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Increased structural connectivity in grapheme-color synesthesia

Romke Rouw & H Steven Scholte

Diffusion tensor imaging allowed us to validate for the first time the hypothesis that hyperconnectivity causes the added sensations in synesthesia. Grapheme-color synesthetes ($n = 18$), who experience specific colors with particular letters or numbers (for example, 'R is sky blue'), showed greater anisotropic diffusion compared with matched controls. Greater anisotropic diffusion indicates more coherent white matter. Anisotropy furthermore differentiated subtypes of grapheme-color synesthesia. Greater connectivity in the inferior temporal cortex was particularly strong for synesthetes who see synesthetic color in the outside world ('projectors') as compared with synesthetes who see the color in their 'mind's eye' only ('associators'). In contrast, greater connectivity (as compared with non-synesthetes) in the superior parietal or frontal cortex did not differentiate between subtypes of synesthesia. In conclusion, we found evidence that increased structural connectivity is associated with the presence of grapheme-color synesthesia, and has a role in the subjective nature of synesthetic color experience.

Synesthesia is a condition in which certain stimuli (for example, hearing particular music) trigger an unusual additional sensory experience (for example, seeing metallic green). The associations are automatic in the sense that they are fast and seemingly effortless; highly consistent, as the same associations persist from early childhood; and specific, as a particular stimulus elicits a highly specific synesthetic sensation. Synesthesia most often results from a developmental (possibly genetic[1]) predisposition. A common and well studied type of synesthesia is grapheme-color synesthesia[2], in which specific graphemes elicit particular colors (for example, "the letter R is sky blue").

Different accounts of the neurobiological mechanisms of synesthesia coexist, diverging mainly on two fundamental issues[3]. The first is whether unusual structural connectivity in the brain sets synesthetes apart from non-synesthetes. Some models presume that synesthetes have excess anatomical connections, owing to a failure to prune connections that were present at birth[4,5]. This issue is as yet unclear, mainly because up to this point structural connectivity in synesthetes as compared with non-synesthetes has not been measured. The second issue is that although it is clear that some explanation must be provided for the coactivation of an induced or synesthetic sensation as a result of a concurrent stimulus, theories differ on the functional (timing) and neurological site of this process. One proposal is that crossactivation occurs between relevant perceptual areas, such as grapheme and color areas adjacently located in the fusiform gyrus[4,6,7]. Alternatively it has been proposed that anomalous processing in the temporal cortex occurs when abnormal feedback from processing graphemic meaning in the anterior fusiform gyrus travels back to posterior inferior temporal regions and area V4 (ref. 8). Feedback can also flow back from a multisensory nexus such as the superior temporal sulcus, posterior parietal lobe, intraparietal cortex or temporo-parietal-occipital junction[9–11]. Models proposing activation flowing back from a multisensory nexus tend to be couched in terms of disinhibited feedback[9], whereas models involving feedback from areas involved in the processing of graphemic meaning tends to be formulated in terms of reentrant processing[8], and local cross-connection theory assumes anomalous structural connectivity[3].

The current study aimed to provide insight into these issues by examining structural connectivity in synesthetes as compared with non-synesthetes. We employed diffusion tensor imaging[12,13] (DTI), wherein a magnetic resonance signal measures the diffusion properties of water molecules. DTI is of particular value for addressing these issues, as it allows the measurement of microscopic properties of cerebral white matter *in vivo*. More, or more coherent, white matter structures will lead to increased anisotropic (directionally dependent) diffusion. We also employed functional magnetic resonance imaging (fMRI), to measure brain activation in synesthetes while they viewed graphemes that elicited a synesthetic color experience, and we compared this activity to that of non-synesthetes presented with the same graphemes. This allowed us to compare in a single group of subjects the location of brain activation in response to the experience of synesthetic color with the location of increased structural connectivity in synesthetes as compared with non-synesthetes. Recently, a sharp increase in neuroimaging studies has provided data on the neural basis of grapheme-color synesthesia[14]; however, the results do not provide a coherent picture. Although many factors may underlie these inconsistencies, an important one to take into account is the presence of individual differences between synesthetes[6,9,15]. We therefore presented participants with a questionnaire that allowed us to

Department of Psychology, University of Amsterdam, Roetersstraat 15, 1018WB Amsterdam, The Netherlands. Correspondence should be addressed to R.R. (R.Rouw@uva.nl).

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Table 1 Location of increased anisotropy in synesthetes as compared with non-synesthetes

	Cluster size (mm^3)	MNI coordinates (mm)			Maximum effect size
		x	*y*	*z*	
Superior frontal left	53	–20	–25	55	3.7
Superior frontal right	100	21	–21	57	4.4
Parietal left	44	–17	–61	55	4.8
Temporal right	67	36	–40	–21	4.8

MNI coordinates are those of the gravity point of each cluster; the maximum effect size is the maximum value of the permutation test. Thresholding $t > 3$, minimum cluster size 40 mm^3.

differentiate between 'associators' and 'projectors'[16]. Although both experience colors when presented with graphemes, projectors experience the synesthetic color in the outside world, whereas associators experience the synesthetic color in their mind only.

We were particularly interested in structural connectivity in the inferior temporal cortex in synesthetes as compared with non-synesthetes. This brain area is crucial for recognition of visual categories[17–19], which includes categorizing certain shapes such as the visual shape of a word[20]. Some models[4,6,7] regard this region as crucial to grapheme-color synesthesia, as this 'word form' area lies adjacent to a brain region that responds preferentially to color perception[21]. The current study examined whether differences in structural connectivity in this brain area were correlated with the presence of grapheme-color synesthesia. Furthermore, we examined whether structural connectivity in this region influenced the nature of synesthetic color experience. This was tested by comparing 'projector' with 'associator' synesthetes.

Results showed clusters of greater anisotropic values in synesthetes as compared with non-synesthetes. Thus we have demonstrated for the first time that the brains of synesthetes, as compared with non-synesthetes, have abnormal structural connectivity. Furthermore, such increased structural connectivity was found both in 'lower level' (visual) areas and in 'higher level' brain areas. We found clusters of increased connectivity in inferior temporal, as well as parietal and frontal, cortex. Notably, increased connectivity in inferior temporal cortex was involved not only in the existence but also in the type of synesthetic color experiences elicited by graphemes. 'Projector' synesthetes showed a stronger increase in anisotropy in this cluster as compared with 'associator' synesthetes. The other clusters of greater anisotropy (in synesthetes as compared with non-synesthetes) showed no relation between strength of anisotropy and subtype of grapheme-color synesthesia. These findings indicate that increased structural connectivity in brain areas involved in perceptual categorization (inferior temporal cortex) may influence the nature of synesthetic experiences.

RESULTS

Increased structural connectivity in synesthesia

For each subject we calculated in each voxel the mean fractional anisotropy (FA) value, which quantifies how strongly directional the local tract structure is. All subjects' FA data were projected onto the most typical FA tract skeleton of all subjects[22], before applying voxel-wise cross-subject statistics (permutation testing[23]). Synesthetes showed higher FA values as compared with control subjects in four clusters (**Table 1**). There were no significantly lower FA values in the synesthetic group when compared with the control group.

A bilateral cluster of higher FA was found in a white matter tract beneath the central sulcus. Synesthetes furthermore showed higher FA values, as compared with controls, in the left superior parietal cortex and the right inferior temporal cortex (**Fig. 1**; see **Supplementary Fig. 1** online for the directions at these clusters in synesthetes and non-synesthetes). The cluster of greater FA values in the inferior temporal cortex was located in the white matter next to the fusiform gyrus. An analysis of variance of the three angles of the first vector (that is, main direction) at each cluster showed only one marginally significant difference ($F_{1,34} = 3.58$, $P = 0.067$) between synesthetes and non-synesthetes at the parietal DTI cluster in the first vector. In line with this finding, tractography from each of these four clusters showed no substantial differences in the general direction of pathways of the white matter tracts between synesthetes and non-synesthetes (**Supplementary Fig. 2** online). Tractography showed that the area of greater FA in the right temporal lobe was part of a network of local corticocortical association fibers in the inferior temporal lobe. These connections were in the area of, but not the same projections as, the inferior longitudinal fasciculus (ILF). The ILF runs between occipital areas (such as V2 and V4) and the temporal lobe[24]. Tractography furthermore showed that strongest connectivity for the cluster of greater FA in the parietal lobe is located in the same brain region, in superior parietal cortex. In contrast, the two clusters in the frontal lobe were part of tracts projecting medially toward the corpus callosum (**Supplementary Fig. 2**).

Brain activation during synesthetic experiences

Participants viewed graphemes that induced strong synesthetic colors, graphemes that induced weak synesthetic colors and graphemes that did not induce synesthetic colors. For stimuli that induced (weak or strong) synesthetic colors as compared with those that did not, we found differences in brain activation, as measured by fMRI blood oxygenation level–dependent (BOLD) contrast, between synesthetes and non-synesthetes. We did not find greater activation in response to synesthetic color–evoking stimuli in any brain region in controls relative to synesthetes. In synesthetes relative to controls, however, we

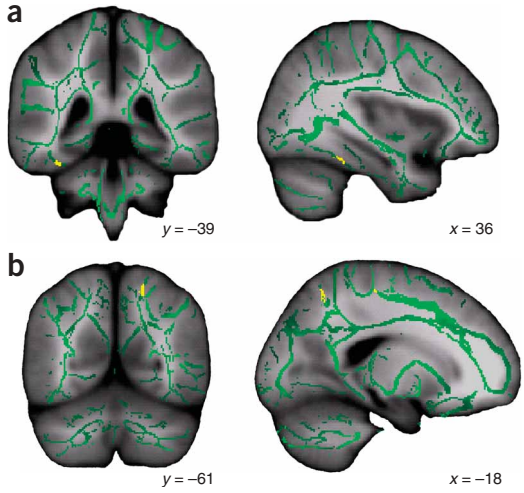

Figure 1 Increased anisotropy in synesthetes as compared with non-synesthetes. On an MNI brain (grayscale) the white matter skeleton is projected (green), as well as the location of higher FA values in synesthetes as compared with non-synesthetes (yellow). (**a**) Higher FA value in the right inferior temporal cortex. (**b**) Higher FA value in the left parietal cortex and (bilateral) frontal cortex (only left hemisphere shown; see also **Supplementary Fig. 1**).

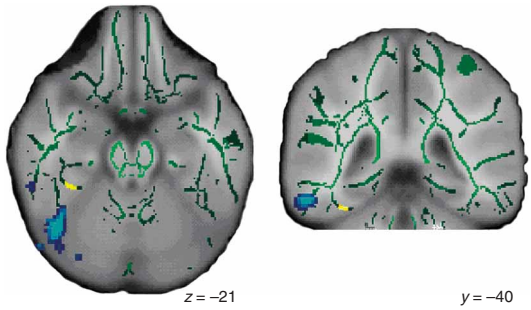

Figure 2 Increased brain activation and increased anisotropy in the inferior temporal cortex in grapheme-color synesthetes. The white matter skeleton (green) is projected on an MNI brain (grayscale). Greater BOLD signal (blue) as well as greater anisotropy (yellow) underlie synesthetic experiences in color-grapheme synesthesia. BOLD data were transformed on the basis of a nonlinear transformation from MNI to tracts-based space (the transformation used in DTI analysis) to allow comparison of DTI and fMRI results. Transversal and coronal views.

found greater activation in the left frontal cortex (center of gravity –44 (*x*), 25 (*y*), 28 (*z*); z_{max} 3.58, cluster size 2,944 mm^3), right cerebellum (43, –69, –24; z_{max} 3.98, 984 mm^3), an inferior region in the right middle temporal gyrus (57, –47, –15; z_{max} 3.8, 864 mm^3) posteriorly located in the right temporal region, in the fusiform gyrus (48, –68, –16; z_{max} 3.5, 1,136 mm^3). All coordinates are given in MNI[25] (Montreal Neurological Institute) coordinates. Thus, both increased BOLD signal in response to synesthetic color experience and a structural difference (higher FA values) between synesthetes and non-synesthetes was found in inferior temporal cortex (**Fig. 2**).

Projector versus associator synesthetes

We next examined whether individual differences in synesthetic experience could be explained by individual differences in properties of the white matter structure. Specifically, we hypothesized that white matter structures in the temporal lobe would have an important role in determining the nature of synesthetic color experience.

Synesthetes indicated their personal experiences on 'projector' type questions and 'associator' type questions (see Methods). One question of the 'projector' type showed low correlation ($r(17) = 0.04$) with the total projector score and was removed from further analysis. The remaining associator or projector questions correlated ($r(17) = 0.27$ to $r(17) = 0.90$) with their total score. The total PA (projector associator) score, defined as mean associator score minus mean projector score, ranged from –3.1 to 3.8. The mean PA score was consistent with answers on open-ended questions; the score thus showed large individual differences in the degree to which synesthetes experience the synesthetic color in the outside world or only in the 'mind's eye'.

We examined the role of the cluster in the inferior temporal cortex of greater FA in synesthetes as compared with controls (**Fig. 3a**). Looking at individual differences between synesthetes, we found that a tendency to 'see' synesthetic color in the outside world (projector) was related to the strongest increase in FA values in the cluster in the right temporal cortex (nonparametric correlation $r_s(17) = 0.548$, $P = 0.009$). No such relation was found between PA scores and the FA value in the cluster in the left parietal cortex $r_s(17) = –0.098$, $P = 0.349$ (**Fig. 3b**). The PA score also did not correlate with FA values in the clusters in the left ($r_s(17) = 0.298$, $P = 0.115$) and right ($r_s(17) = 0.321$, $P = 0.097$) frontal cortex.

Individual differences between synesthetes, as measured in PA score, did not correlate with strength of BOLD signal in any of the regions responding to synesthetic color. (Correlations between BOLD signal in these regions, FA values in the four clusters and PA score are presented in **Supplementary Table 1** online). Of course, a negative finding is not necessarily informative. Increasing statistical power might result in significant correlations. Individual differences in BOLD signal might also be concealed by intersubject variance in size and exact location of the brain area responding to synesthetic color.

Other studies have reported a correlation between subtypes of grapheme-color synesthesia and fMRI responses in early visual areas[6]. Certainly intersubject variability can be found in early visual areas[26]. Perhaps individual differences are more easily obtained in early visual areas—for example, if the two groups are more easily compared because the functional boundaries are more coherent between individual subjects in early visual areas than in higher visual areas.

In our data, DTI measurements were more sensitive to subtypes of grapheme-color synesthesia than fMRI measurements. It is possible that FA provides a better predictor of projector versus associator subtype than BOLD signal. Notably, both the questionnaire and DTI reflect structural and hence stationary individual differences, whereas the BOLD signal is variable and influenced by experimental settings.

As an explorative study, we tested the (nonparametric) correlation between FA value in the right temporal cluster and BOLD signal in the region responding to synesthetic color in the temporal region (the most anterior cluster in temporal cortex). We examined in this test each of the three fMRI conditions separately and found significant correlations for synesthetes, but not for non-synesthetes, between FA value and 'strong' and 'weak' synesthetic color condition (**Supplementary Table 2** online). This indicates a relation between the higher FA values near the fusiform gyrus and activation in the same brain region (though not in the same gyrus) in temporal cortex.

The exact mechanism underlying this influence is yet unknown. We performed symmetric fiber tracking between the BOLD activation in

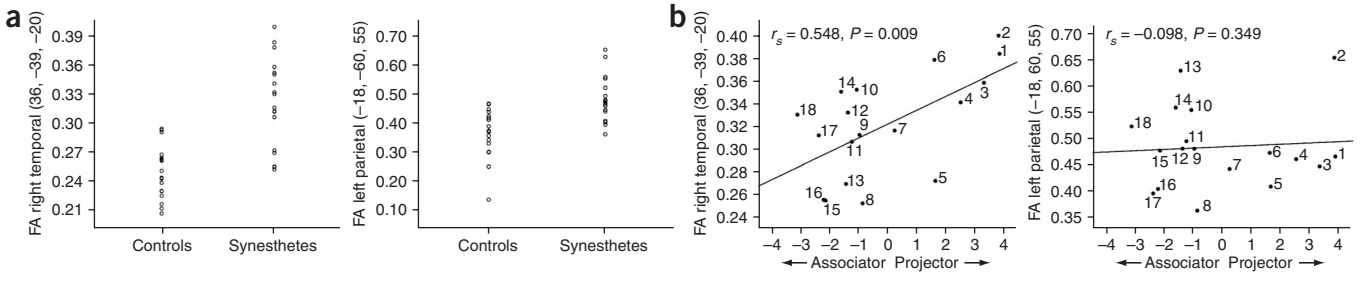

Figure 3 Anisotropy differentiates subtypes of grapheme-color synesthesia. (**a**) A cluster of greater anisotropy in synesthetes as compared with non-synesthetes in the right temporal cortex. As a comparison, a similar cluster of greater anisotropy found in the superior parietal cortex is depicted. (**b**) Projector subtype of grapheme-color synesthesia has a stronger increase in FA values in the right temporal cortex than associative subtype ($r_s(18) = 0.548$, $P = 0.009$). No difference between subtypes is present in the left parietal cortex ($r_s(18) = –0.098$, $P = 0.349$).

the temporal cortex and the cluster of greater FA in the temporal cortex. Results did not yield connectivity in 29 of the 36 subjects (15 synesthetes and 14 controls). This indicates that the relation between greater FA and BOLD signal is not through a direct structural connection. These results are not unexpected, as they show that functional connectivity does not necessarily imply direct structural connectivity from white matter to gray matter. Further research is needed to shed light on the exact relationship between structural factors (as measured with DTI) and functional factors (as measured with fMRI) in synesthesia.

DISCUSSION

Using diffusion tensor imaging, we have shown for the first time that the extraordinary sensory experiences in synesthesia are associated with abnormalities in white matter structure. Grapheme-color synesthetes have, compared with non-synesthetes, greater diffusion anisotropy at various locations in the brain. Increased diffusion anisotropy reflects increased or more coherent connectivity due to microstructural aspects such as degree of myelination, axonal diameter, and density and coherence in fiber orientation[13] as well as macrostructural features such as intravoxel fiber-tract coherence. In a coherent fiber pathway increased diffusion anisotropy is likely to be due to presence and strength of connections (such as density of axons and myelination), whereas in a crossing fiber population it is more likely to be due to directional coherence.

Although a variety of white matter properties can therefore have a role in our finding of higher FA values in synesthetes, we found no significant differences between synesthetes and non-synesthetes in main directions or pathways of the white matter tracts at these four locations. Thus, microstructural properties of the white matter or more local coherence of directions of white matter tracts seem more likely candidates for the structural differences between synesthetes and non-synesthetes than does a general difference in the main direction of the pathways of the white matter tracts. Our finding of clusters of higher FA values in synesthetes as compared with non synesthetes can therefore be viewed as increased strength of connectivity at those particular locations. Further study would be needed to decipher exactly which factors underlie the differences in diffusion anisotropy between synesthetes and non-synesthetes.

The present finding directly shows that synesthesia is related to an increase in structural connectivity. Of course, increased connectivity in synesthetes does not rule out the possibility that the functional aspects of relevant brain areas (for example, a failure of inhibition) are different in synesthetes as compared with controls[8,9].

The changes in white matter structures of synesthetes could be due to a genetic predisposition, such as decreased pruning of connections[4,5]. The present results show that if synesthesia is indeed caused by decreased pruning, the resulting excess connectivity can have very different specific consequences. Of particular interest is the cluster of greater connectivity found in inferior temporal cortex near the fusiform gyrus. The fusiform gyrus is involved in the perception and categorization of visual stimuli[17–19]. Furthermore, an area specialized in the perception of the (form of) graphemes[20] in the fusiform gyrus is located adjacent to an area specialized in the perception of colors[21]. This has spurred the idea that grapheme-color synesthesia is caused by cross-connections between adjacently located grapheme and color areas in the fusiform gyrus[4,6,7].

In line with this theory, we found greater connectivity in synesthetes than in non-synesthetes near the fusiform gyrus. Furthermore, the strength of structural connectivity (as measured in higher FA values) correlates with brain activation in one cluster in temporal cortex responding to graphemes that elicit synesthetic color in synesthetes. More research would be needed to map the exact pathways involved in synesthesia, and the exact relationship between structural connectivity and brain activation. The neural pathways involved might be more complicated than a single cross-connection between a word and a color area. We found greater BOLD signal in response to synesthetic color experience at a number of locations in the inferior temporal cortex. This is in line with previous findings[11,27,28] of increased brain activation at various locations in the inferior temporal cortex. Furthermore, we found greater structural connectivity in white matter adjacent to the fusiform gyrus. As we examined major white matter tracts only, we could not examine possible differences in connectivity within the fusiform gyrus. However, it is now clear that white matter connectivity underlies synesthetic experience. The additional color sensations characteristic of grapheme-color synesthetes are at least partially based in cortical-to-cortical connections between category-specific (perceptual) areas in the ventral visual pathway, specifically in the inferior temporal cortex.

So far, we have been discussing the cluster of greater connectivity in the inferior temporal cortex as the explanation of how a sensation (seeing a grapheme) can involuntarily trigger an additional synesthetic (color) experience. We furthermore propose that this cluster of greater connectivity has a role in individual differences in the nature of synesthetic color experiences. More brain areas, such as early visual areas[6], might however be involved in this distinction. In the present study, we found that grapheme-color synesthetes with the strongest increased diffusion anisotropy at this cluster in the inferior temporal cortex report 'seeing' the synesthetic color in the outside world, as if it were projected on or near the grapheme ('projector' subtype), rather than experiencing the synesthetic color in their mind only ('associator' subtype[16]). That is, hyperconnectivity in this brain area, implicated in categorizing shape and color experiences, leads to synesthetic color experiences more similar to 'real' color experiences. Thus, greater structural connectivity in the inferior temporal cortex relates to both the existence and the (subjective) nature of synesthetic experience.

The greater connectivity in the parietal or frontal cortex of the synesthete brain has a different role in synesthesia than the greater connectivity in the inferior temporal cortex. Although we found greater connectivity in the former brain areas, there was no difference between the projector and associator subtypes. Brain areas where information from different modalities converges, particularly the parietal lobe, have been proposed to underlie anomalous binding of sensations in color-grapheme synaesthesia[10,11]. This hypothesis is based on the implication of the parietal cortex in feature binding in normal perception[29], and on findings indicating the role of associative brain areas (left intraparietal sulcus[11], superior parietal lobe bilaterally[30] and left angular gyrus[27]) in grapheme-color synesthesia. Inhibition of the right posterior parietal lobe with repetitive transcranial magnetic stimulation attenuates interference of synesthetic color on the naming of real color of a grapheme[10]. The authors furthermore suggest that projectors might show greater parietal involvement, as in this case the synesthetic percept is tightly bound spatially to the inducing grapheme. An alternative, however, is that associators still bind a color to a grapheme but that the spatial reference frame evoked differs between these synesthetic subtypes[31]. We found that greater anisotropy in the parietal cortex did not differentiate projector from associator grapheme-color synesthetes. Although greater connectivity in the parietal cortex is likely to have a role in anomalous binding of sensations in grapheme-color synesthesia, this connectivity does not seem to underlie this differential nature of synesthetic color experience.

These findings are interesting in the light of the discussion of how dorsal prefrontal and parietal areas might contribute to conscious visual experience (for example, refs. 32–34). Parietal and frontal regions, which have been implicated in the control of spatial attention, also

show activity that reflects perceptual transitions, such as fluctuations in awareness during binocular rivalry[35,36] or perceptual transitions that occur while viewing bistable figures such as Rubin's face-vase figure[37]. Research on synesthesia might shed further light on this issue, as the particular neurological mechanisms of synesthetes produce a conscious sensory experience in the absence of that particular sensory stimulus. The present results show that increased connectivity in parietal and frontal areas is related to synesthetic experience. Therefore, research on synesthesia might provide insight into the role of fronto-parietal connectivity in the generation of (synesthetic) conscious experience.

Greater diffusion anisotropy in the inferior temporal cortex was found in the right hemisphere only, whereas greater anisotropy in the parietal cortex was found in the left hemisphere only. These results seem to be evidence for lateralization effects. However, although a difference in diffusion anisotropy reflects structural differences between the two groups of subjects at this location in the brain, a negative finding does not guarantee that no differences exist between the two groups. For example, insufficient coherence between individual synesthetes in the exact location of increased connectivity could obscure differences between the synesthetes and non-synesthetes. Another reason to be cautious in interpreting lateralization results is that previous research does not provide a coherent picture; for example, there are conflicting results showing left lateralized fMRI activations in V4 (ref. 27) or no evidence of lateralization[6,28] in response to seeing synesthetic color.

The clusters of greater connectivity in the brains of synesthetes as compared with non-synesthetes indicate that structural characteristics of the brain have a role in grapheme-color synesthesia. We found greater structural connectivity in synesthetes as compared with non-synesthetes. Furthermore, in grapheme-color synesthetes, increased structural connectivity in inferior temporal cortex was related to the perceptual nature of the sensory experience. How, in the brain, an initial perceptual stimulation results in a sensory experience has so far been mainly studied in terms of different patterns of brain activation (firing of neurons). Current results show that variations in structural connectivity are also directly involved in the nature of sensory experiences.

METHODS

Subjects. Subjects were 18 women with synesthesia (mean age = 28.7 years; range 18–43 years) and 18 controls matched for age, sex and level of education. Subjects were invited to participate in the study based on the consistency of their synesthetic grapheme-color reports: over 90% consistency on an unexpected retest which took place at least three weeks after the initial test. No subject had a history of neurological or psychiatric disease or was taking psychoactive drugs. All subjects gave informed consent.

Associators versus projectors. Each synesthete filled in a questionnaire with 24 five-point Likert scale questions and 9 open questions. For each subject, a PA score was calculated by subtracting the mean score on six 'associator' questions (for example, "When I look at a particular letter/digit, the color appears only in my mind and not somewhere outside my head such as on the paper") from the mean score on six 'projector' questions (for example, "the color seems to be projected on the letter/digit"). Overall, the PA scores had a mean of –0.08 but ranged from –3.1 to 3.8 (maximum range –4 to 4).

Procedure. We used a 3-T scanner (Philips) to acquire magnetic resonance images. DTI and fMRI (grapheme and color task) measurements were acquired in a single scanning session, together with a three-dimensional T1 anatomical scan. Total scanning time was about 50 min. We immobilized the subject's head using foam pads to reduce motion artifacts and used earplugs to moderate scanner noise.

Structural connectivity: DTI. We calculated fractional anisotropy, which reflects the degree of diffusion anisotropy within a voxel, on the basis of the acquisition of diffusion-weighted spin-echo echo-planar imaging (EPI) measurements (TR 7,720 ms, TE 94 ms, flip angle 90°, FOV 224 × 224 mm, matrix size 128 × 128, 40 slices, b0 = 1,000 s mm^{-2}, 94 ms. The degree of diffusion anisotropy in a voxel is determined by microstructural features of the tissue in that particular voxel, including fiber properties (for example, fiber diameter and density) and fiber tract coherence. Diffusion was measured four different times in 32 noncollinear directions. The start of each series of directions was preceded by acquisition of a non–diffusion-weighted volume for purposes of registration for motion correction. Total acquisition time was 9.8 min. Data analysis and preprocessing were performed with FSL[38]. Correction for eddy currents and head movement was done by means of affine registration on a reference volume. Using FLIRT (FMRIB's linear image registration tool), DTI images were registered to the structural images through a single-shot spin-echo, echo-planar image without diffusion weighing. FA was calculated for each voxel with FDT[39]. A larger FA value indicates a distortion of brownian motion, which signifies the presence of coherent white matter tracts. Values closer to 0 correspond to more isotropic diffusion of water molecules (showing brownian motion), and absence or little coherence of white matter. Voxelwise statistical analysis of the FA data was carried out using TBSS (tract-based spatial statistics, part of FSL)[22]. FA images were created by fitting the diffusion tensor to the raw diffusion data using FDT after brain-extraction using BET[40]. All subjects' FA data were then aligned into a common space using the nonlinear registration IRTK[41]. The mean FA image was then created and thinned to create a mean FA skeleton representing the centers of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton. The significance of the comparison between synesthetes and non-synesthetes was determined by means of permutation testing. We considered activation significant at a *t*-value higher than 3, with a minimum cluster size of 40 mm^3.

We calculated group maps of the primary directions of the white matter skeleton by performing the transformations of each of the three motion vectors of the primary direction to the tract-based space per subject (with TBSS) and recombining and averaging these after the transformation. For each of the areas where the synesthetes showed a significant FA difference from controls, we tested whether the groups differed significantly from each other in direction, by extracting per subject the average direction per vector in an area and testing this between groups.

We also performed tractography with FDT[39] by first running Markov-chain Monte Carlo sampling to build up distributions on diffusion parameters at each voxel in the individual's subject space. Then we transformed the clusters having higher FA values and the anterior cluster of BOLD activation to synesthetic color in temporal cortex to the space in which the DTI data were acquired per subject. Next, we sampled repetitively (per subject), from the seed masks of the DTI clusters, the distribution on voxelwise principal diffusion directions to generate a probabilistic sample from the distribution. By taking many such samples it was possible to build up the *a posteriori* distribution of the connectivity distribution from that seed.

To compare the resulting tracts between subject groups we transformed each of the individual tracts to MNI space and generated an 'average' distribution map. In each subject, we set a fixed threshold to determine the presence (1) or absence (0) of a tract at each voxel. We then added the 1s and 0s at each voxel from all subjects, but for synesthetes and non-synesthetes separately. In the resulting map a value of 18 indicated a voxel in which each of the subjects from that group had positive results from fiber tracking for that location from the current seed. The maximum overlap in **Supplementary Figure 2** is 17 because there was not a perfect overlap between the registrations from individual space to standard space in the seed locations.

We also performed symmetric fiber tracking between the temporal BOLD and the temporal DTI cluster, in which we kept only the fibers that ran from the BOLD cluster to the DTI cluster and also from the DTI cluster to the BOLD cluster.

Brain activity: fMRI. Subjects performed a grapheme task. Stimuli were projected on a screen at the front end of the scanner table. The subject viewed them in a mirror placed above her head and recorded her response using a magnet-compatible response box. In the synesthetic grapheme task, each synesthete was presented with a personalized set of stimuli: eight graphemes (digits, letters or symbols) that elicited a strong synesthetic color, eight graphemes that elicited a weak synesthetic color, and eight graphemes with

no synesthetic color for that particular synesthetic subject. The synesthete and her matched control saw the same set of stimuli. Each stimulus was presented for 500 ms and was followed by a gray isoluminant screen that lasted between 2,500 and 9,000 ms. The stimuli were presented in randomized order for four times per category over two runs (one run lasting for approximately 5.5 min). Graphemes subtended a visual angle of 2°. Subjects were asked to press with their index finger a key on the response box if a letter, digit or symbol was italic and with their middle finger if the stimulus was in normal font. The BOLD signal was measured, while the paradigm was being presented, with a T2* gradient-echo EPI sequence (TR 2.3 s, TE 28 ms, 35 slices, slice thickness 3.3 mm, FOV 220 × 220 mm, in-plane resolution 96 × 96, duration 333 s), and the measurement was repeated once.

Data were analyzed with FSL[38] and Matlab (Mathworks, Inc). The functional images were slice-time aligned, motion corrected, high-pass filtered (0.01 Hz), temporally smoothed with a gaussian filter (full-width at half maximum of 2.4 s), and spatially smoothed (5 mm). After this, the functional images were aligned to the structural image acquired at the start of each scanning session (T1 turbo field echo, 182 coronal slices, flip angle 8°, TE 4.6 ms, TR 9.7 s, slice thickness 1.2 mm, FOV 256 × 256 mm, matrix 256 × 256) and transformed, on the basis of this structural image, to MNI space. Furthermore, the BOLD data were transformed on the basis of a nonlinear transformation from MNI to tracts-based space (the transformation used in the DTI analysis). Explanatory variables were constructed for the three different types of stimuli and their first order derivatives. Time-series statistical analysis was carried out using FILM with local autocorrelation correction[42]. Higher-level analysis was carried out using FLAME (FMRIB's local analysis of mixed effects) stage 1 and 2 (refs. 43,44). We thresholded z-statistic images using clusters determined by $z > 2.3$ and a (corrected) cluster significance threshold of $P = 0.05$ (ref. 45).

Statistics for projectors and associators. We used nonparametric tests to correlate the PA score of an individual synesthete with her FA value in each of the four clusters found to have higher FA values in synesthetes as compared with non-synesthetes.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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