Author Information Page

Full title: Why human color vision cannot reliably detect CSF xanthochromia

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Acknowledgments and Funding Page

We thank Mr Robert Ludlow for excellent photographic support, Mr Miles Chapman for discussion and advice on the practical experiments and Prof EJ Thompson, Dr GT Plant and Dr M Smith for comments on the manuscript.

Conflict of interest statement

None declared.

Financial interest statement

None declared.

Funding

None.

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Cover title: Xanthochromia

Tables and Figures:

1. Number of Tables: 1
2. Number of Figures: 1

Key words: colour vision, spectralphotometry, xanthochromia, cerebrospinal fluid

Abstract

Background: Visual assessment of cerebrospinal fluid (CSF) for xanthochromia (yellow color) is practiced by the majority of laboratories world wide as a means of diagnosing intracranical bleeds.

Methods: Colorimetric and spectralphotometric analysis of CSF samples for recognising the presence of bilirubin either in low concentrations or in the presence of haemolysed blood.

Results: The experiments provide the physiological and colorimetric basis for abandoning visual assessment of CSF for xanthochromia.

Conclusion: We strongly recommend relying on spectrophotometry as the analytical method of choice.

Article

Subarachnoid haemorrhage (SAH) is one of the most striking conditions in medicine with potentially fatal outcome. The analysis cerebrospinal fluid (CSF) is a crucial diagnostic tool. The sensitivity detecting a bleed by CT falls from 95% on day one to <10% three weeks after the event [1], whilst the sensitivity of CSF analysis remains close to 100% [2].

The presence of pigments in CSF alters its visual appearance. Oxyhaemoglobin makes it appear red or orange, whilst bilirubin gives the yellow coloration of true xanthochromia. Oxyhaemoglobin arises both from a traumatic tap and a true SAH. Importantly the conversion of oxyhaemoglobin to bilirubin can only happen in *in vivo*, allowing distinction between a true intracranial bleed and one due to a traumatic tap. Here we provide physiological evidence that the commonly practised [3,4] visual assessment of CSF should be abandoned and replaced by spectrophotometry.

Methods

To simulate the conditions in which bilirubin may be observed, a set of experiments were designed. Firstly, to reproduce contamination by oxyhaemoglobin as it may occur with a traumatic tap, a series of tubes containing doubling dilutions of haemolysed blood (series 'A' inset in Fig 1A) into CSF containing the same amount of bilirubin was prepared. Secondly, to determine the lowest concentration of bilirubin that could be confidently detected, we prepared a series of tubes containing doubling dilutions of bilirubin alone (series 'B' inset in Fig 1A).

All tubes were examined visually for xanthochromia, in normal daylight or cool white fluorescent light, the typical viewing conditions, by 11 analysts, comprising clinical scientists, biomedical scientists or clinical neurology staff within the Department of Neuroimmunology at National Hospital for Neurology and Neurosurgery. The tubes were presented in a random order and the

analysts were naive to their actual concentrations.

Once the visual assessments were complete, all samples were scanned between 350 and 740 nm using an Ultrospec 4300 pro (Amersham Biosciences). The same analysts were then asked to indicate whether bilirubin was present or absent in the scan. The proportions of subjects finding a positive result by visual or spectroscopic assessment were compared using a χ^2 test.

Finally, the x-y chromaticity coordinates of the spectrophotometric scans were calculated, according to the standard procedures of specification for visual assessment established by the Commission Internationale de l'Eclairage (CIE, the International Commission on Illumination). This involves multiplying the spectral transmittances of the samples, converted from their optical densities, by the spectral concentration of the radiant power of the source illuminating them and then multiplying the product by each of the three color matching functions, which define the CIE standard colorimetric observer [5]. The resulting x-y chromaticity coordinates of the samples can then be plotted in the CIE 1931 chromaticity diagram for the standard 2° field of view (Fig 1 A & B), and their dominant wavelengths, which correspond to hue, and excitation purities, which correspond to saturation, can be geometrically calculated (see explanations in the Table and Figure legends and values in Table 1). The calculations were made for two CIE illumination or lighting standards: "D65," which equates to average daylight; and "A", which is for tungsten light.

Results

Samples in series 'A' , when calculated for viewing by standard illuminant D65, varied in their dominant wavelength from 572 nm (which falls near the hue category 'pure yellow' [6]) to 615 nm (red). They also varied in their excitation purity from 97.9% (highly saturated) to 34.1% (moderately saturated). In contrast, the samples in series 'B' all had the same dominant wavelength, 572 nm (yellow) but differed in their excitation purity from 0.62

(very desaturated) to 36.6% (Table 1). The optical density for bilirubin (450-460 nm) ranged from 3.5 to 0.36 for samples A1 to A14 and from 3.2 to 0.002 for samples B1 to B8.

A significantly higher proportion of the analysts detected traces of bilirubin spectrophotometrically than visually, both when the xanthochromic CSF samples were contaminated by the presence of haemolysed blood (Series 'A') and when they were desaturated (Series 'B'). In Series 'A', visual detection failed for CSF samples with dominant wavelengths greater than 574 nm (samples A1 – A7), most of which fall considerably outside the color category 'pure yellow'. In Series 'B', bilirubin could not be reliably detected in CSF specimens with excitation purity levels below 2.4% (samples B5 – B8). In contrast, in both Series 'A' and 'B', bilirubin could be reliably detected in all the samples by examining the spectrophotometric scans.

This study confirms that spectrophotometry is superior to color vision for analysing CSF samples for the presence of bilirubin [7]. Most critical CSF samples are either contaminated by oxyhaemoglobin or have only low levels of bilirubin. Under such conditions, detection of xanthochromia becomes unreliable, especially when viewed under an incandescent lighting or a tungsten desk lamp (corresponding to CIE standard illuminant A). Indeed, a lower proportion of the assessors were able to detect xanthochromia for samples 'B4' (7/11, χ^2=4.88, p<0.05), 'B5' (3/11, χ^2=9.21, p<0.01) and 'B6' (0/11, χ^2=6.47, p=0.01, see insets in Fig. 1B) under tungsten light than under daylight conditions. Colorimetric analysis revealed that all of the samples now fell completely outside the 'pure yellow' category. This condition represents a 'worst-case scenario', such as may be encountered during a night on-call.

Discussion

The implications of these findings can be judged from our previous analysis of spectrophotometric scans of CSF samples, which did not appear yellow in almost 80% of the cases routinely encountered at the National Hospital for

Neurology and Neurosurgery [8].

About 80% of all CSF samples with significant amounts of bilirubin appear rather 'reddish' than 'yellow' [8] but that 99.7% of over 3,500 laboratories participating in two recent American surveys [3,4] still assess samples by color vision. The observations presented here provide a physiological basis for abandoning the visual assessment of CSF for xanthochromia.

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Table legend

Table 1. Color specifications (CIE 1931 x-y chromaticity coordinates, dominant wavelengths and excitation purities), calculated with respect to CIE illuminant D65, of 14 samples derived by double diluting haemolysed blood (Series ‘A’) and 8 samples derived from a double dilution of xanthochromic CSF in H2O (Series ‘B'). The dominant wavelengths [3] were geometrically derived by drawing lines from the white-point (D65) through the x-y chromaticity coordinates of the samples to the corresponding spectral wavelengths indicated on the outer boundary (spectral locus) of the chromaticity diagram (Figure 1). The excitation purities [3] are the ratios of distances in the chromaticity diagram, indicating how far the chromaticity points of the samples are displaced from the white-point towards the spectral locus. Also shown are the observed frequencies of assessors who correctly identified the presence of bilirubin in the vision and spectrophotometry experiments and the results of the χ^2 analysis.

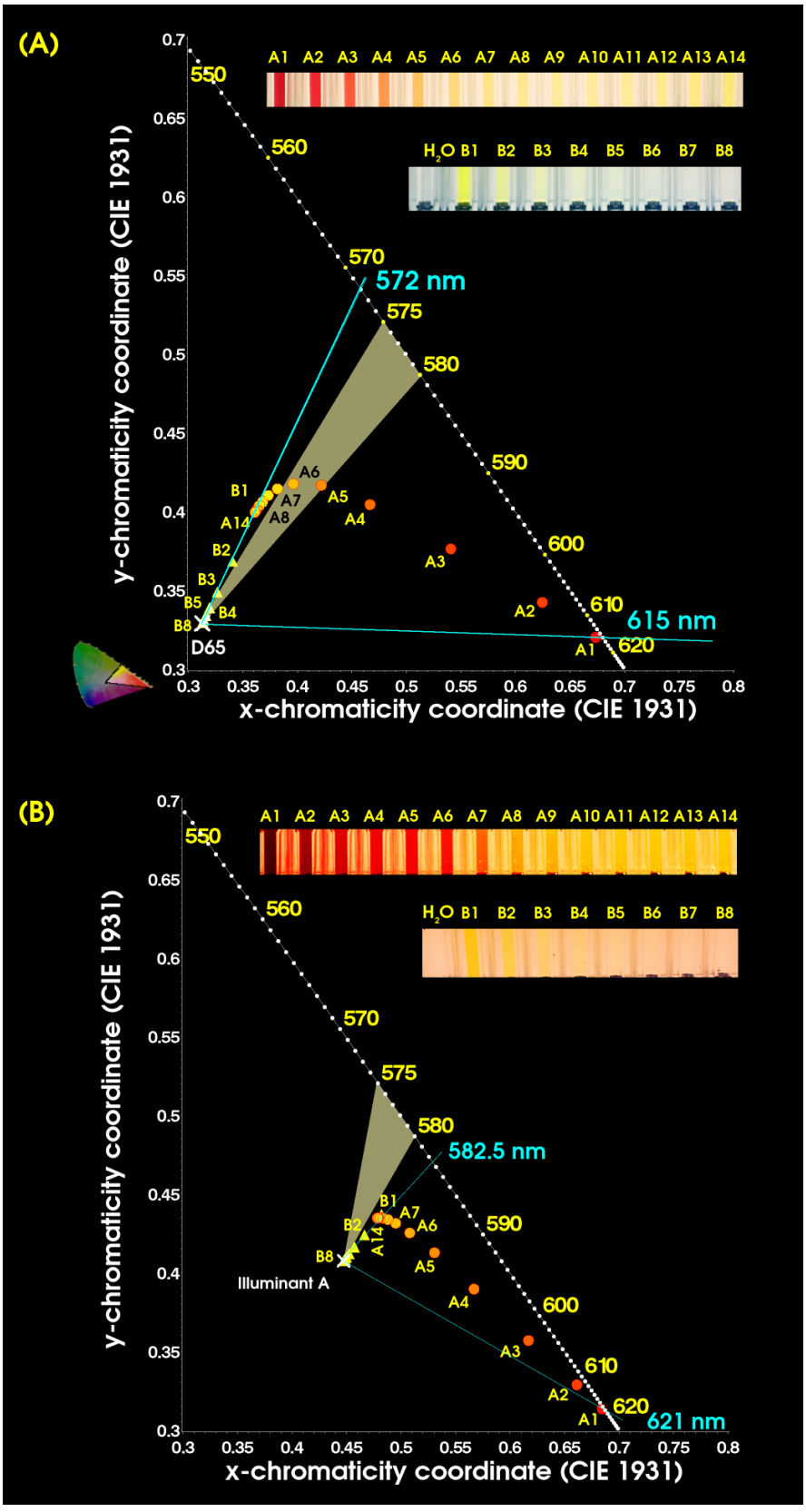
Figure legend

Figure 1 (A). A section of the CIE 1931 chromaticity diagram (the full diagram is shown in the lower left-hand corner) displaying the x-y chromaticity coordinates of 14 samples simulating a traumatic tap derived by double diluting haemolysed blood into xanthochromic CSF sample B1 (Series ‘A’, circles) and 8 samples derived from a double dilution of xanthochromic CSF in H2O (Series ‘B’, triangles). The coordinates have been calculated with respect to CIE illuminant D65 (white cross). For reference, the x-y chromaticity coordinates and wavelengths of the spectral colors from 550 nm (‘yellowish green’) to 620 nm (‘red’) are also indicated. The dominant wavelengths (turquoise lines) of samples 'B1-B8' and sample 'A1' are shown. The region corresponding to the color category 'pure yellow', which ranges between 575 and 580 nm, is indicated in pale grey (see summary table in [4]). The photographic insets show the Series ‘A’ and ‘B’ samples illuminated by cool white fluorescent light.

(B). The x-y chromaticity coordinates of the 22 samples from Series ‘A’ (circles) and 'B' (triangles) calculated for CIE illuminant A (white cross). The dominant wavelengths (turquoise lines) of samples 'B1-B8' and sample 'A1' are shown. The photographic inset shows Series ‘A’ and 'B' illuminated by a tungsten halogen source.

Table 1

Sample	*Chromaticity-coordinates (x, y)*	*Dominant wavelength (nm)*	*Excitation purity (%)*	*Colour vision (observed frequency)*	*Spectrophotometry (observed frequency)*	χ^2	*p-value*
A1	0.3824, 0.3990	615	97.9	0.00	0.72	12.57	<0.001
A2	0.3828, 0.3993	607	91.2	0.00	0.72	12.57	<0.001
A3	0.3836, 0.4002	596	77.3	0.00	0.72	12.57	<0.001
A4	0.3847, 0.4011	586	64.3	0.00	0.72	12.57	<0.001
A5	0.3868, 0.4030	580	55.4	0.09	0.72	9.21	<0.01
A6	0.3903, 0.4058	576	48.0	0.18	0.90	11.73	<0.001
A7	0.3961, 0.4097	574	42.8	0.63	1.00	4.88	<0.05
A8	0.4052, 0.4138	573	39.5	1.00	1.00	--	N.S.
A9	0.4209, 0.4166	572	36.7	1.00	1.00	--	N.S.
A10	0.4478, 0.4142	572	36.0	1.00	1.00	--	N.S.
A11	0.4922, 0.4000	572	34.8	1.00	1.00	--	N.S.
A12	0.5619, 0.3709	572	34.6	1.00	1.00	--	N.S.
A13	0.6354, 0.3383	572	34.3	1.00	1.00	--	N.S.
A14	0.6769, 0.3180	572	34.1	1.00	1.00	--	N.S.
B1	0.3883, 0.4052	572	36.6	1.00	1.00	--	N.S.
B2	0.3615, 0.3702	572	18.2	1.00	1.00	--	N.S.
B3	0.3474, 0.3518	572	9.3	1.00	1.00	--	N.S.
B4	0.3403, 0.3425	572	4.8	0.90	1.00	--	N.S.
B5	0.3366, 0.3378	572	2.4	0.45	1.00	8.25	<0.01
B6	0.3348, 0.3354	572	1.66	0.00	1.00	22.0	<0.001
B7	0.3338, 0.3341	572	1.04	0.00	1.00	22.0	<0.001
B8	0.3334, 0.3336	572	0.62	0.00	1.00	22.0	<0.001


(A)
A1 A2 A3 A4 A5 A6 A7 A8 A9 A10 A11 A12 A13 A14
H2O B1 B2 B3 B4 B5 B6 B7 B8
550
560
570
572 nm
575
580
590
600
610
615 nm
620
D65
y-chromaticity coordinate (CIE 1931)
x-chromaticity coordinate (CIE 1931)
(B)
582.5 nm
621 nm
Illuminant A