes? This is indeed conceivable: since virtually all grapheme-colour synaesthetes have acquired their associations in childhood, and consistent with the late maturation of frontal cortex during development (Sowell et al., 1999; Sowell et al., 2001), late dlPFC development may indeed promote the development of grapheme-colour synaesthesia in children during a critical period of development, much like transient dlPFC-stimulation in non-synaesthetic adults supports learning (i.e., development) of synaesthesia-like letter-colour associations. This is not to suggest that an immature dlPFC is the cause of synaesthesia, but simply that it may constitute an important element defining the critical period for forming such associations.

Could PPC and dlPFC then show a double-dissociation in terms of expression versus acquisition of synaesthesia? While this thesis cannot conclusively respond to this question, the lack of PPC effects here may suggest that, in contrast to grapheme-colour synaesthetes, this brain region is not normally involved in the learning of automatic letter-colour associations by adult trainees. Future studies would need to probe for a double-dissociation between dlPFC- and PPC-involvement during and after training of synaesthesia-like associations in non-synaesthetes. Nevertheless, investigating this point may also prove difficult, given that the differences in PPC involvement between synaesthetes (previous studies) and non-synaesthetes (this thesis) may simply reflect observed differences in phenomenology (i.e., presence vs. absence of conscious colour concurrents), rather than binding of colours to letters, per se.

This thesis set out to test two prominent models of synaesthesia: the “synaesthesia continuum” hypothesis (see (Eagleman, 2012; Martino & Marks, 2000)) and the “discrete synaesthesia” hypothesis: may we all be potential synaesthetes, or is synaesthesia confined to a (unique) fraction of the normal population? In our first series of three experiments, we did not find evidence

supporting the existence of a "synaesthesia continuum;" nevertheless, it was revealed that learned letter-colour associations in non-synaesthetes follow principles of letter-colour binding in synaesthetes (i.e., they are automatic, arbitrary, of a perceptual nature, and show behavioural interference effects). In addition, we showed involvement of the prefrontal cortex (but not of parietal areas) in the acquisition of these synaesthesia-like associations, and we argue that this area may also be crucial for the development of (real) synaesthesia. Interestingly, the two mechanistic accounts of synaesthesia, the Disinhibition model (Grossenbacher & Lovelace, 2001) and the Cross-Activation model (Ramachandran & Hubbard, 2001a), have been associated with the "synaesthesia continuum" and the "discrete synaesthesia" hypotheses, respectively. The argument is grounded on the central difference between the two models: that the former reflects functional changes and the latter structural changes, and thus it is the former (Disinhibition) model that may explain a possible continuum of synaesthetic correspondences across the general population.

Irrespective of whether there exists a "synaesthesia continuum" or not, we conducted an MEG study in order to test these two mechanistic account of grapheme-colour synaesthesia. In addition, investigating the underlying brain mechanisms of (real) synaesthesia would help to further elucidate the similarities and/or differences between the mechanisms driving learned associations in adult trainees and real synaesthetic associations in synaesthetes (*note: MEG acquisition and analysis of data from adult trainees started but not completed yet for inclusion in this thesis). Establishing the when and where of the induced, synaesthetic percept in synaesthetes is a necessary step for further understanding how learned letter-colour associations by adult trainees may relate to real synaesthesia. Given the current lack of consensus regarding the mechanisms of grapheme-colour synaesthesia (i.e., Cross-Activation theory versus Disinhibited Feedback theory), we conducted an MEG study aimed primarily at extracting the temporal (and spatial) characteristics of the induced, synaesthetic percept in synaesthetes.

Using ICA for the blind extraction of the dominant patterns present in our MEG signal, we probed the resulting components for differences in the evoked fields of inducing versus non-inducing graphemes (via non-parametric cluster-

based permutation analyses). We provide evidence that the induced, synaesthetic percept most likely occurs after the initial, feed-forward sweep of activity in the visual processing stream, peaking between 180-210 ms and localizing to extrastriate visual cortex areas. This "late" timing rules out direct, anatomical connections between grapheme and colour processing areas. Together with information on the time course of grapheme-processing (i.e., occurring in their entirety <145 ms following onset, see (Rey et al., 2009)), it is unlikely that the cross-activation model be the best candidate for grapheme-colour synaesthesia (at least, in our sample of synaesthetes, who were all associator synaesthetes). Rather, our results more strongly support a model involving feedback or recurrent connections to extrastriate visual cortex (i.e., like the Disinhibited Feedback model, see (Grossenbacher & Lovelace, 2001); or Re-entrant Theory of Processing model, see (Smilek et al., 2001)). Since these models implicate functional differences (rather than structural connectivity differences), they imply that synaesthesia-learning may indeed be possible following even short training periods. This hypothesis needs further testing, for example by analysis of MEG data recorded from non-synaesthetic learners of letter-colour associations, or alternatively behavioural measures of letter-colour binding in synaesthetes following PPC-stimulation.

Outstanding Questions and Future Outlook

The case for "weak synaesthesia" has recently sparked a *separatist view* that claims that synaesthetic correspondences should not be equated to synaesthetic associations, and that they should be studied in their own right without an assimilation to real synaesthesia (Deroy & Spence, 2013). According to this view, synaesthetic correspondences are different from synaesthesia in that they are acquired, malleable, relative, and transient, and are normally observed across a large portion of the general population. Additionally, because they lack the conscious concurrent fundamental to synaesthesia, the authors claim that they should not even be compared to synaesthesia. While it is important to draw a distinction between canonical synaesthesia and synaesthesia-like cross-modal associations, we must bear in mind that synaesthesia itself may merely be an umbrella term for variations of similar phenomena with distinct phenotypes, underlying neural mechanisms, and genetic predispositions (Novich, Cheng, & Eagleman, 2011). Thus, understanding

the relationship of synaesthesia to the inherent, as well as acquired (or trained), associations in non-synaesthetes may prove to further understand the nature of the phenomenon. Additionally, the existence of synaesthetes with different kinds of concurrents (i.e., projector versus associators) calls into question what "conscious concurrent" should (or must) entail.

One related, outstanding question regarding the synaesthesia-like associations here trained in adult non-synaesthetes is whether they are truly perceptual, rather than conceptual, in nature. While the observed colour-opponency effects replicate the colour-opponency effects first observed by Nikolic et al. (2007) in grapheme-colour synaesthetes, and thus suggest that the colour pairs employed here were indeed opponent, a calibration of colour, as well as luminance, should be considered in future studies.

Regarding our tDCS study, the obtained results provide information on the areas implicated in the learning of synaesthesia-like associations (in the context of letter-colour binding). More specifically, they also suggest that dlPFC-stimulation enhances binding of multisensory letter-colour information. We are currently setting up a tDCS study to test whether dlPFC-stimulation during the learning of crossmodal associations may enhance memory in one sensory modality by strengthening the multisensory context.

Regarding our MEG study, one further step would be to examine the time-frequency domain in order to examine the role of oscillations in the induced, synaesthetic percept. Analysis of frequency bands may reveal differences in power between the inducing and non-inducing graphemes, or differences in connectivity (in different frequency bands) between the implicated brain areas. Examining the activity patterns associated with the induced, synaesthetic percept could help elucidate whether long-range interactions between distant brain areas are involved (i.e., as predicted by the Dininhibited Feedback model) (von Stein, Chiang, & Konig, 2000).

Finally, in order to help further reconcile the differences (or similarities) between grapheme-colour synaesthetes and adult trainees, we could examine the temporal (and spatial) characteristics of the learned associations (i.e., following training) using MEG. Similarly to the analyses of our MEG study (as well

as proposed future analyses for grapheme-colour synaesthetes), we could investigate whether the mechanisms involved in the perception of achromatic (or congruently versus incongruently coloured) letters resemble those observed in grapheme-colour synaesthetes, whether there is still involvement of prefrontal cortex once the associations have been learned, and/or whether there is transfer to parietal areas, and/or signs for feedback mechanisms.

Appendices

Synaesthesia Screening Questionnaire

(adapted from Banissy et al. (2009))

Take a look at the following questions to help determine whether you are a synaesthete. If so, you may be able to help us in better understanding how this extraordinary phenomenon works.

Please answer YES, NO, or SOMETIMES to the following statements:

1. I experience colours when I look at written numbers.
2. I experience colours when I look at written letters.
3. I experience colours when I look at written words.
4. I experience colours when I hear people say numbers.
5. I experience colours when I hear people say letters.
6. I experience colours when I hear people say words.
7. I experience colours when I hear people's voices.
8. Each number/ letter/ word has a specific colour.
9. I associate numbers to colours.
10. I associate letters to colours.
11. I associate words to colours.

8. I experience touch on my own body when I look at someone else being touched (i.e., I feel touch sensations on my own body when I observe them on another person's body).

9. I experience touch on my own body when I look at something else being touched (i.e., I feel touch sensations on my own body when I observe them on objects).

10. I experience touch in response to body postures.

11. Do these experiences have specific locations (i.e., on your body, on words or objects, in front of your eyes) or not (i.e., you just "know" or they feel as though they are in your "mind's eye")? Please describe.

12. Do you think about ANY of the following being arranged in a specific pattern in space (i.e., in a line, a circle, etc.)?

ALPHABET
CALENDAR YEAR
DAYS OF THE WEEK
WEEKS
TIME
NUMBERS (NUMBER LINE)

13. Do you think about numbers/ letters/ words as having personalities or genders?

14. Do you experience colours in response to:

SOUNDS
MUSIC
VOICES
TOUCH

Please match the triggers on the left with the experiences on the right IF you ever experience the two together. For example, if you experience colours in response to numbers, then write "Numbers – Colour" below, OR draw a line between them.

TRIGGERS	EXPERIENCES
Letters of the alphabet	Colours
Words	Shapes
Numbers	Touch
Days of the week	Taste
Months of the year	Smell
Pain	Sounds
Touch	Music
Body postures	Pain
Voices	
Music	
Sounds	
Colours	
Shapes	
Taste	
Smell	
Emotions	
Fingers	
Faces	
Places	
Other	

Minimum Norm Estimates

(Adapted from, revised by, and written in collaboration with Dr. Giorgos Michalareas, *Ernst Strüngmann Institute* (ESI) for Neuroscience in Cooperation with Max Planck Society, Frankfurt, Germany.)

The following subsections contain detailed information and specifics regarding the Minimum Norm approach used in this thesis. While they are not fundamental to the main goals of this thesis, they provide explanations of a novel approach used here in the source reconstruction analyses. These are placed here for the benefit (and interest) of the reader, as they cannot yet be found in the published literature.

Minimum Norm: Theory

The MEG sensor time series can be represented as $\mathbf{x}(t)$, the actual brain sources time series as $\mathbf{y}(t)$ and the estimated brain sources time series as $\hat{\mathbf{y}}(t)$. The inverse problem can be simply formulated as the estimation of a set of projection vectors, $\mathbf{W}$, which linearly transform the sensor time series into estimated brain source time series $\hat{\mathbf{y}}(t)$:

$$\hat{\mathbf{y}}(\mathbf{t}) = \mathbf{W} \cdot \mathbf{x}(\mathbf{t})$$

so that the difference between these estimated brain time series and the actual ones is minimised according to a chosen criterion.

According to the model of magnetic field propagation (called Forward Problem) from current sources inside the brain to the sensor locations, the sensor time series are related to the actual brain sources time series by:

$$\mathbf{x}(\mathbf{t}) = \mathbf{\Lambda} \cdot \mathbf{y}(\mathbf{t})$$

where $\mathbf{\Lambda}$, termed the Leadfield Matrix, is the solution of the free source orientation forward problem (Lin et al., 2004). As the actual brain sources time series are unknown, the core question of the inverse problem can be simply

stated as the derivation of modelled estimated brain source time series $\hat{\mathbf{y}}(t)$ so that they are as close as possible to the unknown actual ones. According to the forward model, these source estimates are directly transformed to modelled sensor measurements as:

$$\hat{\mathbf{x}}(t) = \Lambda \cdot \hat{y}(t).$$

So, the core inverse problem can be mathematically formulated, in the least-squares sense, as the minimisation of the square of the difference between the actual and the modelled sensor measurements.

$$\left\|(x - \hat{x})\right\|_2^2$$

or by substituting for $\hat{\mathbf{x}}(t)$

$$\left\|(x - \Lambda \cdot \hat{y}(t))\right\|_2^2$$

where $\| \ \|_2$ represent the L_2 norm.

If the noise profile in the MEG sensors is known, then the effect of this noise can be extracted from the above minimization problem by normalizing with the noise covariance. Mathematically, this leads to the minimization problem:

$$\left\|C^{-1/2}(x - \Lambda \cdot \hat{y})\right\|_2^2$$

where the matrix $\mathbf{C}$ denotes the MEG sensors' noise covariance matrix.

The above least squares problem is underestimated if the number of brain sources in $\hat{\mathbf{y}}(t)$ is much larger than the number of sensors in $\mathbf{x}(t)$, which is the case with MEG data (i.e., 248 sensors), when the entire cortical sheet or brain volume is used as the source space. Without any other constraining criteria, there is an infinite set of solutions $\hat{\mathbf{y}}(t)$ that can lead to minimization of the above criterion.

In order to constrain this infinite set of solutions, various additional constraints can be used. One of the most common such criteria is that the resulting estimated brain source activity time series should have the least possible power. This is physically translated into the assumption that the brain uses the least possible power in order to produce the magnetic field time series measured by the MEG sensor array. As the power is the square of the norm of the time series, this approach is termed Minimum Norm solution.

This is formulated mathematically as the composite minimization criterion:

$$\left\| \boldsymbol{C}^{-1/2}(\boldsymbol{x} - \Lambda \cdot \hat{\boldsymbol{y}}) \right\|_2^2 + \left\| \hat{\boldsymbol{y}} \right\|_2^2$$

The inverse solution to the above minimization problem is (Lin et al., 2004):

$$\mathbf{W} = \hat{\boldsymbol{R}} \cdot \Lambda^T \cdot (\Lambda \cdot \hat{\boldsymbol{R}} \cdot \Lambda^T + \boldsymbol{C})^{-1}$$

Here $\hat{\boldsymbol{R}}$ is the covariance of the estimated brain source time series. It is evident that in order to derive the above solution, an a-priori assumption about the covariance of the brain source time-series to be estimated is necessary, termed $\mathbf{R}_a$. As such an assumption might not reflect the variance of the actual brain sources, a regularisation parameter is typically used in order to be able to adjust this a-priori assumption. This is performed by representing the brain source time-series covariance $\hat{\mathbf{R}}$ as a scaled version of the a-priori assumed source time-series covariance $\boldsymbol{R}_a$ as:

$$\hat{\boldsymbol{R}} = \frac{\boldsymbol{R}_a}{\lambda^2}$$

By replacement, the minimum norm solution becomes:

$$\mathbf{W} = \boldsymbol{R}_a \cdot \Lambda^T \cdot (\Lambda \cdot \boldsymbol{R}_a \cdot \Lambda^T + \lambda^2 \cdot \boldsymbol{C})^{-1}$$

In this solution the unknown current amplitude variance is interpreted in terms of the regularization parameter λ^2. The selection of the regularisation parameter is typically based on the data characteristics. A typical computation is :

$$\lambda^2 = \frac{trace(A \cdot R \cdot A)}{trace(C) \cdot SNR^2}$$

where SNR is the signal-to-noise ratio at the sensor level. This ratio can be interpreted as the ratio of the power of the a priori assumed current distribution in the brain projected to sensor space, divided by the expected level of power of the actual brain signals measured by the sensors. This latter expected level is defined as the power level of the noise, multiplied by the assumed Signal-to-Noise Ratio.

From the above equations, it is evident that the Minimum Norm inverse solution does not use the measured sensor data. It uses the forward model, a priori assumed current density distribution in the brain, the estimated noise profile at the sensor level. Thus, this inverse solution is model-driven.

As the data covariance is not used for this type of solution, Minimum Norm is suitable for projecting sensor data representing very few mixed sources, in which case the data covariance matrix is rank-deficient. In such cases, all sensor time series are highly co-linear as they are weighted versions of the same few components.

Minimum Norm: Practice

As seen above, in "Minimum Norm: Theory," the weighted Minimum Norm Least Squares solution is computed according to:

$$\mathbf{W} = R_a \cdot \Lambda^T \cdot (\Lambda \cdot R_a \cdot \Lambda^T + \lambda^2 \cdot C)^{-1}$$

where

Λ : Leadfield matrix
R_a : a priori assumed brain source covariance
C : noise covariance in MEG sensor array
λ : Minimum Norm regularisation parameter

In our work, we followed a novel approach for the computation of the regularization parameter for each IC, according to the following equation:

$$\lambda = 0.5 + \frac{\sqrt{\boldsymbol{trace}(\Lambda \cdot \boldsymbol{R}_{\boldsymbol{scaled}} \cdot \Lambda)}}{\sqrt{\boldsymbol{trace}(\boldsymbol{C})} \cdot \boldsymbol{pseudoSNR}}$$

where $\boldsymbol{R}_{\boldsymbol{scaled}}$ is the a priori assumed brain source covariance matrix scaled as : $\boldsymbol{R}_{\boldsymbol{scaled}} = \boldsymbol{R}_{\boldsymbol{a}} \cdot \frac{\boldsymbol{trace}(\boldsymbol{C})}{\boldsymbol{trace}(\Lambda \cdot \boldsymbol{R}_{\boldsymbol{a}} \cdot \Lambda)}$.

so that

$$\frac{\boldsymbol{trace}(\Lambda \cdot \boldsymbol{R}_{\boldsymbol{scaled}} \cdot \Lambda)}{\boldsymbol{trace}(\boldsymbol{C})} = 1$$

Consequently, the regularization parameter formula is reduced to:

$$\lambda = 0.5 + \frac{1}{\boldsymbol{pseudoSNR}}$$

and the inverse solution becomes:

$$\mathbf{W} = \boldsymbol{R}_{\boldsymbol{scaled}} \cdot \Lambda^{T} \cdot (\Lambda \cdot \boldsymbol{R}_{\boldsymbol{scaled}} \cdot \Lambda^{T} + \lambda^{2} \cdot \boldsymbol{C})^{-1}$$

where $\boldsymbol{pseudoSNR}$ is a scalar parameter used to represent a pseudo Signal-to-Noise ratio for a single Independent Component.

As the noise power within a single IC component is unknown, here we chose to derive an empirical measure of how well an ICA is representing a few strong focal brain sources or widely distributed noise. For an ICA representing a strong focal brain dipole, the squared ICA unmixing weights have a skewed distribution, with high values at the sensors close to the underlying sources, and all the rest of the sensors (further from the underlying sources) having much lower values. In the case of an ICA component capturing widely distributed noise, the squared ICA unmixing weights have more comparable values. Consequently, the upper, i.e.,70 %, and lower, i.e. 30 %, distribution percentiles are expected to be more distant in the case of a brain activity ICA component than in the case of a noise ICA component.

This parameterization has been used in order to estimate a pseudo Signal-to-Noise Ratio for a single ICA component. If the squared unmixing ICA coefficients for a single ICA are represented by $Uica^2$, then the $pseudoSNR$ is computed as:

$$pseudoSNR = \sqrt{\frac{prctile(Uica^2,70\%) - prctile(Uica^2,30\%)}{prctile(Uica^2,30\%)}}$$

This parameter has a lower bound of 0. The higher the distance between the percentiles, the higher the value of this parameter. The closer the upper and lower percentiles get, the closer this parameter is to this lower bound.

From the formula for the regularisation parameter, the latter term $1/pseudoSNR$ varies in an inverse fashion, from 0 to high positive values. This means that for independent components representing strong dipolar sources, little regularization is used, as the unmixing matrix contains a clear dipole representation. For independent components representing noise, a higher regularization is used as the unmixing matrix represents a more complex and distributed pattern.

Having very small regularisation values close to 0 for very strong dipoles can lead to instability in the derivation of the inverse solution. In order to avoid such instabilities, a scalar value of 0.5 has been added to $1/pseudoSNR$ in the derivation of the regularization parameter. This value represents the $1/pseudoSNR$ ratio when the difference ratio between the upper and lower percentiles under the square root in $pseudoSNR$ is equal to 4.

With this final formulation, the regularisation parameter varies between 0.5 (for ICs representing strong brain sources) and infinity (for ICs representing noise). Infinity here just represents very high values. This is because in ICA unmixing matrices, the 30 % and 70 % percentiles cannot have the exact same values, as this would require that all the in between weights in the distribution should be identical.

The above described regularisation parameter has a lower bound, which hedges against instabilities of the inverse solution, and no upper bound, which allows for high regularisation when ICA components representing noise are localised.

The above described inverse solution procedure was applied to each of the ICA components, for which a significant statistical difference was found in the comparison between the compared conditions, both for synaesthetes and controls. No a priori brain sources covariance was assumed, so $\mathbf{R}_{\alpha}$ was the identity matrix with dimensions Nsources xNsources. As the level of noise in the single ICA components was also unknown, the noise covariance matrix $\mathbf{C}$ was the identity matrix as well, with dimensions Nsensors x Nsensors. The source localization was performed and plotted on the 3-dimentional template grid with 6mm resolution, warped to each subject's brain volume.

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