Individual differences provide psychophysical evidence for separate on- and off-pathways deriving from short-wave cones

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Distinct neural populations carry signals from short-wave (S) cones. We used individual differences to test whether two types of pathways, those that receive excitatory input (S+) and those that receive inhibitory input (S–), contribute independently to psychophysical performance. We also conducted a genome-wide association study (GWAS) to look for genetic correlates of the individual differences. Our psychophysical test was based on the Cambridge Color Test, but detection thresholds were measured separately for S-cone spatial increments and decrements. Our participants were 1060 healthy adults aged 16–40. Test–retest reliabilities for thresholds were good ($\rho = 0.64$ for S-cone increments, 0.67 for decrements and 0.73 for the average of the two). "Regression scores," isolating variability unique to incremental or decremental sensitivity, were also reliable ($\rho = 0.53$ for increments and $\rho = 0.51$ for decrements). The correlation between incremental and decremental thresholds was $\rho = 0.65$. No genetic markers reached genome-wide significance ($p < 5 \times 10^{-7}$). We identified 18 "suggestive" loci ($p < 10^{-5}$). The significant test–retest reliabilities show stable individual differences in S-cone sensitivity in a normal adult population. Though a portion of the variance in sensitivity is shared between incremental and decremental sensitivity, over 26% of the variance is stable across individuals, but unique to increments or decrements, suggesting distinct neural substrates. Some of the variability in sensitivity is likely to be genetic. We note that four of the suggestive associations found in the GWAS are with genes that are involved in glucose metabolism or have been associated with diabetes.

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1. INTRODUCTION

Although congenital tritanopia is far rarer than is red–green color deficiency, the short-wave (S) cones—or the neural pathways that carry their signals—are disproportionately affected in many conditions that impair the adult retina, such as glaucoma and diabetes [1]. However, when S-cone sensitivity is measured, attention is seldom paid to the possibility that there might be independent variation in the S-on and S-off channels that exist downstream of the receptors. In the present study we establish the range of variation in S-cone sensitivity in a large normal population. We measure separately the thresholds for increments and for decrements.

Existing evidence, both physiological and psychophysical, suggests that increments and decrements of S-cone contrast are signalled in separate pathways. Anatomical and electrophysiological studies of the primate retina are revealing an increasing number of cell types that receive S-cone input. At least two types of retinal ganglion cell respond to S-on stimuli: the small [2,3] and the large [4] bistratified ganglion cells. S-off ganglion cells have proved more elusive, and there seem to be no S-off bipolar cells [4]. However, the melanopsin-containing giant monostratified ganglion cells [5] are thought to receive an S-off input, as are the midget cells [6,7]. Beyond the retina, cells that receive a strong S-cone input are predominantly found in the koniocellular layers of the lateral geniculate nucleus (LGN). Differences between geniculate cells with S-on input and those with S-off input have been found in their long (L) and middle-wave (M) cone inputs, in their sensitivity, in their susceptibility to adaptation and in their spatial resolution for achromatic modulation [8,9]. It is not known how the distinct signals of these S-on and S-off channels are maintained in the cortex.

Psychophysical evidence for independent S-on and S-off subsystems has come from experiments that attempt to adapt differentially one of the two pathways. Shinomori *et al.* [10] used adaptation to temporal "sawtooth" flicker, an adapting stimulus that either ramps on slowly and off quickly, or vice versa. Such adaptation reduced sensitivity to sawtooth stimuli of the same polarity as the adapting sawtooth. Psychophysical sensitivity to stepped S-cone increments or decrements also exhibits a reduction that is specific to the polarity of sawtooth adaptation [11,12].

Another psychophysical method used to isolate S-on and S-off pathways is the "probe-flash" paradigm. Using probe

stimuli visible only to S-cones, Hughes and DeMarco [13] measured sensitivity either to an incremental or to a decremental probe at various latencies after an earlier conditioning flash that also stimulated only S-cones. The reduction in threshold was greatest if the conditioning flash and the probe had the same polarity. This was not the case for stimuli that were increments or decrements in the ratio of the signals of the L and M cones. Hughes and DeMarco also found that the time course of recovery from the conditioning flash was different for S-cone increments and S-cone decrements.

Measures of spatial summation provide further psychophysical evidence for independence of S-on and S-off subsystems. Vassilev *et al.* [14] used a modified version of Stiles' two-color threshold technique. At eccentricities greater than 5°, they found that the area of spatial summation was greater for S-cone decrements than that for S-cone increments, suggesting that receptive fields are larger for the S-off subsystem.

A study of individual differences can reveal the extent to which different psychophysical measures are affected by common sources of variance and thus we can gain insight into the underlying neural organization. Such analyses of individual differences are under-used [15–17]. From the present measurements of individual differences, we argue for independent S-on and S-off postreceptoral channels.

The experiments we report here were conducted as part of the PERGENIC genome-wide association study of the genetic basis of individual differences in perception [18,19]. We report the results of the GWAS for sensitivity to S-cone isolating stimuli.

2. METHODS

A. Stimuli

Our stimuli were based on those of the Cambridge Color Test [20,21]. They were large (diameter 6.1°) C-shaped figures composed of small disks (diameters 0.04°–0.59°). The gap in the C-shaped stimulus subtended 2.1° and could appear either at the top, bottom, left or right. A representation of our stimuli is shown in Fig. 1. The disks comprising the background were metameric with equal-energy white. The chromaticity of the disks comprising the figure differed from that of the disks comprising the background either by an increment of S-cone contrast (purplish, represented in the left-hand panel of Fig. 1), or by a decrement of S-cone contrast (yellowish–green, represented in the right-hand panel of Fig. 1).

The chromaticities of our stimuli were constructed using the cone fundamentals of Smith and Pokorny [22].

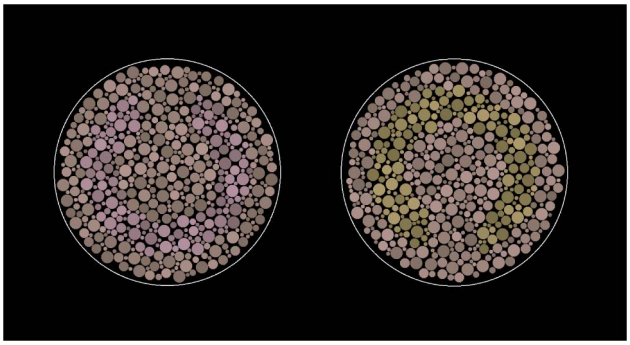

Fig. 1. Representation of the stimulus used to measure sensitivity to S-cone increments and decrements. An S-cone increment appears violet (left) and an S-cone decrement appears chartreuse (right).

The stimulus and surround were, on average, isoluminant for the standard observer, with an average luminance of 27 $cd \cdot m^{-2}$, but we introduced 42% luminance jitter in the disks making up the surround and the test figure, in order to mask any small luminance difference between the figure and the ground that the observer might have been able to detect.

B. Procedure

The task was four-alternative spatial forced-choice. On each trial, the participant was required to press one of four buttons to indicate the position of the gap in the C-shaped target. The stimulus was present for 3 s or until the participant had made a response. Depending on the participant's response, ZEST [23,24] staircases altered the S-cone contrast of the figure on subsequent trials. In each block, two randomly interleaved staircases tracked the participant's threshold. There were four blocks, two measuring thresholds for S-cone increments, and two measuring thresholds for S-cone decrements. Each block terminated after 31 trials.

C. Participants

1060 participants aged 16–40 took part in the PERGENIC study. 413 were male, and 647 were female. All participants were of European origin, to reduce population stratification for our genetic analysis. 105 participants were randomly selected to return for a second testing session, at least a week after their first, and it is the data from these participants that form the basis of our test–retest reliabilities. All participants had normal or corrected-to-normal visual acuity. They completed the experiment monocularly using their dominant eye. The study was approved by the Cambridge Psychology Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki. All participants gave written informed consent before taking part.

D. GWAS Methods

Each participant gave a saliva sample midway through the testing session, using Oragene OG-500 DNA kits (DNA Genotek Inc, Ottawa, Canada). Following DNA extraction, 1008 of our samples were genotyped using Illumina HumanOmniExpress arrays. This BeadChip kit allowed for the characterization of 733,202 single nucleotide polymorphisms (SNPs). Genotype calling was by custom clustering.

20 individuals were excluded from our genetic dataset following genotyping. Our criteria for exclusion were sex anomalies (3), low call rate (1), relatedness or sample duplication (15), and population outliers (1). Genetic data from the remaining 988 individuals were used in the GWAS. We excluded 12.3% of our genotyped SNP markers from the association analysis. These were markers with greater than 2% missing genotypes (12,706) or markers with a minor allele frequency of less than 1% (77,738). 642,758 SNP markers remained in the GWAS.

For each SNP, we performed a quantitative trait analysis using the software PLINK [25]. To control for any residual population stratification, we used Eigensoft [26] to extract the top three principal components (PCA) accounting for genetic variation. The three PCA axes were entered along with sex as covariates in the regression model. The association analysis was carried out on five phenotypic variables: log

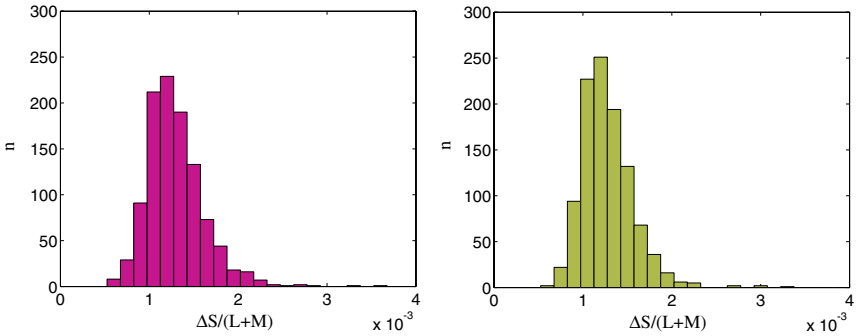

Fig. 2. Histograms of thresholds for S-cone increments (left panel) and thresholds for S-cone decrements (right panel). The units are the difference in S/(L + M) value of the increment or decrement from that of the background. S/(L + M) is as defined in the standard MacLeod–Boynton chromaticity diagram [29].

S-cone increment sensitivity, log S-cone decrement sensitivity, the average sensitivity across S-cone increments and decrements, regression scores (see Fig. 5) for incremental thresholds, and regression scores for decremental thresholds. Any suggestive loci ($p < 10^{-5}$) were then imputed over a region of 2.5 Mbp centered on the SNP of interest. This was achieved using the software IMPUTE2 [27,28] with the 1000 genomes phased haplotypes. Association analysis of these imputed regions was then carried out on the genotype probabilities using the dosage association function of PLINK. The three PCA axes and sex were added as covariates as in the first stage analysis.

The final stage of our genetic analysis was clustering. Here we used PLINK's clumping function, with a significance threshold for index SNPs of 0.00001, a significance threshold for clustered SNPs of 0.01, an linkage disequilibrium (LD) threshold for clustering of 0.1, and a physical distance threshold for clustering of 1250 kbp. Clustering defines a region that is in LD with the locus of interest, and which contains other SNPs (the "clustered" SNPs) that are associated with the trait with a specified p-value. The clustered region therefore defines a region in which the polymorphism causally associated with the phenotype is likely to lie.

3. RESULTS

A. Individual Differences in Sensitivity to S-Cone Increments and Decrements

Psychophysical results are based on an analysis of the data from 1058 participants. 1060 participants completed the PERGENIC study, but the data from one were missing owing to an equipment failure, and the data from another were eliminated because three of the four staircases failed to converge. In three further cases, one out of a pair of staircases used to measure a threshold was eliminated for failing to converge.

Histograms showing the distributions of thresholds for S-cone increment and decrement detection are shown in Fig. 2. The distributions are not normal, but are positively skewed. There is no significant difference in the mean threshold for increments and the mean threshold for decrements when each is expressed as S-cone Weber contrast. Distributions of log thresholds for S-cone increments and decrements appeared more normally distributed, but still deviated significantly from the normal distribution. Log thresholds were used for our GWAS.

Test–retest reliabilities for S-cone incremental and decremental thresholds are shown in Fig. 3. Each panel shows the session 2 scores of our 105 returning participants plotted against their session 1 scores. The test–retest reliability for S-cone incremental thresholds is $\rho = 0.65$, and for S-cone decremental thresholds is $\rho = 0.67$. We also calculated average thresholds, taking the average of the threshold for S-cone increments and the threshold for S-cone decrements for each participant. The test–retest reliability of the average is higher than for either increments or decrements alone, at $\rho = 0.73$.

The relationship between thresholds for S-cone increments and thresholds for S-cone decrements is shown in Fig. 4. The correlation is $\rho = 0.65$. Thus, 42% of the variance in thresholds (within a session) for S-cone isolating stimuli is shared between S-cone increments and S-cone decrements.

To extract the variability unique to increments and decrements, we used linear regression [30]. Separately for

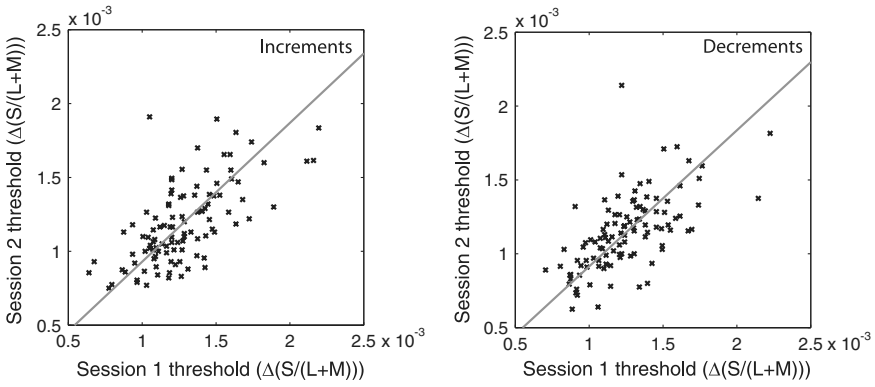

Fig. 3. Test–retest reliabilities for our 105 returning participants for sensitivity to S-cone increments (left panel) and sensitivity to S-cone decrements (right panel). The units are the difference in S/(L + M) from that of the background. The gray lines show orthogonal linear regressions.

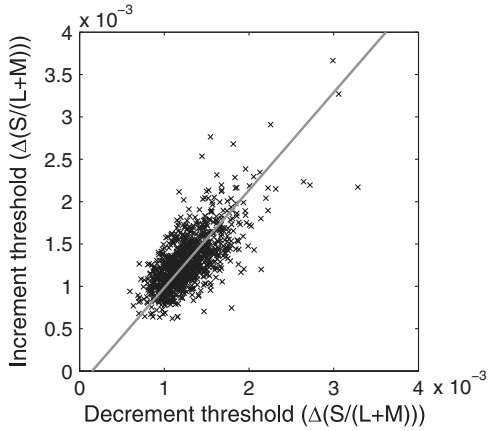

Fig. 4. Correlation between sensitivity to S-cone increments and sensitivity to S-cone decrements across individuals. The units are the difference in S/(L + M) from that of the background. The gray line shows the orthogonal linear regression through the data.

increments and decrements we calculated "regression scores." Regression scores for increments, for example, are the residuals of a linear regression of increments on decrements (Fig. 5). An individual's regression score for increments is a best estimate of how his increment threshold differs from those of other individuals with the same decrement threshold as he has. The test–retest reliabilities of the regression scores (based on our 105 returning participants) were 0.53 for incremental thresholds and 0.51 for decremental thresholds (Fig. 5). Thus, the proportion of the variability in S-cone incremental thresholds that is unique to incremental thresholds (rather than shared between incremental and decremental thresholds) is 28%, and the proportion of the variability in decremental thresholds that is unique is 26%.

To make a fair comparison between the test–retest reliabilities for incremental and decremental regression scores (0.53 and 0.51, respectively) and the proportion of variance common to incremental and decremental sensitivities, it is necessary to calculate an equivalent statistic for the latter relationship. Fig. 4 shows the correlation between incremental and decremental thresholds for our full sample of 1058 participants, but these data were gathered in a single session, whereas the reliability of regression scores correlates data gathered across two different sessions. There are factors that vary across sessions and that may affect performance, such as the alertness and motivation of participants and the time of day. We therefore calculated what we call *inter-task reliabilities* [18]. Here, we correlate decremental thresholds in session 1 against incremental thresholds in session 2, and incremental thresholds in session 1 against decremental thresholds in session 2. The resulting reliabilities have values $\rho = 0.47$ and $\rho = 0.52$, respectively.

The inter-task reliabilities are lower (though not significantly lower) than the within-session correlations between sensitivity to increments and to decrements, which are 0.59 and 0.62 for our 105 returning participants. This indicates that there may be some time-varying factors that cause variability in the data.

The inter-task reliabilities are similar in size to the test–retest reliabilities of the regression scores for incremental thresholds and for decremental thresholds. The proportion of the variance shared between sensitivity to decrements in session 1 and sensitivity to increments in session 2 is 27%. Thus, the proportion of the variance *not* shared between the two subsystems (28% for increment thresholds and 26% for decrement thresholds) is of a similar magnitude to the proportion that is shared.

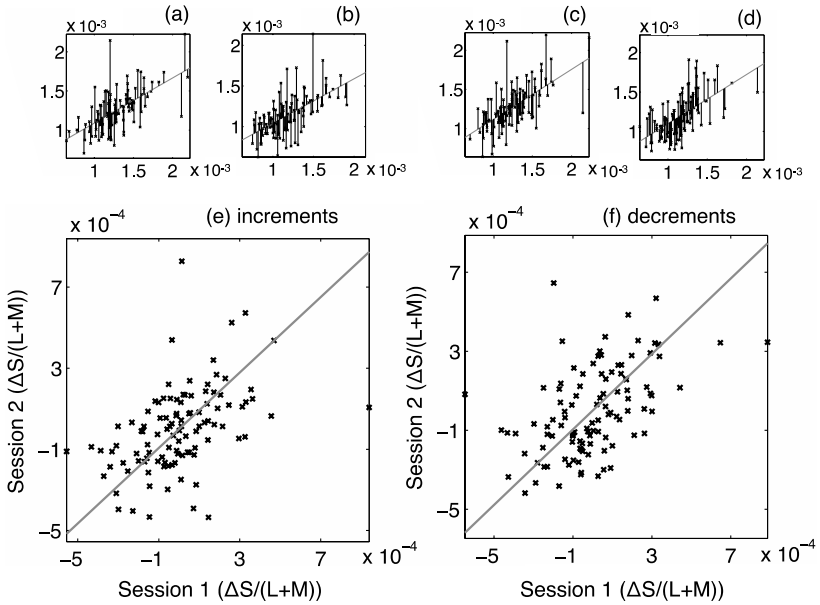

Fig. 5. Test–retest reliabilities of regression scores for incremental and decremental thresholds. Panels (a)–(d) show the residuals for incremental and decremental thresholds in sessions 1 and 2. All axes are ΔS/(L + M). Panels (a) and (b) show linear regressions of incremental thresholds on decremental thresholds, panel (a) for session 1, and panel (b) for session 2. Panels (c) and (d) show linear regressions of decremental thresholds on incremental thresholds; panel (c) for session 1, and panel (d) for session 2. Panel (e) shows the test–retest reliability of regression scores for incremental thresholds; the residuals shown in panel (b) are plotted against the residuals shown in panel (a). Panel (f) shows the test–retest reliability of regression scores for decremental thresholds: the residuals shown in panel (d) are plotted against the residuals shown in panel (c). The gray lines in panels (e) and (f) show orthogonal linear regressions through the data.

Table 1. Correlations between Our Three Measures of Sensitivity to S-Cone Isolating Stimuli and Four Measures of Achromatic Contrast Sensitivity

Contrast Measure	Test–Retest Reliability	Log Decrement Threshold	Log Increment Threshold	Average Log Threshold
Frequency doubled gratings	0.73	$\rho = 0.26$; $n = 1055$; $p = 1.4 \times 10^{-17}$	$\rho = 0.24$; $n = 1055$; $p = 8.7 \times 10^{-16}$	$\rho = 0.28$; $n = 1055$; $p = 4.9 \times 10^{-20}$
Gratings of low spatial frequency and high temporal frequency	0.52	$\rho = 0.28$; $n = 1057$; $p = 7.1 \times 10^{-21}$	$\rho = 0.26$; $n = 1057$; $p = 7.4 \times 10^{-18}$	$\rho = 0.29$; $n = 1057$; $p = 1.4 \times 10^{-22}$
Pelli–Robson	0.58	$\rho = 0.11$; $n = 1057$; $p = 4.9 \times 10^{-4}$	$\rho = 0.13$; $n = 1057$; $p = 1.1 \times 10^{-5}$	$\rho = 0.13$; $n = 1057$; $p = 1.8 \times 10^{-5}$
Gratings of 3 cpd	0.74	$\rho = 0.17$; $n = 1002$; $p = 5.7 \times 10^{-8}$	$\rho = 0.16$; $n = 1002$; $p = 1.5 \times 10^{-7}$	$\rho = 0.18$; $n = 1002$; $p = 3.8 \times 10^{-9}$

B. Correlations with Other Variables

As part of the PERGENIC study we gathered data on many more perceptual traits using psychophysical tests, and we also obtained demographic data by means of an online questionnaire. Here, we report the results of correlations between sensitivity to S-cone isolating stimuli and other relevant variables.

1. *Anatomical Factors*

There is a relationship between sensitivity to S-cone isolating stimuli and the pupil size of the dominant eye used for the experiments. The absolute sizes of the correlations are small, but they are highly significant owing to our large sample: $\rho = 0.13$, $p = 2.9 \times 10^{-5}$ for increments; $\rho = 0.085$, $p = 0.0055$ for decrements. For both S-cone increments and for S-cone decrements, the trend was that participants with larger pupils had lower thresholds. Unexpectedly, neither the chromaticity nor the lightness of the iris was significantly correlated with sensitivity either to S-cone increments or to S-cone decrements ($0.009 \le \rho \le 0.053$; $0.08 \le p \le 0.76$).

2. *Demographic Factors and Red–Green Color Deficiency*

There was no significant correlation between sensitivity to S-cone isolating stimuli and age, in our population of normal healthy adults aged 16–40 ($\rho = 0.04$, $p = 0.19$ for decrements; $\rho = 0.002$, $p = 0.94$ for increments). Nor was there a significant effect of sex on thresholds for S-cone increments ($t = 1.84$, $p = 0.07$) or on those for S-cone decrements ($t = 0.07$, $p = 0.94$). We identified probable red–green color deficiency in our sample by including in our battery 3 plates from the Ishihara test [31]. 57 subjects were categorized as red–green color deficient on the basis of their readings. For decremental thresholds, the difference between red–green color deficient participants and normals was marginally significant (Mann Whitney $U = 2.5 \times 10^4$, $Z = 2.03$, $p = 0.043$), with red–green color deficient participants 0.3 standard deviations poorer than normals. The difference was in the same direction but insignificant for incremental thresholds ($p = 0.17$), and for the average of the two ($p = 0.053$). The relative similarity in S-cone thresholds between color deficient participants and normals is notable, since there are reasons for expecting a difference in either direction [20].

3. *Contrast Sensitivity*

We included four other measures of contrast sensitivity in our battery. These were sensitivity to frequency-doubled gratings [32], sensitivity to gratings of low spatial frequency (0.2 cpd) and high temporal frequency [33], the Pelli–Robson test of contrast sensitivity [34] and sensitivity to gratings of medium spatial frequency (3 cpd). Methods for our contrast sensitivity measures are available in Goodbourn *et al.* [18], with the exception of sensitivity to gratings of 3 cpd. Our procedure for this measure was four-alternative spatial forced-choice. Participants were required to identify the location of a sinusoidal grating of 3 cpd presented as a 3° square patch at an eccentricity of 3.6° from a central fixation point. Stimuli were presented on a Clinton Monoray CRT monitor (Clinton electronics, Loves Park, Illinois) running at 150 Hz. Contrast threshold was measured separately for the right and left eyes, by means of two interleaved ZEST staircases for each eye. Monocular presentation was achieved using Cambridge Research Systems FE-1 shutter goggles synchronized to the monitor's refreshes. The measure of contrast sensitivity we present here is averaged across the two eyes.

We correlated our three S-cone measures—log decrement threshold, log increment threshold and average log threshold—with each of our four measures of achromatic contrast sensitivity. Results are shown in Table 1, together with the test–retest reliabilities for our four measures of contrast sensitivity. There are modest but significant correlations between all three measures of S-cone thresholds and the thresholds for achromatic contrast sensitivity, ranging from $\rho = 0.11$ ($p = 4.9 \times 10^{-4}$) to $\rho = 0.29$ ($p = 1.4 \times 10^{-22}$). In all cases these correlations are in the expected positive direction: subjects who perform better on one measure of contrast sensitivity also tend to perform better on the others.

C. GWAS Results

No genotyped SNP reached our criterion for genome-wide significance ($p < 5 \times 10^{-7}$). However, in Table 2 we list 18 suggestive loci ($p < 10^{-5}$): 6 for incremental thresholds, 5 for decremental thresholds, 2 for the average of incremental and decremental thresholds, 4 for regression scores for incremental thresholds and 1 for regression scores for decremental thresholds. Two SNPs (on chromosome 1 and chromosome 14) that were associated with both increment sensitivity and average sensitivity are listed in Table 2 only under increment sensitivity. For each suggestive association we give position, minor allele frequency, p-value, the position and size of the clustered region, and any genes situated inside the clustered region. The strongest suggestive association listed in Table 2 is between rs1891931 and sensitivity to S-cone increments ($p = 6.3 \times 10^{-7}$). The association would account for 2.4% of the variability in sensitivity to S-cone increments.

Table 2. Details of the 15 Suggestive Loci Identified by the GWAS

SNP	Chr	Position	MAF	p	Clustered Region	Center of Clustered Region	Genes Inside Clustered Region
Decremental thresholds							
rs2382987	19	17934018	0.27	1.52×10^{-6}	19 kbp	17925567	*B3GNT3; INSL3*
rs254775	16	77883374	0.28	8.23×10^{-6}	12 kbp	77885270	*VAT1L*
rs6531596	4	37905601	0.12	8.40×10^{-6}	13 kbp	37908382	*TBC1D1*
rs7038842	9	3701667	0.49	8.54×10^{-6}	36 kbp	3719825	N/A
rs6956493	7	51420327	0.018	9.16×10^{-6}	891 kbp	51228626	*GRB10; COBL*
Incremental thresholds							
rs1891931	1	165160578	0.34	6.28×10^{-7}	56 kbp	165155806	*LMX1A*
rs2240342	14	71373128	0.012	1.44×10^{-6}	292 kbp	71504358	*PCNX*
rs6871461	5	2999174	0.37	2.53×10^{-6}	9 kbp	2996002	N/A
rs7095238	10	69913203	0.43	5.71×10^{-6}	55 kbp	69939237	*MYPN*
rs1386212	12	129582658	0.38	8.12×10^{-6}	49 kbp	129568325	*TMEM132D*
rs2013879	2	162176044	0.16	8.53×10^{-6}	N/A		
Sensitivity averaged across increments and decrements							
rs6898100	5	155742820	0.32	4.84×10^{-6}	64 kbp	155776278	*SGCD*
rs2311780	4	131629960	0.33	9.24×10^{-6}	239 kbp	131742160	*N/A*
Regression scores for incremental thresholds							
rs1286887	13	28806513	0.030	6.43×10^{-6}	144 kbp	28765134	*PAN3*
rs17705297	7	95609297	0.18	3.76×10^{-6}	12 kbp	95613770	*DYNC1I1*
rs6831381	4	7904431	0.25	7.36×10^{-6}	108 kbp	7901247	*AFAP1*
rs2931130	2	8434948	0.14	8.25×10^{-6}	125 kbp	8486472	*LINC00299*
Regression scores for decremental thresholds							
rs10048253	18	22631477	0.20	9.15×10^{-6}	113 kbp	22632690	*ZNF521*

4. DISCUSSION

A. Psychophysical Results

Many shared sources of variance might be expected to contribute to individual differences in both incremental and decremental S-cone sensitivity. These are likely to include: (i) differences in the number or the sensitivity of the short-wave cones themselves, (ii) differences in prereceptoral factors such as the yellowness of the lens, and (iii) any non-visual factors that determine how efficiently the participant carries out the psychophysical task.

However, the fact that the regression scores for S-cone incremental thresholds and for S-cone decremental thresholds show significant test–retest reliabilities means that some of the variance across individuals is unique to detection of S-cone increments or to detection of S-cone decrements, rather than being shared. The existence of this independent variance implies that the neural substrates are not wholly identical for the two tasks. The source of the nonshared portion of the variance is unlikely to be the S-cones themselves, but rather downstream structures such as the bipolar cells, the different populations of retinal ganglion cells that process S-on and S-off stimuli, or pathways beyond the retina in the LGN or the cortex.

We found a small positive correlation between sensitivity to our incremental and decremental stimuli and pupil size. The factor driving this correlation is likely to be the amount of light entering the eye, since we found those with larger pupils to be more sensitive on average.

Despite results reported in the literature that sensitivity to S-cone isolating stimuli declines with age [35–37], we found no correlation between age and sensitivity. This was perhaps unsurprising because our population was of young adults aged 16–40, with a low mean age of 22.1 years. Mullen *et al.* [38] found that thresholds for S-cone increments and thresholds for S-cone decrements remained stable until age 50, when both began declining.

We found positive correlations between sensitivity to S-cone isolating stimuli and sensitivity to four different measures of achromatic contrast sensitivity. For example, at least 8% of the variance in S-cone thresholds is shared with thresholds for achromatic gratings of low spatial, and high temporal, frequency. This common variance may arise from common neural substrates within early visual pathways or from more central aspects of psychophysical detection or from both. One plausible source for some of the common variance would be the personality trait of conscientiousness, but we found no significant correlations between our S-cone thresholds and conscientiousness measured using the mini IPIP [39] ($\rho = 0.001$; $p = 0.96$).

B. GWAS

Since none of our loci reached genome-wide significance, we do not want to make any strong claims about the possible contribution of particular genes to individual differences in sensitivity to S-cone isolating stimuli. However, we should like to make some observations about the suggestively associated genes that have emerged.

Four of the genes we have identified are involved in pathways for glucose metabolism, or have been associated with diabetes. These are INSL3 [40], *TBC1D1* [41,42], *GRB10* [43,44] and *LMX1A* [45]. Sensitivity to S-cone isolating stimuli is known to be reduced in diabetes [46,47], and to correlate with acute changes in blood glucose [48]. It is plausible that individual differences in metabolic stress contribute to the overall individual differences that we observe in sensitivity to S-cone stimuli within our normal population.

The SNP most strongly associated with sensitivity to S-cone isolating stimuli is rs1891931, which is associated with incremental sensitivity with a p-value of 6.4×10^{-7}. This SNP is also associated with sensitivity to decrements ($p = 0.0002$), and with sensitivity averaged over increments and decrements ($p = 1.6 \times 10^{-6}$). Fig. 6 is a regional Manhattan plot for this

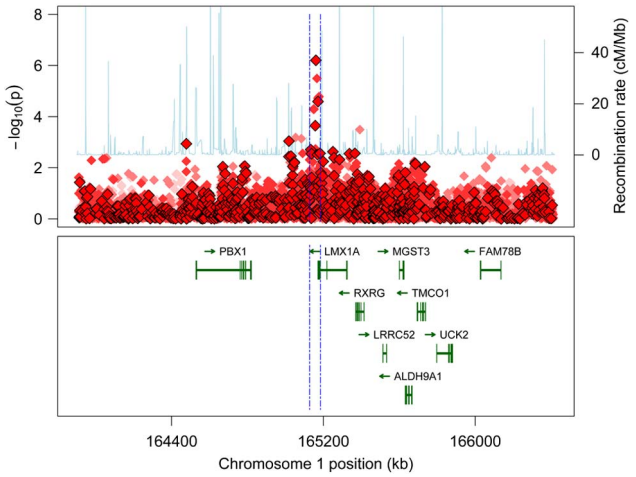

Fig. 6. Regional Manhattan plot for the association between sensitivity to S-cone increments and the region around rs1891931. The association results for genotyped SNPs are shown with black borders, and results for imputed SNPs are shown without borders, with saturation indicating the imputation quality. The recombination rate is plotted with a solid blue line. The lower panel shows the genomic context of the region. Vertical green lines indicate exons. The dashed blue line in both panels indicates the clustered region identified by clumping analysis.

locus. Although the clustered region identified in a clumping analysis includes only *LMX1A*, we note that the nearby gene *RXRG* is known to be critical for the differentiation of short-wave versus long- and middle-wave cones [49]. Independently, we suggest a tentative genetic association between *RXRG* and the ratio of numbers of L and M cones, estimated from settings on the OSCAR test [31].

Other suggestively associated genes are involved in neural development. The protein encoded by *COBL* facilitates neural tube closure [50], acts as an actin nucleation factor, and enhances induction of neurite and neurite branching in neurons [51]. *LMX1A* is critical in the development of midbrain dopamine-producing neurons [52]. A candidate that has been associated specifically with the eye is *SGCD*, which encodes a protein forming a link between the cytoskeleton and the extracellular matrix, and has been associated in a GWAS with age-related macular degeneration [53].

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