



RESEARCH ARTICLE

REVISED Comparison of color discrimination in chronic heavy smokers and healthy subjects [version 3; referees: 2 approved]

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Abstract

Background: Cigarette smoke is probably the most significant source of exposure to toxic chemicals for humans, involving health-damaging components, such as nicotine, hydrogen cyanide and formaldehyde. The aim of the present study was to assess the influence of chronic heavy smoking on color discrimination (CD). *Methods:* All subjects were free of any neuropsychiatric disorder, identifiable ocular disease and had normal acuity. No abnormalities were detected in the fundoscopic examination and in the optical coherence tomography exam. We assessed color vision for healthy heavy smokers ($n = 15$; age range, 20-45 years), deprived smokers ($n = 15$, age range 20-45 years) and healthy non-smokers ($n = 15$; age range, 20-45 years), using the psychophysical forced-choice method. All groups were matched for gender and education level. In this test, the volunteers had to choose the pseudoisochromatic stimulus containing a test frequency at four directions (e.g., up, down, right and left) in the subtest of Cambridge Colour Test (CCT): Trivector. *Results:* Performance on CCT differed between groups, and the observed pattern was that smokers had lower discrimination compared to non-smokers. In addition, deprived smokers presented lower discrimination to smokers and non-smokers. Contrary to expectation, the largest differences were observed for medium and long wavelengths. *Conclusions:* These results suggests that cigarette smoking, chronic exposure to its compounds, and withdrawal from nicotine affect color discrimination. This highlights the importance of understanding the diverse effects of nicotine on attentional bias.

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REVISED Amendments from Version 2

The study was adapted according to other publications involving Trivector and English edition. The "cut-off" section of the Ms methods was removed, and some lines in introduction and methodology were added (and corrected).

See referee reports

Introduction

Cigarette smoking is still a major source of exposure to chemicals that are toxic for humans. The compounds in cigarettes and cigarette smoke, such as nicotine, oxygen dioxide and formaldehyde, are highly harmful to health¹. Data from the World Health Organization (WHO) hypothesize that by 2030, cigarettes could kill nearly 9 million people a year around the world^{2,3}.

Cigarette nicotine deprivation in chronic users may impair cognitive and attentional abilities even after long time of cessation^{4,5}. The neurotoxic effects of chronic use and smoking abstinence on the nervous system have not been extensively studied⁶⁻⁸. However, chronic cigarette smoking increases cardiovascular response⁹, which, in turn, affects retinal responses through altered blood flow¹⁰. In addition, tobacco compounds may increase free radical that would cause macular degeneration along with the action of ischemia¹¹. Whereas smoking effects on color vision are understudied, the existing data are controversial and highlights the importance of a rigorous testing procedure that measures color discrimination^{12,13}. Thus, to identify the mechanisms underlying neurotoxic smoking effects on multi-sensory integration, we need to understand how smoking may alter early visual processing.

A visual percept may consist of stimuli that vary over the space (spatial contrast), time (temporal contrast) or direction of motion, and vary in luminance (achromatic) and chromaticity (saturation and hue color)^{12,14,15}. Thus, chromatic contrast involves chromaticity differences, which can be expressed by the distance in the CIE 1976 uniform chromaticity scale diagram and assessed by thresholds of vectors on the Cambridge Color Test (CCT), for example^{16,17}. It has the advantage of being used to evaluate in detail whether these anomalies are due to congenital factors or acquired conditions^{16,17}.

We base our rationale on the premise that chronic exposure to nicotine will led to receptor desensitization and not suffer influence of arousal and increase in attentional resources in smokers¹⁸. The purpose of the present study was to assess the influence of chronic heavy smoking on color discrimination (CD).

Methods

Participants

In this study, 15 non-smokers (mean age = 32.5 years; $SD = 9.1$; 7 male), 15 cigarette smokers (mean age = 32.1 years; $SD = 5.7$; 7 male) and 15 deprived smokers (mean age = 31.9 years; $SD = 6.3$; 7 male) who were staff or students at the Federal University of Paraiba were recruited through newspaper advertisements. The participants were 25–45 years old. The participants were excluded if they had any one of the following criteria: younger than 20 and older

than 45 years (since effects of aging in the human visual system could superestimate the results^{19,20}); current history of neurological or cardiovascular disease; a history of head trauma, color blindness, current or previous drug abuse and current use of medications that may affect visual processing and cognition. Subjects were required to have good ocular health, with no abnormalities on fundoscopic examination or optical coherence tomography examination. All of the subjects were screened for color blindness using the test of Ishihara for color deficiency, and had normal or corrected-to-normal vision as determined by a visual acuity of at least 20/20.

Smokers reported a smoking history of at least 8 years, currently smoked > 20 cigarettes/day and had a score > 5 on the Fagerstrom Test for Nicotine Dependence (FTND)²¹. Generally, smokers and deprived smokers began smoking at an average of 16.5 years of age ($SD = 3.25$) and had been smoking for an average 15 years ($SD = 6.45$). Smokers were allowed to smoke until the beginning of experiment. An abstinence period of 6 h was chosen based on previous studies (Bailey, Goedecker, & Tiffany, 2010; Fernandes, Almeida, & Santos, 2017; Kunchulia, Pilz, & Herzog, 2014). Non-smokers had never smoked a cigarette. No significant differences were found between depression and anxiety symptoms before and after the study, as measured by the Hamilton Scale for Depression and Hamilton Anxiety Rating Scale.

This research followed the ethical principles from the Declaration of Helsinki and was approved by the Committee of Ethics in Research of the Health Sciences Center of Federal University da Paraiba (CAAE: 60944816.3.0000.5188). Written informed consent was obtained from all participants.

Color discrimination test

The stimuli were presented on a 19-inch LG CRT monitor with 1024×786 resolution and a sampling rate of 100 Hz. Stimuli were generated using a VSG 2/5 video card (Cambridge Research Systems), which was run on a microcomputer Precision T3500 with W3530 graphics card. All of the procedures were performed in a room at $26^\circ\text{C} \pm 1^\circ\text{C}$, with the walls covered in gray for better control of luminance during the experiments. All of the measurements were performed with binocular vision. Monitor luminance and chromatic calibrations were performed with a ColorCAL MKII photometer (Cambridge Research Systems).

The color vision test was performed using CCT, version 2.0, with Trivector subtest (Cambridge Research Systems; <http://www.crsLtd.com/tools-for-vision-science/measuring-visual-functions/cambridge-colour-test/>). The CTT was performed in a darkened room with illumination that was provided only by the monitor that was used to present the visual stimuli. Trivector provides a clinical assessment of color vision deficiencies as a rapid means screening of the existence of congenital or acquired deficits¹⁶. CCT uses pseudoisochromatic stimuli (Landolt C) defined by the test colors that are to be discriminated, on an achromatic background. The figure and the background are composed of grouped circles randomly varying in diameter and having no spatial structure (variation of 5.7° arcmin of external diameter and 2.8° arcmin of internal diameter). The luminance variation in each response avoids learning effects or use of tricks to respond correctly.

The four-alternative forced-choice^{16,22} (4-AFC) method was used, and the subjects' task was to identify, using a remote control response box, whether the Landolt 'C' stimulus was presented at the left, right, up or down side of the monitor screen. The participants were also instructed to respond whether they could not identify the stimulus gap¹⁶. After each correct answer, the chromaticity of the target proceeded closer to that of background, while each wrong answer or omission was followed by the presentation of the target at a greater chromatic distance from the background. After each correct answer, the chromaticity of the target proceeded closer to the background. Each incorrect answer or omission was followed by presentation of the target at a greater chromatic distance from the background. The experiment ended after 11 reversals for each axis and the threshold was estimated from the six final reversals²³.

The trivector testing protocol estimates sensitivity for the short, medium and long wavelengths through the protanopic, deuteranopic, and tritanopic confusion axes, respectively^{23,24}. Trivector protocol uses vectors as central measurement. The advantage of this brief test is that it can be performed in about 5 minutes and provides a reliable result¹⁶. The three confusion axes converge at a co-punctual point, and the u'v' coordinates (CIE 1976) used were: protan (0.6579, 0.5013), deutan (-1.2174, 0.7826) and tritan (0.2573, 0.0000) (for more details, see 17).

In general, we used a default setting where the Landolt 'C' had an opening at 1° of visual angle, minimum luminance of 8 cd/m², maximum luminance of 18 cd/m², 6 s of response time for each trial and distance of 269 cm between participant and monitor screen.

Data analysis

The distributions for each group were compared with Shapiro-Wilk. Both groups showed non-normal distribution; therefore, nonparametric statistical methods were used to analyze the data. For group comparisons, the non-parametric univariate analysis was used, with pairwise comparisons by Mann-Whitney *U* test. Spearman's rank correlation coefficients (ρ) were conducted to assess the relationship between outcomes of color discrimination data and biosociodemographic variables, such as age, gender and level of education. All the calculations were made using SPSS®, version 21.0.

The effect size (*r*) estimation was used from the conversion of *z*-scores^{25,26}. Values above .50 are considered as large effect size.

Results are presented as medians. Center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by SPSS software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles (ends of the whiskers are the

maximum and minimum values). When presented, errors bars represent standard deviations (SD) of the median based on 1000 bootstrap resamplings. Bonferroni correction was the method of adjusting the *P*-value that we used. *P* < 0.016 was accepted as statistically significant for multiple comparisons and *P* < 0.025 for pairwise comparisons.

Results

Color discrimination thresholds were obtained in u'v' units of the CIE 1976 color diagram, for protan, deutan, and tritan axes, respectively. Nonparametric analysis were carried out showing that there were significant differences in discrimination thresholds between groups along the protan ($\chi^2(2) = 26.53$, *P* < 0.001), deutan ($\chi^2(2) = 22.40$, *P* < 0.001) and tritan ($\chi^2(2) = 14.93$, *P* < 0.001) confusion axes. Thresholds for the smokers and deprived smokers were higher than the normative values observed in other studies. Therefore, there was a reduction in color discrimination in both groups. The results of the trivector measurements are shown in Figure 1.

Along protan vectors (Figure 1A), pairwise comparisons showed that discrimination thresholds were higher in the group of smokers compared to non-smokers (*U* = 132, *P* = 0.002, *r* = -.61). In addition, deprived smokers had the highest thresholds compared to the group of non-smokers (*U* = 105, *P* < 0.001, *r* = -.85) and smokers (*U* = 136, *P* = 0.002, *r* = -.58).

Along deutan vectors (Figure 1B), when compared with the control group, smokers (*U* = 136, *P* = 0.001, *r* = -.58) and deprived smokers (*U* = 108, *P* < 0.001, *r* = -.83) presented higher discrimination thresholds, with high effect size. There was statistically significant differences between smokers and deprived smokers (*U* = 154, *P* = 0.024, *r* = -.43).

Along tritan vectors (Figure 1C), when compared with the control group, smokers (*U* = 140, *P* = 0.003, *r* = -.55) and deprived smokers (*U* = 126, *P* < 0.001, *r* = -.67) presented higher discrimination thresholds. There was no statistically significant differences among smokers vs. deprived smokers (*P* = 0.250).

Correlations

There is no relationship between color discrimination and gender ($\chi^2 = 72$, *df* = 39, *P* > 0.05). A spearman correlation showed no correlation between FTND and trivector data (*P* > 0.050), color discrimination and education years [$\rho = .078$, *P* = 0.515], and color discrimination and age [$\rho = .096$, *P* = 0.347].

Dataset 1. Patient demographics and Trivector results

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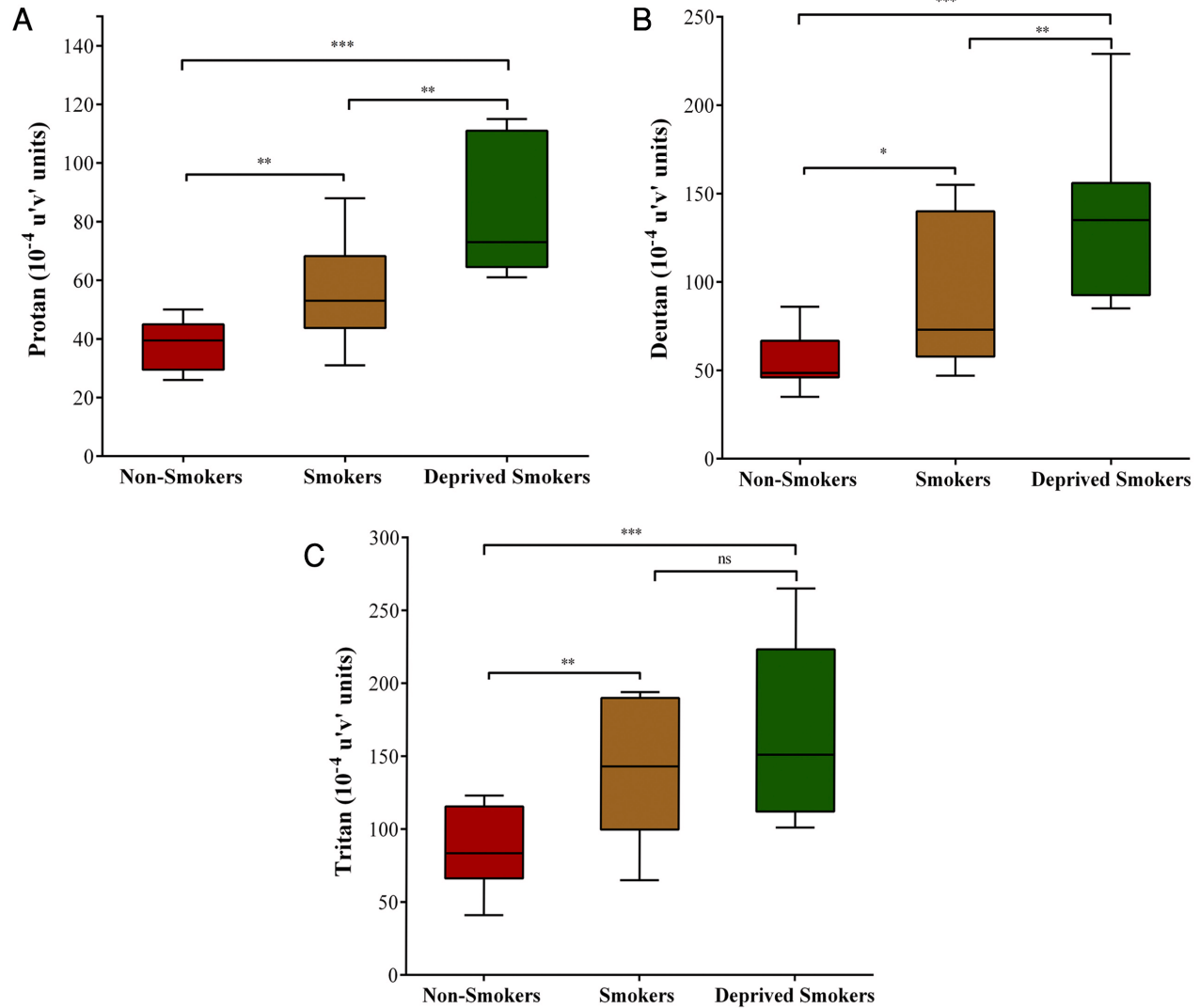


Figure 1. Trivector test: box-and-whiskers plots for protan (A), deutan (B) and tritan (C) confusion lines. Data are presented in 10^{-4} u'v' units. Each box-and-whiskers plot is based on results for 45 participants. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Discussion

The data indicated that smokers groups, as a whole, had higher discrimination thresholds when compared to non-smokers ($P < 0.05$), indicating the existence of a diffuse impairment in visual processing. Results showed good agreement between the normative data of control groups, being the protan and deutan thresholds lower than tritan thresholds, a pattern repeatedly observed in adults tested with the CCT¹⁷. Moreover, the higher thresholds observed in the group of smokers and deprived smokers are in agreement with the differences observed in other studies using CCT. The effect sizes reached medium to high values.

Small differences in blue-yellow color processing suggest that sensor neurons responsive to the short wavelength may differently operate from those responding to medium and long wavelengths²⁷. Indeed, the koniocellular pathway may not suffer from the influences of tobacco components.

Along the trivector protocol, smokers had more errors in protan and deutan confusion axes (Figure 1). An effect size analysis confirmed that smokers had the largest discrimination errors for protan ($r = -85$) and deutan ($r = -82$) confusion axes when comparing against non-smokers. As stated, this result does not support the idea of channel selectivity. However, we base our rationale on the existence of diffuse processing impairment, which may include magno- and parvocellular pathways²⁸.

Nicotine enhances neurotransmission release through modulation of nicotinic acetylcholine receptors (nAChRs) located in the cortex^{18,29}. There are also nAChRs and dopamine receptors on the retina; therefore, the chronic use of cigarette would enhance attentional resources³⁰⁻³². However, there were no improvements in color discrimination. One may argue this could be due to desensitization effect, one of many brain changes caused by addiction³³. Chronic nicotine exposure leads to nAChRs desensitization through brain

upregulation^{34,35}. The more exposure, the greater the need for it activate the receptors^{36,37}. Whereas nicotine enhancing effects remain unchanged after chronic exposure, this may explain the lower discrimination, but the small similarity, between smokers and non-smokers in some of our data (Figure 1).

Then, why did the deprived smokers group have less discrimination? The withdrawal effect, which affect neurotransmission release^{38,39}, reflecting both visual processing⁴⁰⁻⁴² and brain reward function⁴³, may explain this. Visual attention plays a role for detection of environmental stimuli⁴⁴.

As stated, impairments observed at color discrimination can occur due to cones saturation, amplification of the noise that reach visual cortex or by the action of nicotine in parvocellular pathway⁴⁵. In agreement with studies, color vision impairments may be related to ventral stream, which processes color⁴⁶. However, our tests used pseudoisochromatic stimuli. Thus, color discrimination may have occurred through dorsal and ventral stream²⁸. Too soon to conclude anything, but there may be nAChRs in both dorsal and ventral stream⁴⁷ and both streams may suffer from the action of neurotransmission hypofunction, affecting directly visual processing^{39-41,43}.

Knowing the existence of the expression of nAChRs in bipolar, amacrine and ganglionar cells^{29,47,48}, we suggest that smoking affects visual processing, regardless of deprivation. Although the differences between smokers and non-smokers were small, we could not ignore the existence of many harmful compounds to vision in cigarettes. As noted in others studies, exposure to cigarette smoking⁴⁹⁻⁵⁵ and solvents^{56,57} affects vision. Thus, smoking can be harmful even for passive smokers.

Our limitations need to be considered. We evaluated cigarette smoking as a whole, not the nicotine-only effects^{51,52}. Which

brings us to the idea of further studies, using nicotine gum and the same paradigm used here. Clearly, further work is needed, but this study highlights the relationship between smoking and color discrimination, involving short, medium and long wavelengths²⁷. We conclude that cigarette compounds affect vision^{54,55} more than nicotine separately⁵⁸⁻⁶¹.

Data availability

Dataset 1: Patient demographics and Trivector results. Raw data of the subjects biosociodemographic and trivector (protan, deutan and tritan) results. doi, [10.5256/f1000research.10714.d15005962](https://doi.org/10.5256/f1000research.10714.d15005962)

Author contributions

TM: design of the work, data collection and interpretation, and drafting the article. NL: data reanalysis and interpretation and revision of the updated manuscript. NA: design of the work, data analysis and interpretation, and critical revision of the article. All authors approved the final version to be published.

Competing interests

No competing interests were disclosed.

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Open Peer Review

Current Referee Status:  

Version 3

Referee Report 07 August 2017

doi:[10.5256/f1000research.13276.r24707](https://doi.org/10.5256/f1000research.13276.r24707)



Goro Maehara

Department of Human Sciences, Kanagawa University, Yokohama, Japan

The authors have addressed all my concerns. Thanks.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Referee Report 27 April 2017

doi:[10.5256/f1000research.12149.r21404](https://doi.org/10.5256/f1000research.12149.r21404)



Marine Raquel Diniz da Rosa

Neuroscience and Behavior Graduate Program , Federal University of Paraíba , João Pessoa, Brazil

From my point of view, the authors have made relevant changes to the paper and have met all the requests previously suggested. In a way that was reviewed by the authors, it was clarified the cut-off points used for the color discrimination, contributing to a better understanding of the results. In addition, the discussion has improved. Thus, I believe that now the paper should be accepted for publication.

Competing Interests: I am affiliated with The Federal University of Paraíba, where all 3 of the authors, Thiago Monteiro de Paiva Fernandes, Natalia Leandro Almeida and Natanael Antonio dos Santos are also affiliated.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 03 April 2017

doi:[10.5256/f1000research.12149.r21405](https://doi.org/10.5256/f1000research.12149.r21405)

**Goro Maehara**

Department of Human Sciences, Kanagawa University, Yokohama, Japan

I found the manuscript was improved but still have some concerns.

1. Outcome Cutoffs

There are some difficulties to understand the Outcome Cutoffs section. The authors should state clearly how they determined and calculated the cutoff points. I also suggest explaining what the values in parentheses represent and what IQR means (interquartile range). Although the authors mentioned '(mean + median + IQR + upper and lower limits)', the plus signs in this phrase seem a bit odd.

2. The Results Section

Please clearly compare the thresholds with the cutoffs values. Not only the median thresholds for smokers, but those for normal observers also exceeds the cutoff values in the deutan and tritan tests, don't they? I think that the cutoff values might be too strict (low).

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 03 Apr 2017

Thiago P Fernandes, Federal University of Paraiba, Brazil

Adding the cutoff values was just one way to meet the review specifications. If you take a brief look at all papers using Trivector as test, there are no specifications for cut-off values. However, the paper of Paramei *et al.* (2012) compared the color discrimination across four life decades, and used data obtained in Brazil. If it is possible, at least briefly, to read this and other papers using Trivector, you will notice a little of what I am talking about.

In addition, when reading other articles about Trivector, you will observe a simple pattern: **generally, both the control and the observed (experimental) conditions may have values below 100** for the protan or deutan (eg) confusion axes. The values obtained by Mollon & Reffin **unfortunately do not match experimental practice**. The reasons are more varied: type of computer, type of processor used at the time, condition of luminance or characteristic of the study. The following Mollon & Reffin studies used the commercial version called Cambridge Color Test, where we tried to standardize and avoid differences between studies. This standardization could be observed when Paramei *et al.* (2012) **observed that there are not many differences in results for controls both in Brazil and around the world.**

No matter how small the visual processing loss is, the differences that will be classified as impairments will be the statistically significant differences. If it was not clear, I will summarize. Conjecturing: If in one study, for the protan axis, the control group had 50 10^{-4} u' v' units and the experimental one had 80 10^{-4} u' v' units, having a value of $p < .001$, this is considered color impairment. Thus, the experimental group would present damage in color processing. **This is what indicates most, if not 90% of all articles that used the Trivector as a measure.**

I hope it has helped in understanding how the Trivector test works. Even so, I will try to answer your questions below:

1. Outcome Cutoffs

- **There are some difficulties to understand the Outcome Cutoffs section. The authors should state clearly how they determined and calculated the cutoff points.**

This was explained. The mean or median (+ SD or + IQR) for the control group of all the studies that used the Trivector were summed and divided by the number of studies (a simple grand mean / median).

- **I also suggest explaining what the values in parentheses represent and what IQR means (interquartile range).**

"Based on previous studies, cutoff points (median + IQR/2) for the trivector test were established to designate color vision impairment." As stated, median + IQR/2 was established by Paramei *et al.* (2012). This was also explained. In my opinion, there is no need to explain why using IQR/2 or IQR, since they are simply representing the standard deviation in a non-parametric data

- **Although the authors mentioned '(mean + median + IQR + upper and lower limits)', the plus signs in this phrase seem a bit odd.**

I understand your point of view. However, the reason for inserting this comment into the text was to explain to the reader that there are normative values for the Trivector. These values would be means, medians, tolerance values, etc. They can be observed in the reference article quoted in the cutoffs outcome section.

2. The Results Section

- **Please clearly compare the thresholds with the cutoffs values. Not only the median thresholds for smokers, but those for normal observers also exceeds the cutoff values in the deutan and tritan tests, don't they? I think that the cutoff values might be too strict (low).**

As explained, the use of cutoff values was only one way to meet the requests of the reviewers. However, in no study using trivector cutoff values are imposed. This is due to the fact that to consider impairment in color vision it is only necessary to have statistically significant differences and to have medium or high effect size.

Nevertheless, the medians of our results for the control group are "beating" with the cutoff values + IQR/2.

So, the purpose of this comment was to try to show how the trivector works. Just read one or two papers that made use of the test and you will note that the differences need not exceed the set values of 100, 100 and 150 10^{-4} u'v 'units to prove impairments at color vision. Even differences observed in values smaller than 100 10^{-4} u'v 'units are significant differences, since this is a robust and reliable test.

According to Trivector's studies, there is no large or small differences, there is statistically significant differences, with large or small effect sizes. And this was what we observed in our study.

Thank you for the review.

Kind Regards,

Competing Interests: None

Referee Response 06 Jun 2017

Goro Maehara, Kanagawa University, Japan

Dear Thiago,

The authors have solved the major concern in my 1st response through the revision process. Mollon & Regan (2000) set the cutoff values relatively high probably because the test was originally designed for detecting color blindness.

Here we need to tackle the issues about the present cutoff values.

I agree with another reviewer in that the standard of normality is useful for making a comparison between the present and previous studies. Any analysis in a paper must be logical even if authors think a specific analysis is not very important. In the present study, not only the thresholds for the smoker, but also those for normal controls exceeded the cutoff values for the tritan test (and deutan test, too?). The authors need to somehow solve this logical problem.

Thank you for the explanations on the cutoff values. That is, the cutoff values were defined as the average of normal observers' (median threshold + IQR/2) reported by the previous studies (or only by Paramei?). It will be helpful for readers to rewrite the Outcome Cutoff section as the authors commented above.

The cutoff values of (median threshold + IQR/2) mean that 25 % of the population is classified as abnormal. It seems reasonable for me to increase the cutoff values because 25 % of the population is too much. This might be a possible solution.

Another possible solution could be discussing why the thresholds for normal observers exceeded the cutoff values in the Discussion section. The authors could also emphasize the importance of statistical differences there.

If the authors have any idea for workable solutions, please feel free to let me know.

Minor concerns,

1. My previous comment on IQR was misleading. I would like to suggest inserting '(interquartile range)' just after IQR when the authors first use the abbreviation.
2. If the authors used data reported by researchers other than Paramei (2012) for the cutoff value calculation, I suggest including the citations of the studies.

Best wishes,

Goro

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 17 March 2017

doi:10.5256/f1000research.11553.r20562

**Marine Raquel Diniz da Rosa**

Neuroscience and Behavior Graduate Program , Federal University of Paraíba , João Pessoa, Brazil

The article investigates and compares color discrimination in chronic smokers and healthy individuals. The authors found a lower significant color discrimination in chronic smokers.

However, I believe that in order to clarify the cut-off point of color discrimination, authors should write in Methods the standard of normality used to rate low or high color discrimination. In addition, in the description of the results, for better understanding, the authors should explain better the results (which are below the expected) and then the significance of them.

The results suggest a possible, even small, important change to the color discrimination of smokers which deserves attention and should be better studied. Therefore, I believe that the paper should be accepted for publication with the aforementioned suggestions.

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Competing Interests: I am affiliated with The Federal University of Paraíba, where all 3 of the authors, Thiago Monteiro de Paiva Fernandes, Natalia Leandro Almeida and Natanael Antonio dos Santos are also affiliated.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 24 Mar 2017

Thiago P Fernandes, Federal University of Paraíba, Brazil

Dear Marine,

First of all, many thanks for the reading and suggestions for our manuscript.

We will try to answer your questions below:

- *However, I believe that in order to clarify the cut-off point of color discrimination, authors should write in Methods the standard of normality used to rate low or high color discrimination.*

We appreciate the suggestion. These changes were made. We stand by calculating the average of the control group of all studies using Trivector in Brazil and using the normative data for age groups used by Paramei et al.¹

- *In addition, in the description of the results, for better understanding, the authors should explain better the results (which are below the expected) and then the significance of them.*

We appreciate the suggestion. Although the description of Trivector's results is quite directive (for details, see ²⁻⁵), we agreed that the way the results were presented was below expectations. These changes were made.

- *The results suggest a possible, even small, important change to the color discrimination of smokers which deserves attention and should be better studied.*

In agreement. Based on this suggestion, we added a few paragraphs on the importance of the present study, see Introduction and Discussion.

References:

1. Paramei GV. Color discrimination across four life decades assessed by the Cambridge Colour Test. *J Opt Soc Am A Opt Image Sci Vis*. 2012;29(2):A290-297.
2. Lacerda EM da CB, Ventura DF, Silveira LC de L: Visual assessment by psychophysical methods of people subjected to occupational exposure to organic solvents. *Psicol USP*. 2011;22(1):117-145. 10.1590/S0103-65642011005000011
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Competing Interests: None.

Referee Report 10 March 2017

doi:10.5256/f1000research.11553.r20559



Goro Maehara

Department of Human Sciences, Kanagawa University, Yokohama, Japan

The authors measured color discrimination thresholds in chronic smokers and non-smokers using the Cambridge Color Test. The color discrimination thresholds were significantly higher for chronic smokers than non-smokers. Although their methods were scientifically sound, the thresholds for chronic smokers were not high enough to conclude that smoking impairs their color discrimination abilities.

Major concern

1. The thresholds for chronic smokers

According to Mollon & Regan (2000)¹, “normal limits for performance for first examination on the basic “Trivector” test are 100 (protan), 100 (deutan) and 150 (tritan).” The threshold medians for chronic smokers were lower than those values, except for the deutan threshold median for deprived smokers. Although there were statistically significant difference between chronic smokers and non-smokers, the thresholds for non-smokers were very low (about 40, 50, and 80 in 10^{-4} u' v' units for protan, deutan, and tritan, respectively). In addition, the differences in thresholds (about 60) make little change in color appearance.

Taken together, it seems difficult to conclude that smoking impairs color discrimination abilities.

Minor concern

1. Equation 1

I am not sure why Weber contrast (equation 1) needs to be explained in the Methods section. The authors should state clearly how they used the equation in their experiment.

2. Results

The authors just listed the statistical results in the Results section. I suggest describing the results in the more detailed way (ex. thresholds were higher for smokers than non-smokers, $U = 132$, $P = 0.002$, $r = -.61$).

3. 3rd paragraph in the Discussion section

The authors stated that “smokers were more sensitive to protanopic and deutanopic confusion axes.” This sentence is confusing. Which does this sentence mean, “more sensitive than non-smokers” or “more sensitive than to tritanopic axes”?

References

1. Mollon JD, Regan BC: Cambridge Colour Test Handbook (version 1.1). *Cambridge Research Systems Ltd.*2000. [Reference Source](#)

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 10 Mar 2017

Thiago P Fernandes, Federal University of Paraiba, Brazil

Dear Goro Maehara,

We respectfully thank you for the reading and responding to our manuscript.

If I may contest your decision of this manuscript, we respectfully do not agree that it can not be accepted as an acceptable scientific standard. We believe that the data contribute in terms of scientific validity, and because we know that there are few studies on color vision in chronic smoking (and abstinence).

We will try to answer your questions below:

- "Although their methods were scientifically sound, the thresholds for chronic smokers were not high enough to conclude that smoking impairs their color discrimination abilities."

Although we did not observe large differences between the thresholds of the control group and the group of smokers, we agree with Lakens (2013) and Field (2013) noting that the statistically significant differences are consonant with the related effect sizes which fluctuated between mid-to-high values (r values between .50 and .61; chronic smokers x controls). Moreover, when comparing the group of smokers with deprived smokers, we observed that these differences were not so large (r values reaching .50). We do not know if the smoking habit, cigarette compounds or smoking *per se*, are responsible for the decrease in color discrimination.

But there was a loss of color discrimination, suggesting the idea that visual color processing may be diffusely impaired in smokers (Besson et al., 2007; Vallejo, Buisson, Bertrand, & Green, 2005; Zhang, Dong, Doyon, & Dani, 2012). We base this hypothesis on the fact that the many cigarette compounds, including organic solvents in the cigarette smoke, impairs color processing *per se*.

Major Concern

- 'The threshold medians for chronic smokers were lower than those values, except for the deutan threshold median for deprived smokers. Although there were statistically significant difference between chronic smokers and non-smokers, the thresholds for non-smokers were very low (about 40, 50, and 80 in 10–4 u' v' units for protan, deutan, and tritan, respectively). In addition, the differences in thresholds (about 60) make little change in color appearance. Taken together, it seems difficult to conclude that smoking impairs color discrimination abilities.'

Many thanks for the review.

Based on our expertise in the use of Cambridge Colour Test: **the minor the threshold, better discrimination**. If a group (in this case, smokers group) has a higher threshold, this means that they needed more chromatic contrast to detect the stimuli. **Thus, higher thresholds means lower discrimination along confusion axes** (Hasrod & Rubin, 2015).

After the publication of Mollon and Reffin's about the CCT (2000), several studies using Trivector have been published. Including preliminary norms for the use of CCT (Ventura et al., 2003), which was considered by the creators of the CCT on the software website (<http://www.crsLtd.com/tools-for-vision-science/measuring-visual-functions/cambridge-colour-test/>)

We re-checked our Trivector data and compared to several studies and we observed that we're with similar values for control groups. If another group (such smokers or deprived smokers) had higher thresholds, it means that they differ from the standard values and are likely to have color vision impairments.

As shown in the other studies, values for control subjects have fluctuated precisely in the values that we obtained in our data (Costa et al., 2007; Goulart et al., 2008; Paramei, 2012, 2014; Ventura et al., 2002).

Thus, the raising of the threshold of smokers is possibly connected with smoking conditions, since

we've matched all possibly intervenient variables.

Taken together, we can not ignore that, although not as large, the differences were significant in this sample. Based on previous studies, even though there are small differences, they need to be punctuated, since we agree that this is an important area that requires further research.

Minor concern

- 1. Equation 1: I am not sure why Weber contrast (equation 1) needs to be explained in the Methods section. The authors should state clearly how they used the equation in their experiment.

Ops! Many thanks! Since CCT already uses this default setting, we strongly agreed with your review. These changes will be in the second version of the manuscript (we will remove it).

- 2. Results: The authors just listed the statistical results in the Results section. I suggest describing the results in the more detailed way (ex. thresholds were higher for smokers than non-smokers, $U = 132$, $P = 0.002$, $r = -.61$).

Many thanks again. We believe that the way you suggested will facilitate the reader's understanding and will enhance the scientific level of our writing. We fixed it. They will be more descriptive in the next version

- 3. 3rd paragraph in the Discussion section: The authors stated that "smokers were more sensitive to protanopic and deutanopic confusion axes." This sentence is confusing. Which does this sentence mean, "more sensitive than non-smokers" or "more sensitive than to tritanopic axes"?

We appreciate the suggestion and agree that the use of two forms of explanation may actually confuse the reader. We will correct this.

However, when we mention that the smoking group was more sensitive to the protanopic or deutanopic axes, we simply mean that they made more errors than the control group, for example. That is, they needed more chromatic contrast (they were more sensitive) than the comparison group. The confusion axes refer to the red (protanopic), green (deutanopic) and blue (tritanopic) axes. Thus, if any group was more sensitive to the red confusion axis, for example, it means that they possibly had impairments in the processing of this wavelength.

In this way, based on the appointments above, the '**not approved**' status is honestly inconsistent with the content of the work. We ask you to reconsider your decision and we are grateful for the comment, reading, and review of the manuscript.

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Competing Interests: No competing interests were disclosed.

Referee Response 19 Mar 2017

Goro Maehara, Kanagawa University, Japan

Dear Thiago,

I am happy to review the revised manuscript.

Please make it clear that the thresholds of normal observers were comparable with those reported by previous studies using the Cambridge color test.

According to Thornton, Edwards, Mitchell, Harrison, Buchan & Kelly (2005), there is a strong association between current smoking and age-related macular degeneration. This line of studies could strengthen your paper.

Regards,

Goro

Competing Interests: No competing interests were disclosed.

Author Response 24 Mar 2017

Thiago P Fernandes, Federal University of Paraiba, Brazil

Dear Goro,
Many thanks for the quick answer.

Based on your suggestions, substantial changes were made.

We inserted a subsection in the methods where we explained the cutoff points (where the results would be normal and where the discrimination losses would be).

In addition, we better describe the results section, making it clear to the reader that the higher the threshold, the lower the color discrimination. Also, based on your last suggestion, we added a few paragraphs about the relationship between the harmful cigarette compounds and the damage they cause to the retina, and consequently, visual processing.

I hope we have answered the suggestions. Again, we ask you to reconsider your decision about the status of our work.

Best regards,

Competing Interests: None.
