2013

Effects Of Nicotine On The Human Visual System: 1) Color Perception; 2) Processing At The Cortical Level

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EFFECTS OF NICOTINE ON THE HUMAN VISUAL SYSTEM: 1) COLOR PERCEPTION; 2) PROCESSING AT THE CORTICAL LEVEL

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A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

BIRMINGHAM, ALABAMA

2013

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ABSTRACT

The visual system is sensitive to various types of chemical substances, including nicotine. Nicotinic acetylcholine receptors (nAChRs) are found in the nervous system, including the retina. These receptors play a role in modulating signals within the visual system. It has been shown that smoking affects color vision, and visual evoked potentials (VEPs). But few studies have been devoted to understanding the effects of nicotine alone.

The purpose of this research was to examine the effects of oral administration of nicotine in human adults, who are non-smokers, on color vision, and visual cortical processing. Previous studies on adult smokers revealed that smokers differ in their color discrimination threshold compared to non-smokers, with higher error scores and thresholds on color tests when compared to light smokers or non-smokers. Also, smoking alters the responses of the cortical visual pathways measured by VEP, showing shorter latencies, and amplitudes increased.

A group of non-smokers participated in this study. Subjects were given a comprehensive eye examination to determine eligibility. All subjects attended a minimum of two visits. Two doses of Nicorette nicotine gum (2 and 4 mg) were given to each subject, one dose per visit. We conducted two separate experiments: 1) color vision; 2) VEP. For each experiment subjects were tested twice at each visit: 1) baseline; 2) 30 minutes after chewing nicotine gum.

Color testing revealed an overall improvement in sensitivity to the Farnsworth-Munsell 100 hue test (FM-100) with nicotine. Total error score (TES) decreased on the FM-100. Also, by using an increment threshold test, sensitivity increased with nicotine. Visual evoked potential recordings revealed no changes in latency or amplitude of the P100 peak of the transient VEP. Nicotine had both inhibitory and excitatory effects on lateral interactions in the cortex (1^{st} and 2^{nd} harmonics). In addition, increases in amplitudes were observed only in the magnocellular pathway. Model fits to the data also indicated in cortical shunting inhibition and initial conductance. We therefore assume that nicotine differentially alters the projected signals from the different visual pathways and systems. Also, the findings demonstrate that nicotine plays an effect in modulating both excitatory and inhibitory responses.

Keywords: nicotine, visual cortex, color vision, visual evoked potentials, intraocular pressure, cardiovascular.

ACKNOWLEDGMENTS

I am grateful to my advisor and mentor, Dr. E. Eugenie Hartmann, for her encouragement and support throughout the years. I am thankful for her continuous efforts and guidance.

I am thankful for the continuous support of my parents, my wife Hala, and my children Talal and Tuleen. I am especially thankful for their support throughout the tough times I have encountered in the previous years. Being grateful, I am hoping that I can reimburse them for every minute of our journey. I could not do it without them. They are the ones that kept me going!

I also thank my committee members for their guidance, patience, and efforts, to make this project work, by devoting their time and resources. I am privileged to be able to do this work side-by-side with some of the best and most professional individuals in the field. To all of you, thank you.

I thank the University of Alabama and the School of Optometry Clinical Research Council and the various faculty and staff members for providing additional support.

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LIST OF ABBREVIATIONS

φ_0	initial phase
AC	amacrine cell
ACh	acetylcholine
AChRs	acetylcholine receptors
BC	bipolar cell
Bgt	Bungarotoxin
BP	blood pressure
BPM	beats per minute
CGC	contrast gain control
C_{mean}	contrast mean
CNS	central nervous system
d_0	depth of threshold
DFT	discrete fourier transform
DOM	depth of modulation
EEG	electroencephalogram
ERG	electroretinogram
F1	1^{st} harmonic
F2	2^{nd} harmonic
FI	facilitation index
FM-100	farnsworth munsell 100- hue test

g_0	initial conductance
GABA	gamma-aminobutyric acid
GC	ganglion cell
HC	horizontal cell
HR	heart rate
H.R.R.	Hardy-Rand-Rittler
IC	isolated-checks
IOP	intra ocular pressure
LGN	lateral geniculate nucleus
LWS	long-wave sensitive
m	shunting coefficient
M	magnocellular
mAChRs	metabotropic acetylcholine receptors
MB	magno-bright
MD	magno-dark
MWS	medium-wave sensitive
nAChRs	nicotinic acetylcholine receptors
P	parvocellular
P100	positive peak at 100 ms.
PB	parvo-bright
PD	parvo-dark
PNS	peripheral nervous system
PRs	photoreceptors

PVN	paraventicular neurons
PW	partial-windmill
RF	receptive field
RGC	retinal ganglion cell
RT	red test
SI	suppression index
SNR	signal to noise ratio
SON	supraoptic nucleus
SSVEP	steady-state vep
SWS	short-wave sensitive
TES	total error score
V1	visual cortex
VEP	visual evoked potential
WD	windmill-dartboard

GENERAL INTRODUCTION

Retinal Information Processing

Vertical connections

The retina, a light sensitive tissue lining the back of the eye, has two types of photoreceptors (PRs): rods and cones. These receptors capture photons of light and initiate a cascade of signals resulting in changes in membrane potential. Photoreceptors contact bipolar cells (BCs) and release glutamate in a graded fashion by an amount proportional to their degree of depolarization. Glutamate binds to specific receptor sites on the BCs, which depolarize or hyperpolarize depending on the specific glutamate receptors. The main role of BCs is to transmit the signals from the PRs to the ganglion cells (GCs). The BCs produce only graded potentials and like the rods and cones, they release glutamate in an amount proportional to their degree of depolarization. Ganglion cells produce action potentials when the release of glutamate by the BCs that contact them is sufficient to reach threshold. These action potentials are transmitted to the brain via the axons of the optic nerve. In addition to these straight-through vertical connections, the retina has lateral connections via the horizontal cells (HCs) and amacrine cells (ACs).

Lateral connections

Both HCs and ACs process signals in a lateral direction. Horizontal cells receive input from one or more PRs and contact BCs that are innervated by other PRs. They also

provide feedback onto PRs. Horizontal cells release the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) in a graded fashion, when depolarized by PRs. The inhibitory neurotransmitter GABA hyperpolarizes the BCs, causing them to release less glutamate onto the GCs that they contact. Similarly, ACs contribute to the modulation of the vertical pathways by mediating lateral interactions between terminals of BCs and dendrites of GCs (Kolb et al., 1992). Amacrine cells receive input from one or more BCs and contact GCs that receive input from other BCs. Similar to HCs, ACs release inhibitory neurotransmitters in a graded fashion, hyperpolarizing the GCs they contact, making them less likely to fire an action potential. The various stages of retinal processing ends with different classes of GCs, some of which increase their firing rate in response to light and some which decrease their firing rate in response to light.

Signal transmission

The retina collects and transduces light to generate visual information. The signal that is transmitted to the brain goes through multiple stages of processing before it enters the optic nerve. Signals from multiple rods converge onto BCs and then to a population of GCs. This process of amplification with convergence results in a reduction of acuity.

Cones in the fovea provide one-to-one input, and then to a small number of GCs. This preserves information about the details of a visual image. Light energy from different parts of the visual field falls on different parts of the retina. Therefore, each retina contains a two-dimensional representation of visual space. This visual space of a neuron, called the receptive field (RF), is the region of space in which a stimulus will affect the response of that receptor or neuron (Hartline, 1938). While rods and cones are

the major PRs with light sensitive pigments in the eye, other parts of the eye and brain contribute to visual processing.

Rods and Cones

The detection of light occurs in a PR organelle called the outer segment, a membranous structure where light absorbing proteins, opsins, are embedded. There are two major types of PRs in all vertebrate eyes, rods and cones. Rods are very sensitive to light but slow, and their response saturates at light levels where cones function optimally. Rods are not used much in modern society where artificial illumination adequate for cone vision is ubiquitous. Cones are less sensitive to light, but are fast and can adapt to the brightest lights, being almost impossible to saturate. Cones evolved before rods, undoubtedly in areas of strong sunlight where vision was a great advantage (ref or delete). In broad sunlight, shadows are strong and more important to detect than increments of lights. Differences in contrast depolarize cones leading to a release of a transmitter that influences second order retinal neurons (BC, AC, HC). The appearance of light hyperpolarizes cones leading to a curtailing of this transmitter. To preserve spatial details, cones transmit their information through relatively convergent pathways (cone to midget BC to midget GC). This private line involves two midget cells (Kolb, 1970). The axons of these BCs extend in parallel to contact the arbors of ON and OFF midget GCs (Polyak, 1941; Boycott and Dowling, 1969).

Retinal Ganglion Cells (RGCs)

Retinal GCs transmit information via the optic nerve tract to the thalamus and other areas in the brain. There are at least five classes of RGCs that differ in morphology, size, connections, and response to visual stimulation (midget, parasol, photosensitive GCs, bistratified, and others projecting to the superior colliculus) (Leicester and Stone, 1967; Boycott and Dowling, 1969; Lin et al., 2004). Gouras (1968) was the first to show that the primate retina contains two populations of RGCs. He reported that some population discharges were phasic while others were tonic. In 1981, Leventhal et al. reported that phasic cells corresponded to parasol cells, while tonic cells corresponded to midget cells of RGCs.

These names (midget, parasol) refer to the sizes of the dendritic trees and cell bodies. The majority (80%) are midget P cells (Polyak, 1941) that project to the P layers of the lateral geniculate nucleus (LGN) (Goodchild et al., 1996). Parasol M cells, on the other hand, project to the M layers of the LGN (Polyak, 1941) and account for 10% of RGCs (Leventhal et al., 1981; Perry and Cowey, 1984). Both cell types can be either ON or OFF cells. The dendritic fields of human ON-center parasol and midget cells are 30-50% larger in diameter than their OFF-center counterparts, suggesting a distinct asymmetry in the human ON-OFF visual pathways (Dacey and Petterson, 1992).

Magno- (M) & Parvocellular (P) Pathways: Characteristics of M & P Cells

The visual system from the retina to the LGN and to the cortex has been shown to be composed of several parallel pathways, the main two being the M and P pathways. Cells of the M pathway respond optimally to motion, low contrast and high temporal

frequencies, while P cells respond to high spatial frequencies, convey spatial resolution, color information, and respond well to higher levels of contrast. Previous research has shown that most P cells are color opponent (De Valois et al., 1966; De Valois, 1977; Wiesel and Hubel, 1966; Gouras 1968, 1969), while M cells are more sensitive to luminance contrast (Scobey, 1981; Shapley et al., 1981; Kaplan & Shapley, 1982).

Recent data indicate that the ratio of M to P is constant across the retina (Perry & Cowey, 1984; Silveira & Perry, 1991). The larger receptive field size of M cells compared to P cells is the reason for their increased sensitivity to luminance contrast (Kaplan & Shapley, 1986). Both M and P cells have nonlinear temporal response properties, although nonlinearity is more pronounced in the M cell population (Kaplan & Shapley, 1986; Benardete & Kaplan, 1997, 1999).

The LGN

The LGN is the nucleus of the thalamus that receives the bulk of the retinal output. The LGN has six layers and each layer receives input from one hemifield of each eye: three layers for the left eye and three for the right eye. Cells of the left eye project to layers 1, 4 & 6 of the right LGN, and the right eye GCs project to its layers 2, 3 & 5 (Nicholls et al., 2001).

The ventral part of the LGN is comprised of two M layers receiving input from the M pathway, while P layers occupy the 4 dorsal layers and receive input from the P pathway. Both M and P layers contain a mixture of ON and OFF cells, and both layers send their signals to the visual cortex (V1).

Organization

V1 consists of 6 layers with layer 4 divided into 4A, 4B, 4Cα, and 4Cβ. Among the cells in layers 2-6, 20% are GABAergic interneurons (Hubel and Weisel, 1972). Perceptual, anatomical and physiological studies suggest some degree of separation in the M and P pathways up to the level of V1 (Kaplan & Shapley, 1986; Kaplan et al., 2008). Cells in the M layers of the LGN project to layer 4Cα of V1. Cells in 4Cα then project to layer 4B, which in turn project to area MT. Cells in the P layers of the LGN project to layers 4Cβ and 4A. Cells of 4Cβ layer project to V2 and V4.

Nicotine & nAChRs

Acetylcholine (ACh) and Acetylcholine Receptors (AChRs)

Acetylcholine is a major neurotransmitter in both the central nervous system (CNS) and the peripheral nervous system (PNS) in humans and other organisms. There are two main classes of AChRs named for the ligands used to activate the receptors: nicotinic AChRs and muscarinic acetylcholine receptors (mAChRs). Nicotinic acetylcholine receptors are expressed in non-neuronal cells, as well as in cells of the CNS and PNS (Grando et al., 1995; Macklin et al., 1998). These receptors are ionotropic receptors that can be activated by the endogenous neurotransmitter ACh or exogenous drugs such as nicotine.

nAChRs: Subtypes and Subunits

Nicotinic acetylcholine receptors are classified into two subtypes based on their sites of expression (muscle type and neuronal type). The receptors are composed of five subunits (pentamers) (Purves et al., 2008). Muscle types of nAChRs have α, β, and three

other subunits; neuronal type can be homomeric (α*7,* α*9)* or heteromeric combination of α and β **(**α*2*- α6 and β2- β4) (McGehee, 1999; Lindstrom, 2000; Dani, 2001).

The neuronal nicotinic receptor α*7* is usually homomeric. The homomeric type has lower ACh affinity (Severance et al., 2004) and is sensitive to α-Bungarotoxin (Bgt), a krait snake venom (Albuquerque et al., 1974). The heteromeric type usually has higher ACh affinity and is insensitive to αBgt. Much of our knowledge about the functions of discrete subtypes of nAChRs is based on indirect clues obtained from behavioral studies of agonists and antagonists on animal models.

Expression of nAChRs

Animal studies have revealed the expression of subunit compositions of nAChRs on the retinal afferents of the LGN and superior colliculus (Gotti et al., 2005), the retina (Feller, 2002), the nucleus of the supraoptic (SON) (Zaninetti et al., 2000), the hippocampus (Paylor et al., 1998), and V1 (Schröde et al., 1989). In V1, both mAChRs (Tigges et al., 1997) and nAChRs (Han et al., 2000) are expressed at significant levels. Studies have shown that nAChRs are located at five primary locations on neurons: cell soma, dendrites, preterminal axon regions, axons terminals, and myelinated axons on the neurons (Albuquerque et al., 1985; Alkondon and Albuquerque, 2004; Gotti et al., 2005). Combined electrophysiological and pharmacological studies have shown that nicotinic agonists acting on different presynaptic nAChR subtypes can produce an increase in GABAergic as well as glutamatergic transmission in the LGN (McMahon et al., 1994; Guo et al., 1998).

Effects of Ach & Nicotine on Cortical Activity

Gotti et al.'s studies in 2005 and 2007 have shown that nAChRs exist in the retina. Multiple nAChR subtypes were found to be expressed on retinal afferents of the superior colliculus and LGN**,** mainly α6β2 and α4 (nonα6)β2. Another study, by Zaninetti (2002), looked at whether paraventricular neurons (PVN) possess functional AChRs by exploring nicotinic agonists' sensitivity in M and P cells of PVN and SON in rats. Acetylcholine, when locally applied by pressure microinjection in the presence of the muscarinic antagonist atropine, evoked a rapid inward current in the PVN magno endocrine neurons. The current persisted but was reversibly suppressed by selective antagonist of α7-containing nAChRs, but insensitive to antagonist acting on non-α7 nAChRs. Nicotinic currents could not be evoked in PVN P cells, suggesting they are devoid of functional nAChRs. The M cells in both PVN and SON were found to be similar, and Zaninetti concluded that M but not P cells in PVN and SON are sensitive to nicotinic agonists. Extracellular recordings (Barker et al., 1971; Dreifuss and Kelly, 1972; Gribkoff et al., 1988) suggested that ACh could increase excitability of SON and these effects could be mimicked, in part, by nicotine or blocked by nicotinic antagonists. Armitage et al. (1969) examined the effects of nicotine on electrocortical activity and ACh release from the cat cerebral cortex. They reported that large doses of nicotine administered intravenously caused either an increase or a decrease in cortical activity, accompanied by an increase or decrease in ACh release. The effect of nicotine on cortical activity could be due to changes in ACh output, which were small but prolonged. Armitage et al. reported that high dose of nicotine injection (90ug/kg) caused a decrease in ACh output, which may be associated with convulsing activity. It has previously been

reported, in cats, that convulsing drugs such as leptazol causes an increase in the release of ACh (Mitchell, 1963; Beleslin et al., 1965).

Exploring nAChRs in V1

In 2007, Disney et al. examined localization and function of nAChRs in V1 of the macaque monkey. The authors reported that nAChRs were expressed in V1, with layer 4C of V1 the principal recipient from the thalamus onto excitatory cells. Application of nicotine showed enhanced response gain in layer 4C with both dose dependent and transient changes. They reported that 50% of the 18 sites of layer 4C recorded reflected M pathway input. These excitatory changes were seen in both 4Cα and 4Cβ layers. Low contrast detection was enhanced with nicotine, and β2 nAChRs, were the receptor affected in 4C layers. On the contrary, nicotine suppressed activity in layers 2, 3, and 5. The extent to which the effect seen was from α7 was unknown, but the authors observed α7 nAChR subunits in a band of layer 4C. α7 has 5 times lower affinity for Ach compared to β2, but because they have lower affinity, it may be possible that only few cells at a time were desensitized and therefore α7 receptors could have contributed to the overall effect.

Disney et al. concluded that their observations were mostly due to ionotropic nAChR activation because the responses were elicited in less than 200 milliseconds, which would be too slow for mAChRs. Data from Disney's study were consistent with conclusions made by Mrzljak et al. (1996) that ACh may suppress activity in the P-pathway, which arrives in layer 4cβ. Mrzljak (1996), studying V1 in macaque monkeys

revealed mAChRs located in layer IV originating from the P pathway and in cytochrome oxidase compartments in layers II and III in V1.

Nicotine Absorption

As nicotine enters the body, it is distributed quickly through the bloodstream and crosses the blood-brain barrier. The effects of nicotine are influenced by the rate and route of dosing and by the development of tolerance. On average it takes about seven seconds for inhaled nicotine to reach the brain (Benowitz, 1996). The amount of nicotine released into the body tends to be much greater for chewing and dipping tobacco than smoking tobacco (Benowitz, 1996).

The amount of nicotine absorbed by the body from smoking depends on the type of tobacco, whether the smoke is inhaled, and whether a filter is used. The elimination half-life of nicotine averages 2-3 hours (Macdonald & Olson, 1994). Thus, nicotine levels accumulate over 6-8 hours during regular smoking or nicotine dosing (Benowitz, 1996). However, there is also a very long terminal half-life, 20 hours or more (McDonald & Olson, 1994), which reflects the slow release of nicotine from body tissues.

After inhalation, nicotine passes rapidly into the arterial bloodstream and to the brain. Rapid delivery of nicotine to the brain allows the smoker to titrate the dose of nicotine from a cigarette to achieve a particular desired effect without toxicity (Benowitz, 1996). Benowitz reported that nicotine from chewing gum and nasal spray are absorbed more rapidly than from transdermal patches. Differences between individuals in nicotine absorption may be due to a variety of factors including individual differences in saliva pH, rate of salivation and expectoration, and differences in mucosal characteristics (Fant

et al., 1999). Henningfield et al. (1990) found that rinsing with acidic beverages such as coffee or cola before chewing nicotine nearly eliminated nicotine absorption.

Benowitz et al. (1988) compared absorption and vascular effects between cigarettes and smokeless tobacco use. Ten male subjects who were habitual smokers were asked to abstain from smoking overnight. Two pieces of nicotine gum were given to each subject to chew over 30 minutes (2 mg x 2). Absorption of nicotine from nicotine gum occurs primarily across mucous membranes and is maintained at a low, more constant rate during most of the period of chewing. Even though the gum was similar in nicotine concentration to that of a cigarette, the absorption from gum showed slower increase over 90 minutes compared to that of cigarettes.

Effects of Smoking on the Visual System

Impact of Smoking

Smoking is a serious public health problem worldwide. The mood altering and rewarding effects of nicotine are the primary reason people use tobacco products (Benowitz, 1986). Smoking is a risk factor for many disorders and systemic diseases, some of which affect the eye such as age-related macular degeneration (The Eye Disease Case-Control Study Group, 1992), Grave's disease (Hagg & Asplund, 1987), hypertension and cataract (Clayton et al., 1982; Harding &Van Heyningen, 1988).

Tobacco Smoke Composition

Inhaled smoke contains many harmful substances, such as tar, carbon monoxide, hydrogen cyanide, heavy metals and free radicals (National Research Council (U.S.),

1978). Each of these damages the body in various ways. Tar is sticky and brown, containing many chemicals known to be carcinogenic, including benzopyrene. The thousands of substances that result from tobacco consumption makes it difficult to identify specific substances responsible for causing diseases related to smoking.

Receptor Occupancy and Desensitization

Because prolonged binding of nicotine to nAChRs is associated with desensitization and up-regulation of these receptors (increase in the number of receptors), Brody et al. (2006) suggested that the full occupancy of nAChRs caused by smoking also maintains the receptors in the desensitized state.

Effects of Smoking on the Brain

Studies on human adult smokers have shown that nAChRs tend to up-regulate or increase in their expression when chronically exposed to nicotine (Celie et al., 2004; Perry, 1999). In the smoker's brain, up-regulation can increase high-affinity binding sites 4-fold (Perry, 1999). The increased expression of this receptor accounts for the increase in nicotine binding sites following nicotine administration (Celie et al., 2004). It is interesting that nAChRs are the first measurable receptor type to show a decline in the brain of older mammalians, and in degenerative disorders such as Alzheimer's disease (Kellar et al., 1987). It has been shown that nAChRs can be involved in inhibitory and disinhibitory mechanisms such that GABA release can increase inhibition, and blocking nAChRs expressed by GABAergic cells can result in disinhibition (Alkondon et al., 2000). Specifically, interneurons of the cerebral cortex express α*7* and α4β2-like

receptors and activation of the preterminal or presynaptic terminals of these cells triggers post-synaptic currents of GABA suggesting a role in inhibitory circuits (Alkondon et al., 2000).

Cholinergic Impact at the Cortical Level

Armitage et al. (1966) examined the effects of nicotine and some nicotine- like compounds (e.g. nicotine hydrogen tartrate, neostigmine, nicotine monomethiodide, nornicotine) when injected into the cerebral ventricles of cats. They noticed an increase in ACh release and desynchronization of electroencephalographic (EEG) signals. In a similar study, Armitage et al. (1969) examined the effects of nicotine on electrocortical activity and ACh release from the cat cerebral cortex. Given intravenously, a higher dose of nicotine (4 mg/kg every min for 20 min), given less frequently, caused desynchronization of the electrocorticogram (recorded from the exposed surface of the brain), indicating cortical activation. This larger dose leads to an increase, in some experiments, or a decrease, in others, in cortical activation (therefore, an increase or a decrease in Ach release output release). Armitage's study suggests that opposite effects could be explained by the depression of different areas or as a result of stimulation of inhibitory mechanisms (i.e. GABAergic).

Effects of Tobacco Products and Nicotine on the Vascular System

Nicotine causes blood vessels to constrict (Benowitz, 1986). The resulting restriction of blood flow through constricted vessels causes an increase in blood pressure (BP) and an elevation of the heart rate (HR) (US Department of Health and Human

Services, 1988). The constricting effect of nicotine magnifies the risks of plaque accumulation. Nicotine and carbon monoxide, which are absorbed during smoking, have opposite vasoactive properties. Nicotine produces constriction of the peripheral vasculature and increases the peripheral resistance to flow, however carbon monoxide is a cerebral vasodilator. It was reported that HR usually levels off or declines about 15 minutes after administration of nicotine, despite continued increases in nicotine plasma concentration (Fant et al., 1999; Benowitz et al., 1988).

Smoking or Exposure to Smoke

Smoking causes morphological and functional changes to the lens and retina. It also enhances the generation of free radicals (a molecule with one or more non-paired electron in its outer orbit making it active and unstable) and decreases the levels of antioxidants in the blood circulation, aqueous humor and ocular tissue.

Cigarette smoking reduces plasma antioxidant levels, a substance in the blood stream that protects retinal cells. Smoking also causes the protective layer between the retina and blood vessels to erode, resulting in poor circulation, irritation and scarring (Kaspari, 2006). Smoking exposes the body to free radicals, which causes cellular damage and can lead to decreased circulation and exchange of nutrients to the retina and lens. Smoking also oxidizes cholesterol, causing fat to build up in the arteries, which can lead to heart attack, stroke, and possibly macular degeneration.

Effects of Smoking on Intra Ocular Pressure (IOP)

Intra ocular pressure is the pressure inside the eye. Normal IOP is in the range of 10-21 mmHg. In a study involving a sample of women with primary open angle glaucoma, Zanon-Moreno et al. (2009) reported significant increased levels of interleukin-6 (IL-6) as an inflammation marker as well as an increase in the levels of apoptosis markers in the aqueous humor and plasma samples of those who continued to smoke compared with ex-smokers and non-smokers.

Nasal snuff is a finely ground, flavored tobacco, taken in the form of a sniff into the nostrils. In 2002, using nasal snuff, Iyamu et al. studied IOP changes in two groups: smokers and non-smokers. Smokers abstained for 24 hours before testing. After sniffing 40 mg of smokeless tobacco, IOPs peaked at one minute and then gradually decreased a few minutes later. The difference in mean IOP between smokers and non-smokers before inhaling smokeless tobacco was significant. Iyamu et al. concluded that smokers are at higher risk of developing glaucoma than non-smokers. They also suggested that the transient IOP elevation in response to nasal snuff might be enough to destroy the optic nerve over time and lead to glaucoma. Furthermore, it would be difficult to observe transient hypertension during regular examinations.

The Blue Mountains Eye Study (Lee et al., 2003) examined 3654 residents and found that smokers had slightly higher, yet significant mean IOP (16.34 mm Hg) difference than non-smokers (16.04 mm Hg). Timothy and Nneli (2007) recruited young students who were non-smokers (18-30 years of age) to test the relationship between smoking and IOP. Subjects were given 56 cigarettes to smoke, at a rate of two cigarettes per day at three hours intervals for one month. Their IOPs were monitored daily in the

clinic, before and after smoking, for 30 days. They reported a strong correlation (r= 0.96) between increased IOPs and smoking. Similar reports by Tamaki et al. (1995) found that smoking increases tissue blood velocity in the optic nerve head and possibly in the choroid in habitual smokers whose length of smoking history is on average 10 years.

Effects of Smoking on HR/BP

Benowitz (1982) reported that in healthy individuals, smoking increases HR by 10-20 beats per minute (BPM) and BP by 5-10 mmHg. No correlation was found between nicotine blood concentration and HR. Using nicotine gum, HR increased by 12 BPM, and systolic BP increased by 10 mmHg (Nyberg et al., 1982). Similarly, Benowitz injected healthy male subjects with either nicotine or salt water (Benowitz, 1986). Heart rate reached a maximum 5 min after low volume nicotine infusion and then plateaued, which is consistent with adaptation to nicotine.

Sympathetic neural stimulation by nicotine results in HR acceleration and a transient increase in BP, associated with an increase in cardiac output (Nyberg et al., 1982; Metz et al., 2004). Blood flow to various vascular beds is differentially affected, with constriction of blood vessels in the skin and dilation of skeletal muscle blood vessels. Tolerance to HR acceleration develops fairly rapidly. Some in vitro studies have suggested that nicotine reduces vascular synthesis of prostacyclin, an endothelial cell-derived local vasodilator and antiplatelet hormone (Benowitz, 1996). Reduced prostacyclin synthesis could aggravate tissue ischemia. Nyberg et al. (1982) tested the effects of chewing nicotine gum in healthy non-smokers. They found that nicotine mostly affects systolic pressure and HR. They also reported that 72-96% of the nicotine in the

gum gets absorbed and that peak levels were reached 15-90 minutes from the time chewing started. Based on the previous information, it is clear that nicotine alone or smoking can have an impact on cardiovascular measurements.

The Color System

Detection of Contrast: Wavelength-opponent vs. Non-wavelength Opponent Cells

Wavelength contrast can detect objects when photon absorption contrast is absent or minimal. An object can reflect the same number of photons but seldom reflects the same wavelength composition as its background. Color vision combines both photon capture and wavelength contrasts to detect objects and this advantage must have appeared early in the evolution of vision. Svaetichin and MacNichol (1959), Wagner et al. (1960), and De Valois et al. (1982) have demonstrated the existence of two general classes of neural units: (i) wavelength-opponent cells, which increase their response to one part of the spectrum and decrease their response to another part, showing an inhibitory interaction of two classes of cones, and (ii) non-wavelength opponent cells, which show increased or decreased response to light from all parts of the spectrum.

How Many Types of Cones and Rods Do We Need?

In order to detect objects by differences in spectral reflectance, two or more different types of cones are needed. This is an important concept for understanding color vision. For dichromatic color vision, two cone types must exist and be sensitive to different parts of the visible spectrum. The range of the visible spectrum depends on the ability of light to penetrate the eye and be absorbed by the PRs. Ultra-violet light is

absorbed by the anterior segment of our eyes and seldom reaches the PRs. Infra-red light penetrates our eye readily but its quantal energy may be too small to activate opsins. Therefore early in the evolution of color vision, opsins sensitive to the middle of our visible spectrum evolved, near spectral yellow, and a short-wavelength opsin evolved in a second type of cone, near spectral blue. These have been called LWS and SWS cones, respectively.

The Color System in Humans and Non-human Primates

The visual system of the macaque monkey resembles the human's in its structure and function, from the retina through to V1, including trichromatic vision and multilayered LGN (Polyak, 1957). Both humans and monkeys possess PRs with specific wavelength sensitivities. (Schnapf and Schneeweis, 1999). Different color mechanisms exist in V1 in macaque monkey that contribute separately to the perception of color. Neural signals carrying information about color arrive at the cortex from the retina. Early segregation offers several advantages, one being that a single channel would require compromise and thus be suboptimal (Kingdom and Mullen, 1995). Therefore, it was critical to understand the various channels we possess.

Perception of Color

Color as we perceive it can be described in terms of hue (the aspect of appearance which is most affected by wavelength), saturation (the aspect of appearance which is most affected by wavelength purity, e.g. red vs. pink), and brightness (the aspect of appearance which is most affected by intensity). But there is no color in the external

world, rather, our perception of color is a delusion created by the interactions of billions of neurons in our brain. The typical human perceives color because the visual system utilizes two properties of light, energy and frequency of vibration, or wavelength. We know much about the nature of light and the subjective impressions of color, defined by physical standards, but ultimately color should be explained at the level of single cells in our brain. Ultimately, the perception of color can be explained by arrays of such neurons that can provide the best insights into the physiology of color vision.

Color Opponency

The pattern of connectivity between the LWS, SWS and short medium-wavelength sensitive (MWS) cones and various RGC types, which determines how color signals are transmitted in parallel pathways to the brain, remains incompletely understood (Derrington et al., 1984; Martin et al., 2001; Reid & Shapley, 2002; Chatterjee, & Callaway, 2002). Opponent color signals travel from the retina to cortex by way of the P pathway (De Valois et al., 1966). Color-opponent neurons in the P layers of the visual cortex compute the difference between two opponent cone signals and therefore respond in opposite directions to different wavelengths (Reid & Shapley, 1992; Lee et al., 1998; Martin et al., 2001). In contrast to the P neurons, cells in the M layers are somewhat "color blind." Therefore, when cone inputs in the RF center are "equal" and opposite in sign, M cells are insensitive. (Lee et al., 1988; Shapley et al., 1991).

De Valois (1966) relates the activity of the wavelength-opponent cells to hue discrimination behavior, the ratio of the activity of wavelength-opponent to non-

wavelength opponent cells to saturation discrimination, and the non-wavelength opponent activity to the "luminosity" or "brightness" discrimination function.

Color Channels

A cone responds only to the number of photons it absorbs. A single cone produces univariate responses reflecting only the number of photons absorbed. Two wavelengths of light can produce identical and equal responses based on the photons being absorbed. Different psychophysical “channels” mediate our perception of fine spatial patterns and color. The spatial channel compares the photon catch between adjacent cones, regardless of type, and thus supports perception of patterns as fine as the cone mosaic (Williams, 1986). Color channels compare the photon catch between different cone types (Hurvich & Jameson, 1957; Krauskopf et al., 1982). The “red/green” channel compares the photon catch in LWS and MWS cones (Thornton & Pugh, 1983; Calkins et al., 1992). Signals segregate early for the fine spatial channel and the blue/yellow color channel; LWS + MWS vs. SWS. Signals probably also segregate early for the red/green channel via a dedicated RGC—whose morphology remains to be identified (Calkins and Sterling, 1999). Early segregation offers several advantages, one being that a single channel would require compromise and thus be suboptimal (Atick, 1992; Kingdom & Mullen, 1995).

Color vs. Luminance

While Watson et al. (1983) examined what the eye sees best by using stimuli that only varied in luminance, Chaparro et al. (1993) used stimuli that varied in both luminance and color by using a small test spot that fell on the central fovea where there

are only long L and medium M cones. The best detected color stimulus can be seen 5-9 folds better than luminance stimulus and 3-8 fold better than Watson's best luminance stimulus. This was attributed to the prevalence and high color-contrast gain of P cells.

Since humans have only one type of rod cell, these cells alone cannot report chromaticity at low levels and therefore these cells are considered "color blind."

Color Vision Tests

A number of tests have been developed to identify individuals with abnormal color vision. These tests include: 1) FM-100 hue test, uses four trays with 21 or 22 caps per tray (total 85 caps). There is only minimal separation of hue between caps. Each tray has an anchored cap at both ends. The total error score is a measure of accuracy in arranging the loose caps between the two anchored caps. 2) The D-15, also called an arrangement test, uses a set of 15 colored discs. The individual's task is to arrange the disks based on hue. The type of deficit as well as its severity can be calculated based on mistakes and the resulting confusion vector. 3) The Roth-28 is similar to the D-15 but is more sensitive. In this test, every cap is a third color cap from the FM-100 test. Protans (defective or missing LWS cones), deutans (defective or missing MWS cones), and tritans (missing or mutated SWS cones) exhibit slightly different confusion axes on the Roth 28-hue test and the Farnsworth D-15 test.

Studies on Color Vision and Smoking

Erb et al. (1999) tested the influence of smoking on color vision in two groups of smokers using the Roth-28 (desaturated) color test. Group one was composed of 20 light

smokers, i.e. individuals with a smoking consumption of less than one pack of cigarettes per day. Group two consisted of 32 heavy smokers, i.e. individuals with a smoking consumption of one or more packs of cigarettes per day. Normal non-smokers served as a control group (n=76). All smokers were asked to abstain from smoking the night before testing. Testing was performed on the dominant eye. The calculation of error score followed Verriest (1963). The authors reported that only the group of heavy smokers differed in their threshold color discrimination compared to non-smokers, with elevated scores on the Roth-28 (higher = more errors; TES = 102 (heavy) vs. 42 (controls). The increase in TES did not show a preference for any particular color-axis. The authors attributed the changes to the toxic effect of smoke on the retinal pigment epithelium. During burning of one cigarette, about 4,000+ different chemical substances are produced, some of them carcinogenic or toxic. These toxic chemicals can accumulate in the blood and might alter the retinal pigment epithelium. As a consequence, all three types of cones could be affected, and this could be the cause of the general increase of error score. Smoking also has a clear effect on the ocular vascular system. In chronic smokers (Morgado et al., 1994), reduced retinal blood flow has been observed. Therefore, the color vision disturbance found in this study could be a sign of the effects of smoking on cones, either by way of toxicity, vascular, or both. Other studies have suggested similar conclusions.

In 2004, Bimler and Kirkland examined the possible effects of smoking on color vision using the D-15 (saturated and desaturated versions). Smokers in this study had a history of smoking at least one pack per day for at least one year. Their interest came from the fact that retinal cells have high metabolic demands, and tobacco smoke contains

a range of toxins that could affect the cells in the retina. They were particularly interested in whether smokers perceive a different pattern of "suprathreshold" color dissimilarities compared to non-smokers. Subjects (males and females) were presented with a series of triads of color stimuli (pigment swatches), and were asked which stimulus pair (of the three pairs in each triad) was the most similar, that is, more similar than the other two. These "triadic data" were treated as ordinal dissimilarity comparisons. They were asked to compare dissimilarities among 32 pigmented stimuli (the caps of the saturated and desaturated versions of the D15 panel test). The authors applied multidimensional scaling to quantify individual variations in the salience of the axes of color space. They reported that on average, the group of smokers was significantly less sensitive to red-green differences. They reasoned that the acquired red-green deficits, according to Köllner's law (in which colors along a red–green axis of color space become harder to discriminate, while differences along that axis contribute less to perceived color dissimilarities) are associated with insults to the optic nerve rather than to the retina. The authors reported that female smokers performed worse in discriminating red-green differences while male smokers were lower on overall performance. Their results differ from Erb et al. (1999), which did not concentrate along any specific direction in color space. On the other hand, their conclusions could be supported by suggestions from Fletcher and Voke (1985) that tobacco and carbon oxide can be potential causes of red–green disturbances. Conclusions from this study are different from Hood and Greenstein, 1988; and Sperling and Mills (1991). The latter studies explained that damage to PRs from smoking would more probably reduce blue–yellow salience (acquired tritanopia) because SWS cones are

considered relatively more fragile among all cones types. It remains elusive as to which PR cells are damaged by smoking.

Contrast Gain Control (CGC)

Contrast gain is the relative change in a cell's response to a given change in contrast. M cells are larger than P cells and therefore, their contrast gain is typically higher than P cells (Kaplan & Shapley, 1986; Croner & Kaplan, 1995), especially at low contrast levels. Rudvin et al. (2000) believe it is unlikely that the contribution of the M population disappears at high contrast because M cells continue to increase their responses to higher contrast levels. The high contrast gain of M cells is most likely due to their large RFs compared to P cells, which may be why they are relatively poor at identifying fine patterns (Kaplan & Shapley, 1982). Baccus (2004) noted that there is strong contrast adaptation in M cells of the primate LGN but much less in P cells. Baccus believes that the gain changes in M cells can be as much as 250% compared to 30%-50% in P cells.

It is important to remember the difference in spatial resolution for the two pathways. The larger M GCs pool signals of many more BCs than do P GCs (Baccus, 2004). Contrast adaptation is known to arise most strongly at sites where there is pooling over many inputs (Solomon et al., 2004). Not much additional adaptation occurs in LGN relay cells, which pool over only one or a few GC inputs. The P pathway does not pool many inputs together until the level of the cortex. In V1, additional pooling in the P pathway then coincides with additional adaptation. It is worth noting that sites of strong pooling coincide with strong modulation in sensitivity to inputs (Solomon et al., 2004).

At any given stage, the strength of contrast adaptation is a cumulative effect of modulation at preceding stages. Adaptation was largely missed in previous recordings from the LGN, maybe because some of the prior studies used changes in a neuron's firing rate as the measure of adaptation (Baccus, 2004). However, as shown by Solomon et al. (2004), cells can shift their contrast sensitivity without substantially changing their firing rates and a simple measurement of firing rate would ignore large changes in gain. As expected, the size and morphology of neurons play an important role in their adaptation, luminance contrast response, and sensitivity (Frazor & Geisler, 2006).

The dendritic fields of parasol cells are larger in humans than in monkeys. The difference in dendritic field size increases from 20% in the retinal periphery to 90% at 5 degree eccentricity (Dacey & Petersen, 1992). This result predicts that the human parasol cells should show a lower resolving ability and an increased sensitivity to luminance contrast compared to their cell equivalent in the macaque (Dacey & Petersen, 1992). It is well known that neurons of the P pathway have small color-opponent RFs and are necessary for the perception of color and fine detail (Schiller et al.,1990; Merigan et al., 1991). On the contrary, neurons of the M pathway have larger non-color-opponent RFs that appear to compromise their spatial resolving ability and extend their range of vision to low levels of contrast and higher temporal resolution (Schiller et al., 1990).

VEPs

What is a VEP?

Evoked potentials are the electrophysiological activity of the nervous system elicited by specific stimuli. The VEP evaluates the function of the visual pathway from

the retina to the occipital cortex. Stimulus patterns used in VEP testing can vary along two dimensions: temporal and spatial. Transient responses are evoked by some relatively rapid change in the visual stimulus. On the other hand, sustained responses are elicited by the continuance of a visual stimulus. The most common transient stimulus used is the contrast-reversal checkerboard, because the responses elicited by this pattern are typically very robust. The VEP waveform generated in response to a contrast- reversal checkerboard is characterized by an initial positive peak around 60 msec (P60), an initial negative peak (N75), followed by a positive peak at around 100 msec (P100, or P). This class of stimuli is based on low repetition rate. They are called transient because the slow rate of stimulation allows the sensory pathway to reset or recover before the next stimulus appears. But when the visual stimuli are presented at a constant rate that is rapid enough to prevent the neuronal activity from returning to baseline, the elicited response is sustained and becomes continuous and is called the steady-state visual evoked potential (SSVEP). At more rapid stimulation rates, the brain response to the same stimulus becomes sinusoidal and is typically modulated at two times the fundamental stimulus frequency (or the second harmonic) (Regan, 1989).

VEP Techniques

Conventional waveforms are commonly referred to as "P100" responses because the P100 peak is the first major positive component and the peak is normally observed around 100 ms. The P100 responses generated vary with check size. For example, a decrease in check size typically leads to a decrease in amplitude. In addition, pupil size, gender, and age all affect the VEP and should always be considered in research studies.

Certain drugs have also been shown to affect VEP responses. For example, Yuksel et al. (1995) studied the effect of carbamazepine and sodium valproate on VEPs in epileptic children. Pattern-reversal VEPs were obtained prior to and after 1 year of drug therapy. P100 amplitudes showed no consistent changes 1 year after therapy, but latencies were significantly increased.

Some VEP techniques focus on separating signals of the visual pathways (Zemon & Ratliff, 1982; Zemon & Ratliff, 1984; Zemon, Gordon & Welch, 1988; Zemon & Gordon, 2006). The authors presumed that a conventional contrast-reversing pattern cannot segregate responses from the different pathways (e.g. M vs. P, and ON vs. OFF). The authors noted that the direct-through pathways send signals from one area of visual processing to another while the lateral pathways transmit signals between neighboring cells within a given level, locally. And so, by modulating specific spatial regions they were able to show that lateral interactions also contribute significantly to the VEP (Zemon & Ratliff, 1982; Zemon & Ratliff, 1984). They suggested that what is called the "*superimposed"* VEP condition emphasizes contributions from the direct-through excitatory pathways and that the "*lateral condition"* emphasizes contributions from the lateral inhibitory pathways to the VEP.

The ON-OFF dendritic field size asymmetry in the human retina would predict that OFF-center cells have smaller RFs, and thus have greater resolving power than ON-center cells (Dacey & Petersen, 1992). Study of human VEPs generated by positive and negative contrast stimuli giving rise to perception of brightness and darkness concluded that the response to stimulation of the OFF pathway showed finer spatial tuning than the ON-pathway response (Zemon et al., 1988). It has been reported that the large difference

in contrast sensitivity is due not to LGN circuitry but to differences in sensitivity of the RGCs that provide excitatory inputs to the LGN neurons (Kaplan & Shapley, 1986).

Effects of Smoking on VEPs

In 2006, Durukan et al. measured the acute effects of cigarette smoking on pattern VEPs in male smokers. The study included two groups: individuals who had been smokers for less than 10 years and individuals who had been smokers for more than 10 years. Pattern VEPs were measured under two conditions: real vs. sham smoking. VEPs were recorded before, immediately after, and 5 minutes after smoking. Significant changes in P100 latencies (decreased) and amplitudes (increased) were reported in the group of subjects who had smoked for more than 10 years.

Thompson et al. (2000) studied the effects of smoking on the topography of the 13Hz SSVEP in male and female smokers. The amplitude and latency of the SSVEP elicited by an unstructured sinusoidal 13 Hz flicker following a <. 05 mg nicotine cigarette were compared to those following a 0.8 mg nicotine cigarette. The Fourier components were used to determine the amplitude and phase of the SSVEP. Again decreases in latencies and increases in amplitude responses were observed after cigarette smoking. Similarly, Woodson et al. (1982) compared P100 amplitudes before and after smoking 0.14 and 1.34 mg cigarettes in 10 male smokers. Testing showed larger amplitudes post smoking the 1.34 mg cigarette. This led them to believe that the changes in amplitudes resulted from the pharmacological effects of smoking. Most of these studies tested smokers to study the effects of smoking. However, it would be difficult to

attribute the changes seen in VEP waveforms to nicotine alone since tobacco products are composed of over 4,000 substances.

Research Aims and Hypotheses

Nicotine is considered the main addicting chemical ingested from smoking tobacco. However, there are thousands of other substances in tobacco smoke, which may contribute to the effects of smoking including increased attentiveness and deleterious health effects.

Previous studies compared groups of individuals who are smokers with individuals who are non-smokers, but the findings cannot be attributed to any specific chemical. In order to enhance our understanding of the pharmacology of nicotine itself and its effects on the visual system, we have developed protocols administering nicotine in the form of gum.

The overall goal of the project was to examine the effects of oral administration of nicotine on two aspects of visual processing: color vision, and cortical responses measured by VEPs. Additionally, because nicotine can have non-neuronal effects, IOP, BP, and HR measurements were monitored and reported. The studies were conducted using human adults with no history of smoking. We compared our findings with the current understanding of the pharmacology of nicotine to further elucidate its function within the visual system.

Research Aim 1

The first aim was to examine how nicotine affects color perception when a subject was exposed to nicotine via gum. We evaluated the changes in color perception using the

FM-100 to assess hue discrimination and the red-test (RT) to assess sensitivity to light increments.

Research Aim I Hypotheses

Several studies have reported deteriorations of color vision in chronic smokers. We assessed the effects of nicotine alone by having adults who are non-smokers chew two different doses of nicotine gum at different testing sessions. Even though the effects of nicotine can be excitatory or inhibitory, we predicted that nicotine alone will have different effects on non-smokers compared to what has been reported in smokers in previous studies. In most cases, nicotine has been shown to act as a stimulant, acting on nAChRs in the nervous system. Therefore, we predict that nicotine administered as gum, will produce excitatory effects in color vision in our participants.

Research Aim II

The second aim was to examine the effects of nicotine on cortical responses measured by VEPs.

Research Aim II Hypotheses

Most of the earlier studies recruited smokers as their subjects and only looked at amplitudes and latencies of the VEP using contrast-reversing checkerboards. The Windmill-Dartboard (WD) and Partial-Windmill (PW) stimuli (Zemon & Ratliff, 1982) are helpful in exploring the possible local lateral inhibition, while the isolated-checks (IC) should be helpful in isolating responses of the M and P pathways (Zemon & Gordon,

2006). We will use these conditions to reveal antagonistic contributions to the VEP, both direct-through excitatory and lateral inhibitory interactions (Zemon & Ratliff, 1984). Our study was designed to examine how oral nicotine ingestion (via nicotine gum) can affect VEP responses from the M and P pathways as well as its possible effects on local inhibitory interactions.

EFFECTS OF NICOTINE ON COLOR VISION IN NON-SMOKERS

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In preparation for (*Vision Research)*

Format adapted for dissertation

Abstract

The purpose of this project was to examine the effects of oral nicotine on color vision in non-smokers. We recruited twelve non-smokers. All subjects had their vision evaluated before enrolling in the study and were determined to be visually normal. Nicotine gum (2 and 4 mg) was administered to each of the subjects, one dose per testing session. We tested the color vision of all subjects prior to and 30 minutes after chewing nicotine gum. We tested subjects with: 1) FM-100 hue test and 2) RT; increment threshold test using both "white" and "red" lights).

Color testing revealed significant improvement in both FM-100 TES and RT increment thresholds following nicotine administration in individuals who were non-smokers. The TES improved with both 2 and 4 mg nicotine gum; RT thresholds improved with the 4 mg dose for both red and white light stimuli, with more improvement for red stimulus.

In contrast to previous studies reporting worse color vision sensitivity in smokers compared to non-smokers, our study shows that nicotine alone improved color vision discrimination and threshold detection in non-smokers. It is possible that nicotinic receptors expressed by neurons in the visual system are responsible for the changes seen in color vision observed in this study.

Introduction

For decades, smoking has been reported to cause various disorders and diseases, some of which affect the eye such as Grave's disease (Hagg et al., 1987), age-related macular degeneration (The Eye Disease Case-Control Study Group, 1992), and cataract (Clayton, 1982; Harding & Heyningen, 1988). Smoking has also been implicated in elevated IOP (Iyamu et al., 2002; Timothy & Nneli, 2007), BP, and HR (Benowitz, 1986; Benowitz et al., 1988). Nicotine is considered to be the major addictive component in tobacco (Metz et al., 2004). Previous studies showed that smokers, especially heavy smokers, score more errors when tested with the D-15 and Roth-28 color vision tests (Erb et al., 1999; Bimler & Kirkland, 2004). Although such changes have been attributed to nicotine, effects of the thousands of substances in tobacco products on the eye make it difficult to determine which of the substances in tobacco smoke may be responsible for the changes seen in the color vision of smokers.

ACh is a neurotransmitter that is synthesized and released by neurons of the brain, including the retina. The effects of ACh are mediated in part by nAChRs, which can also be activated by nicotine. The nAChRs are pentameric ligand-gated ion channels, with different subtypes based on subunit composition. nAChRs have been reported to be expressed throughout the nervous system. Studies have reported expression of various neuronal nAChR subtypes in the retina (Feller, 2002), on the retinal afferents of the LGN

and superior colliculus, in neurons of the SON, hippocampus (Paylor et al., 1998), and in V1 (Schroder et al., 1989). In the mammalian retina, nAChRs are expressed by subsets of cone bipolar, amacrine, and GCs (Keyser et al., 2000; Dmitrieva et al., 2007).

There are a variety of color vision tests available for clinical or research use. Among these are the FM-100, the D-15, and the Roth-28. All three assess color vision using the subject's ability to place color caps in order of hue. The FM-100 is highly effective and sensitive enough to differentiate individuals with normal color vision into classes of superior, average and low color discrimination ability and to measure the zones of color confusion of individuals with color vision anomalies (Fine, 1990; Arden & Wolf, 2004; Papaconstantinou et al., 2009). The caps used in the D-15 change in larger steps compared to the FM-100. The Roth-28 hue test uses every third color cap from the FM-100.

Erb et al. (1999) used the Roth-28 to examine the effects of smoking on color vision in light and heavy smokers, compared with non-smokers (controls). The authors reported that only heavy smokers displayed elevated TES on the Roth-28 color test with scores of 102 (heavy smokers) vs. 42 (controls). A higher score indicates more errors. In 2004, Bimler and Kirkland assessed color vision in smokers and non-smokers using the D-15. They reported that female smokers "placed less weight on red-green differences" while male smokers performed worse overall compared to non-smokers. Both studies demonstrated that smoking affects color vision and leads to increases in color discrimination errors in human adults who smoke.

Recent work in our laboratory has demonstrated changes in electroretinographic (ERG) responses in human adults who are non-smokers when they have chewed nicotine

gum (Varghese et al., 2011). Specifically, the photopic b-wave amplitudes were significantly higher after subjects had chewed nicotine gum, compared to baseline measures using a within-subjects design. These findings are intriguing, but do not convey explicit information about possible changes in visual perception experienced by the subjects. The literature on color vision in humans who are smokers led us to question what might happen to a non-smoker's perception of color when exposed to nicotine alone.

Conventional tests of color vision measure color discrimination ability. The RT (York & Loop, 2008) measures increment thresholds or color detection sensitivity. We chose to use the FM-100 and the RT to examine changes in both color discrimination and sensitivity in non-smokers who chewed nicotine gum.

The purpose of this study was to examine whether nicotine *alone*, delivered in nicotine gum, has an impact on color vision. We hypothesized that the administration of nicotine alone will not result in the degraded color vision discrimination compared to the effects reported in studies of individuals who smoke.

Materials and Methods

Subjects

Twelve naïve individuals (non-smokers) participated in this study (males: 8, females: 4; W: 9, AA: 3). The age range of subjects was 19-35 years (mean = 26.5). The research study was approved by the University of Alabama at Birmingham Institutional Review Board for human use and was in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects after explanation of the nature and possible consequences

of the study. All subjects completed a questionnaire on general health history at the beginning of the study. In addition, female subjects were required to take a pregnancy test. Exclusion criteria included: ex-smokers, women who were pregnant, planning to become pregnant or nursing, subjects with history of high BP, abnormal heart rhythm, heart palpitations/murmurs, history of strokes, arteriosclerosis, diabetes, asthma, ulcer, dental work that would prevent the subject from chewing gum, stomach ulcers, overactive thyroid, history of seizures, diabetes, or jaw problems (temporomandibular joint disorder). Some medicines may interact with nicotine gum, especially the following: asthma medicines (e.g., theophylline), insulin, antidepressants (e.g., imipramine), opioids, inderal, or bronchodilators (http://smoking-tobacco.blogspot.com/2011/06/nicotine-gum-for-nrt-nicotine.html). Subjects with a history of any of the above medical conditions or who were on any of the above medications were also excluded from the study.

Eligible subjects were given a comprehensive eye examination including visual field testing (24-2 SITA standard) and color vision screening, using the Hardy-Rand-Rittler (H.R.R., 4th ed; binocular) screening procedure, to ensure that they were free of any retinal abnormalities, eye diseases or disorders. Those who had an eye exam within the last 12 months were only given the H.R.R. and the visual field test. All subjects included in the study had healthy retinas and visual function within the normal range.

Apparatus and Testing Procedures

1- FM-100. Each of the four trays was set in front of the subject one at a time, with the color caps placed randomly in front of the tray. Subjects were asked to arrange the caps in order of color between the two anchored caps. Color vision was determined by the ability of the subject to place the color caps in order of hue. Subjects took the FM-100

on a black background, illuminated by a Macbeth lamp. The TES was calculated at the end of each testing session (www.torok.info). [Web-based platform independent scoring for the FM- 100-Hue vision test. After pressing the "Calculate results" button the patient's data, date, the order of the color caps, a polar diagram, the TES, midpoint and color defect type are presented in the same window].

2- RT. The RT measures increment threshold for a 2 cm stationary, red or white light increment of adjustable intensity on a white background (150 cd/m^2) (York & Loop, 2008). Briefly, the increment and background lights are inside a 30.5 cm × 30.5 cm × 45.7 cm wooden box. One end of the box is a piece of translucent white plastic, which serves as the viewing screen. The stimulus increment is provided by a white (7750K) light-emitting diode (LED) (L7114PWC, Kingbright, Taiwan) mounted in a small single lens projection system that produces a circular spot of light on the viewing screen. A small filter holder is mounted in front of the LED projector which always contains either a 35-mm projection slide that is empty (white stimulus) or holds a Wratten No. 26 filter (dominant wavelength: 620.6 nm, excitation purity: 100%; red stimulus). The LED flickers at 1000 Hz. The range of duty cycles used is 0-99%. No flicker is seen, so duty cycle determines increment luminance. Duty cycle readings are converted into cd/m^2 by measuring the increments' luminance with a Minolta luminance meter. The increment luminance is controlled by a log potentiometer, which controls stimulus pulse width. The white background (7100K) is produced by a circular daylight fluorescent bulb (FC8T9D/RS, 22watts, Damar, USA).

Subjects were seated 57 cm from the stimulus using a chinrest to stabilize their heads. The subject turned the knob on the potentiometer slowly increasing the luminance

of the light until it was just barely visible (method of adjustment). The tester recorded the subject's setting, and to prevent adaptation, turned the intensity down to below threshold and the procedure was repeated. Three detection increment thresholds for both red and white lights were obtained.

Administration of Nicotine Gum and Sequence of Testing

Nicotine has a half-life of two hours (Benowitz et al., 1982) and nicotine absorption peaks between 15-90 minutes (Benowitz et al., 1988). We expected that testing subjects 30 minutes after chewing nicotine would be close to the optimal time for most subjects. Therefore, non-smokers chewed nicotine gum for 30 minutes at each of the two testing sessions. Subjects were assigned to chew either 2 or 4 mg nicotine gum at the first testing session following a randomized block design with half of the subjects receiving the lower dose first. For the second testing session they were assigned the other dosage level. The 4 mg nicotine gum has been shown to yield blood nicotine levels similar to smoking one cigarette (Russell et al., 1976). The two testing sessions were separated by at least one week to ensure complete nicotine elimination. Both the subject and examiner were masked to the dose of nicotine gum at each testing session, and each subject was tested around the same time of day for both sessions. Each testing session lasted between 60-90 minutes. Testing (FM-100, RT, chewing period) was done with both eyes open in a darkened room.

Testing Sessions

During each of the testing sessions, subjects took the FM-100 test first (baseline), before chewing any gum. There was no time limit and the test was performed with both eyes open. Subjects then performed the RT before chewing nicotine gum (baseline). Since gum nicotine absorption is slow and gradual, we were interested in looking at possible time-course changes in color vision responses as nicotine plasma levels build up within 30 minutes. After the baseline RT, subjects chewed the nicotine gum for a total duration of 30 minutes. Subjects took the RT after chewing the gum for 10 and 20 minutes. At the end of 30 minutes subjects discarded the gum and took the RT test again and then performed the FM-100 test.

The FM-100 score is calculated by adding the sum of the difference between the number of a cap and the numbers of the caps adjacent to it. The TES is obtained by summing the errors of each cap (Farnsworth, 1957). The TES usually improves by 30% between the first and the second time a naïve subject takes the FM-100 test (Farnsworth, 1957). Nine out of the 12 subjects took the FM-100 hue color test on the day of the comprehensive eye exam before any dilation (practice session + to eliminate learning effect). These subjects took the FM-100 four additional times in two paired sessions (Testing session 1: baseline and 30 minutes after chewing nicotine gum; testing session 2: baseline and 30 minutes after chewing nicotine gum). Three of the subjects had already had a comprehensive eye exam before participating in the study, and therefore did not take the FM-100 practice trial. Therefore, the baseline TES at the second testing session (their 3rd out of 4 tests) was used as the baseline measure for both sessions for these subjects.

Data Analysis

Scoring of FM-100 tests was performed using a web-based platform (www.torok.info). Normative data for different age groups are incorporated into this on-line scoring system from Kinnear and Sahraie (Kinnear & Sahraie, 2002). Cap numbers were manually inserted into the appropriate boxes and the results were calculated. The results of the test were in html and Scalable Vector Graphics file format. The TES at baseline (not the practice session) was used to determine percent change for each subject. Individual threshold values from the RT were converted to cd/m^2 and baseline thresholds for each subject were used to determine percent change. Normalizing the data for both the FM-100 and RT tests was done to facilitate comparisons across doses for both the FM-100 and RT, between colors for the RT, as well as across subjects. Statistical comparisons among testing conditions for both the FM-100 and the RT were conducted by repeated measures analysis of variance (ANOVA) with SPSS® 17.0 (Chicago, IL, USA) and post-hoc paired t-test. The level of significance was set at $p < .05$ for all statistical tests.

Results

1- FM-100

Paired t-test comparison between TES at the two baseline measures was not significant ($p = .78$; $n = 9$; $r = .83$). The TES for all twelve subjects ranged from 0 to 76 (both baseline and post-gum scores). The average TES was 38.6 (SEM ±5.7) and 34.8 (SEM ± 5.6) at baseline, respectively; and the average TES was 24.4 (SEM ± 4.7) and 16.7 (SEM ± 3.5) post-gum for 2 and 4 mg respectively. The TES improved by 37% post

2 mg dose ($p < .001$) and 52% post 4 mg dose ($p < .001$). As shown in Table 1 and Figure 1, the difference in means between baseline and post-gum tests (for both doses) was highly significant [$F(1,11) = 40.25$, $p \leq .001$].

2- RT

The RT thresholds to white light at baseline ranged from 3.92 to 19.65 cd/m^2; thresholds to white light measured 30 minutes post-gum ranged from 3.75 to 14.88 cd/m^2. Thresholds to red light at baseline ranged from 1.03 to 5.86 cd/m^2 while thresholds to red light measured 30 minutes post-gum ranged from 0.66 to 4.63 cd/m^2. Our analysis showed significant threshold differences only between the 30 minute measurement and the baseline, 10, and 20 minute measurements [p (< .001, .003, <.001)]. The changes seen in thresholds for red vs. white 30 minutes post-gum for the 4 mg nicotine dose were significant ($F(1,11) = 104$, $p \leq .001$; Figure 2). Paired t-test comparison between subjects' responses to red vs. white at 30 minutes post-gum revealed that threshold detections decreased by 16% for white ($p = .037$), and 37% for red ($p \leq .001$), with the 4 mg dose. Little to no effects were seen with the 2 mg dose (white: $p = .48$; red: $p = .86$; Table 2, Figure 3).

Discussion

Previous research has shown that human adults who are smokers have higher error scores than non-smokers on color vision assessment tests (Erb et al., 1999; Bimler & Kirkland, 2004). In contrast, we report that nicotine alone administered to individuals who are not smokers significantly improved color vision. Even though three of our subjects did not take the FM-100 practice test in a similar fashion to the rest of the subjects (on the day of the eye exam), their 1st FM-100 test on the first testing session

was considered a practice test. Their 2nd baseline test (without nicotine) on the 2nd testing session, was taken as a baseline measure and included in our data analysis. Therefore, no differences in our results were observed due to this methodological testing conflict. Also, our testing protocol was slightly different from others that have been used with individuals who smoke. Therefore, we decided to test three smokers to verify that our findings were not based on idiosyncrasies of the specific color vision assessments. The three smokers were screened for any significant visual abnormalities and trained on the FM-100 test, following the protocol used for the non-smokers. These three individuals came in for one session only. Each smoker served as his/her own control. The three smokers were tested twice in a session, with immediate exposure to nicotine through smoking a cigarette between the two assessments. Average FM-100 TES from the three smokers we tested was 114 and 118 (pre- vs. post-smoking). Similarly, average RT measurements were higher in smokers compared to non-smokers prior to smoking and did not change after smoking (white: 27.27 cd/m^2 pre-smoking vs. 28.14 cd/m^2 post-smoking; red: 5.28 cd/m^2 pre-smoking vs. 5.53 cd/m^2 post-smoking). Our findings with smokers were consistent with previous studies, showing that in general, chronic smokers show deficits in color vision compared to non-smokers.

Tobacco smoke contains thousands of compounds (U.S. Department of Health and Human Services How Tobacco Smoke Causes Disease, 2010). Therefore, it is impossible to attribute the changes seen in smokers to any single factor. The high error scores on color vision tests reported in smokers from previous studies, and similar data collected in our limited sample of smokers, are likely due to a combination of substances. Furthermore, lenticular changes are likely to contribute to the results observed in smokers

(Kelly et al., 2005). Oxidative damage appears to play a major role in cataract formation (Taylor et al., 1995), and smoking causes oxidative stress through reducing endogenous levels of antioxidants (Chow et al., 1986). Smoking may indirectly impose additional oxidative stress on the lens through reducing the levels of systemic antioxidants. Furthermore, tobacco smoke contains heavy metals such as cadmium, lead and copper, which both accumulate in the lens and cause direct toxicity. In addition, we cannot rule out any vascular changes that might have contributed to the results observed as nAChRs are also expressed by vascular epithelial cells.

For many years, the FM-100 color test has been used as a tool for the diagnosis of possible color vision deficits and disorders. Farnsworth (Farnsworth, 1957) emphasized the importance of re-tests when the subject is inexperienced, showing that the mean improvement between the first and the second tests was about 30%, while the improvement between the second and third tests was about 10%. In our study, subjects completed the FM-100 five times: once for screening and four times during the testing sessions. Almost all showed improvement on the first re-test. There was no significant improvement between the two baseline assessments in TES for the 9 subjects who were given the practice test. Therefore, we are confident that all the changes reported are due to the effects of nicotine, not practice. Both doses of nicotine affected TES, with the higher dose resulting in greater improvement in color discrimination (improvement: 2 mg: 37% vs. 4 mg: 52%). The results we obtained from subjects who smoke (n = 3) clearly showed higher TES on the FM-100 and higher increment thresholds on the RT (worse performance) compared to non-smokers both at baseline and post-gum. This

performance could be due to long-term effects of tobacco smoke, as well as the short-term effects of cigarette smoking.

Results from the RT indicate that nicotine effects on thresholds are larger for colored (red) than non-colored stimulus (white). The results from the RT are consistent with direct effects of nicotine on neurons within the visual system. Our use of "red" and "white" test stimuli was intended to select wavelengths that would maximize differences between the color and luminance systems' detection thresholds (Chow et al., 1986). The RT test uses a large, long duration colored increment presented on a bright white background. Under these conditions, color increments are detected at a lower luminance than luminance increments (Sperling & Harwerth, 1971; King-Smith, 1975; Schwartz & Loop, 1982). De Valois et al. (1977) suggested that the saturation of color is based on relative responses by wavelength opponent vs. non-wavelength opponent cells (in the LGN). Since the improvement in threshold detection was greater for the red stimulus, we speculate that the color processing pathways may be more sensitive to nicotine than the luminance pathway. It is also possible that the color system alone contributed to the increased sensitivity, since wavelength-opponent cells can be sensitive to both intensity and wavelength (Lennie, 1984). We suggest that the TYPE II color-opponent system, along with the parvo-cellular pathway and V4, may be possible loci for the changes we observed. Our study involved nicotine-naïve subjects, however, we speculate that the improvement in color discrimination may apply as well to individuals who use nicotine regularly (as opposed to smokers).

Although we have no quantification of nicotine levels for our subjects, the blood nicotine concentration resulting from chewing of nicotine gum has been well established

(Benowitz, 1996). Rusted & Warburton (1992) investigated blood nicotine levels in cigarette smoking vs. nicotine gum and reported maximum blood plasma nicotine levels 30 minutes after chewing 4 mg nicotine gum, which was comparable to the effect of smoking one cigarette. We expect that our subjects' serum nicotine levels were similar to those found in previous studies. Based on these studies, we estimated that nicotine would reach the maximum level at 30 minutes, and we established our time-points accordingly.

We know that nAChRs are expressed in multiple locations within the nervous system, including the retina. Therefore, it is likely that nicotine affects the visual system at multiple levels of visual processing. The choice of nicotine gum ensured gradual intake of nicotine by all subjects. Nicotine uptake and metabolism varies among individuals based on their body mass index and other physiological factors. A better understanding of the concentration of nicotine and its time course for individual participants would enhance the interpretation of our data. Further studies incorporating nicotine blood level determinations would provide useful information with regard to degree of response vs. nicotine blood concentration. Our data only apply to acute administration of nicotine in naïve subjects.

The improvement in TES and RT thresholds in non-smokers support the interpretation that nicotine is involved in modulating the responses of the color system. The effects could be mediated at the level of the retina, or anywhere in the color vision pathway. Since the changes in the red stimulus threshold varied depending on dosage in non-smokers who chewed nicotine gum, we speculate that the changes we observed were neuronal rather than lenticular. The fact that nicotine alone improved color vision discrimination and detection in our subjects contrasts with data from previous studies

showing deterioration in color vision discrimination in individuals who smoke. However, our results with smokers were consistent with previous studies reporting that these individuals have impaired sensitivity to color compared to non-smokers. It is possible that exposure to smoke toxins, not nicotine, is responsible for the deterioration of color vision in smokers. Alternatively, it is possible that chronic nicotine consumption causes different effects than acute exposure.

Our results suggest that additional studies into the possible therapeutic or medical use of nicotine would be useful. However, the benefits of nicotine intake may not outweigh the already known side effects such as increases in IOP, HR, and BP. Cholinergic agents are used therapeutically for patients with Alzheimer's (Levin et al., 1992) and Parkinson (Morens et al., 1995) diseases. The effects of these drugs on visual processing remain to be explored.

Acknowledgments

-We thank Christianne E. Strang, PhD, of The University of Alabama at Birmingham Vision Science department, for assistance with manuscript edits.

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- This work was supported by the Clinical Research Advisory Committee funds awarded by the Department of Optometry and National Institutes of Health Grants P30 EY003039 and R01 EY07845.

References

Arden GB, Wolf JE. Colour vision testing as an aid to diagnosis and management of age related maculopathy. Br J Ophthalmol. 2004; 88(9):1180-5.

Benowitz NL, Jacob P, Jones RT, Rosenberg J. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther. 1982; 221(2):368-72.

Benowitz NL. Clinical pharmacology of nicotine. Annu Rev Med. 1986;37:21-32.

Benowitz NL, Porchet H, Sheiner L, Jacob P. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther. 1988; 44(1):23-8.

Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol. 1996; 36:597-613.

Bimler D, Kirkland J. Multidimensional scaling of D15 caps: color-vision defects among tobacco smokers? Vis Neurosci. 2004; 21(3):445-8.

Chow CK, Thacker RR, Changchit C, Bridges RB, Rehm SR, Humble J, Turbek J. Lower levels of vitamin C and carotenes in plasma of cigarette smokers. J Am Coll Nutr. 1986; 5, pp. 305-312.

Clayton RM, Cuthbert J, Duffy J, Seth J, Phillips CI, Bartholomew RS, et al. Some risk factors associated with cataract in S.E. Scotland: a pilot study. Trans Ophthalmol Soc U K. 1982;102 Pt 3:331-6.

De Valois RL, Snodderly DM, Yund EW, Hepler NK. Responses of macaque lateral geniculate cells to luminance and color figures. Sens Processes. 1977; 1(3):244-59.

Dmitrieva NA, Strang CE, Keyser KT. Expression of alpha 7 nicotinic acetylcholine receptors by bipolar, amacrine, and ganglion cells of the rabbit retina. J Histochem Cytochem. 2007; 55(5):461-76.

Erb C, Nicaeus T, Adler M, Isensee J, Zrenner E, Thiel HJ. Colour vision disturbances in chronic smokers. Graefes Arch Clin Exp Ophthalmol. 1999; 237(5):377-80.

Farnsworth, D. The Farnsworth-Munsell 100-hue test for the examination of color

discrimination: Manual; Munsell Color, Macbeth, Division of Kollmorgen Instruments Corp. 1957.

Feller MB. The role of nAChR-mediated spontaneous retinal activity in visual system development. J Neurobiol. 2002; 53(4):556-67.

Fine B. Farnsworth-Munsell 100-hue test and learning: reestablishing the priority of a discovery. Applied Optics. 1990; 29(2):186.

Hagg E, Asplund K. Is endocrine ophthalmopathy related to smoking? Br Med J (Clin Res Ed). 1987; 295(6599):634-5.

Harding JJ, Van Heyningen R. Drugs, including alcohol, that act as risk factors for cataract, and possible protection against cataract by aspirin-like analgesics and cyclopenthiazide. Br J Ophthalmol. 1988;72(11):809-14.

http://smoking-tobacco.blogspot.com/2011/06/nicotine-gum-for-nrt-nicotine.html

Iyamu E, Ekure E, Oghre E. The effect of smokeless tobacco on intra-ocular pressure in a Nigerian population. Health and Allied Sciences. 2002.

Kelly SP, Thornton J, Edwards R, Sahu A, Harrison R. Smoking and cataract: review of causal association. J Cataract Refract Surg. 2005; 31(12):2395-404.

Keyser KT, MacNeil MA, Dmitrieva N, Wang F, Masland RH, Lindstrom JM. Amacrine, ganglion, and displaced amacrine cells in the rabbit retina express nicotinic acetylcholine receptors. Vis Neurosci. 2000; 17(5):743-52.

King-Smith PE. Visual detection analyzed in terms of luminance and chromatic signals. Nature. 1975; 255(5503):69-70.

Kinnear PR, Sahraie A. New Farnsworth-Munsell 100 hue test norms of normal observers for each year of age 5-22 and for age decades 30-70. Br J Ophthalmol. 2002; 86(12):1408-11.
Lennie P. Recent developments in the physiology of color vision. Trends Neurosci. 1984;5:243–248.

Levin ED, Briggs SJ, Christopher NC, Rose JE. Persistence of chronic nicotine-induced cognitive facilitation. Behav Neural Biol. 1992; 58(2):152-8.

Metz CN, Gregersen PK, Malhotra AK. Metabolism and biochemical effects of nicotine for primary care providers. Med Clin North Am. 2004;88(6):1399-413, ix.

Morens DM, Grandinetti A, Reed D, White LR, Ross GW. Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology. 1995; 45(6).

Papaconstantinou D, Georgalas I, Kalantzis G, Karmiris E, Koutsandrea C, Diagourtas A, et al.

Acquired color vision and visual field defects in patients with ocular hypertension and early glaucoma. Clin Ophthalmol. 2009; 3:251-7.

Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. Learn Mem. 1998; 5(4-5):302-16.

Russell MA, Wilson C, Feyerabend C, Cole PV. Effect of nicotine chewing gum on smoking behavior and as an aid to cigarette withdrawal. Br Med J. 1976; 2(6032):391-3.

Rusted JM, Warburton DM. Facilitation of memory by post-trial administration of nicotine: evidence for an attentional explanation. Psychopharmacology (Berl). 1992; 108(4):452-5.

Schroder H, Zilles K, Maelicke A, Hajos F. Immunohisto- and cytochemical localization of cortical nicotinic cholinoceptors in rat and man. Brain Res. 1989; 502(2):287-95.

Schwartz SH, Loop MS. Evidence for luminance and quasi-sustained color mechanisms. Vision Res. 1982;22(4):445-7.

Sperling HG, Harwerth RS. Red-green cone interactions in the increment-threshold spectral sensitivity of primates. Science. 1971; 172(3979):180-4.

Taylor A, Jacques PF, Epstein EM. Relations among aging, antioxidant status, and cataract. Am J Clin Nutr. 1995; pp. 1439S-1447S.

The Eye Disease Case-Control Study Group. Risk factors for neovascular age-related macular degeneration. Arch Ophthalmol 1992;110:1701-8.

Timothy CO, Nneli RO. The effects of cigarette smoking on intraocular pressure and arterial blood pressure of normotensive young Nigerian male adults. Niger J Physiol Sci. 2007; 22(1-2):33-6.

U.S. Department of Health and Human Services How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking Attributable Disease*: A Report of the Surgeon General*. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010.

Varghese SB, Reid JC, Hartmann EE, Keyser KT. The effects of nicotine on the human electroretinogram. Invest Ophthalmol Vis Sci. 2011; 52(13):9445-51.

www.torok.info

York YC, Loop MS. Red light increment threshold as a measure of deficient color vision. Optom Vis Sci. 2008; 85(2):106-11.

Table 1.

Comparison of mean FM-100 TES: baseline vs. post gum.

n = 12	Baseline (SEM)	Nicotine (SEM)	p
2 mg	38.6 (5.7)	24.4 (4.7)	$\leq$.001
4 mg	34.8 (5.6)	16.75 (3.5)	$\leq$.001

Table 2.

Mean detection thresholds from Red Test (cd/m^2).

	n = 12	Baseline (cd/m^2) (SEM)	Post-gum (cd/m^2) (SEM)	p value
2 mg	White	8.48 (1.00)	8.16 (0.77)	.48
	Red	2.6 (0.21)	2.6 (0.29)	.86
4 mg	White	10.50 (1.18)	8.44 (0.54)	.037
	Red	3.11 (0.44)	1.85 (0.26)	$\leq$ .001

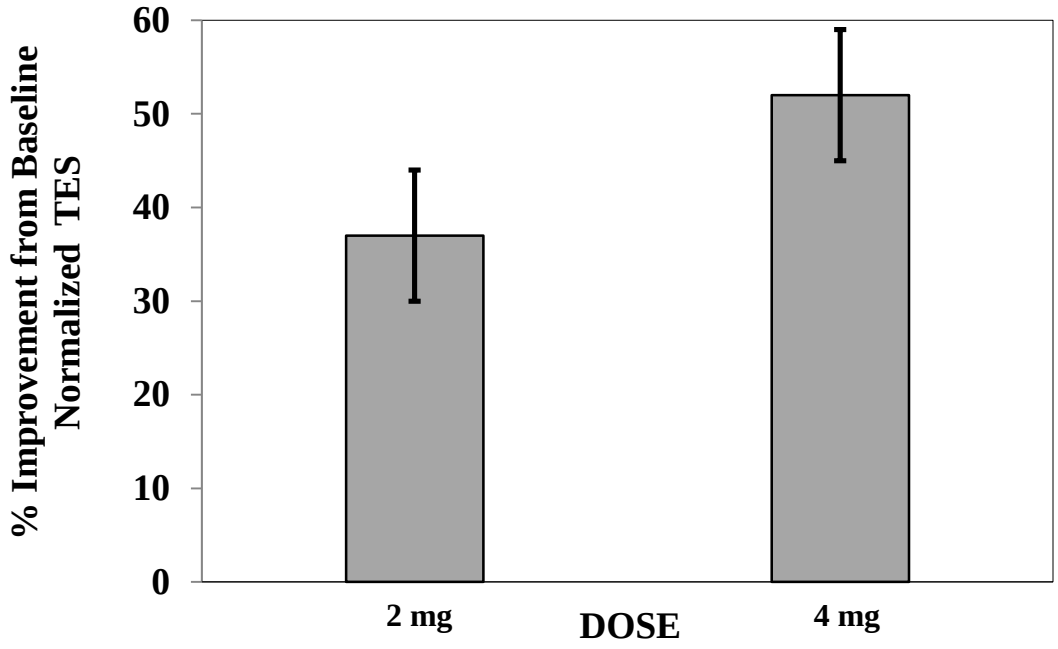

Figure 1. FM-100: Percent improvement in FM-100 TES 30 minutes after chewing 2 or 4 mg nicotine gum (n = 12). Error bars: SEM. * $p \leq .05$

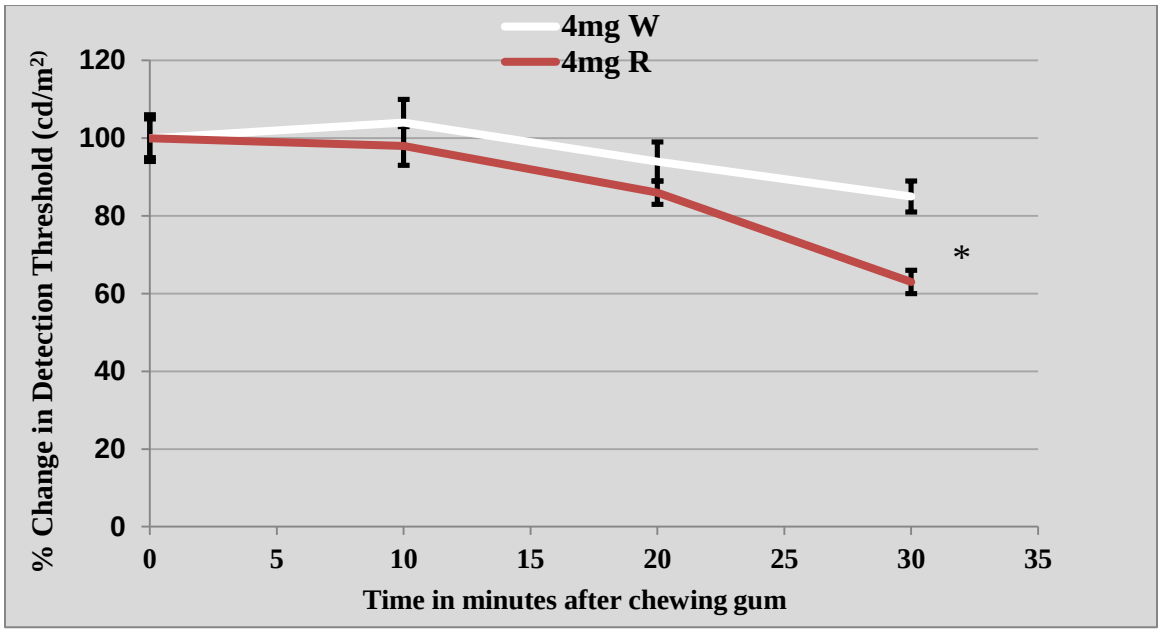

Figure 2. Red Test: Normalized data from non-smokers (n = 12) under 4 mg condition. [Baseline, indicated by time 0 reflects normalized thresholds for white or red light before chewing nicotine gum. Post-gum, indicated by time 30 reflects percent change in thresholds for white or red light 30 minutes after chewing nicotine gum. Times 10 and 20 reflect thresholds 10 and 20 minutes after start of gum chewing. Effect of nicotine increased over time and was larger for red vs. white stimuli. Significant difference between effects of nicotine on red vs. white color can be seen 30 minutes after chewing gum. The changes in average thresholds between baseline, 10, and 20 minutes post-gum, vs. 30 minutes post-gum (4 mg) were not linear]. Error bars: SEM. * $p \leq .05$

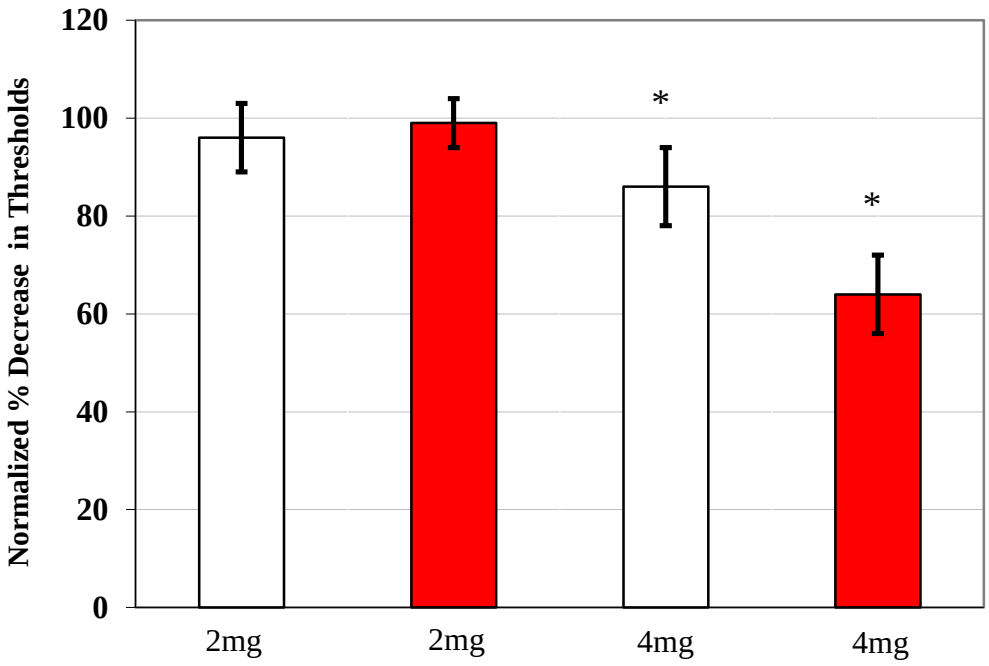

Figure 3. Normalized percent decrease in average thresholds to RT in non-smokers.[Percent change in thresholds 30 minutes after chewing 2 or 4 mg nicotine gum. W: responses to white light; R: Responses to red light. Significant decreases in thresholds were only observed with the 4 mg dose. Notice that the changes to the red increment were more robust than the changes to the white increment]. Error bars: SEM. * $p \leq .05$

EFFECTS OF NICOTINE ON CORTICAL VISUAL PROCESSING IN NON-SMOKERS

by

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In preparation for submission to (*Documenta Ophthalmologica)*

Format adapted for dissertation

Abstract

The purpose of this project was to examine the effects of oral nicotine on visual cortical processing in human adults who are non-smokers by means of VEPs. Twelve non-smokers and three smokers participated in this study. All subjects had their vision evaluated before enrolling in the study and were determined to be visually normal. Nicotine gum (2 and 4 mg) was administered to each of the subjects, who were non-smokers, one dose per testing session. Subjects who were non-smokers were tested before and after chewing the gum. Smokers were tested prior to and after smoking cigarettes. In order to explore the impact of nicotine on various response functions [e.g. latency, amplitude, excitatory and inhibitory interactions, responses of ON/OFF M and P pathways] we utilized various types of stimuli.

We observed no changes in P100 latencies or amplitudes in response to nicotine. Changes in responses to stimuli designed to emphasize contributions of M and P pathways were observed, mainly in increased amplitudes in ON responses of the M pathway. Both excitation and inhibition in local lateral inhibitory interactions, reflected mainly in the 1^{st} (F1) and 2^{nd} (F2) harmonic frequency components of SSVEPs to windmill-dartboard stimuli were also observed with nicotine. Our study shows that nicotine administered alone had an effect on both the M and P pathway. We also posit that the changes in lateral inhibitory responses are due to the involvement of nicotinic receptors expressed by inhibitory (GABAergic) neurons in the cortex.

Introduction

Nicotine is considered the main addicting chemical ingested from smoking tobacco (Metz et al., 2004). However, there are thousands of other substances in tobacco smoke (U.S. Department of Health and Human Services, 2010). Therefore, studies comparing groups of individuals who are smokers with individuals who are non-smokers cannot attribute their findings to any specific chemical. In order to enhance our understanding of the pharmacology of nicotine itself and its effects on the visual system, we have developed protocols administering nicotine in the form of gum to naïve individuals who are non-smokers.

nAChRs

Nicotine binds to and activates neuronal nAChRs. We know that nAChRs are widely expressed in the retina (Feller, 2002), LGN (Gotti et al., 2005), SON (Zaninetti et al., 2000), and V1 of the visual cortex (Schroder et al., 1989) in both animals and humans. Thus, systemic nicotine would be expected to affect responses within the visual system at multiple levels. Human studies have shown that nAChRs can be involved in inhibition and disinhibition at the cortical level via GABA mechanisms (Alkondon et al., 2000). Disney et al. (2007) examined the impact of nicotine in V1 of the macaque monkey. They showed that nicotine increased responsiveness and lowered contrast threshold in neurons of layer 4C.

Mrzljak (1996) reported expression of nAChRs in the parvo area of V1 in macaque monkeys; Zaninetti (2000) found functional nAChRs in neurons of the SON. Studies using extracellular recordings (Barker et al., 1971; Dreifuss & Kelly, 1972; Gribkoff et al., 1988) suggested that ACh increases excitability within these neurons, which could be mimicked, in part, by nicotine. Strang and colleagues (Strang et al., 2007) showed modulation of directionally selective RGCs responses by activation of nAChRs. These studies support the strong involvement of ACh in modulating responses of the visual system, and indicate mechanisms by which nicotine can change visual responsiveness.

VEP Studies

The VEP is a non-invasive electrophysiological measure of the brain's electrical activity in response to visual stimuli. Many early studies have reported consistent differences in VEPs from individuals who smoke compared to individuals who are non-smokers. Woodson et al. (1982) used five different flash intensities to examine the P100 responses in smokers after smoking either 0.14 mg or 1.34 mg cigarettes (current cigarettes yield an average of about 1-3 mg of absorbed nicotine, Wynder and Hoffman, 1967). They found increases in amplitudes with the 1.34 mg nicotine cigarette, but not the 0.14 nicotine cigarette. Thompson et al. (2000) measured VEP responses to a 13Hz SSVEP flicker in smokers after administering 0.05 and 0.8 mg cigarettes. Amplitudes and latencies in smokers following 0.05 mg and 0.8 mg nicotine cigarettes were compared to controls. The 0.8 mg nicotine condition showed increased amplitudes and decreased latencies when compared to the 0.05 mg. Similarly, Durukan et al. (2006) investigated

pattern VEPs in a group of smokers in separate real and sham smoking sessions. They classified their subjects by duration of smoking: 1) less than 10 years, 2) more than 10 years. During each session, pattern VEPs were recorded before smoking, immediately after smoking, and five minutes after smoking. P100 latencies decreased significantly immediately after smoking compared with before smoking. P100 amplitudes increased immediately after smoking but were not significant until five minute after smoking. Statistically, no significant difference was observed in sham smoking sessions. Changes were observed only in the 2nd group (smoked for more than 10 years), suggesting possible long-term changes in receptor response.

VEP Stimuli

The contrast-reversing checkerboard pattern yields robust P100 responses, but it lacks specificity with regard to the underlying neural pathway. Zemon and Ratliff (1982, 1984) used specific stimuli to separate contributions of the different pathways. Specifically, Zemon and Ratliff (1984) utilized specific stimuli to compare direct-through excitatory and lateral inhibitory responses. Later using isolated-check patterns, Zemon, Gordon, and Welch (1988) were able to emphasize VEP contributions to the VEP from ON or OFF and M or P pathways.

Zemon and Gordon (2006), used shunting inhibition as the nonlinear neural mechanism in a model to fit the contrast response function. This mechanism appears to be controlled by the GABA. Application of this nonlinear model provides a close correspondence between the properties of single cells and the extracellular signals that produce the VEP.

Methods

Subjects

Twenty non-smokers (ages 19-35; 11 males, 9 females) participated in this study. The research was approved by the University of Alabama at Birmingham Institutional Review Board for human use and was in accordance with the tenets of the Declaration of Helsinki. This project also complied with HIPAA (Health Insurance Portability and Accountability Act) privacy regulations. Written consent was obtained from all subjects after the nature and possible consequences of the study were fully discussed. All subjects completed a general health history questionnaire at the beginning of the study. Female subjects were required to take a pregnancy test. Exclusion criteria included: ex-smokers, women who were pregnant, planning to become pregnant, or nursing, subjects with history of high BP, abnormal heart rhythm, heart palpitations/murmurs, history of strokes, diabetes, asthma, ulcer, dental work that would prevent the subject from chewing gum, stomach ulcers, overactive thyroid, history of seizures, jaw problems (temporomandibular joint disorder), or blood vessel problems. Subjects were also excluded if they were taking any medications that might interact with nicotine gum [e.g. antidepressants, opioids, inderal, or bronchodilators.

Eligible subjects were given a comprehensive eye exam including dilation, tonometry, visual acuity, and visual field tests as well as color vision screening using the H.R.R. test (4^{th} ed; binocular). Those who had had an eye exam within the last 12 months were only given the H.R.R. and the visual field test. Subjects included in the study had no ocular abnormalities and their visual functions were within normal limits.

Administration of Nicotine Gum and Sequence of Testing

Non-smokers chewed nicotine gum for 30 minutes at each of the two testing sessions. Nicotine has a half-life of two hours (Benowitz et al., 1982) and nicotine absorption peaks between 15-90 minutes (Benowitz et al., 1982). We expected that testing subjects 30 minutes after chewing nicotine would be close to the optimal time for most subjects. Subjects were assigned to chew either 2 mg or 4 mg nicotine gum at the first testing session following a randomized block design with half of the subjects receiving the lower dose first. For the second testing session they were assigned the other dosage level. The 4 mg nicotine gum has been shown to yield blood nicotine levels similar to smoking one cigarette (Benowitz, 1986). The two testing sessions were separated by at least one week. Both the subject and examiner were masked to the dose of nicotine gum at each testing session, and each subject was tested around the same time of day for both sessions. Each testing session lasted between 60-90 minutes.

VEP Apparatus & Testing Procedures

We tested subjects at baseline (pre-nicotine) and 30 minutes after chewing nicotine. Subjects were tested with best-corrected vision. Scalp electrodes were placed at Oz (active), Cz (reference), and Pz (ground) following the International 10-20 system (Jasper, 1958). VEP recordings were obtained using the Neucodia system (VeriSci Corp, NJ). Testing was conducted in a dark room. An infrared camera was used to monitor patient fixation. Subjects were instructed to fixate carefully at the cross in the center of the display during the period the stimulus pattern was displayed. Viewing distance was 114 cm (10 x10 degree visual field) and the stimulus display was 20 x 20 cm.

Stimuli

The VEP stimuli used are detailed in Table 1. The transient stimulus was a contrast-reversing checkerboard. In addition, we used the windmill-dartboard (WD) and partial-windmill (PW) patterns (Zemon & Ratliff, 1982) (Figure 1) to examine direct-through excitatory and lateral inhibitory interactions. WD and PW patterns were modulated sinusoidally in time at a frequency of 4.22 Hz (duration of 1 minute). Figure 2 shows our 3rd stimulus was an isolated-check (IC) pattern. The ICs were either brighter or darker than the background to emphasize contributions from the ON and OFF pathways respectively. In the stimulus condition that is designed to emphasize the M pathway, the luminance of the checks was modulated from 0% contrast to a maximum positive or negative contrast of 32% (ON or OFF). This resulted in appearance and disappearance of bright or dark checks. In the condition used to emphasize the P pathway contribution, the checks never disappeared, but were modulated about a mean contrast level and remained either bright or dark throughout the experimental run (pedestal condition). In the appearance–disappearance condition, contrast mean (C_{mean}) and depth of modulation (DOM), are varied, whereas in the pedestal condition only DOM is varied. Both the M and P stimuli were presented following a sweep VEP paradigm. There were six steps for each sweep. We averaged 10 sweeps per condition. The DOM steps for the M were 1, 2, 4, 8, 16, and 32%. For the pedestal condition the mean contrast was 32% and the DOM = 1, 2, 4, 8, 16, 32%.

Data Analysis

Transient (P100) responses were averaged across the entire recording period. We

determined latencies and amplitudes (Figure 3) of the P100 response. For steady-state responses, averaged EEG signals were analyzed using the discrete Fourier Transform (DFT). Confidence limits of the Fourier components were determined using the T^2circ statistic (Victor & Mast, 1991). For each mean fundamental response, a circular 95% confidence region was established (Figure 4) and its confidence limits are plotted for both amplitude and phase values (Figure 5). A signal-to-noise (SNR) value could be derived for each response by dividing the mean amplitude of the fundamental component by the radius of the confidence circle. Statistical comparisons among testing conditions were conducted by repeated measures ANOVA with SPSS® 17.0 (Chicago, IL, USA) and post-hoc paired t-tests. For all statistical tests, the level of significance was set at $p \leq .05$.

Results

All study participants were run under all conditions. Figure 3 shows VEP response comparison for an individual subject for pattern-reversal checkerboard with baseline and under nicotine condition (4 mg). Average latencies and amplitudes were averaged across subjects for both baseline and under nicotine conditions. Comparisons between baseline and nicotine conditions yielded no significance in either measure. Latency: 101.08 vs. 101.58 (2 mg, SEM: $\pm$0.57); 101.57 vs. 101.75 (4 mg, SEM: $\pm$0.45); amplitudes were 19.19 vs. 18.38 (2 mg, SEM: $\pm$0.88); 19.84 vs. 20.04 (4 mg, SEM: $\pm$0.77).

Figure 6 shows a comparison of responses to PW stimulus between baseline and 4 mg nicotine conditions for one of the subjects. We observed changes within F1 and F2 of WD and PW stimuli (Table 2). We observed increases in amplitudes with both doses in

the F1 component of both WD [2mg: 2.78 to 3.23 (p = .001); 4mg: 2.81 to 3.48 (p = .002)] (Figure 7) and PW VEPs [2mg: 0.753 to 0.766 (.05); 4mg: 0.928 to 0.939 (p = .05)]. Changes in amplitudes in F2 of WD showed decrease in amplitudes with the 4 mg [2.03 to 1.68 (p = .05)] (Figure 8) dose and increases under the PW 4 mg [2.99 to 3.44 (p = 0.01)] (Figure 9).Changes were also seen in phase in response to WD and PW stimuli, in both F1 and F2 (Table 2). For the WD, there was a phase lead in F1 [4 mg: -401 to -374 (p = .008)] (Figure 10), and a bigger attenuation phase lead in F2 [2 mg: -366 to -319 (p = .005); 4 mg: -375 to -309 (p = .004)]. For the PW, there was a phase lag in F1 [4 mg: -372 to -409 ($p \leq .001$)], and a phase lead in F2 [2 mg: -302 to -261 (p = 0.01)] (Figure 11). Looking at SNR values (Table 2), the only significant change was an increase in F1 of the PW stimulus [2 mg: 0.67 to 0.90 (p = .001); 4 mg: 0.67 to 0.92 (p = .001)]. Other changes in SNR were not statistically significant. The SNR values were lower after nicotine for F1 and F2 of WD and increased for F2 of PW.

We expect that the PW stimulus to generate even-order harmonics, while the WD stimulus should generate both even and odd harmonics. With the WD, we can look at both 1^{st} and 2^{nd} harmonics. A "facilitation index" (FI) can be derived by looking at the ratio of F1 and F2 from WD pattern, while a "suppression index (SI) can be derived from ratio of F2 from PW pattern divided by F2 from WD pattern. With FI one can look at the relative measure of facilitation with regard to the fundamental component that gets generated (FI=F1 WD/F2 WD), while with SI we can examine the relative changes in F2's when going from a PW to a WD pattern. In other words, FI looks at how big F1 (one type of lateral interaction) is compared to the F2 (another type of lateral interaction) in the WD condition, which is a measure of the strength of the attenuation of the lateral

interaction. With SI, we can examine whether F2 amplitudes have become smaller or larger when the pattern changes from PW to WD (SI = F2 PW/F2 WD). We report increases in both rations with the 4 mg only: FI [1.33 to 2.43 ($p \leq .05$)] and SI [1.17 to 2.44 ($p \leq .001$)] (Table 3, Figure 12, Figure 13).

Figure 4 shows a comparison of a sweep response for one subject for baseline and nicotine under the IC-MB condition (2 mg). The IC-M (appearance-disappearance) or P (pedestal) conditions revealed changes in amplitudes of the MB (magno bright) condition: 1.17 vs. 1.77 (2 mg, $p = .001$); 2.25 vs. 4.65 (4 mg, $p = .002$) (Table 4). No significant changes were observed under the MD (magno dark), PB (parvo bright), or PD (parvo dark). Similarly, no significant changes were observed in the phase under any of the conditions. Measures of SNR were not significantly different, but tended to be higher under nicotine conditions, especially with the 4 mg.

The biophysical model developed by Zemon and Gordon (2006) was applied to fit the IC M and P VEP data. This nonlinear model (Zemon and Gordon, 2006) fits estimates of these 4 parameters: initial membrane conductance (g_0), initial phase (φ_0) coefficient of shunting inhibition (m), and threshold depth of modulation (d_0). Then, time constants of the system can be derived. We were more interested in looking at changes in the m and g_0 parameters.

Changes were also observed in the model parameters. We found changes in MB, MD, PB, and PD (Table 5). Changes in g_0 were especially seen in the MB and PD conditions. While there were decreases in g_0 with the 2 mg dose for both MB (-0.39, $p \leq .01$) and PD (-1.42, $p \leq .05$), there were increases with the 4 mg dose for MB (1.95, $p \leq .005$) and PD (.28, $p \leq .05$). Increases in do were seen in: MD with the 2 mg (.70, $p \leq$

0.05), PD condition [2 mg: .70, $p \leq .05$; 4 mg: 1.32, $p \leq .05$], and PB condition (2 mg: 2.48, $p \leq .05$). Decreases were observed in MD (4 mg: -1.05, $p \leq .05$) and PB (4 mg: -.49, $p \leq .05$) conditions under the 4 mg dose. Changes in shunting coefficient *m* were the most robust and were observed under all conditions, mostly increases. We report changes in: MB [(2 mg: .23, $p \leq .05$), (4 mg: 0.28, $p \leq .05$)], MD [4 mg: 1.93, $p \leq .01$), PB [(2 mg: .74, $p \leq .005$), 4 mg: 2.11, $p \leq .005$)], and PD (2 mg: 42.12, $p \leq .005$), 4 mg: 3.1, $p \leq 0.005$]. Little or no changes were seen in time constants (Tau^{1} and Tau^{32}).

Discussion

As early as the 1970's the presumptive effect of nicotine on VEP responses was documented by Philip Morris tobacco company in unpublished studies. Gullotta and Ryan (1979) found that cigarette smoking produced an increase in amplitudes of the late components of evoked response potentials elicited by light flashes. Gullotta (1981) also found that smoking a high delivery cigarette (1.34 mg nicotine/cigarette) but not a low delivery cigarette (0.14 mg nicotine/cigarette) produced decreases in N1 amplitude and P100 latency in response to a checkerboard stimulus. In addition, there is evidence that nicotine has an effect on retinal blood flow (Klein et al., 2004). Therefore, we cannot rule out such effect on results obtained from our study. The current study explored changes at the cortical level in response to oral administration of nicotine in naïve adults who are non-smokers. We used stimuli designed to elicit responses from specific pathways within the visual system. Specifically, we used the WD/PW stimuli to examine lateral interactions in response to nicotine (Zemon & Ratliff, 1984). Further, we used bright and dark ICs, which allowed us to examine the effects of nicotine on the ON and OFF

systems. Also, by controlling the DOM for the IC stimuli (appearance-disappearance & pedestal), we were able to stimulate responses from the M and P pathway respectively. Finally, we fit our data using a nonlinear model (Zemon & Gordon, 2006), which incorporates shunting inhibition to characterize the VEP responses.

Several studies have demonstrated that GABA is a major contributor to VEPs (Daniels & Pettigrew, 1975; Rose & Blakemore, 1974; Zemon et al., 1980). GABA contribution to VEPs has been documented in studies on cats (Zemon et al., 1980; Ts'o et al., 1986) and humans (Zemon & Ratliff, 1984). These studies suggest that both excitatory and inhibitory intracortical synaptic potentials involving GABA contribute to the VEP. Disney (2007) found that nicotine increased responsiveness and lowered contrast thresholds in neurons of layer 4C in V1 of macaque monkeys. The primary neurons in layer 4C receiving inputs from the thalamus of the LGN. Disney et al. (2007) reported the expression of nAChRs pre-synaptically, at thalamic afferents onto receiving neurons in layer 4C. It is possible that those receiving neurons could be the spiny stellate neurons, which are excitatory. They then, could stimulate inhibitory interneurons, as well as other excitatory neurons, such as pyramidal cells.

A study by Zemon et al. (1986) suggests that the generation of a significant fundamental component in the VEP elicited by the WD stimulus used in our study may arise from lateral interactions, possibly by action of GABAergic synapses. The increase we report in FI is due to either an increase in amplitudes of F1 of the WD or a decrease in F2 of the WD. We observed changes in both F1 (increases) and F2 (decreases) of the WD pattern, with nicotine. It is suggested that generation of this F1 component reflects GABAergic local inhibitory interactions over short distances, while attenuation of the F2

is evidence for lateral interactions over long distances. The changes we observed with nicotine indicate a stronger facilitation. We also report increases in the ratio of suppression index, as a result of an increase in F2 of the PW pattern. This is an indication of a greater suppression. These results suggest that nicotine exerted its effects on both long and short-range interactions.

Biophysical Model

We decided to use a nonlinear model to fit our data and relate it to some neurophysiological parameters. This model, developed by Zemon & Gordon (2006) has 4 parameters. We were interested in exploring the main two of the parameters: g_0, which is the initial conductance at low contrast levels (a measure of CG); the coefficient of shunting inhibition "m". This m parameter is a measure of CGC, which determines the magnitude of amplitude compression and phase advance. In this model, the nonlinearity is based on what is known about shunting inhibition, reflected in results of this CGC phenomenon (Zemon & Gordon, 2006; Carandini et al., 1997; Carandini & Heeger, 1994). In a linear system, the slope of amplitudes is usually constant (straight line), while in the nonlinear visual system the slope (or CG) of amplitudes decreases with increase in contrast (amplitude compression). Phase also advances (speeding up the system). Without CGC, the response magnitude would saturate at low contrast, and so CGC enables high gain at low contrasts without saturation the system.

One way of moderating the responses in the cortex is by shunting inhibition. Shunting inhibition is a type of nonlinear CG mechanism that has a divisive effect which can stabilize and speed up signal processing. So, in case of an excitation at a certain point

(e.g. soma), an inhibitory input at a close by point, would cause ion channels to be open, leading to current leak, and therefore a lower resistance current ground to path.

Contrast gain control was first described in RGCs in cats (Shapley & Victor, 1978, 1979; Shapley & Victor, 1979; Shapley et al., 1981). This CGC phenomenon has since been demonstrated in the M pathway of primates (Kaplan & Shapley, 1986; Benardete et al., 1992). Previous studies on single-cell in the cochlea were based on a shunting mechanism (Furman, 1964). And so, this nonlinear mechanism has been used and was demonstrated to be accountable for CGC as reflected in both cortical simple cell data (Carandini et al., 1997; Carandini & Heeger, 1994) and VEP data (Zemon & Ratliff, 1999; Zemon et al., 1993). This nonlinear model demonstrates how ICs might be processed separately by cortical neurons. Borg-Graham et al. (1998) reported that visual signals increased the conductance of cortical neurons, most likely by increases in GABA-mediated shunting inhibition. Results from Borg-Graham's study indicate an involvement of GABAergic synapses. We demonstrated a significant effect of nicotine on the ON and OFF pathways of the visual M- and P-cellular pathways. The impact of nicotine on the F1 and F2 suggests a strong inhibition effect on the visual system, possibly GABAergic ones.

Shunting inhibition is more likely mediate by GABA receptors, and so, an increase in contrast results in increases in cellular conductance. Increases in *m* (shunting coefficient), especially with 4 mg, with all conditions, indicate a significant increase in CGC with nicotine, presumably due to shunting inhibition, a stronger nonlinear inhibition effect with increase in contrast. This greater shunting inhibition produced greater conductance, leading to more compressed responses (CGC).

In this model, the ON and OFF stream remain segregated at their initial cortical projection sites in layer 4C (Gilbert & Wiesel, 1985). Somogyi (1989) demonstrated that there is a wide variety of GABAergic interneurons present in the visual cortex. Some of these GABAergic interneurons express nAChRs, and so, might provide the basis for this shunting inhibition, one possible mechanism for this CGC behavior.

Since both the m and g_0 parameters were enhanced with nicotine, and since FI and SI ratios became larger, it is likely that both effects are due to GABAergic interneurons that are involved in both long and short-range interactions. It is possible that the effects we observed are due to a combination of many GABAergic cell types (chandelier cells, basket cells, stellate cells, all could mediate different types of interactions). Nicotine could be playing two major roles. It can be increasing the excitation by its action on excitatory cells; nicotine can exert its effects on GABAergic interneurons, leading to inhibition; nicotine can also act on GABAergic cells, which themselves could act on other nearby GABAergic cells, causing disinhibition. The fact that nAChRs are found to be expressed on GABAergic interneurons, which are inhibitory, can possibly mean that these interneurons can be involved in shunting inhibition. There are various types of GABAergic interneurons, some of which are not involved in shunting inhibition, so there could be other possibilities to explain our results. Therefore, nicotine acts as a neuromodulator facilitating the GABAergic inhibition, making the CGC stronger, which is indicated by our results (the increase in the g_0 and m with the 4 mg).

Our results show that the most significant changes in the initial conductance g_0 were mostly on the MB and PD conditions. The literature tells us that nAChRs are expressed on ON-cone BPs in the retina. And even though it is possible that the effects of

nicotine are taking place in either the MB or PB systems, it is difficult to attribute our results to one specific pathway. It is likely that these changes are related to the differences in the M and P pathways, but there are other possibilities that we can consider. Also, under both 2 and 4 mg, the shunting coefficient *m* significantly increased in almost every condition. We believe that this fits with Disney's findings in that you can modulate GABAergic activity in the cortex using nicotine. This extends to previous studies, which suggested the role of higher affinity nAChRs expressed by GABAergic neurons in the cortex.

Although we observed decreases in g_0 delta values with the 2 mg, we observed increases with the 4 mg. It might be that the effects of nicotine are more dominant on inhibitory mechanisms with lower dose, and shifts to dominance on excitatory mechanisms at higher doses. The increases in g_0 mean amplified responses at low contrasts. The increases in the shunting inhibition, quantified by the increases in the m parameter suggest stronger CGC.

Cholinergic agents are used therapeutically for patients with Alzheimer's and Parkinson diseases (delay its onset). But, cholinergic agents, such as nicotine, also have side effects (increases in HR, BP, IOP). Therefore, additional studies into the possible therapeutic or medical use of nicotine would be useful.

References

Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. J Neurosci. 2000 Jan;20(1):66-75. PubMed PMID: 10627582. eng.

Barker JL, Crayton JW, Nicoll RA. Supraoptic neurosecretory cells: adrenergic and cholinergic sensitivity. Science. 1971 Jan;171(967):208-10. PubMed PMID: 4395231. eng.

Benardete EA, Kaplan E, Knight BW. Contrast gain control in the primate retina: P cells are not X-like, some M cells are. Vis Neurosci. 1992 May;8(5):483-6. PubMed PMID: 1586649. eng.

Benowitz NL. Clinical pharmacology of nicotine. Annu Rev Med. 1986;37:21-32. PubMed PMID: 3518606. eng.

Benowitz NL, Jacob P, Jones RT, Rosenberg J. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther. 1982 May;221(2):368-72. PubMed PMID: 7077531. eng.

Benowitz NL, Kuyt F, Jacob P. Circadian blood nicotine concentrations during cigarette smoking. Clin Pharmacol Ther. 1982 Dec;32(6):758-64. PubMed PMID: 7140139. eng.

Borg-Graham LJ, Monier C, Frégnac Y. Visual input evokes transient and strong shunting inhibition in visual cortical neurons. Nature. 1998 May;393(6683):369-73. PubMed PMID: 9620800. eng.

Carandini M, Heeger DJ. Summation and division by neurons in primate visual cortex. Science. 1994 May;264(5163):1333-6. PubMed PMID: 8191289. eng.

Carandini M, Heeger DJ, Movshon JA. Linearity and normalization in simple cells of the macaque primary visual cortex. J Neurosci. 1997 Nov;17(21):8621-44. PubMed PMID: 9334433. eng.

Daniels JD, Pettigrew JD. A study of inhibitory antagonism in cat visual cortex. Brain Res. 1975 Jul;93(1):41-62. PubMed PMID: 166731. eng.

De Valois RL, Yund EW, Hepler N. The orientation and direction selectivity of cells in

macaque visual cortex. Vision Res. 1982;22(5):531-44. PubMed PMID: 7112953. eng.

Disney AA, Aoki C, Hawken MJ. Gain modulation by nicotine in macaque v1. Neuron. 2007 Nov;56(4):701-13. PubMed PMID: 18031686. Pubmed Central PMCID: PMC2875676. eng.

Dreifuss JJ, Kelly JS. The activity of identified supraoptic neurones and their response to acetylcholine applied by iontophoresis. J Physiol. 1972 Jan;220(1):105-18. PubMed PMID: 5059232. Pubmed Central PMCID: PMC1331692. eng.

Durukan AH, Gundogan FC, Erdem U, Kilic S, Sobaci G, Bayraktar MZ. The acute effect of cigarette smoking on pattern visual evoked potentials. Doc Ophthalmol. 2006 Jan;112(1):23-9. PubMed PMID: 16633722. eng.

Fatt P, Katz B. An analysis of the end-plate potential recorded with an intracellular electrode. J Physiol. 1951 Nov;115(3):320-70. PubMed PMID: 14898516. Pubmed Central PMCID: PMC1392060. eng.

Feller MB. The role of nAChR-mediated spontaneous retinal activity in visual system development. J Neurobiol. 2002 Dec;53(4):556-67. PubMed PMID: 12436420. eng.

Furman GGF, L.S. Model of neural inhibition in the mam-maliancochlea. J.acoust.Soc.Am. 1964. 36,2194-2201.

Gilbert CD, Wiesel TN. Intrinsic connectivity and receptive field properties in visual cortex. Vision Res. 1985;25(3):365-74. PubMed PMID: 3895724. eng.

Gotti C, Moretti M, Zanardi A, Gaimarri A, Champtiaux N, Changeux JP, et al. Heterogeneity and selective targeting of neuronal nicotinic acetylcholine receptor (nAChR) subtypes expressed on retinal afferents of the superior colliculus and lateral geniculate nucleus: identification of a new native nAChR subtype alpha3beta2(alpha5 or beta3) enriched in retinocollicular afferents. Mol Pharmacol. 2005 Oct;68(4):1162-71. PubMed PMID: 16049166. eng.

Gribkoff VK, Christian EP, Robinson JH, Deadwyler SA, Dudek FE. Cholinergic excitation of supraoptic neurons in hypothalamic slices of rat. Neuropharmacology. 1988 Jul;27(7):721-7. PubMed PMID: 3419551. eng.

Gullotta. 1981. (Unpublished).

Gullotta FP, Ryan FJ. 1979. (Unpublished).

Hubel DH, Wiesel TN. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol. 1962 Jan;160:106-54. PubMed PMID: 14449617. Pubmed Central PMCID: PMC1359523. eng.

Hubel DH, Wiesel TN. Receptive fields and functional architecture of monkey striate cortex. J Physiol. 1968 Mar;195(1):215-43. PubMed PMID: 4966457. Pubmed Central PMCID: PMC1557912. eng.

Jasper HA. The ten–twenty system of the International Federation. . Electroencephalography and Clinical Neurophysiology, 10, 371–375. 1958.

Kaplan E, Shapley RM. The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. Proc Natl Acad Sci U S A. 1986 Apr;83(8):2755-7. PubMed PMID: 3458235. Pubmed Central PMCID: PMC323379. eng.

Klein R, Klein, BE, Fielding JE, Riva CE, Peautkau ME, Moss SE, Rand LI, Ballard DJ, Morgado PB, Patel V, Guyton AC, Damon DN, Petrig BL, Engvold O, Mohlhauser L, Winship G, West KM, Brinchmann HO, Grace M, Sinclair SH, Grunwald JE, Duling BR, Ross JM, Carrier O, Walker JR, Kuhner, EM, Erdrich LS, Stober JA, Krolewski AS, Dwyer MS, Herbert L. The Effect of Nicotine on Retinal Blood Flow and Autoregulation. Unit Royal Postgraduate Medical School. 2004.

Metz CN, Gregersen PK, Malhotra AK. Metabolism and biochemical effects of nicotine for primary care providers. Med Clin North Am. 2004;88(6):1399-413, ix.

Movshon JA, Thompson ID, Tolhurst DJ. Receptive field organization of complex cells in the cat's striate cortex. J Physiol. 1978 Oct;283:79-99. PubMed PMID: 722592. Pubmed Central PMCID: PMC1282766. eng.

Mrzljak L, Levey AI, Rakic P. Selective expression of m2 muscarinic receptor in the parvocellular channel of the primate visual cortex. Proc Natl Acad Sci U S A. 1996 Jul;93(14):7337-40. PubMed PMID: 8692994. Pubmed Central PMCID: PMC38985. eng.

Rose D, Blakemore C. Effects of bicuculline on functions of inhibition in visual cortex. Nature. 1974 May;249(455):375-7. PubMed PMID: 4842746. eng.

Ryan Ga. 1979. (Unpublished)

Schröder H, Zilles K, Maelicke A, Hajós F. Immunohisto- and cytochemical localization of cortical nicotinic cholinoceptors in rat and man. Brain Res. 1989 Nov;502(2):287-95. PubMed PMID: 2819467. eng.

Shapley R, Kaplan E, Soodak R. Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque. Nature. 1981 Aug;292(5823):543-5. PubMed PMID: 7254350. eng.

Shapley R, Victor JD. The contrast gain control of the cat retina. Vision Res. 1979;19(4):431-4. PubMed PMID: 473613. Eng.

Shapley RM, Victor JD. The effect of contrast on the transfer properties of cat retinal ganglion cells. J Physiol. 1978 Dec;285:275-98. PubMed PMID: 745079. Pubmed Central PMCID: PMC1281756. eng.

Shapley RM, Victor JD. Nonlinear spatial summation and the contrast gain control of cat retinal ganglion cells. J Physiol. 1979 May;290(2):141-61. PubMed PMID: 469742. Pubmed Central PMCID: PMC1278829. eng.

Somogyi P, Takagi H, Richards JG, Mohler H. Subcellular localization of benzodiazepine/GABAA receptors in the cerebellum of rat, cat, and monkey using monoclonal antibodies. J Neurosci. 1989 Jun;9(6):2197-209. PubMed PMID: 2542489. eng.

Spitzer H, Hochstein S. A complex-cell receptive-field model. J Neurophysiol. 1985 May;53(5):1266-86. PubMed PMID: 3998809. eng.

Strang CE, Renna JM, Amthor FR, Keyser KT. Nicotinic acetylcholine receptor expression by directionally selective ganglion cells. Vis Neurosci. 2007 2007 Jul-Aug;24(4):523-33. PubMed PMID: 17686198. eng.

Thompson JC, Tzambazis K, Stough C, Nagata K, Silberstein RB. The effects of nicotine on the 13 Hz steady-state visually evoked potential. Clin Neurophysiol. 2000 Sep;111(9):1589-95. PubMed PMID: 10964069. eng.

Ts'o DY, Gilbert CD, Wiesel TN. Relationships between horizontal interactions and functional architecture in cat striate cortex as revealed by cross-correlation analysis. J Neurosci. 1986 Apr;6(4):1160-70. PubMed PMID: 3701413. eng.

U.S. Department of Health and Human Services How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking Attributable Disease*: A Report of the Surgeon General*. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2010.

Varghese, SB, Reid, JC, Hartmann, EE, & Keyser, KT. The effects of nicotine on the human electroretinogram. Invest Ophthalmol Vis Sci. 2011. 52(13), 9445-9451. doi: iovs.11-7874 [pii]10.1167/iovs.11-7874

Victor JD, Mast J. A new statistic for steady-state evoked potentials. Electroencephalogr Clin Neurophysiol. 1991 May;78(5):378-88. PubMed PMID: 1711456. eng.

Woodson PP, Baettig K, Etkin MW, Kallman WM, Harry GJ, Kallman MJ, et al. Effects of nicotine on the visual evoked response. Pharmacol Biochem Behav. 1982 Nov;17(5):915-20. PubMed PMID: 7178205. eng.

Wynder, E. L. and Hoffman, D. Tobacco and Tobacco Smoke. Academic Press, New York. 1967. Pp. 375 and 379.

Zaninetti M, Blanchet C, Tribollet E, Bertrand D, Raggenbass M. Magnocellular neurons of the rat supraoptic nucleus are endowed with functional nicotinic acetylcholine receptors. Neuroscience. 2000;95(2):319-23. PubMed PMID: 10658610. eng.

Zemon V, Conte MM, Camisa J. Stimulus orientation and contrast constancy. Int J Neurosci. 1993 1993 Mar-Apr;69(1-4):143-8. PubMed PMID: 8083001. eng.

Zemon V, Gordon J, Welch J. Asymmetries in ON and OFF visual pathways of humans revealed using contrast-evoked cortical potentials. Vis Neurosci. 1988;1(1):145-50. PubMed PMID: 3154786. eng.

Zemon V, Kaplan E, Ratliff F. Bicuculline enhances a negative component and diminishes a positive component of the visual evoked cortical potential in the cat. Proc Natl Acad Sci U S A. 1980 Dec;77(12):7476-8. PubMed PMID: 6938987. Pubmed Central PMCID: PMC350527. eng.

Zemon V, Kaplan E, Ratliff F. The role of GABA-mediated intracortical inhibition in the generation of visual evoked potentials. In: Cracco RQ, Bodis-Wollner I, editors. Evoked Potentials. Frontiers of Clinical Neuroscience. 1986. Vol. 3. New York: Liss. pp. 287–295.

Zemon V, Ratliff F. Intermodulation components of the visual evoked potential: responses to lateral and superimposed stimuli. Biol Cybern. 1984;50(6):401-8. PubMed PMID: 6487677. eng.

Table 1.

Stimuli used during VEP testing.

Pattern	Type	Measure	Emphasis
Checkerboard	Transient	Amplitude, latency	Overall response, P100
Windmill-Dartboard (WD)	Steady state; Fourier Analysis	Amplitude, phase, SNR, SI, FI	Lateral interactions
Partial-Windmill (PW)	Steady state; Fourier Analysis	Amplitude, phase, SNR, SI, FI	Lateral interactions
Isolated Checks (IC)	Steady state (bright or dark); Fourier Analysis	Amplitude, phase, SNR	M & P, ON/FF

Table 2.

Amplitude, phase, and SNR for both F1 and F2 under the Windmill-Dartboard and Partial-Windmill conditions.

Condition	**Windmill-Dartboard**			**Partial-Windmill**		
Harmonic	Amplitude	Phase	SNR	Amplitude	Phase	SNR
F1	2↑*** 4↑*	4↑**	NS	2↑*, 4↑*	4↓**** 2↓**	2↑**** 4↑****
F2	4↓*	2↑** 4↑**	NS	4↑**	2↑*	NS

F1: 1st harmonic; F2: 2nd harmonic; 2: 2 mg; 4: 4 mg;
↓: decrease; ↑: increase. * $p \leq .05$, ** $p \leq .01$, *** $p \leq .005$, **** $p \leq .001$,
NS: not significant

Table 3.

FI and SI indices.

Harmonic	2 mg	4 mg
FI	NS	*↑
SI	NS	****↑

Indices for quantifying VEP contributions (different types of lateral interactions):

Facilitation index (FI) = F1 WD/F2 WD.

Suppression index (SI) = F2 PW/ F2 WD.

* $p \leq .05$; **** $p \leq .001$, NS: not significant

Table 4.

Changes in amplitudes and SNR of isolated-checks stimuli : MB, MD, PB, & MD conditions.

Condition	Amplitude	SNR
MB	2↑**** 4↑***	NS
MD	NS	NS
PB	NS	NS
PD	NS	NS

MB: Magno bright; MD: Magno dark; PB: Parvo bright; PD: Parvo dark
2: 2 mg; 4: 4mg; ↑**:** increase; ↓**:** decrease; *** $p \leq .005$; **** $p \leq .001$,
NS: Not significant

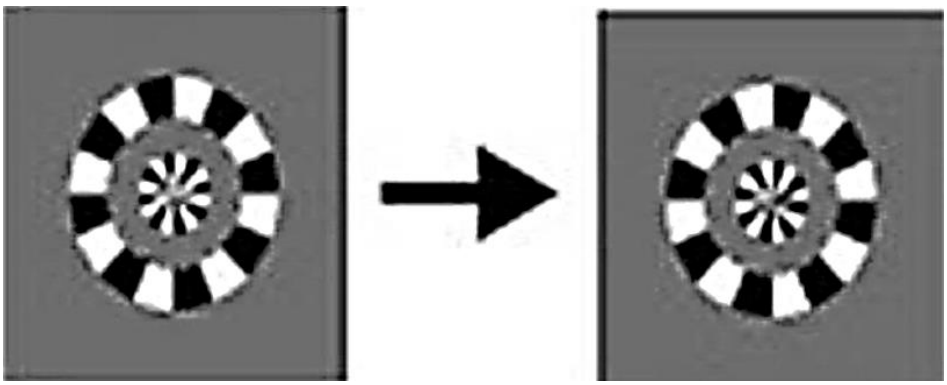
Table 5.

Statistically significant changes in model parameters 30 minutes post nicotine ingestion.

Condition	**Initial Conductance (g_0)**	**Initial Phase (ϕ_0)**	**Threshold (d_0)**	**Shunting Coefficient (m)**
MB	2↓**, 4↑***	NS	NS	2↓*, 4↑*
MD	NS	NS	2↑*, 4↓*	4↑**
PB	NS	NS	2↑*, 4↓*	2↑***, 4↑***
PD	2↓*, 4↑*	4↓**	2↑*, 4↑*	2↑***, 4↑***

MB: Magno bright; MD: Magno dark; PB: Parvo bright; PD: Parvo dark
2: 2 mg; 4: 4mg; *: statistically significant; ↑**:** increase; ↓**:** decrease;
* $p \leq .05$; ** $p \leq .01$; *** $p \leq .005$

A



B

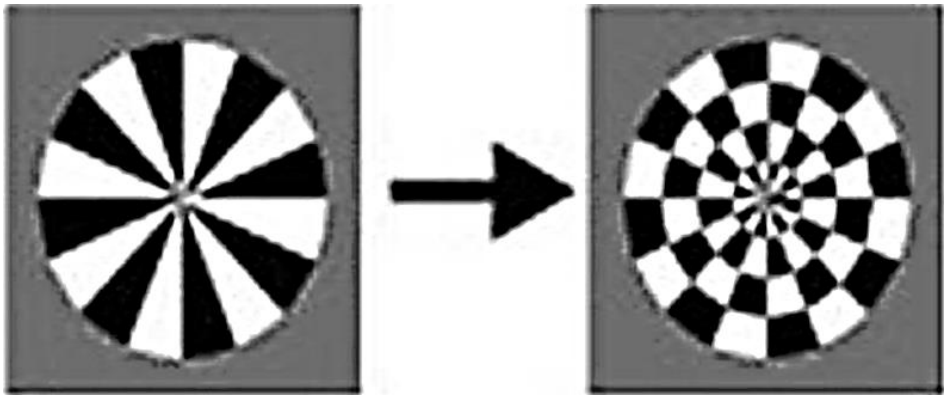


Figure. 1 (A) Partial-Windmill pattern. (B) Windmill-Dartboard pattern.

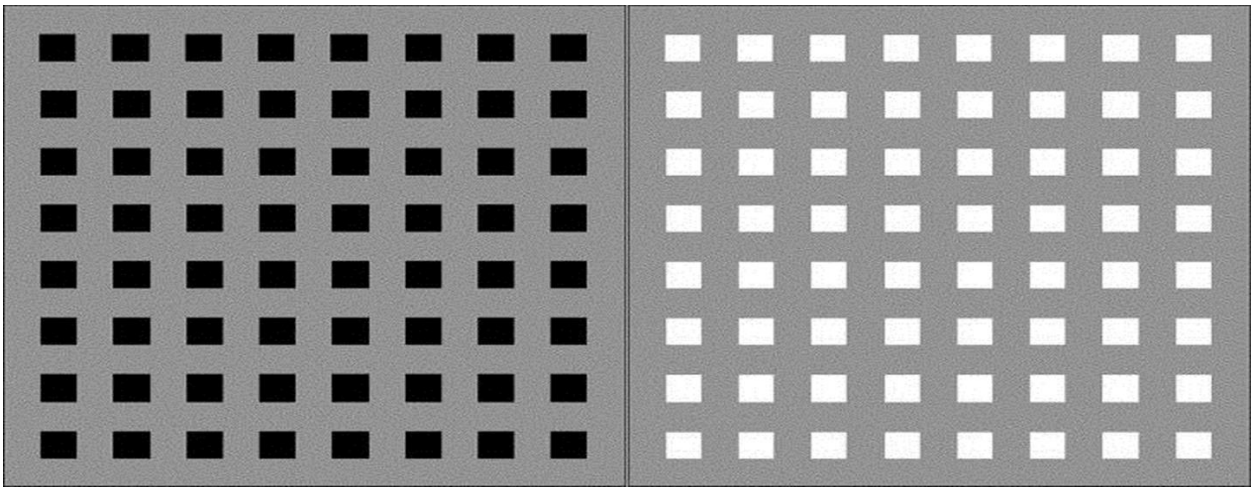


Figure. 2 Left, dark-check condition (negative contrast); right, bright-check condition (positive contrast).

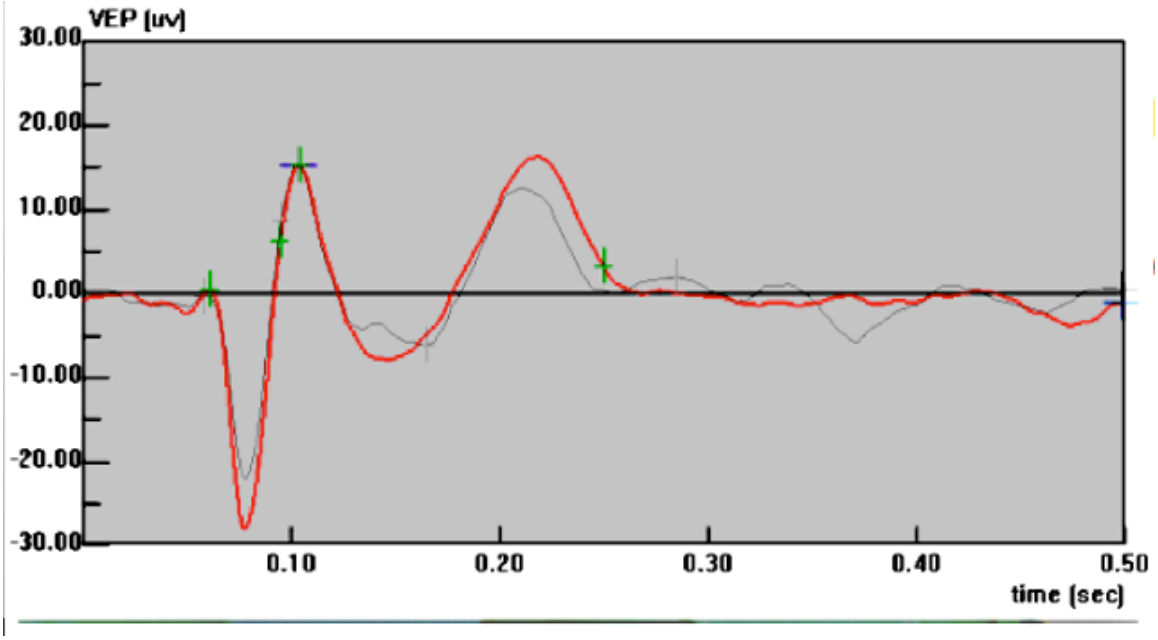


Figure. 3 P100 transient response: Date obtained from a single subject showing superimposed transient VEP wave comparison of baseline and 4 mg nicotine condition. Light grey: baseline; red: nicotine condition.

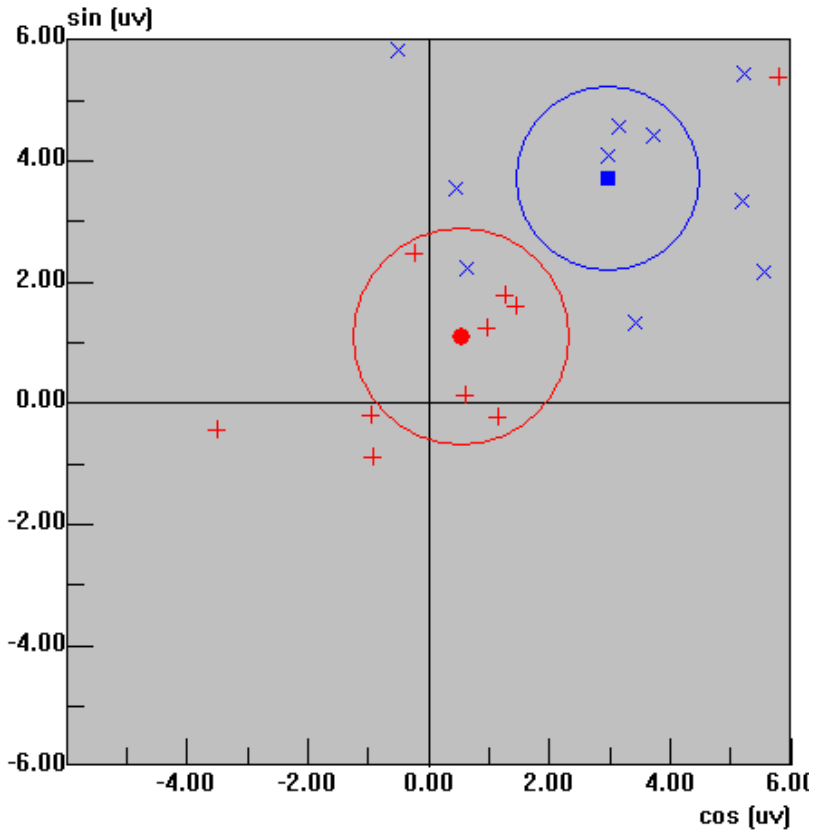


Figure. 4 Fourier components for F1 responses from IC-VEP under the MB condition. [Red: pre-nicotine; blue: post nicotine. The circle represents the 95% confidence region based on the T^2circ statistic. (Red circle: includes the origin and therefore in the noise. Blue circle: does not include the origin and therefore above the noise).]

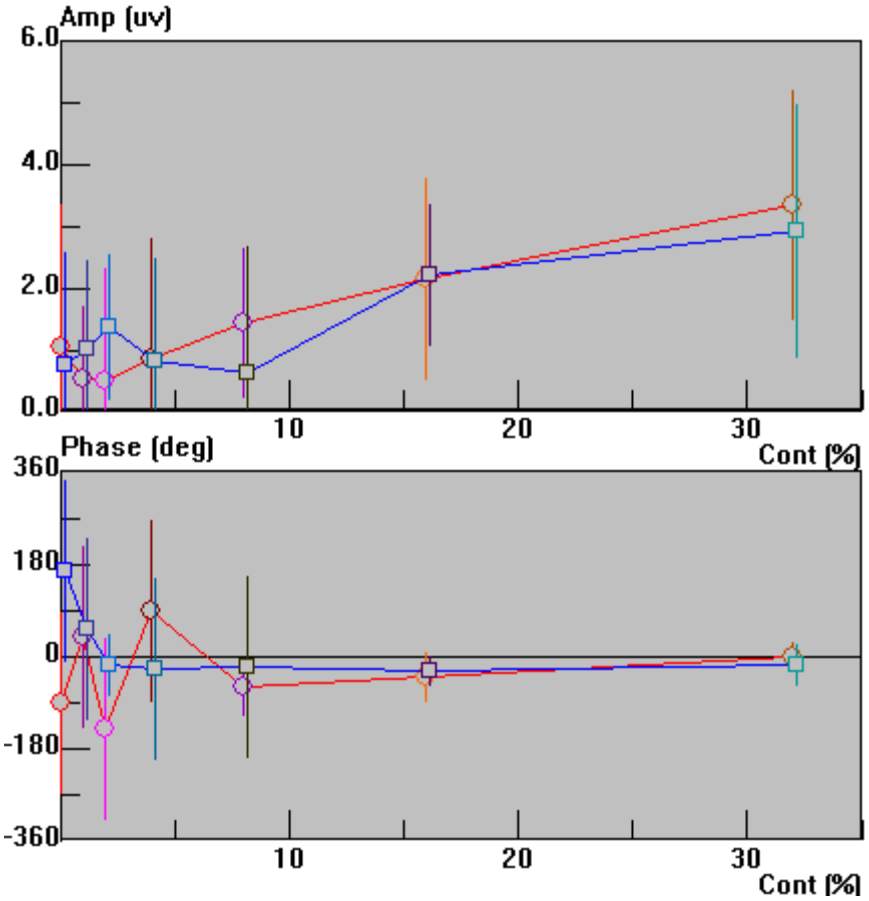


Figure. 5 Example showing amplitude vs. contrast (top) and phase vs. contrast (bottom). [Red: pre nicotine, Blue: post nicotine. (MB condition used as an example)].

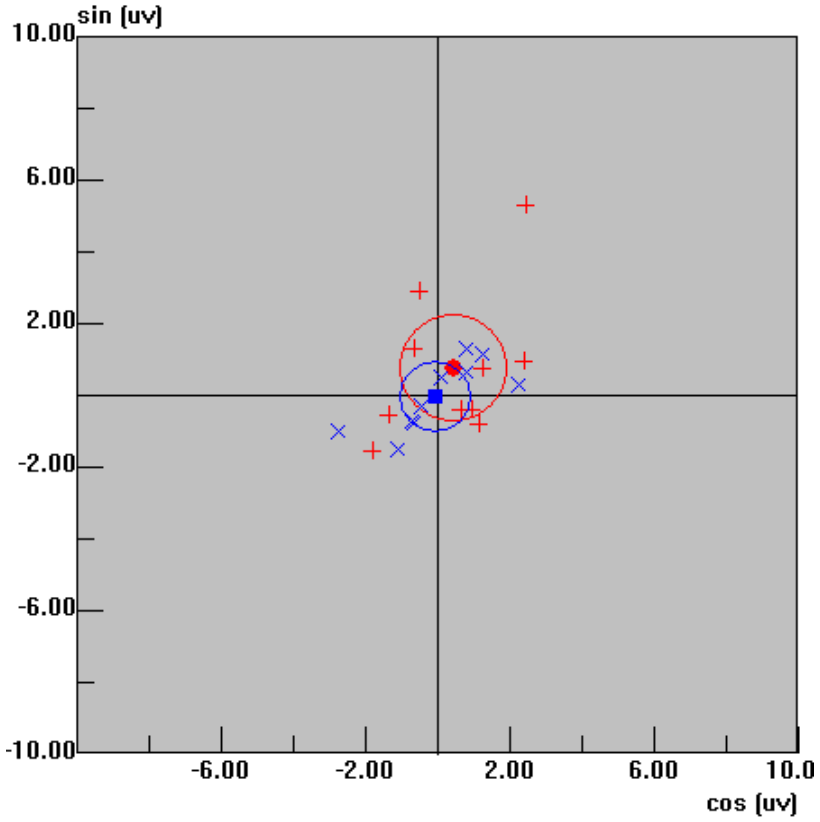


Figure. 6 PW condition: Red: pre nicotine, blue: post nicotine. [Example: 1st harmonics, both pre and post nicotine SNRs are below 1 and therefore in the noise (both circles include the origin)].

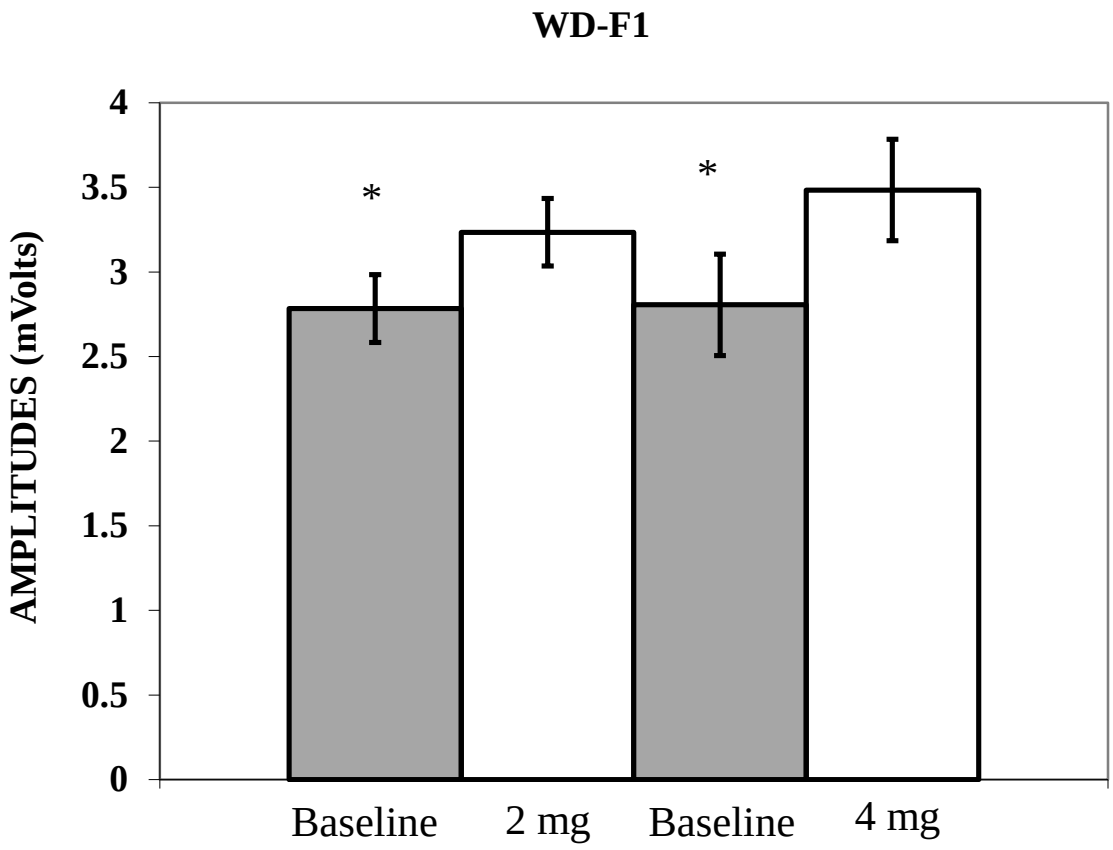


Figure. 7 F1 Amplitudes for WD VEPs post 2 and 4 mg nicotine. Error bars: SEM. * $p \leq .05$

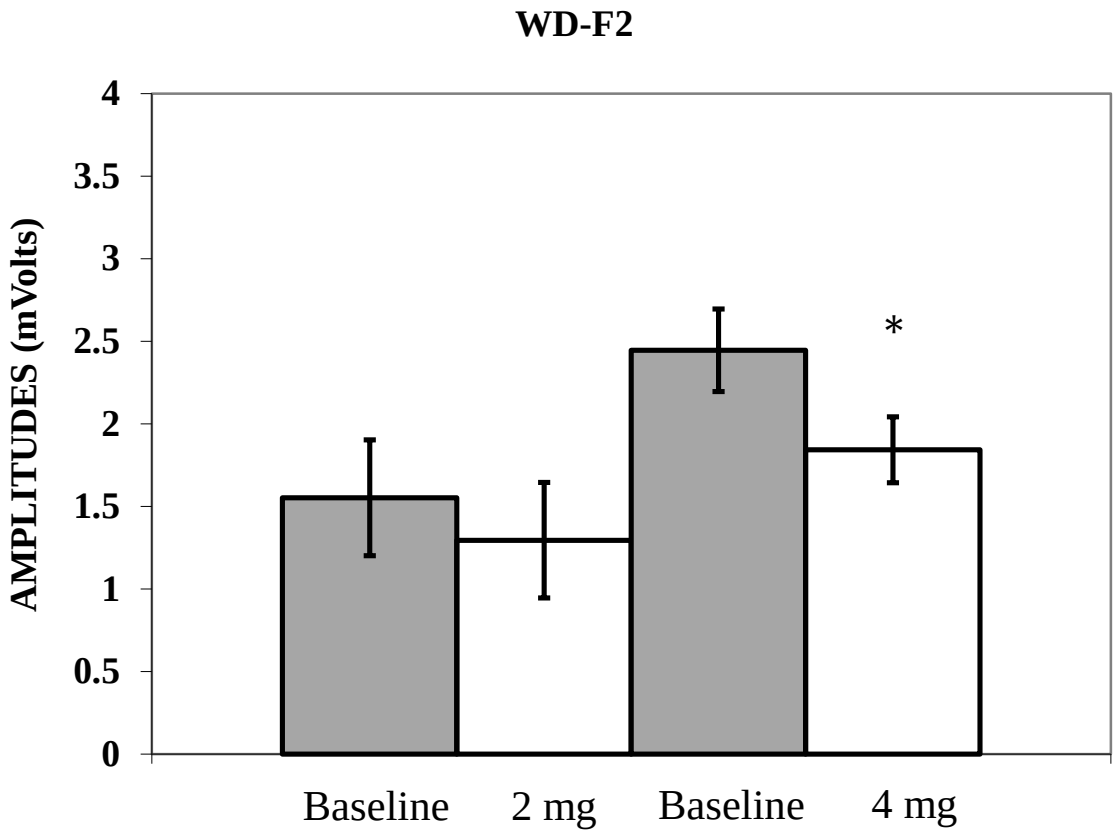


Figure. 8 F2 amplitudes for WD VEPs post 2 and 4 mg nicotine. Error bars: SEM. * $p \leq .05$

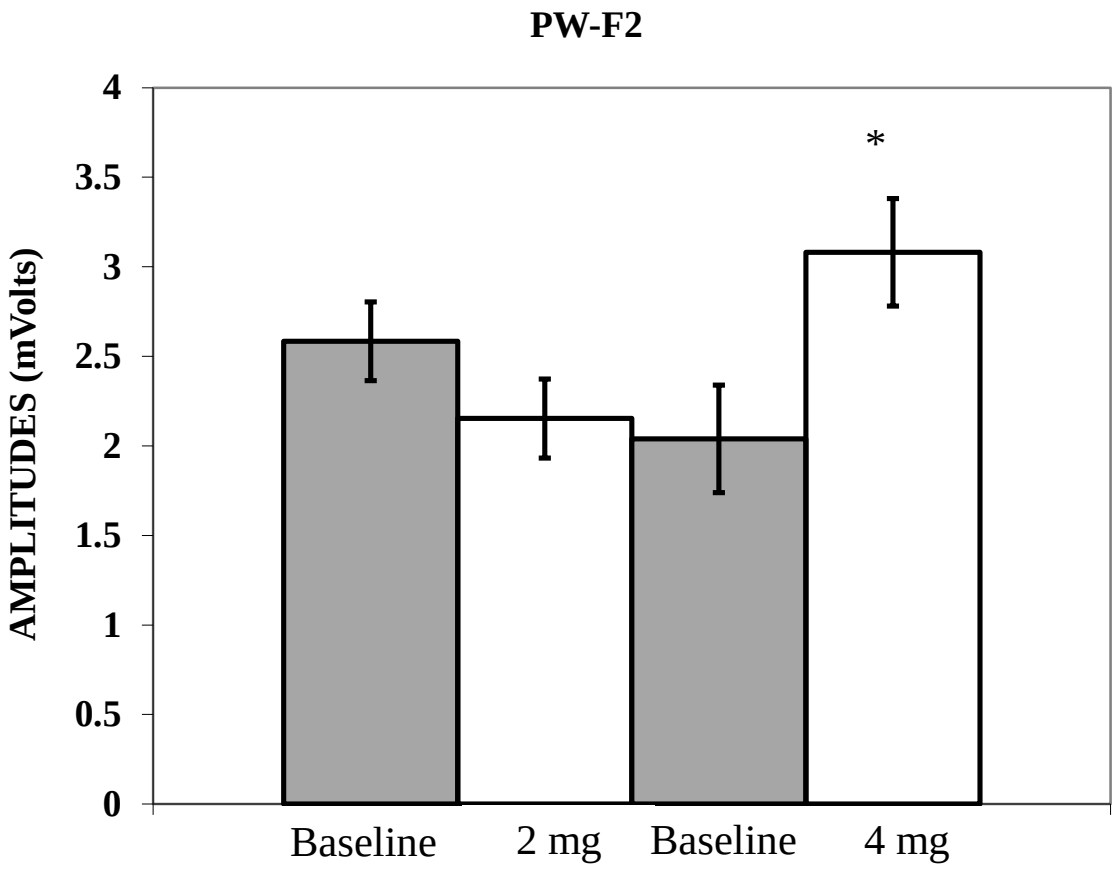


Figure. 9 F2 amplitudes for PW VEPs post 2 and 4 mg nicotine. Error bars: SEM. * $p \leq .05$

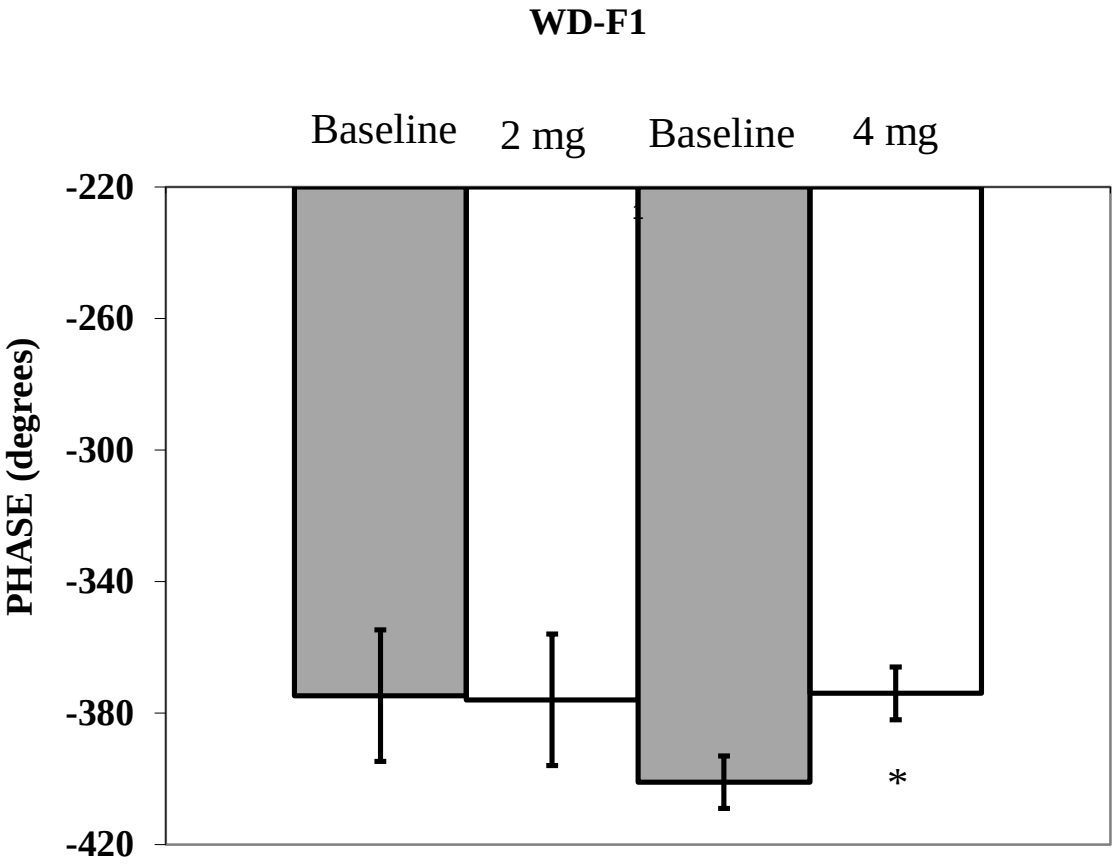


Figure. 10 F1 Phase changes for WD VEPs post 2 and 4 mg nicotine. Error bars: SEM. * $p \leq .05$.

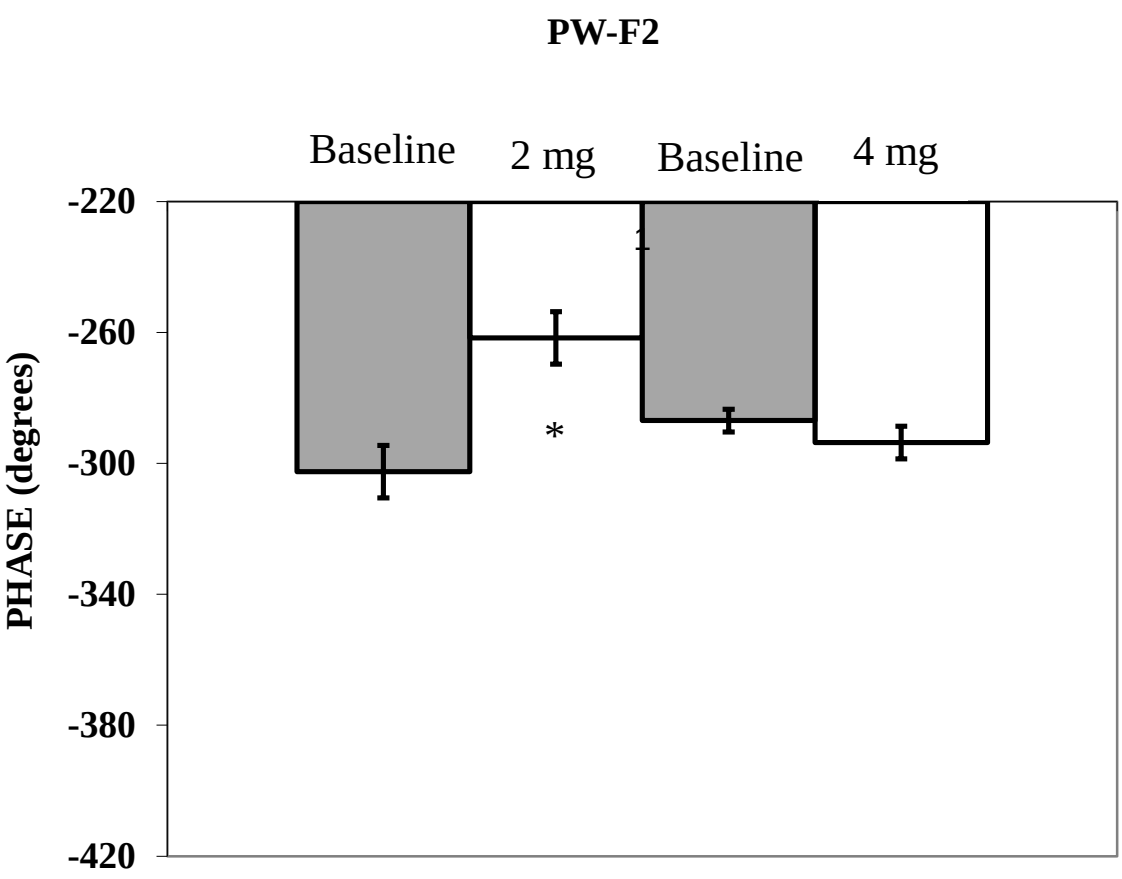


Figure. 11 F2 Phase changes for PW VEPs post 2 and 4 mg nicotine. Error bars; SEM. * $p \leq .05$.

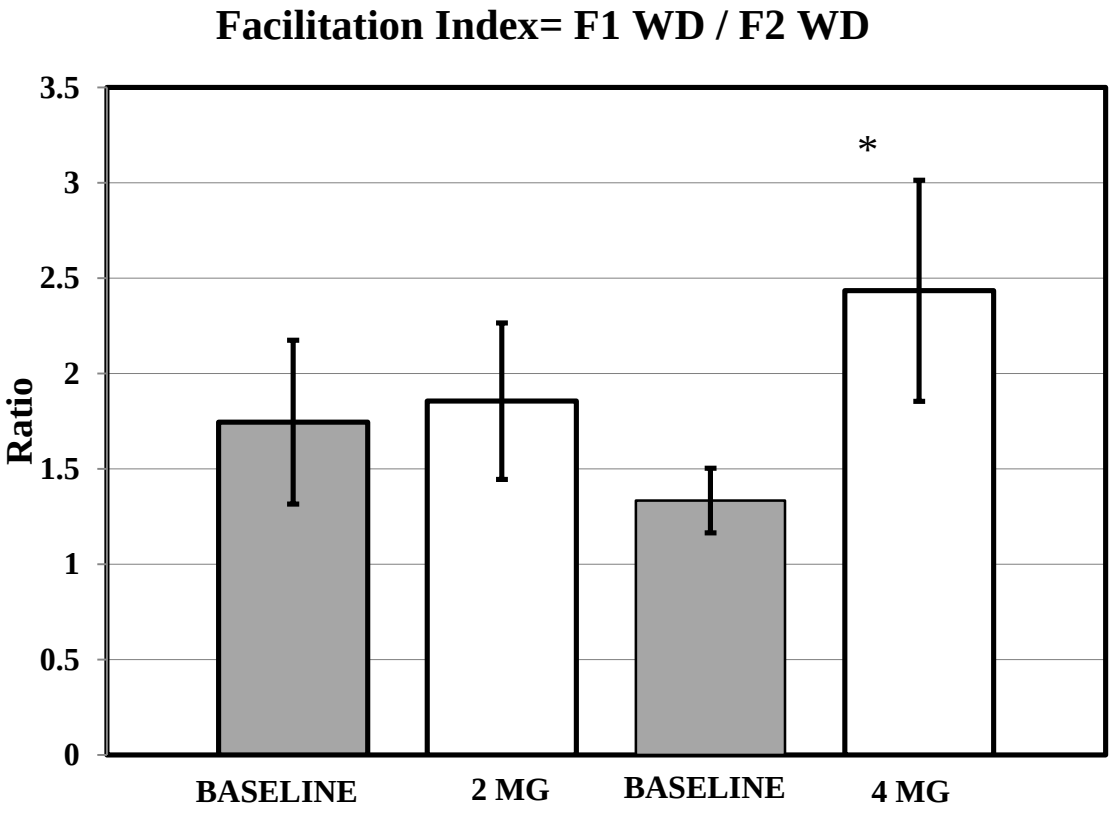


Figure. 12 Changes in facilitation index (FI) between baseline and 2 and 4 mg nicotine conditions. Error bars; SEM. * $p \leq .05$

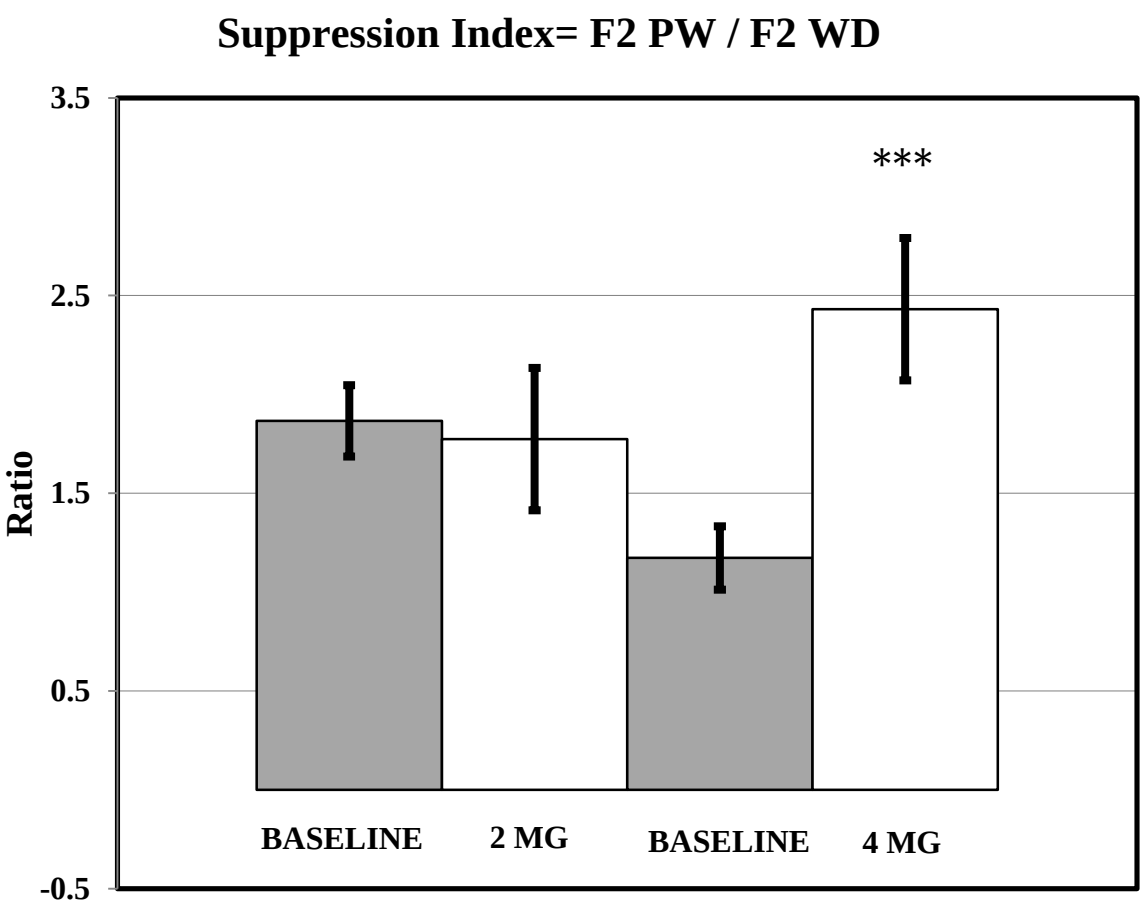


Figure. 13 Changes in suppression index (SI) between baseline and 2 and 4 mg nicotine conditions. Error bars: SEM. *** $p \leq .001$

GLOBAL DISCUSSION

This study brings important and significant findings regarding the effects of nicotine on the visual pathways. There are marked differences between the results obtained from this study and those in the literature. We incorporated possible excitatory and inhibitory mechanisms to justify for such actions by nicotine on the visual pathways.

Relationship to Previous Studies

Color Vision

The results from this study contradict previous reports examining the effects of smoking on color vision discrimination. In general, our subjects, who are non-smokers, showed improvements in color discrimination and increment thresholds compared with the reported deteriorations in color vision in smokers. Erb et al. (1999) and Bimler & Kirkland (2004) found that the TES was higher in smokers compared to controls. However, in both studies, no isolated effect of nicotine was documented. It is difficult to attribute the changes observed from both studies to nicotine alone.

In the present study we found that the TES of our subjects improved with both 2 and 4 mg nicotine gum, and the increment threshold to white and red light stimuli

improved with the 4 mg dose. Therefore, we believe that the effects observed from our study appear to be governed by the action of nicotine alone on the color vision pathway. In addition, since the improvement to increment threshold was more robust with the colored (red) than the non-colored (white) stimuli, and was dose dependent, and even though it is possible that the effect is both neuronal and vascular, we believe it to be more likely to be neuronal, mediated by the action of nicotine on nAChRs in the brain and the retina.

VEPs

Various studies have investigated the effects of smoking on responses of the VEP. While these studies have reported conflicting data with regard to the effect on latencies and amplitudes, most reported increases in amplitudes and decreases in latencies. We found no study examining the possible effects of nicotine alone on responses of the M and P pathways. We also could not find any studies that investigated the possible cortical lateral inhibition change that could be exerted by the action of nicotine alone.

To date, this is the first study to examine the effects of nicotine on responses of the M and P pathways by means of VEPs. Results indicate that the effect of nicotine on amplitudes is on MB. In addition, attenuation in amplitude and phase of F1 and F2 of the WD and PW clearly indicate the role of nicotine in modulating highly localized interactions. The enhancement in *m*, the shunting coefficient, indicates that nicotine plays a major role in shunting inhibition. An increase in shunting inhibition causes a decrease in the time constant of the cell. It is possible that GABAergic modulation of such

inhibition plays a role in modulating responses of the visual system. This would agree with Disney's study (2007). By incorporating the IC data we obtained, into a nonlinear biophysical model (Zemon & Gordon, 2006), we were able to demonstrate that the coefficient of shunting inhibition (*m*) has significantly increased under all conditions (MB, MD, PB, PD). The results we obtained clearly demonstrate that nicotine could have an excitatory as well as an inhibitory effect on neurons.

We know that neurons of the M pathway are more sensitive to low contrast and non-colored stimuli, while neurons of the P pathway prefer colored stimuli and respond optimally to high contrast. We suspected that the effect of nicotine alone on non-smokers would be different than the effect of smoking on smokers, and it turned out that the effect was an improvement in color vision thresholds rather than a decline in performance, in both color vision tests, and from both doses. Studies have reported that nAChRs are expressed on ON-cone BP cells in the retina in many species, and our results are consistent with effects on some of the conditions. In addition, the model-based change in conductance, and hence in shunting inhibition, was significantly altered with both bright and dark IC, especially with the MB and PD conditions, indicating a strong effect on both pathways.

We speculate that the effect of nicotine could be established via various routes. It is possible that nicotine is exciting glutamatergic neurons, and those neurons excited nAChRs expressed by GABAergic cells. It also is possible that nicotine directly affected nAChRs expressed by GABAergic cells, and that led to more inhibition. Another possibility is that nicotine did bind to GABAergic cells expressing nAChRs, and those GABAergic cells in turn inhibited other neighboring GABAergic cells, which led to dis-

inhibition of those neighboring GABAergic cells, and therefore an excitation. All possibilities lead us to believe that nicotine is modulating the visual responses by way of balancing both excitatory and inhibitory inputs. The increase in shunting inhibition, amplitudes under the MB condition, and the increase in F1 and F2 of the WD and PW, all tells us that nicotine has improved subjects' ability to detect contrast. Overall, we believe that nicotine has impacted our visual perception.

GENERAL LIST OF REFERENCES

Albuquerque EX, Barnard EA, Porter CW, Warnick JE. The density of acetylcholine receptors and their sensitivity in the postsynaptic membrane of muscle endplates.Proc Natl Acad Sci U S A. 1974. Jul;71(7):2818-22.

Albuquerque EX, Deshpande SS, Kawabuchi M, Aracava Y, Idriss M, Rickett DL, and Boyne AF. Multiple actions of anticholinesterase agents on chemosen- sitive synapses: molecular basis for prophylaxis and treatment of organophosphate poisoning. *Fundam Appl Toxicol.* 1985. 5:S182–S203.

Alkondon M, Albuquerque EX. The nicotinic acetylcholine receptor subtypes and their function in the hippocampus and cerebral cortex. Prog Brain Res. 2004;145:109-20.

Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. J Neurosci. 2000 Jan;20(1):66-75. PubMed PMID: 10627582. eng.

Armitage AK, Hall GH, Sellers CM. Effects of nicotine on electrocortical activity and acetylcholine release from the cat cerebral cortex. Br J Pharmacol. 1969 Jan;35(1):152-60.

Armitage AK, Milton AS, Morrison CF. Effects of nicotine and some nicotine-like compounds injected into the cerebral ventricles of the cat. Br J Pharmacol Chemother. 1966 May;27(1):33-45.

Baccus SA, Meister M.Retina versus cortex; contrast adaptation in parallel visual pathways.Neuron. 2004. Apr 8;42(1):5-7.

Barker JL, Crayton JW, Nicoll RA. Supraoptic neurosecretory cells: adrenergic and cholinergic sensitivity. Science. 1971 Jan;171(967):208-10. PubMed PMID: 4395231. eng.

Beleslin D, Polak RL, Sproull DH. The effect of leptazol and strychnine on the acetylcholine release from the cat brain. J Physiol. 1965;181(2):308-16.

Benardete EA, Kaplan E. The receptive field of the primate P retinal ganglion cell, I: Linear dynamics. Vis Neurosci. 1997. Jan-Feb;14(1):169-85.

Benardete EA, Kaplan E. Dynamics of primate P retinal ganglion cells: responses to chromatic and achromatic stimuli. J Physiol. 1999. Sep 15;519 Pt 3:775-90.

Benowitz NL, Jacob P, Jones RT, Rosenberg J. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. J Pharmacol Exp Ther. 1982 May;221(2):368-72. PubMed PMID: 7077531. eng.

Benowitz NL, Kuyt F, Jacob P. Circadian blood nicotine concentrations during cigarette smoking. Clin Pharmacol Ther. 1982 Dec;32(6):758-64. PubMed PMID: 7140139. eng.

Benowitz NL, Porchet H, Sheiner L, Jacob P. Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum. Clin Pharmacol Ther. 1988; 44(1):23-8.

Benowitz NL. Clinical pharmacology of nicotine. Annu Rev Med. 1986;37:21-32. PubMed PMID: 3518606. eng.

Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol. 1996; 36:597-613.

Bimler D, Kirkland J. Multidimensional scaling of D15 caps: color-vision defects among tobacco smokers? Vis Neurosci. 2004. 21(3):445-8.

Blakemore C, Vital-Durand F. Organization and post-natal development of the monkey's lateral geniculate nucleus. J Physiol. 1986 Nov;380:453-91.
Boycott BB, Dowling JE. Organization of the primate retina: light microscopy. Phil Trans R Soc B. 1969; 255:109–184.

Boycott BB, Wässle H. Morphological Classification of Bipolar Cells of the Primate Retina.Eur J Neurosci. 1991 Oct;3(11):1069-1088.

Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, et al. Cigarette smoking saturates brain alpha 4 beta 2 nicotinic acetylcholine receptors. Arch Gen Psychiatry. 2006;63(8):907-15.

Calkins DJ, Thornton JE, Pugh EN Jr. Monochromatism determined at a long-wavelength/middle-wavelength cone-antagonistic locus. Vision Res. 1992. Dec;32(12):2349-67. Erratum in: Vision Res 1993 May;33(8):1151.

Calkins DJ, Sterling P. Evidence that circuits for spatial and color vision segregate at the first retinal synapse. Neuron. 1999. Oct;24(2):313-21.

Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK. Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. Neuron. 2004;41(6):907-14.

Chaparro A, Stromeyer CF 3rd, Huang EP, Kronauer RE, Eskew RT Jr. Colour is what the eye sees best. Nature. 1993. Jan 28;361(6410):348-50.

Chatterjee S, Callaway EM. S cone contributions to the magnocellular visual pathway in macaque monkey. Neuron. 2002. Sep 12;35(6):1135-46.

Clayton RM, Cuthbert J, Duffy J, Seth J, Phillips CI, Bartholomew RS, et al. Some risk factors associated with cataract in S.E. Scotland: a pilot study. Trans Ophthalmol Soc U K. 1982;102 Pt 3:331-6.

Croner LJ, Kaplan E. Receptive fields of P and M ganglion cells across the primate retina. Vision Res. 1995. Jan;35(1):7-24. PubMed PMID: 7839612. eng.

Crook JM, Lange-Malecki B, Lee BB, Valberg A. Visual resolution of macaque retinal ganglion cells. J Physiol. 1988. 396:206 –224.

Dacey DM, and Petersen M. Dendritic field size and morphology of midget parasol ganglion cells of the human retina. Proc. Natl. 1992. Acad Sci. USA. 89: 9666-9670.

Dani JA. Overview of nicotinic receptors and their roles in the central nervous system. Biol Psychiatry. 2001. Feb 1;49(3):166-74. Review.

Derrington AM, Krauskopf J, Lennie P. Chromatic mechanisms in lateral geniculate nucleus of macaque. J Physiol. 1984. Dec;357:241-65.

De Valois RL, Abramov I., & Jacobs GH. Analysis of response patterns of LGN cells. J.opt. Soc. Am. 1966. 56,966-977.

De Valois RL, Yund EW, Hepler N. The orientation and direction selectivity of cells in macaque visual cortex. Vision Res. 1982;22(5):531-44. PubMed PMID: 7112953. eng.

Disney AA, Aoki C, Hawken MJ. Gain modulation by nicotine in macaque v1. Neuron. 2007 Nov;56(4):701-13. PubMed PMID: 18031686. Pubmed Central PMCID: PMC2875676. eng.

Dreifuss JJ, Kelly JS. The activity of identified supraoptic neurones and their response to acetylcholine applied by iontophoresis. J Physiol. 1972 Jan;220(1):105-18. PubMed PMID: 5059232. Pubmed Central PMCID: PMC1331692. eng.

Durukan AH, Gundogan FC, Erdem U, Kilic S, Sobaci G, Bayraktar MZ. The acute effect of cigarette smoking on pattern visual evoked potentials. Doc Ophthalmol. 2006 Jan;112(1):23-9. PubMed PMID: 16633722. eng.

Erb C, Nicaeus T, Adler M, Isensee J, Zrenner E, Thiel HJ. Colour vision disturbances in chronic smokers. Graefes Arch Clin Exp Ophthalmol. 1999; 237(5):377-80.

Fant RV, Owen LL, Henningfield JE. Nicotine replacement therapy. Prim Care. 1999;26(3):633-52.

Feller MB. The role of nAChR-mediated spontaneous retinal activity in visual system development. J Neurobiol. 2002. Dec;53(4):556-67. PubMed PMID: 12436420. eng.

Fletcher R, Voke J. Defective Color Vision: Fundamentals, diagnosis, and management. (2nd ed.) New York: HarperCollins. 1985.

Frazor RA, Geisler WS. Local luminance and contrast in natural images. Vision Res. 2006. 46, 1585-1598.

Goodchild AK, Chan TL, Grunert U. Horizontal cell connections with short-wavelength-sensitive cones in macaque monkey retina. Visual Neuroscience. 1996;13(5):833–845.

Gotti C, Moretti M, Zanardi A, Gaimarri A, Champtiaux N, Changeux JP, et al. Heterogeneity and selective targeting of neuronal nicotinic acetylcholine receptor (nAChR) subtypes expressed on retinal afferents of the superior colliculus and lateral geniculate nucleus: identification of a new native nAChR subtype alpha3beta2(alpha5 or beta3) enriched in retinocollicular afferents. Mol Pharmacol. 2005 Oct;68(4):1162-71. PubMed PMID: 16049166. eng.

Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. Heterogeneity and complexity of native brain nicotinic receptors. Biochem Pharmacol. 2007;74(8):1102-11.

Gouras P. Identification of cone mechanisms in monkey ganglion cells. J. Physiol. 1968. 199, 537-547.

Gounas P. Antidromic responses of orthodromically identified ganglion cells in monkey retina. Journal of Physiology (London). 1969. 204,407-41.

Grando SA, Zelickson BD, Kist DA, Weinshenker D, Bigliardi PL, Wendelschafer-Crabb G, Kennedy WR, Dahl MV. Keratinocyte muscarinic acetylcholine receptors: immunolocalization and partial characterization.J Invest Dermatol. 1995. Jan;104(1):95-100.

Gribkoff VK, Christian EP, Robinson JH, Deadwyler SA, Dudek FE. Cholinergic excitation of supraoptic neurons in hypothalamic slices of rat. Neuropharmacology. 1988 Jul;27(7):721-7. PubMed PMID: 3419551. eng.

Guo JZ, Tredway TL, Chiappinelli VA. Glutamate and GABA release are enhanced by different subtypes of presynaptic nicotinic receptors in the lateral geniculate nucleus. J Neurosci. 1998;18(6):1963-9.

Hagg E, Asplund K. Is endocrine ophthalmopathy related to smoking? Br Med J (Clin Res Ed). 1987; 295(6599):634-5.

Han ZY, Le Novere, Zoli M, Hill Jr. JA, Champtiaux N, Changeux JP. Localization of

nAChR subunit mRNAs in the brain of Macaca mulatta. Eur. J. Neurosci. 2000. 12, 3664–3674.

Harding JJ, Van Heyningen R. Drugs, including alcohol, that act as risk factors for cataract, and possible protection against cataract by aspirin-like analgesics and cyclopenthiazide. Br J Ophthalmol. 1988;72(11):809-14.

Hartline HK. The response of single optic nerve fibers of the vertebrate eye to illumination of the retina. *Am J Physiol*. 1938. 121: 400-415).

Henningfield JE, Radzius A, Cooper TM, Clayton RR. Drinking coffee and carbonated beverages blocks absorption of nicotine from nicotine polacrilex gum. JAMA. 1990;264(12):1560-4.

Hood DC, Greenstein VC. Blue (S) cone pathway vulnerability: a test of a fragile receptor hypothesis. Appl Opt. 1988. Mar 15;27(6):1025-9. doi: 10.1364/AO.27.001025.

Hubel DH, Wiesel TN. Laminar and columnar distribution of geniculo-cortical fibers in the macaque monkey. J Comp Neurol. 1972. Dec;146(4):421-50.

Hurvich LM, Jameson D. An opponent-process theory of color vision. Psychol Rev. 1957. Nov;64, Part 1(6):384-404.

Iyamu E, Ekure E, Oghre E. The effect of smokeless tobacco on intra-ocular pressure in a Nigerian population. Health and Allied Sciences. 2002.

Kaplan E, Shapley RM. The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. Proc Natl Acad Sci U S A. 1986 Apr;83(8):2755-7. PubMed PMID: 3458235. Pubmed Central PMCID: PMC323379. eng.

Kaplan E, Shapley RM. X and Y cells in the lateral geniculate nucleus of macaque monkeys. J Physiol. 1982 Sep;330:125-43. PubMed PMID: 7175738. Pubmed Central PMCID: PMC1225290. eng.

Kaplan. The P, M and K Streams of the Primate Visual System: What Do They Do for Vision?. In: Allan I. Basbaum, Akimichi Kaneko, Gordon M. Shepherd and Gerald Westheimer, editors The Senses: A Comprehensive Reference, Vol 1, Vision I, Richard Masland and Thomas D. Albright. San Diego: Academic Press; 2008. p. 369-382.

Kaspari L. No Smoking. http://www.mdsupport.org/library/nosmoke.html.

Kellar KJ, Whitehouse PJ, Martino-Barrows AM, Marcus K, Price DL. Muscarinic and nicotinic cholinergic binding sites in Alzheimer's disease cerebral cortex. Brain Res. 1987;436(1):62-8.

Kingdom FA, Mullen KT. Separating colour and luminance information in the visual system.

Spat Vis. 1995. 9(2):191-219. Review.

Kolb H, Linberg KA, and Fisher SK. The neurons of the human retina: a Golgi study. J. Comp. 1992. Neurol. 318 : 147-187.

Kolb H. Organization of the outer plexiform layer of the primate retina: electron microscopy of Golgi-impregnated cells. Philos Trans. R. Soc. B.1970; 258:261–283.

Krauskopf J, Williams DR, Heeley DW. Cardinal directions of color space.Vision Res. 1982.22(9):1123-31.

Lee AJ, Rochtchina E, Wang JJ, Healey PR, Mitchell P. Does smoking affect intraocular pressure? Findings from the Blue Mountains Eye Study. *J Glaucom. 2003. a* 12, 209–12

Leicester J, Stone J. Ganglion, amacrine and horizontal cells of the cat's retina. Vision Res. 1967;7:695–705.

Leventhal AG, Rodieck RW, & Dreher B. Retinal ganglion cell classes in the Old World monkey: morphology and central projections. Science. 1981. 213(4512), 1139-42.

Lin B, Wang SW, Masland RH. Retinal ganglion cell type, size, and spacing can be specified independent of homotypic dendritic contacts. Neuron. 2004 Aug 19; 43(4):475-85.

Lindstrom JM. Acetylcholine receptors and myasthenia. Muscle Nerve. 2000. Apr;23(4):453-77. Review.

Macdonald RL, Olsen RW. $GABA_A$ receptor channels. Annu Rev Neurosci. 1994. 17:569–602.

Macklin KD, Maus AD, Pereira EF, Albuquerque EX, Conti-Fine BM. Human vascular endothelial cells express functional nicotinic acetylcholine receptors. J Pharmacol Exp Ther. 1998. Oct;287(1):435-9.
Martin PR, Lee BB, White AJ, Solomon SG, Ruttiger L. Chromatic sensitivity of ganglion cells in the peripheral primate retina. Nature. 2001. 410:933–936

McGehee DS. Molecular diversity of neuronal nicotinic acetylcholine receptors. Ann NY Acad Sci. 1999. Apr 30;868:565-77.

McMahon LL, Yoon KW, Chiappinelli VA. Electrophysiological evidence for presynaptic nicotinic receptors in the avian ventral lateral geniculate nucleus. J Neurophysiol. 1994;71(2):826-9.

Merigan WH, Katz LM, Maunsell JH. The effects of parvocellular lateral geniculate lesions on the acuity and contrast sensitivity of macaque monkeys.
J Neurosci. 1991. Apr;11(4):994-1001.

Metz CN, Gregersen PK, Malhotra AK. Metabolism and biochemical effects of nicotine for

primary care providers. Med Clin North Am. 2004;88(6):1399-413, ix.

Mishkin M, Ungerleider LG. Contribution of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. Behav Brain Res. *1982* Sep;6(1):57-77.

Mitchell JF. The spontaneous and evoked release of acetylcholine from the cerebral cortex. J Physiol. 1963;165(1):98-116.

Morgado PB, Chen HC, Patel V, Herbert L, Kohner EM.The acute effect of smoking on retinal blood flow in subjects with and without diabetes. Ophthalmology. 1994. Jul;101(7):1220-6.

Mrzljak L, Levey AI, Rakic P. Selective expression of m2 muscarinic receptor in the parvocellular channel of the primate visual cortex. Proc Natl Acad Sci U S A. 1996 Jul;93(14):7337-40. PubMed PMID: 8692994. Pubmed Central PMCID: PMC38985. eng.

National Research Council (U.S.), (1978).

Nicholls J., et al. From Neuron to Brain: Fourth Edition. Sinauer Associates, Inc. 2001.

Nyberg G, Panfilov V, Sivertsson R, Wilhelmsen L. Cardiovascular effect of nicotine chewing gum in healthy non-smokers. Eur J Clin Pharmacol. 1982 Oct;23(4):303-7.

Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. Alpha7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. Learn Mem. 1998; 5(4-5):302-16.

Perry VH, Cowey A. Retinal ganglion cells that project to the superior colliculus and pretectum in the macaque monkey. Neuroscience 1984; 12: 1125–37.

Perry CL. The tobacco industry and underage youth smoking: tobacco industry documents from the Minnesota litigation. Arch Pediatr Adolesc Med. 1999 Sep;153(9):935-41.

Polyak, SL. The Retina. University of Chicago Press, Chicago. 1941.

Polyak, SL. The Vertebrate Visual System. Univ. Chicago Press, Chicago. 1957.

Purves et al. Neuroscience. 2008. Sinauer 4ed. 754-56.

Ratliff F, Zemon V.Some new methods for the analysis of lateral interactions that influence the visual evoked potential. Ann N Y Acad Sci. 1982. 388:113-24.

Regan D. Orientation discrimination for objects defined by relative motion and objects defined by luminance contrast.Vision Res. 1989. 29(10):1389-400.

Reid RC, Shapley RM. Spatial structure of cone inputs to receptive fields in primate lateral geniculate nucleus. Nature. 1992. Apr 23;356(6371):716-8.

Reid RC, Shapley RM. Space and time maps of cone photoreceptor signals in macaque lateral geniculate nucleus. J Neurosci. 2002. Jul 15;22(14):6158-75.

Rudvin I, Valberg A, Kilavik BE. Visual evoked potentials and magnocellular and parvocellular segregation. Vis Neurosci. 2000. Jul-Aug;17(4):579-90.

Schiller PH, Logothetis NK, Charles ER. Role of the color-opponent and broad-band channels in vision. Vis Neurosci. 1990. Oct;5(4):321-46.

Schnapf, JL, Schneeweis DM. Electrophysiology of primate cone photoreceptors. in Color Vision: From Genes to Perception. Ed. Sharpe, L. & Genenfurter, K., Cambridge:Cambridge University Press. 1999.

Schröder H, Zilles K, Maelicke A, Hajós F. Immunohisto- and cytochemical localization of cortical nicotinic cholinoceptors in rat and man. Brain Res. 1989 Nov;502(2):287-95. PubMed PMID: 2819467. eng.

Scobey RP. Movement sensitivity of retinal ganglion cells in monkey. Vision Res. 1981;21(2):181-90.

Services UDoHaH. Rockville, Maryland 1988.

Severance EG, Zhang H, Cruz Y, Pakhlevaniants S, Hadley SH, Amin J, Wecker L, Reed C, Cuevas J. The alpha7 nicotinic acetylcholine receptor subunit exists in two isoforms that contribute to functional ligand-gated ion channels. Mol Pharmacol. 2004. Sep;66(3):420-9.

Shapley R, Kaplan E, Soodak R. Spatial summation and contrast sensitivity of X and Y cells in the lateral geniculate nucleus of the macaque. Nature. 1981 Aug;292(5823):543-5. PubMed PMID: 7254350. eng.

Shapley R, Reid RC, Kaplan E. Receptive fields of P and M cells in the monkey retina and their photoreceptor inputs. Neurosci Res Suppl. 1991.15:S199-211.

Silveira LC, Perry VH. The topography of magnocellular projecting ganglion cells (M-ganglion cells) in the primate retina. Neuroscience. 1991;40(1):217-37.

Solomon SG, Peirce JW, Lennie P. The impact of suppressive surrounds on chromatic properties of cortical neurons. J Neurosci. 2004. Jan 7;24(1):148-60.

Sperling HG, Mills SL. Red-green interactions in the spectral sensitivity of primates as derived from ERG and behavioral data.Vis Neurosci. 1991. Jul-Aug;7(1-2):75-86.

Svaetichin G, MacnichoL EF Jr. Retinal mechanisms for chromatic and achromatic

vision. Ann N Y Acad Sci. 1959 Nov 12;74(2):385-404.

Tamaki Y, Araie M, Kawamoto E, Eguchi S & Fujii H. Non-contact, two- dimensional measurement of tissue circulation in choroid and optic nerve head using laser speckle phenomenon. Exp Eye Res. 1995. 60, 4, pp.373-383.

The Eye Disease Case-Control Study Group. Risk factors for neovascular age-related macular degeneration. Arch Ophthalmol 1992;110:1701-8.

Thompson JC, Tzambazis K, Stough C, Nagata K, Silberstein RB. The effects of nicotine on the 13 Hz steady-state visually evoked potential. Clin Neurophysiol. 2000 Sep;111(9):1589-95. PubMed PMID: 10964069. eng.

Thornton JE, Pugh EN Jr. Red/Green color opponency at detection threshold. Science. 1983. Jan 14;219(4581):191-3.

Tigges M, Tigges J, Rees H, Rye D, Levey AI. Distribution of muscarinic cholinergic receptor proteins m1 to m4 in area 17 of normal and monocularly deprived rhesus monkeys. J Comp Neurol. 1997 Nov 10;388(1):130-45.

Timothy CO, Nneli RO. The effects of cigarette smoking on intraocular pressure and arterial blood pressure of normotensive young Nigerian male adults. Niger J Physiol Sci. 2007; 22(1-2):33-6.

Ungerleider LG, Galkin TW, Mishkin M. Visuotopic organization of projections from striate cortex to inferior and lateral pulvinar in rhesus monkey. J Comp Neurol. 1983 Jun 20;217(2):137-57.

Verriest G. Further studies on acquired deficiency of color discrimination. J Opt Soc Am. 1963. Jan;53:185-95.

Wagner HG, Macnichol EF Jr, Wolbarsht ML. Opponent Color Responses in Retinal Ganglion Cells.
Science. 1960 Apr 29;131(3409):1314.

Watson AB, Barlow HB, Robson JG. What does the eye see best? Nature. 1983. Mar 31-Apr 6;302(5907):419-22.

Wiesel TN & Hubel DH. Spatial and chromatic interactions in the lateral geniculate body of the rhesus monkey. *J Neurophysiol.* 1966. 29, 1115–1156.

The spatial channel compares the photon catch between adjacent cones, regardless of type, and thus supports perception of patterns as fine as the cone mosaic. 1986.

Woodson PP, Baettig K, Etkin MW, Kallman WM, Harry GJ, Kallman MJ, et al. Effects of nicotine on the visual evoked response. Pharmacol Biochem Behav. 1982

Nov;17(5):915-20. PubMed PMID: 7178205. eng.

Yuksel A, Senocak D, Sozuer D, Keskin G, Dirican A, Cenani A, Yalcin E. Effects of carbamazepine and valproate on brainstem auditory evoked potentials in epileptic children. Childs Nerv Syst. 1995 Aug;11(8):474-7.

Zaninetti M, Blanchet C, Tribollet E, Bertrand D, Raggenbass M. Magnocellular neurons of the rat supraoptic nucleus are endowed with functional nicotinic acetylcholine receptors. Neuroscience. 2000;95(2):319-23. PubMed PMID: 10658610. eng.

Zaninetti M, Tribollet E, Bertrand D, Raggenbass M. Nicotinic cholinergic activation of magnocellular neurons of the hypothalamic paraventricular nucleus. Neuroscience. 2002;110(2):287-99.

Zanon-Moreno V, Garcia-Medina JJ, Zanon-Viguer V, Moreno-Nadal MA, Pinazo-Duran MD. Smoking, an additional risk factor in elder women with primary open-angle glaucoma. Mol Vis. 2009;15:2953-9.

Zemon V, Gordon J, Welch J. Asymmetries in ON and OFF visual pathways of humans revealed using contrast-evoked cortical potentials. Vis Neurosci. 1988;1(1):145-50. PubMed PMID: 3154786. eng.

Zemon V, Ratliff F. Intermodulation components of the visual evoked potential: responses to lateral and superimposed stimuli. Biol Cybern. 1984;50(6):401-8. PubMed PMID: 6487677. eng.

Zemon V, Ratliff F. Visual evoked potentials: evidence for lateral interactions. Proc Natl Acad Sci U S A. 1982. Sep;79(18):5723-6.

APPENDIX
IRB APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

UAB's Institutional Review Boards for Human Use (IRBs) have an approved Federalwide Assurance with the Office for Human Research Protections (OHRP). The Assurance number is FWA00005960 and it expires on January 24, 2017. The UAB IRBs are also in compliance with 21 CFR Parts 50 and 56.

Principal Investigator:	NASER, NASER
Co-Investigator(s):	HARTMANN, E EUGENIE KEYSER, KENT T VARGHESE, STEFANIE BRYANT
Protocol Number:	**F100929011**
Protocol Title:	*Effects of Nicotine on Processing in the Human Adult Visual Pathways [VEP and Color Vision]*

The IRB reviewed and approved the above named project on 12/5/2012. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received FULL COMMITTEE review.

IRB Approval Date: 12/5/2012

Date IRB Approval Issued: 12-6-12

Identification Number: IRB00000196

Albert Oberman, MD, MPH/RC

Albert Oberman, M.D., MPH
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

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