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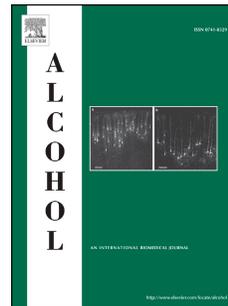
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**No blue-yellow color vision impairment after acute ethanol ingestion**

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## Introduction

Alcohol (ethanol) is the widest used psychoactive substance, known since antiquity. According to the World Health Organization (WHO), 3.3 million people are dying worldwide every year due to harmful alcohol consumption and 5.1% of the global burden of disease can be attributed to alcohol consumption (World Health Organization, 2014). Different alterations of the visual functions are described in chronic alcohol users such as alcohol tobacco amblyopia (Grzybowski, Zulsdorff, Wilhelm, & Tonagel, 2015; Prakash et al., 2011) or acquired color vision impairment. There is no agreement about the the axis of color defects due to alcohol misuse since changes in the red-green and the blue-yellow axis are described in literature (Baron, 1972; Burdick & Chebib, 1982; de Carvalho, Danda, Dantas, Arraes, & Cavalcanti, 2006; Ebran et al., 1988; Francq, Pierart, & Verriest, 1979; Gerhard, 1981; Kapitany et al., 1993; Krumsiek, Kruger, & Patzold, 1985; Krumsiek, Kruger, Wurster, & Patzold, 1985; Mergler, Blain, Lemaire, & Lalande, 1988; Primo, 1988; Sakuma, 1973; Shimozono, Townsend, Ilsen, & Bright, 1998; Smith, 1972; Swinson, 1972; Ugarte, Cruz-Coke, Rivera, Altschiller, & Mardones, 1970).

Only few studies dealt with the acute influence of alcohol ingestion on color vision perception. Adams et al. (Adams, 1976), Russel et al. (Russell, Carney, Feiock, Garrett, & Karwoski, 1980) and Zrenner et al. (Zrenner, Riedel, Adamczyk, Gilg, & Liebhardt, 1986) were able to find a significant color vision impairment after an alcohol intake. Quite contrary to this results, Hill et al. (Hill & Toffolon, 1990) were unable to document any color vision defects in their study.

In the previous studies, blue-yellow color vision was tested by using ordering/arrangement test as the Farnsworth-Munsell 100-Hue test (Adams, 1976; Russell et al., 1980; Zrenner et al., 1986), the Farnsworth 28-Hue-test (Hill & Toffolon, 1990) and the desaturated Panel D-15-test (Zrenner et al., 1986). According to the above-mentioned discordance, this study wants to investigate the acute effect of an alcohol ingestion on blue-yellow color vision in non-alcoholic subjects using short wavelength automated perimetry (SWAP) and an anomaloscope with Moreland equation (Oculus Heidelberg Multi Color anomaloscope).

Anomaloscopes provide a quantitative assessment of color vision. For testing blue-yellow perception/discrimination, the Moreland equation is widely accepted (Mantjarvi, Syrjakoski, Tuppurainen, & Honkonen, 1997; Moreland, 2004; Moreland, Maione, Carta, & Scoccianti, 1978; Muftuoglu, Karel, & Duman, 2007; Rufer et al., 2012; Sommerhalder et al., 1998).

In SWAP, the short wavelenght sensitive S-cones of the retina, which are essential for the perception of blue hues, are isolated by suppressing the other ("red-green") L- and M-cones

and the rods by using a yellow background illumination (Sample, 2000). The S-cones represent the smallest subgroup of cones (approx. 9 %) and are found only parafoveal. They are linked with bistratified retinal ganglion cell projecting in the koniocellular layer of the lateral geniculate nucleus (LGN) (Dacey & Lee, 1994; Szmajda, Grunert, & Martin, 2008). Because of their sparse number, S-cones and the koniocellular pathway, respectively, are suspected to be particularly vulnerable for pathological alteration (Corallo et al., 2005; Makino, 2013; Remky & Elsner, 2005; Terasaki et al., 1999; Zhong, Zhou, Cheng, & Xie, 2010).

## **Material and methods**

18 volunteers (8 male and 10 female) were included. All subjects signed an informed consent prior to experiments. They were aware of the risk of an alcohol ingestion and confirmed by signature not to drive a motor vehicle for 12 hours after the examinations. A pregnancy test was carried out in a urine sample of all female subjects (qualitative immunoassay on human chorionic gonadotropin). Furthermore, the medical history, namely ocular diseases and operations as well as alcohol intolerance or misuse, was collected. A positive pregnancy test, ocular diseases/operations (with the exception of refraction errors) and an alcohol misuse were exclusion criteria.

This study was approved by the cantonal ethics commission of Bern (Decision KEK-Nr. 014/12).

### ***Alcohol ingestion***

The goal of blood alcohol concentration of the subjects was 0.8 g/kg (=‰). The amount of alcohol used to reach 0.8 g/kg was weight-adapted calculated with 1 g per kg bodyweight in males and 0.8 g per kg bodyweight in females. Vodka 37.5 % (Smirnoff®) was used for all subjects. The vodka could be mixed with different soft drinks (e.g. Coke) or orange juice of the subjects' own choice. The alcohol was ingested in a 30 minutes period.

A first blood sample was taken from a cubital vein before alcohol ingestion. In this sample, blood alcohol as well as phosphatidylethanol homologues 16:0/18:1 were analyzed by liquid-chromatography tandem mass spectrometry. Phosphatidylethanol (PEth) is a direct marker for an alcohol misuse. PEth homologues 16:0/18:1 levels >210 ng/mL indicates an alcohol misuse and thus subjects with a PEth concentration above this limit were excluded from the study (Helander & Hansson, 2013). PEth homologues 16:0/18:1 was determined by a validated LC-MS/MS method (limit of quantification 100 ng/mL) (Schröck, Hernandez Redondo, Martin Fabritius, König, & Weinmann, 2016; Schröck, Thierauf-Emberger, Schurch, & Weinmann, 2016).

Alcohol was determined by a fully validated gaschromatographic method in quadruplicate using two GC systems (Aderjan et al., 2011).

The second blood sample was taken before the first examination (perimetry or anomaloscopy) at least 30 min after alcohol ingestion, the third before the second test. Perimetry or anomaloscopy were chosen at random. In all subjects, the right eye was chosen for perimetry and anomaloscopy.

To reduce a learning effect, the subjects could practice – in sober state – perimetry and anomamolscopy for several minutes before performing the study's examinations.

### ***Perimetry***

The SWAP was performed using an Oculus Centerfield 2® perimeter (Goldmann size III, duration of stimulus 0.2 sec, blue stimulus with a transmission of 440 nm, yellow background illumination of 10 cd/m<sup>2</sup> [31.8 asb], threshold mentioned 4/2 strategy, investigation area 10-2, program macula threshold).

The objective refraction used for optimal correction in perimetry was measured by the use of an autorefractometer Nidek® AR-310A. Contact lens wearers were tested with their lenses.

### ***Anomaloscopy***

The blue-yellow color discrimination was examined with an Oculus HMC (Heidelberger Multi Color) anomaloscope using the Moreland equation (blue [436 nm] + blue-green [490 nm] = cyan [480nm] + yellow [589 nm]). The test field was presented under an observation angle of 4°. Every 15 seconds, the examined right eye was adapted on white light (so-called neutral adaptation) for short time. The testing time was not restricted.

### ***Data Analysis***

The data was analyzed using the SPSS Statistics 23 program for Windows (SPSS Inc., Chicago, USA). The results shown in Table 1 and 2 were obtained by using descriptive data analysis. Since not all variables were normally distributed (tested using the Kolmogorov-Smirnov-test), Wilcoxon signed-rank test was used for comparing the tested variables before and after alcohol intake. The level for statistical significance was set to  $p < 0.05$ .

## Results

In 16 of 18 tested subjects, PEth 16:0/18:1 concentration in blood was <210 ng/mL. The 2 subjects with PEth 16:0/18:1 concentration  $\geq$ 210 ng/mL were excluded.

The mean age of the included subjects was 26.3 years (range 20-39). 6 male and 10 female subjects were tested. Table 1 shows the characteristics of the study population.

The mean alcohol concentration was 0.86 +/- 0.20 g/kg while perimetry and 0.84 +/- 0.20 g/kg while anomaloscopy.

In SWAP, no significant difference between the results before and after alcohol intake were found for mean sensitivity (34.00 vs. 34.10,  $p=0.918$ ), mean defect (-0.52 vs. -0.71,  $p=0.351$ ) and for the reliability factor (0.98 vs. 0.98,  $p=0.573$ ). However, the subjects showed a significant higher loss of variance (3.40 vs. 4.63,  $p=0.011$ ). The duration of examination did not differ significantly ( $p=0.642$ ) (cf. Table 2).

In anomaloscopy, the subjects revealed a significant narrower matching range after alcohol intake (43.50 vs. 32.40,  $p=0.017$ ) and as well a significant shorter duration of examination (494 sec vs. 348 sec,  $p=0.014$ ). The match midpoint was just slightly lower (56.23 vs. 53.73,  $p=0.163$ ) (cf. Table 2).

## Discussion

In several studies, the influence of chronic alcohol consumption on color vision has been investigated and acquired color vision deficiencies, both on the red-green and the blue-yellow axis, were described. The reason for this alterations remains unclear: Direct chronic neurotoxic effects of alcohol on the retina and malnutrition in alcoholics are discussed (Brasil et al., 2015).

Four previous studies (Adams, 1976; Hill & Toffolon, 1990; Russell et al., 1980; Zrenner et al., 1986) investigated the acute influence of an alcohol intake on color vision. In all this studies, color vision was tested with ordering/arrangement tests (Farnsworth-Munsell 100-hue test, Farnsworth-Munsell 28-hue test, Lanthony Desaturated Panel D15 test).

Adams et al. (Adams, 1976) in 1976 showed a significant higher amount of errors in the Farnsworth-Munsell 100-hue test after alcohol intake than without any alcohol. Russel et al. (Russell et al., 1980) were also able to detect significantly more errors after alcohol consumption using as well the Farnsworth-Munsell 100 hue test. The errors were predominantly on the blue-yellow-axis.

Zrenner et al. (Zrenner et al., 1986) tested 8 subjects and described higher numbers of errors in the Lanthony Desaturated Panel D15 test and in the Farnsworth-Munsell 100-hue test. The axis of errors was tritanopic.

In opposition to these studies, Hill et al. (Hill & Toffolon, 1990) could not find any color vision defects examining 10 volunteers with the Farnsworth 28-hue test under the influence of acute alcohol ingestion.

Our results support the conclusions of Hill et al.: We could not find a significant alteration in blue-yellow vision using SWAP and an anomaloscope with Moreland equation instead of an ordering/arrangement test.

The mathematically significant higher loss of variance (LV) in SWAP has no practical meaning because an important inhomogeneity of the visual field can be supposed only with values above 25 for the used perimeter (LV before/after alcohol intake 3.40/4.63).

The reliability factor (RF) represents the quality of collaboration of the examined subjects. With acceptable to good collaboration, RF has a value between 0.7 and 1 for the used perimeter (according to the manufacturer's information). In our study, RF was 0.98 in sober

subjects and after alcohol intake. Thus, the collaboration of the examined subjects was good and showed no decrease after alcohol intake.

In anomaloscopy, the subjects showed a significant narrower matching range after alcohol intake (43.50 vs. 32.40,  $p=0.017$ ) and as well a significant shorter duration of examination (494 sec vs. 348 sec,  $p=0.014$ ). The match midpoint was not shifted in a significant degree (56.23 vs. 53.73,  $p=0.163$ ). The narrower matching range and the shorter duration of examination can be interpreted as a result of a learning process. Examinations with anomaloscope using Moreland equation are known to be difficult to perform and can be influenced by factors as a restricted viewing time (Taylor, 1985). It seems that the examined subjects in our study were more familiar with the test in the second turn, perhaps amplified by a higher self-confidence due to the alcohol intake. As mentioned, the good RF in perimetry after alcohol ingestion shows that the collaboration after alcohol intake was not worse than in sober state. The viewing time was not restricted. So we do not doubt the quality of the anomaloscope results.

There are some limitations in the former studies. In ordering/arrangement tests, illumination (spectral composition, illuminance) and testing distance can have an impact on the results. In the above-mentioned studies, conditions of illumination are specified, but different (Hill et al. "standard light conditions", Adams et al. "standard procedures outlined in the instructions", Zrenner et al. "standard illuminant C", Russell et al. "standard illumination using a Mac Beth illuminant C lamp"). One of the advantages of testing color vision with an anomaloscope and with SWAP are the strictly defined and reproducible conditions of testing.

In the studies of Hill et al., Russel et al. (control group), Zrenner et al. and Adams et al., an alcohol misuse in the study subjects was excluded only by using anamnestic information. The objective exclusion of subjects with an alcohol misuse based on an objective parameter – phosphatidylethanol concentration in blood – is a great advantage of our study. We can be sure, that our study subjects do not misuse alcohol. This fact is of importance because of the well-known color vision alterations in chronic alcohol consumers. The minimization of a harmful alcohol consumption is a well-known problem in addiction medicine.

The mean blood alcohol concentrations (BAC) in our study was comparable to this in the paper of Hill et al. (0.28-1.00 g/L) and slightly lower than that in the study of Russell et al. (1.24/1.11 g/L) and Zrenner et al. (0.7-1.6 g/L). Strictly, it can only be concluded that BAC in the range of 0.8 g/L does not have an impact of blue-yellow color vision.

The fact that Adams et al. examined not only the acute effect of alcohol intake but also the effect of marijuana relativizes their results: All tested subjects were social drinkers as well as regular marijuana smokers. Therefore it is not sure if the described color vision deficiency is the result of alcohol intake or if it is at least influenced by the chronic effects of the marijuana. In chronic marijuana users, alterations of color vision are described (color intensification of objects, dimmed color, narrower matching range for red-green discrimination) (Dawson, Jimenez-Antillon, Perez, & Zeskind, 1977; Lerner, Goodman, Rudinski, & Bleich, 2011).

Our study has some limitations. As in all four previous studies dealing with the acute influence of alcohol ingestion on blue-yellow color vision, the number of tested subjects is rather low. Furthermore, the time period between alcohol ingestion and tests is short. But an exaggerated response to alcohol because of the short time for adaption was not observed in our study. We used other methods to evaluate color vision – SWAP and anomaloscope instead of ordering/arrangement tests. This could be a possible explication for different results than Zrenner et al. and Russell et al. Otherwise, results are comparable with those of Hill et al., also using arrangement tests.

## Conclusions

In our study, the influence of an acute alcohol ingestion on blue-yellow vision was examined for the first time by using SWAP and anomaloscopy (Moreland equation). In the range of 0.8 g/kg BAC, no blue-yellow vision deficiencies could be demonstrated. In further studies, the effect of higher BAC on blue-yellow vision should be investigated by different methods. It seems important, to fix the physiological target of such an effect (e.g. photoreceptor, pre/post lateral geniculate nucleus processing), if found.

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<b>Sex, n (%)</b> male female	6 (37.5) 10 (62.5)
<b>Age [years], mean (range)</b> all male female	26.3 (20-39) 23.8 (21-26) 27.8 (20-39)
<b>Weight [kg], mean (SD)</b> all male female	70.5 (13.9) 78.0 (7.4) 66.1 (15.3)

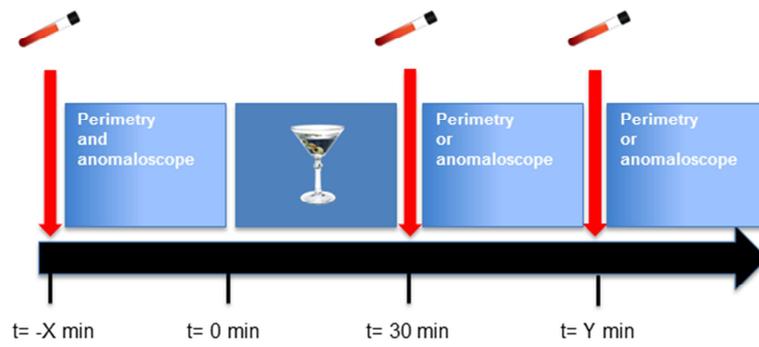
Abbreviations: SD = standard deviation

Table 2 Results

	Before alcohol intake	After alcohol intake
<b><u>Perimetry</u></b>		
<b>Blood alcohol concentration</b> [g/kg], mean (SD)	0.00	0.86 (0.20)
<b>Mean sensitivity (MS)</b> , mean (SD)	34.00 (1.47)	34.10 (1.08)
<b>Mean defect (MD)</b> , mean (SD)	-0.52 (1.50)	-0.71 (1.05)
<b>Loss of Variance (LV)</b> , mean (SD)	3.40 (1.28)	4.63 (1.11) *
<b>Reliability factor (RF)</b> , mean (SD)	0.98 (0.03)	0.98 (0.03)
<b>Duration of examination [sec]</b> , mean (SD)	472 (85)	476 (98)
<b><u>Anomaloscope</u></b>		
<b>Blood alcohol concentration</b> [g/kg], mean (SD)	0.00	0.84 (0.20)
<b>Matching range</b> , mean (SD)	43.50 (17.63)	32.40 (20.74) *
<b>Match midpoint</b> , mean (SD)	56.23 (5.29)	53.73 (5.92)
<b>Duration of examination [sec]</b> , mean (SD)	494 (229)	348 (137) *

Significant differences ( $p < 0.05$ ) between the results before ethanol intake and the results after ethanol intake are indicated by \*

Abbreviations: SD = standard deviation



$t = -X$  min and  $t = Y$  min depend on the required examination time

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- An acute effect of an ethanol ingestion leading to a blood alcohol concentration of app. 0.8 g/kg on the blue-yellow color vision is studied using short wavelength automated perimetry and anomaloscopy with Moreland equation.
- This is the first time that an effect of an acute ethanol ingestion on blue-yellow color vision is studied by using short wavelength automated perimetry and an anomaloscope.
- An misuse of alcohol was excluded by analyzing phosphatidylethanol homologues 16:0/18:1.
- Ethanol ingestion leads not to acute color vision deficiencies on the blue-yellow axis.