THE EFFECTS OF NICOTINE ON THE HUMAN ADULT VISUAL PATHWAY AND PROCESSING

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VISION SCIENCE

ABSTRACT

Nicotine, the primary addictive component found in tobacco/cigarettes, can bind to nicotinic acetylcholine receptors (nAChR), which are found throughout the central nervous system including the retina of the eye. Previous studies have shown nAChR expression in the retina of non-human and human (Hutchins & Hollyfield, 1985; Liu et al., 2009). By use of electrophysiologic measures, i.e. electroretinograms (ERGs), early reports indicated that nicotine and/or cigarette smoking affects vision and visual processing in different species (Jurklies, Kaelin-Lang, & Niemeyer, 1996; Jünemann & Damaske, 1968). Because previous human studies have used only cigarette smokers, there are questions about what role nicotine, itself, plays in vision and visual processing. Tobacco/cigarette smoke has numerous additives and chemicals that could affect measures of visual processing (Rabinoff, Caskey, Rissling, & Park, 2007). The hypothesis that nicotine administered as gum in two different dosages (2mg and 4mg) affects visual processing was tested in this study using humans with no previous smoking history. Dose-related changes were seen in measures of retinal processing, as well as, cortical processing using ERGs, flicker ERGs, and contrast sensitivity measures. To our knowledge, these studies are the first demonstration of nicotine itself having an impact on vision and visual processing in non-smokers.
TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... ii

LIST OF TABLES ................................................................................................................ v

LIST OF FIGURES ............................................................................................................. vi

LIST OF ABBREVIATIONS ................................................................................................. viii

INTRODUCTION .............................................................................................................. 1

Visual Pathways and Processing ...................................................................................... 1
  The Human Retina ........................................................................................................ 1
  The Visual Cortex ......................................................................................................... 4

Electrophysiology and Psychophysics ............................................................................. 5
  The Electroretinogram ................................................................................................. 5
  The Contrast Sensitivity Function .............................................................................. 6
  Critical Flicker Frequency ............................................................................................... 7

Nicotinic Receptors in the Visual System ........................................................................ 8

Hypotheses and Specific Aims ....................................................................................... 9
  Specific Aim One (SA1): Determine the effect of nicotine on specific aspects of retinal function ........................................................................................................ 9
  Specific Aim Two (SA2): Determine the effect of nicotine on temporal vision ......................................................................................................................... 12
  Specific Aim Three (SA3): Determine the effects of nicotine on spatial vision ......................................................................................................................... 13

THE EFFECTS OF NICOTINE ON THE HUMAN ELECTRORETINOGRAM (ERG) ......................................................................................................................... 19

THE EFFECTS OF NICOTINE ON SINE-WAVE FLICKER ERGS: APPLICATION OF DFT AND T² CIRC ................................................................................................................. 48

EFFECTS OF NICOTINE ON SPATIAL AND TEMPORAL CONTRAST SENSITIVITY IN NON-SMOKERS ......................................................................................................................... 80

iii
CONCLUSIONS .................................................................................................................99
  Global summary ........................................................................................................99
  Future directions .......................................................................................................103
GENERAL LIST OF REFERENCES .............................................................................105
APPENDIX: IRB APPROVAL ..................................................................................110
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
</table>

## INTRODUCTION

1. Overview of retinal layers, location of nicotinic receptors, and electrophysiological/psychophysical methods ................................................... 18

## THE EFFECTS OF NICOTINE ON THE HUMAN ELECTRORETINOGRAM (ERG)

1. Dark- and light-adapted ERG measures under ISCEV standard conditions: Placebo vs. Nicotine ................................................................. 41

2. B-wave amplitude Naka Rushton fit parameters for dark- and light-adapted conditions ................................................................. 42

3. Overview of ERG changes with nicotine ................................................................. 43

## THE EFFECTS OF NICOTINE ON SINE-WAVE FLICKER ERGS: APPLICATION OF DFT AND $T^2$ CIRC

1. CFF measures derived from linear regression of F1 flicker ERG magnitudes .......... 77
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Retinal current flow diagram ................................................... 15</td>
</tr>
<tr>
<td>2</td>
<td>Example of a light-adapted ERG waveform ............................. 16</td>
</tr>
<tr>
<td>3</td>
<td>Flicker ERG waveform .......................................................... 16</td>
</tr>
<tr>
<td>4</td>
<td>Contrast sensitivity curve ...................................................... 17</td>
</tr>
<tr>
<td></td>
<td>THE EFFECTS OF NICOTINE ON THE HUMAN ELECTRORETINOGRAM (ERG)</td>
</tr>
<tr>
<td>1</td>
<td>Individual ERG responses for both scotopic and photopic intensity ranges .......... 44</td>
</tr>
<tr>
<td>2</td>
<td>Individual ERG responses for placebo, 2mg nicotine gum, and 4mg nicotine gum under both dark- and light-adapted conditions ........................................ 45</td>
</tr>
<tr>
<td>3</td>
<td>Dark-adapted ERG values for placebo and nicotine conditions .................. 46</td>
</tr>
<tr>
<td>4</td>
<td>Light-adapted ERG values for placebo and nicotine conditions ................... 47</td>
</tr>
<tr>
<td></td>
<td>THE EFFECTS OF NICOTINE ON SINE-WAVE FLICKER ERGS: APPLICATION OF DFT AND T² CIRC</td>
</tr>
<tr>
<td>1</td>
<td>Representative baseline ERG recordings from one subject across the frequency range examined .......................................................... 72</td>
</tr>
<tr>
<td>2</td>
<td>Individual participant data ........................................................ 73</td>
</tr>
<tr>
<td>3</td>
<td>Individual participant data for the F1 magnitude and phase for baseline and 2mg nicotine form Fourier analysis and T² circ analysis ........................................ 74</td>
</tr>
<tr>
<td>4</td>
<td>Mean F1 magnitude and phase for baseline and nicotine (2mg and 4mg) ............ 75</td>
</tr>
<tr>
<td>5</td>
<td>CFF measures obtained from flicker ERG F1 magnitude data (n=10) ............... 76</td>
</tr>
</tbody>
</table>
EFFECTS OF NICOTINE ON SPATIAL AND TEMPORAL CONTRAST SENSITIVITY IN NON-SMOKERS

1  Individual responses for baseline, 2mg nicotine, and 4mg nicotine for spatial and temporal contrast sensitivity .................................................................96

2  Mean normalized sCSF data for baseline and nicotine conditions (2mg and 4mg) ....97

3  Mean normalized tCSF data for baseline and nicotine (2mg and 4mg) conditions ....98
## LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>α7nAChR</td>
<td>alpha-7 nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>ACh</td>
<td>acetylcholine</td>
</tr>
<tr>
<td>CFF</td>
<td>critical flicker fusion</td>
</tr>
<tr>
<td>DFT</td>
<td>discrete fourier transform</td>
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<tr>
<td>ERG</td>
<td>electroretinograms</td>
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<tr>
<td>F1</td>
<td>fundamental harmonic</td>
</tr>
<tr>
<td>F2</td>
<td>secondary harmonic</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamate</td>
</tr>
<tr>
<td>Gly</td>
<td>glycine</td>
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<tr>
<td>LGN</td>
<td>lateral geniculate nucleus</td>
</tr>
<tr>
<td>nAChR</td>
<td>nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>sCSF</td>
<td>spatial contrast sensitivity function</td>
</tr>
<tr>
<td>tCSF</td>
<td>temporal contrast sensitivity function</td>
</tr>
<tr>
<td>V1</td>
<td>primary visual cortex</td>
</tr>
</tbody>
</table>
INTRODUCTION

VISUAL PATHWAYS AND PROCESSING

The Human Retina

The retina is the neural tissue lining the posterior two thirds of the eye. Visual processing begins at the photoreceptor layer of the retina; this layer consists of rods and cones (three types: short- (SWS), medium- (MWS), and long- (LWS) wavelength sensitive) (Rodieck, 1998). Photons of light are captured by rods and cones which initiates the sensory process of vision. Photoreceptors synapse onto horizontal and bipolar cells in the outer plexiform layer (OPL). At this level glutamate release from photoreceptors causes membrane potential changes in bipolar cells. Bipolar cells (rod bipolars, ON and OFF cone bipolars) then synapse with amacrine and ganglion cells at the inner plexiform layer (IPL) (Rodieck, 1998). The IPL is segregated into sublamina A, which contains synapses for the OFF responses, and sublamina B, which contains synapses for ON responses. Bipolar cells release glutamate onto ganglion cells whose axons make up the optic nerve. Horizontal and amacrine cells modulate information flow along the vertical pathways typically through the release of the inhibitory neurotransmitters GABA and glycine (Rodieck, 1998). Retinal neurons form specific circuits, the output of which contributes to visual perception of the world. For instance, the rod pathway is responsible for scotopic or night vision under dimly illuminated conditions. Similarly, the cone pathway is responsible for photopic vision and is optimally responsive under conditions of bright light that occur during the daytime.
(Rodieck, 1998). These cell pathways are the focus of research on retinal functioning and are described in detail below.

**Rod Pathway and Signal Flow**

Retinal current flow is mediated by neurotransmitters and their physiological actions upon ion/protein channels on retinal cells. As mentioned previously, there are three distinct pathways within the retina: the rod pathway which is optimally tuned to night-time vision, the cone ON- and the cone OFF- pathways, which are optimally tuned to day-time vision. A flow diagram of these pathways can be seen in Figure 1.

When the rod photoreceptor absorbs light, it hyperpolarizes and the amount of neurotransmitter released, glutamate, decreases. This decrease in glutamate is relayed to the rod bipolar cell synapses where the signal is inverted, i.e. depolarizes, and is relayed to AII amacrine cells via glutamate. Rod signals are relayed to ganglion cells via the cone ON- and OFF- pathways through AII amacrine cells. Gap junctions between AII and cone ON-bipolar cell terminals cause the cone ON-bipolar cells to depolarize and this signal is relayed to ganglion cells. Chemical synapses, using the neurotransmitter glycine, between AII and cone OFF-bipolar cells cause the cone OFF-bipolar cell to hyperpolarize and this signal is relayed to ganglion cells (Rodieck, 1998).

**Cone Pathway and Signal Flow**

The cone pathway is comprised of three different cone photoreceptors and cone ON- and OFF-bipolar cells. In response to light, cones hyperpolarize and decrease the amount of glutamate released from their terminals. Cone OFF-bipolar cells receive the signals from cones via ionotropic glutamate receptors and hyperpolarize in response to
the decrease in glutamate, thereby conserving the responses from the cones and relaying the information to OFF-center ganglion cells within sublamina A of the IPL (Rodieck, 1998). Cone ON-bipolar cells respond in the opposite manner: As cones hyperpolarize in response to light, glutamate release is decreased and this decrease is detected by cone ON-bipolar cells via metabotropic glutamate receptors. Cone ON-bipolar cells, in turn, depolarize and invert the signal sent from the cones, which is relayed to ON-center ganglion cells in sublamina B of IPL (Rodieck, 1998).

Lateral Pathway Signal Flow

Horizontal cells. Horizontal cells play a pivotal role in the lateral inhibitory pathways in the retina. Horizontal cells synapse with photoreceptors and other horizontal cells in the OPL of the retina. The primary role of horizontal cells is to provide inhibitory feedback via the neurotransmitter GABA onto both rod and cone photoreceptors (Rodieck, 1998). There are two types of horizontal cells: H1 and H2. The dendrites of H1 cells interact with MWS and LWS cones. The axons of H1 cells interact with rods. In addition, H1 cells are coupled with each other via gap junctions (Rodieck, 1998). H2 cells interact primarily with SWS cones and do not appear to be coupled with one another (Rodieck, 1998). An example of signal flow is as follows: as cones hyperpolarize to light, glutamate release is decreased, horizontal cells hyperpolarize in response, and in turn, reduce the amount of GABA released onto the cone pedicles.

Amacrine cells. Amacrine cells are responsible for inhibitory feedback onto both bipolar and ganglion cells. Synapses from amacrine cells onto other retinal cells are found within the IPL of the retina. The role of amacrine cells is to provide lateral signal modulation primarily via inhibitory pathways (Rodieck, 1998).
There are multiple types of amacrine cells in which each type can use different neurotransmitters. The three primary neurotransmitters employed by amacrine cells are glutamate, GABA, and glycine. All amacrine cells utilize the inhibitory neurotransmitter, glycine. Dopaminergic and starburst amacrine cells have a co-localization of neurotransmitters and/or neuromodulators. For example, starburst amacrine cells use both acetylcholine and GABA and respond to both increases and decreases to light (Rodieck, 1998).

The Visual Cortex

Visual processing begins with the capture of photons in the retina. Ganglion cells are the primary contributor to signals from the retina to the visual cortex of the brain. Ganglion cell axons are bundled together to make up the optic nerve. The optic nerve relays information obtained from the retina and transmits signals to the right and left lateral geniculate nuclei (LGN) (Rodieck, 1998). The LGN is made up of 6 layers and is divided into two specific pathways: magnocellular and parvocellular (Rodieck, 1998). The parvocellular pathway comprises the upper four layers of the LGN and is the primary pathway responsible for color vision. ON- and OFF-midget ganglion cells in the retina receive input from long and medium wavelength cones; these signals are transmitted to the parvocellular LGN (Rodieck, 1998). The magnocellular pathway makes up the bottom two layers of the LGN and is primarily responsible for interpreting luminance (Verdon & Adams, 2002). ON- and OFF-parasol ganglion cells receive input from diffuse bipolar cells, and these signals are transmitted to the magnocellular LGN (Rodieck, 1998). Magnocellular cells typically respond to rapid flickering stimuli and are
spectrally nonoppenent (Verdon & Adams, 2002). Both magnocellular and parvocellular pathways relay signals to different layers in the striate visual cortex.

ELECTROPHYSIOLOGY AND PSYCHOPHYSICS

The Electroretinogram

The electroretinogram (ERG) is a non-invasive tool for evaluating the function of the retina. It can be used for research purposes with human subjects, including infants, as well as for the diagnosis of specific retinal disorders. In practice, a bipolar electrode is placed directly on the cornea, i.e. the front of the eye, and a reference electrode is attached to the ear lobe (Coupland, 2006). The ERG waveform is comprised of several discrete components as follows (Figure 2): The *a-wave* has been identified as a measure of the photoreceptor response; the *b-wave* is derived from bipolar cell activity; and the *oscillatory potentials*, located on the leading edge of the b-wave, represent activity of the amacrine cells and other inner retinal processes (Frishman, 2006). The ERG is a powerful diagnostic tool, because variations in the state of the retina (dark- versus light-adapted), and responses to variations in stimulus presentation parameters (high intensity versus low intensity, slow versus fast presentation) can be used to evaluate functioning in different retinal pathways and diagnose retinal diseases such as retinitis pigmentosa (RP), congenital stationary night blindness (CSNB), and Leber’s congenital amaurosis (Birch, 2006; Miyake, 2006).

Flicker ERGs are obtained under full-field stimulus conditions using sinusoidally modulated light (Figure 3). The neural basis underlying the flicker ERG is the firing rate
of retinal neurons (Coletta, 2002). The output of photoreceptors is converted through the activation of glutamate receptors expressed by bipolar cells along two distinct pathways: the ON and OFF pathways. The ON and OFF bipolar cells respond to light increments and decrements, respectively. The two pathways are segregated such that ON and OFF bipolar cells contact specific subpopulations of ganglion cells and amacrine cells in separate sublamina of the inner plexiform layer (IPL). Studies have shown that bipolar cells are the primary contributors to the flicker ERG (Bush & Sieving, 1996).

Odom et al. (1992) investigated the linear and nonlinear components of the flicker ERG to sinusoidal stimuli. They conducted a systematic study manipulating the modulation depth (25%, 50%, and 75%), illuminance (0.02 lux-2000 lux) and retinal eccentricity (0 deg-38 deg). After the application of Discrete Fourier Transform (DFT) analysis, they found distinct differences between the first (F1) and second (F2) harmonics of the response waveforms. Based on the changes in latency, F1 is hypothesized to be generated from the outer retina and F2 has contributors from the inner retina (Odom, Reits, Burgers, & Riemslag, 1992). Additional research demonstrated that F1 of the flicker ERG is altered in patients with photoreceptor diseases and F2 is altered in diseases that affect the inner retina such as transient retinal ischemia (Falsini et al., 1995; Porciatti, Moretti, Ciavarella, & Falsini, 1993).

The Contrast Sensitivity Function

Spatial vision is important in the recognition of patterns of light and dark and identifying shapes in the real world. Differences in luminance between object and background are seen as edges or boundaries, which are readily detectable to retinal and
cortical neurons. These differences in luminance can be quantified in terms of contrast. Contrast sensitivity is a psychophysical measurement of spatial vision (Norton, Lakshaminarayanan, & Bassi, 2002). CSF is measured using sine-wave gratings at different spatial frequencies (Norton et al., 2002). The contrast of the sine waves is varied to obtain a threshold level using psychophysical techniques such as method of adjustment or method of constant stimuli. The human spatial CSF has a band pass shape with a low frequency roll off, a peak contrast sensitivity in the range of 3-10 cycles per degree and a cutoff high spatial frequency (30-60 cycles per degree) that corresponds to the spatial resolution acuity (Figure 4) (Norton et al., 2002).

Sensitivity to contrast can be measured both spatially and temporally. Temporal contrast sensitivity is obtained using stimulus patterns that vary in contrast as well as temporal frequencies. The high frequency limit of temporal vision is typically evaluated by critical flicker frequency (CFF). CFF is normally within the range of 60-75Hz (Coletta, 2002). The temporal contrast sensitivity function (tCSF) is band pass with a peak contrast sensitivity, high and low frequency roll-offs, similar to the spatial CSF (Coletta, 2002). Temporal CSF has become an important diagnostic test in retinal disorders such as glaucoma and age-related macular degeneration (AMD) (Coletta, 2002).

Critical Flicker Frequency (CFF)

Critical flicker/fusion frequency (CFF) is a measure of temporal processing at the temporal “acuity” limit for the visual system (Coletta, 2002). CFF can be defined as the rate at which a flickering stimulus can no longer be seen as changing, but appears to be steady (Cornsweet, 1970). Retinal neurons respond differently to flickering stimuli:
MWS and LWS cones show a CFF threshold of 60-70Hz and SWS cones exhibit a CFF threshold of 35Hz (Zrenner et al., 1990). Bipolar and ganglion cells have higher CFF thresholds compared to cones (Arden, 2006). CFF is typically measured psychophysically using the method of adjustment but can also be obtained using flicker ERGs. CFF thresholds obtained using flicker ERGs are typically higher compared to those obtained by psychophysical methods (Arden, 2006).

NICOTINIC RECEPTORS IN THE VISUAL SYSTEM

Nicotine, the addictive stimulant in cigarettes, is an alkaloid found in tobacco plants that binds to and activates nicotinic acetylcholine receptors (nAChRs) (Dani, 2001). nAChRs, members of the family of ligand-gated ion channels, are pentameric receptors comprised of subunits α2-α6 and β2-β4 in α/β combinations, or of subunits α7-α9 in homomeric forms (Dani, 2001; Lindstrom, 2000; McGehee, 1999). The subunit composition of nAChRs has been shown to determine their pharmacological and functional properties, including agonist/antagonist affinity, open time, and desensitization rate (Dani, 2001). nAChRs are widely distributed throughout the central nervous system and have been detected in cells of the retina, lateral geniculate nucleus (LGN), superior colliculus, and primary visual cortex (V1) in various species (Cox, Marritt, Perry, & Kellar, 2008; Dani, 2001; Disney, Aoki, & Hawken, 2007; Gotti et al., 2007; Hutchins & Hollyfield, 1985; Keyser, Hughes, Whiting, Lindstrom, & Karten, 1988; Keyser et al., 2000; Liu et al., 2009; Marritt et al., 2005). nAChR subtypes have been detected in bipolar, amacrine, and ganglion cells and their processes throughout the inner plexiform
layer (IPL) in the retina of species including mouse, chick and rabbit, (Hutchins & Hollyfield, 1985; Keyser et al., 2000; Liu et al., 2009).

Data from studies on human retina are more limited. One report presented evidence for muscarinic and nicotinic receptor expression in the IPL but the identity of the cells that expressed nAChRs was not entirely clear (Hutchins & Hollyfield, 1985). A recent study of the retina of non-human primates also showed receptor expression in amacrine, bipolar, and ganglion cells (Liu et al., 2009). Nicotine and/or acetylcholine can affect the responses of retinal ganglion cells and amacrine cells expressing nAChRs (Neal, Cunningham, & Matthews, 2001; Strang, Amthor, & Keyser, 2003). Neal et al. (2001) demonstrated, in response to nicotine, activation of nAChRs on GABAergic amacrine cells that resulted in an indirect increase of dopamine release via GABAergic amacrine cells (Neal et al., 2001). Similar results were also reported by Strang et al. in rabbit retinal ganglion cells (Strang et al., 2003). These two studies confirm that nicotine can affect the response properties of neurons in various retinal circuits.

HYPOTHESES AND SPECIFIC AIMS

The purpose of the studies described herein is to evaluate the effects of nicotine, itself, in gum form, on visual processing. The design for all specific aims was limited to testing only non-smokers.

Specific Aim One (SA1): Determine the effect of nicotine on specific aspects of retinal function
Numerous studies with animals and humans have examined the effects of cigarette smoking, nicotine, and/or by-products of cigarette smoke such as carbon monoxide on vision (Gundogan, Erdurman, Durukan, Sobaci, & Bayraktar, 2007; Ingenito, 1979; Jurklies, Kaelin-Lang, & Niemeyer, 1996; Jüinemann & Damaske, 1968). For example, Jüinemann and Damaske (1968) reported a decrease in amplitude of the dark-adapted b-wave in subjects who were smokers compared to subjects who were non-smokers. Since cigarette smoking influences blood flow, the authors concluded that a change in blood flow could explain their results (Jüinemann & Damaske, 1968).

Jurklies et al. (1996) studied ERG responses in the cat retina under the influence of either a cholinergic agonist (acetylcholine; ACh) or a muscarinic ACh antagonist (scopolamine). Their results showed that ACh increased the rod driven b-wave across all concentrations examined (18-1600µM), but maximal increases in the b-wave amplitude were seen at the lower concentrations (18-150µM). Across the same concentration range, ACh induced an increase in the amplitude of the cone driven b-wave for all concentrations. ERG a-waves and b-waves represent the electrical activity of photoreceptors and ON- bipolar cells/Mueller cells, respectively. Jurklies et al. hypothesized that the increase in amplitude of the cone driven b-wave was based on feedback mechanisms in the retina between the amacrine cells and cone ON- bipolar cells (Jurklies et al., 1996).

Other electrophysiological studies have detected significant changes in retinal function in individuals who are smokers (Gundogan, Durukan, Mumcuoglu, Sobaci, & Bayraktar, 2006; Gundogan et al., 2007). Gundogan et al., used the pattern electroretinogram (PERG), which is considered to be generated by retinal ganglion cells
and the optic nerve head (Holder, 2006). Their results showed increased amplitudes and decreased latencies in the PERG for individuals who were smokers compared with individuals who were non-smokers (Gundogan et al., 2006). A more recent study performed by this group evaluated retinal functioning using the multifocal electroretinogram (mfERG). Their data revealed increases in amplitudes and decreases in latencies of the mfERG N1 and P1 components in central retinal regions for individuals who were smokers compared with individuals who were non-smokers (Gundogan et al., 2007). These two studies clearly demonstrated that smoking tobacco alters the responses of both the PERG and mfERG. However because there are many active compounds in cigarette smoke, the observed changes cannot be unequivocally attributed to any one component (Robinson, Petrig, & Riva, 1985).

SA1 aim is to investigate how nicotine affects retinal ERG responses under both dark- and light-adapted conditions. I hypothesize nicotine will enhance the light-adapted ERG b-wave and will not affect the dark-adapted ERG. This hypothesis is based on the results from the previous studies, mentioned above, that investigated the effects of cigarette smoking on the ERG and the effects of acetylcholine on rod- and cone-driven responses (Jurklies et al., 1996; Jünemann & Damaske, 1968). I expect the ERG changes from nicotine to be modulated by retinal neurons that express nAChRs, specifically cone ON-bipolar cells and amacrine cells. I hypothesize that nicotine will increase the excitatory response magnitude of cone ON-bipolar cells via increasing the release of glutamate, and increase the release of glutamate onto amacrine cells, thereby disinhibiting the inhibitory response of amacrine cells. The measurable results will be
shown as an increase in cone-driven b-wave amplitudes and decreased response amplitudes of the oscillatory potentials.

Specific Aim Two (SA2): Determine the effect of nicotine on temporal vision

The purpose of this aim is to expand on SA1 and to evaluate how nicotine affects CFF, a measure of the temporal resolving limits of the visual system. CFF will be quantified by use of full-field flicker ERGs using sinusoidally modulated light stimulus for the purpose of this study. This technique will provide a CFF threshold while also evaluating the neural integrity of the retina. Based on previous reports of nAChR expression in retina and nicotine’s effect on functional properties of retinal neurons, I predict that changes seen in flicker ERG responses and CFF will be a result of the impact of nicotine on inner retinal neurons such as amacrine cells expressing nAChRs (Keyser et al., 2000; Liu et al., 2009; Neal et al., 2001; Strang et al., 2003). I hypothesize that the response magnitudes obtained from flicker ERGs will increase in response to nicotine. I propose nicotine will elevate the release of glutamate from ON-cone bipolar cells containing nAChRs and decrease the inhibitory response of amacrine cells. The CFF thresholds will increase while under the influence of nicotine based on the results from previous studies measuring CFF psychophysically in cigarette smokers (Barlow & Baer, 1967; Leigh, 1982).

To our knowledge there are no reports describing the effect of nicotine on flicker ERG measurements or psychophysical CFF thresholds. However, Barlow and Baer (1967) showed that CFF thresholds, obtained psychophysically, increased in both heavy and light smokers immediately after smoking (Barlow & Baer, 1967). A subsequent
study investigated the combined effects of cigarette smoking and alcohol consumption.
The results revealed an increase in CFF thresholds with cigarette smoking alone (Leigh, 1982). CFF thresholds decreased under the combined effect of smoking and alcohol.

Specific Aim Three (SA3): Determine the effect of nicotine on spatial vision

Little information is available concerning the effects of cigarette smoking and/or nicotine on human visual contrast sensitivity. Fine and Kobrick (1987) measured the effects of cigarette smoking on field dependence (cognitive perception and comprehension) and contrast sensitivity (Fine & Kobrick, 1987). Their results demonstrated a decrease in contrast sensitivity at high spatial frequencies for habitual smokers compared to non-smokers (Fine & Kobrick, 1987). Another study investigated the pharmacological separation of human contrast sensitivity using scopolamine (1.2mg orally), a muscarinic receptor antagonist, and nicotine (1.5mg buccal absorption), a nicotinic receptor agonist. The data revealed differential effects of nicotine on spatial and temporal contrast sensitivity. Nicotine increased thresholds at high spatial frequencies and decreased thresholds at lower frequencies for the spatial CSF. In addition, nicotine was reported to decrease temporal CSF thresholds at high temporal frequencies and increase thresholds at low frequencies (Smith & Baker-Short, 1993).

There is one limitation common to both studies: they were performed with individuals who smoke. As mentioned previously, cigarette smoke contains numerous compounds that can directly or indirectly affect measured responses. In addition, it is important to note that in cigarette smokers/tobacco users, prolonged nicotine exposure can lead to an upregulation of nAChR subtypes (Fenster, Rains, Noerager, Quick, &
Lester, 1997). Furthermore, data from individuals who smoke cannot clearly isolate the effects of nicotine. SA3 is intended to evaluate the effects of “pure” nicotine on spatial and temporal contrast sensitivity in non-smokers.

Contrast sensitivity will be measured at different spatial and temporal frequencies. I hypothesize nicotine will increase contrast sensitivity thresholds at low spatial and temporal frequencies and decrease contrast sensitivity thresholds at higher spatial and temporal frequencies. This hypothesis is based on previous reports of cigarette smoking and CSF, investigation of nAChR expression patterns on retinal neurons and nicotine’s effect in retinal neuron functional properties (Keyser et al., 2000; Neal et al., 2001; Smith & Baker-Short, 1993; Strang et al., 2003).
Figure 1: Retinal current flow diagram. Flow for the rod pathway (light gray), ON-cone pathway (dark gray) and OFF-cone pathway (black). Minus symbols (■) = hyperpolarization, plus symbols (✚) = depolarization, down arrow (▼) = decrease in glutamate (Glu) or glycine (Gly), up arrow (▲) = increase in glutamate or glycine, and bolt (➔) = light. Abbreviations are as follows: rBC= rod bipolar cell, cBC= cone bipolar cell, AII= AII amacrine cell, and rGC= retinal ganglion cell.
Figure 2: Example of a light-adapted ERG waveform.

Figure 3: Flicker ERG waveform. Illustration of a flicker ERG waveform to a 30Hz frequency stimulus at a luminance of 30 cd/m².
**Figure 4:** Contrast Sensitivity Curve. Example of a spatial contrast sensitivity curve with spatial frequencies ranging from 0.5-25 cycles per degree. The vertically shaded area under the curve represents contrasts that are visible; the dotted shaded area above and around the curve are contrasts that are not visible. The labeled sections of the curves are as follows: A- low frequency roll off, B- peak contrast sensitivity, and C- high frequency cutoff.
**Table 1: Overview of retinal layers, location of nicotinic receptors, and electrophysiological/psychophysical methods.**

<table>
<thead>
<tr>
<th>Retinal Layers</th>
<th>Location of Nicotinic Receptors</th>
<th>Method of Assessment</th>
</tr>
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<td>Inner Nuclear Layer (INL):</td>
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<td>Somata of GCs</td>
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a (Liu et al., 2009)

b (Dmitrieva, Strang, & Keyser, 2007)

c (Hutchins & Hollyfield, 1985)
THE EFFECTS OF NICOTINE ON THE HUMAN ELECTRORETINOGRAM (ERG).

by


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Format adapted for dissertation
Abstract

**Purpose:** To examine the effects of nicotine on responses from the human retina measured electrophysiologically.

**Methods:** Electroretinogram (ERG) responses were obtained from ten healthy, visually normal adults who were non-smokers. Nicotine (2mg and 4mg) and a placebo were administered in the form of gum 30 minutes prior to testing in two separate experiments. ERG responses were collected and analyzed using the Espion ERG system. Responses were recorded from one eye of each subject using a bipolar Burian-Allen contact lens electrode. Intensity response curves were obtained under dark- and light-adapted conditions. In Experiment 1, dark- and light-adapted testing was completed sequentially. In Experiment 2, only light-adapted testing was performed. Intensity response functions were analyzed using the Naka-Rushton equation.

**Results:** In Experiment 1, compared to placebo, dark-adapted b-wave amplitude responses decreased significantly after chewing gum containing both 2mg and 4mg of nicotine. Under light-adapted conditions, the peak b-wave amplitude was significantly decreased after chewing gum containing 4mg of nicotine. In Experiment 2, light-adapted b-wave amplitudes were increased after 4mg nicotine. Oscillatory potentials were measured but no significant effects under nicotine were observed.

**Conclusions:** To our knowledge, this is the first demonstration that nicotine by itself affects responses in the human retina. These data support reports of the expression of nicotinic acetylcholine receptors in rabbit and non-human primate retina.
Introduction

Nicotine is an alkaloid found in tobacco plants that binds to and activates nicotinic acetylcholine receptors (nAChRs). nAChRs are members of the family of ligand-gated ion channels. nAChRs are pentameric receptors comprised of subunits α2-α6 and β2-β4 in α/β combinations, or of subunits α7-α9 in homomeric forms.\(^1\) The subunit composition of nAChRs has been shown to determine their pharmacological and functional properties, including agonist/antagonist affinity, channel open time, and desensitization rate. nAChRs have been detected in cells of the retina, lateral geniculate nucleus (LGN), superior colliculus, and primary visual cortex (V1) in various species.\(^2\)\(^4\)\(^10\)

In the retina of mice, chick and rabbit, nAChR subtypes have been identified in bipolar, amacrine, and ganglion cells including processes throughout the inner plexiform layer (IPL).\(^8\)\(^10\)\(^11\) Ligand binding studies in human retina revealed muscarinic and nicotinic binding sites in the IPL, however it was not entirely clear which cell types were involved.\(^6\) A recent immunohistochemical study of the retina of non-human primates also showed receptor expression in amacrine, bipolar, and ganglion cells.\(^10\)

In the mammalian retina, there are subpopulations of amacrine cells including starburst, dopaminergic, and AII amacrine cells. Each amacrine cell type employs a different neurotransmitter i.e. acetylcholine, GABA, glycine, or dopamine. Results from earlier studies have shown that nicotinic agonists (nicotine and epibatidine) affect the release of neurotransmitters from these subpopulations of amacrine cells.\(^12\) The application of nicotine and epibatidine onto GABAergic amacrine cells increased the release of dopamine. However, Neal and colleagues determined this increase was an indirect effect of the nicotinic agonists: The increased dopamine release was a result of
nicotine/epibatidine increasing the amount of GABA. This study revealed that nicotine acts on nAChRs in the retina to alter the function of the retinal cells.

Numerous studies on animals and humans have described the effects of cigarette smoking, nicotine, and/or by products of cigarette smoke such as carbon monoxide on vision. For example, Jünemann and Damaske (1968) reported a decrease in amplitude of the dark-adapted b-wave, after cigarette smoking, in subjects who were non-smokers as well as smokers who had abstained from smoking. Since cigarette smoking influences blood flow, the authors concluded that a change in blood flow could explain their results.

Jurklies et al. studied ERG responses in the cat retina treated with either a cholinergic agonist (acetylcholine; ACh) or a muscarinic ACh antagonist (scopolamine). Their results showed that ACh increased the dark-adapted b-wave across all concentrations examined (18-1600µM), with maximal increases of the b-wave amplitude seen at lower concentrations (18-150µM). Acetylcholine, in the same concentration range, induced an increase in the amplitude of the light-adapted b-wave across all concentrations. In addition, scopolamine decreased both the dark- and light-adapted b-wave across concentrations from 500µM to 1000µM. ERG a-waves and b-waves represent the electrical activity of photoreceptors and OFF- and ON- bipolar cells, therefore, Jurklies hypothesized that the increase in the amplitude of the light-adapted b-wave could be based on feedback mechanisms in the retina between the amacrine cells and ON-bipolar cells.

Other electrophysiological studies have shown significant changes in retinal function in individuals who are smokers. Gundogan and colleagues used the pattern
electroretinogram (PERG), which has contributions from inner retinal cells, retinal ganglion cells, and optic nerve head.\textsuperscript{18} Their results showed increased amplitudes and decreased latencies in the PERG after smoking for individuals who were smokers compared with individuals who were non-smokers.\textsuperscript{17} The most recent study performed by this group compared multifocal electroretinogram (mfERG) responses under photopic conditions obtained from smokers who had abstained for twelve hours. N1 and P1 components in the central retinal regions revealed increases in amplitudes and decreases in latencies.\textsuperscript{16} These two studies clearly demonstrated that smoking tobacco alters the responses of both the PERG and mfERG. However, there are many active compounds in cigarette smoke\textsuperscript{17} and the observed changes could not be unequivocally attributed to any one compound.

Cigarette smoking is a major risk factor for potentially blinding ocular diseases such as age-related macular degeneration (AMD) and glaucoma.\textsuperscript{19, 20} Nicotine is thought to be the primary addictive substance in cigarettes.\textsuperscript{21} However, the number of active compounds in and the mechanisms underlying the correlation between inhalation of and/or exposure to cigarette smoke and eye diseases have not been clearly delineated. The purpose of our study was to observe the effects of nicotine, in gum form, on retinal ERG responses under both dark- and light-adapted conditions in non-smoking adults.

Materials and Methods

\textit{Subjects:}

Ten subjects with no history of smoking participated in this study. Full comprehensive eye exams, including visual field tests, were used to determine ocular and
retinal health. Exclusion criteria included 1) any vision disorders that related to overall systemic health; 2) ocular disorders such as glaucoma or diabetic retinopathy; 3) health issues or prescription medications that contra-indicated the use of nicotine; 4) refractive error of -3.00D or higher, since high myopia has been shown to attenuate ERG responses.\textsuperscript{22} The age range of subjects was 20 to 32 years (mean=24.3). All of our participants were males. Two females volunteered for the study, but were excluded on the basis of health issues and refractive error. Subject refractions ranged from +0.25 D to -2.50 D.

This study conformed to the tenets of the Declaration of Helsinki and was approved by the University of Alabama at Birmingham Institutional Review Board for Human Use. Written informed consent was obtained from all subjects.

**ERG Procedure:**

One eye (the non-dominant eye, determined subjectively by the participant) was tested. The pupil was dilated with tropicamide 1\% (Alcon, Fort Worth, TX) prior to 30 minutes of dark adaptation. A bipolar Burian-Allen lens electrode (Hansen Ophthalmic, Coralville, IA) was used to obtain the ERG recordings. The corneal surface was numbed with proparacaine 0.5\% (Alcon, Fort Worth, TX) and a drop of Celluvisc (Allergan, Inc., Irvine, CA) was applied to the electrode prior to placement. The ground electrode was placed behind the opposite ear on the skin of the mastoid process.

ERG responses were amplified (1-1000 Hz), displayed, digitized, and stored for later analysis using an Espion system (Diagnosys, Lowell, MA). Oscillatory potentials (OP) were filtered using a low frequency cutoff of 100Hz and a high frequency cutoff of
300Hz. Subjects were tested under dark- and light-adapted conditions. Two to fifteen responses were averaged for each condition with a stimulus interval from 5 to 60 seconds. Responses that contained artifacts were manually rejected.

**Dark-adapted ERG**

Subjects were dark adapted for 30 minutes. Responses were produced using a series of brief (≤1ms), full-field 470 nm flashes, generated by an array of LEDs presented in the Espion Colordome. Our retinal illuminance range was -1.96 to +2.95 log scotopic trolands, with 0.3-log unit steps. Responses were also obtained using a stimulus of 0.01 and 3.0 cd.s/m$^2$, which is the International Society for Clinical Electrophysiology of Vision (ISCEV) standard for dark-adapted testing. Pupil diameter measurements ranged from 8mm to 10mm. The average pupil diameter of 9mm was used to calculate trolands for both dark-and light-adapted conditions. OPs were obtained using the ISCEV standard maximal flash (3.0 cd.s/m$^2$). Raw data from one subject are shown in Figure 1. Waveforms were measured from baseline-to-trough for the a-wave amplitude and trough-to-peak for the b-wave amplitude and b-wave implicit time (Figure 1).

**Light-adapted ERG**

Subjects were light-adapted for 10 minutes to a rod saturating background (30 cd/m$^2$). Responses were produced using a 630nm light over a retinal illuminance range from +1.26 to +3.36 log photopic trolands incremented in 0.3-log unit steps. Xenon flashes were used for the highest retinal illuminance levels ranging from +3.05 to +3.36
log photopic trolands. OPs were obtained over the entire intensity range, as well as, for the ISCEV standard light-adapted flash (3.0 cd.s/m²).

Administration of Nicotine:

Two dosages (2mg and 4mg) of nicotine gum (GlaxoSmithKline, Moon Township, PA) and one placebo gum (Laclede, Inc., Rancho Dominguez, CA) were used in this study. Four mg nicotine gum has been shown to yield blood nicotine levels similar to smoking one cigarette. The placebo gum was chosen because of its similarity in taste and appearance to the nicotine gums. Testing sessions were at least one week apart. Order of testing sessions was randomized for both Experiment 1 and 2. The subject was masked to the testing condition. The experimenter was also masked to the testing condition during both data collection and initial analysis.

Experiment 1

Subjects were tested in two separate sessions: one session with the placebo gum and the second session with either 2mg or 4mg nicotine gum. The same subjects were retested at two additional sessions with the alternate dosage of nicotine gum and another placebo session. ERGs were obtained under both dark-adapted and light-adapted conditions, which were completed sequentially. Gum was administered only during the 30 minute dark adaptation and was discarded prior to testing.

Experiment 2

In Experiment 2, we tested each subject in three separate sessions: 2mg and 4mg nicotine gum, and placebo gum. Subjects were not dark-adapted and ERGs were recorded
only under light-adapted conditions. Gum was administered 30 minutes prior to ERG recording and was discarded when recording started.

Data Analysis:

B-wave amplitude data were fit to the Naka-Rushton equation:

\[ R = R_{\text{max}} \left( \frac{I^n}{I^n + K^n} \right) \]

where \( R \) is the response amplitude at stimulus intensity (I), \( R_{\text{max}} \) is the maximal response amplitude, \( K \) is the stimulus intensity (I) that produces a response amplitude that is half of \( R_{\text{max}} \), and \( n \) is the constant that controls the slope of the function. The initial \( K \) value was chosen to be 100 and \( n \) parameter was held at 1. The \( R_{\text{max}} \) and \( K \) parameters were found using PSI Plot (Poly Software International, Pearl River, NY). Individual data were normalized to the placebo \( R_{\text{max}} \) values to minimize the variance. Within subject comparisons among testing conditions (placebo and two levels of nicotine) were made by repeated measures ANOVA (SPSS, Inc., Chicago, IL). Post hoc tests were performed using student t-test. The level of significance was set at \( p<0.01 \) for all statistical tests.

Results

Individual ERG responses-

Dark- and light-adapted ERGs were recorded from ten subjects. Averaged amplitudes and implicit times produced by the ISCEV standard flash are shown in Table 1 for all testing conditions. Figure 1 presents both sets of placebo ERG data from a representative subject. As seen in Figure 1, under dark-adapted conditions, a-wave, b-wave, and OP amplitudes increase, while the implicit times of both components decrease
with increasing stimulus retinal illuminance; under light-adapted conditions, the peak a- and b-wave amplitudes, increase up +3.06 log photopic trolands and then begin to decrease at higher intensities. OP amplitudes increase with increasing retinal illuminance across the entire range tested. B-wave implicit times also increase with increasing retinal illuminance. Figure 2 compares the responses of a single subject for one stimulus retinal illuminance across all three nicotine conditions for Experiment #1 and Experiment #2 including OPs.

*Effects of nicotine on dark-adapted ERGs-*

Dark-adapted ERG responses (n=8) were obtained after thirty minutes of dark adaptation; nicotine/placebo was administered during dark adaptation. A-wave amplitudes were measured at a fixed time point (8ms) to obtain some measure of photoreceptor activity since the leading edge of the a-wave is less contaminated by bipolar cell activity. Repeated measures ANOVA did not indicate significant changes in timing or amplitudes for the a-wave component across conditions. B-wave amplitudes were measured and fit to the Naka-Rushton equation. Rmax and K values are shown in Table 2. Using repeated measures ANOVA, no significant changes between placebo and nicotine Rmax and K values were observed. Implicit times of the b-waves did not change across conditions. B-wave amplitudes were normalized to the placebo Rmax and repeated measures ANOVA indicated a significant effect of condition on the normalized dark-adapted b-wave responses with 2mg, F(1,98)=7.60, p≤0.01, and 4mg, F(1,112)=7.53, p≤0.01 (Figure 3). Summed OP amplitudes and latencies were not significantly different across conditions.

*Effects of nicotine on light-adapted ERGs-*
Experiment #1:

Light-adapted ERGs (n=10) were obtained after ten minutes of light adaptation immediately following dark-adapted testing. Placebo/nicotine had been administered during the thirty minute dark adaptation prior to dark-adapted testing. The a-wave mean amplitude values are seen in Table 1. No significant changes were seen across conditions. B-wave amplitudes were fit to the Naka-Rushton equation and the Rmax and K values are reported in Table 2. Values of Rmax and K as well as implicit times for either a- or b-waves were not significantly different across conditions. Responses obtained in the 4mg condition showed decreased amplitudes (Figure 4). Repeated measures analysis of the normalized b-wave amplitude responses showed a significant effect of 4mg nicotine on light-adapted b-wave amplitudes, F(1,63)=6.68, p=0.01, but did not show a significant effect with 2mg nicotine, F(1,49)=0.07, p ≥ 0.05.

Experiment #2:

Light-adapted ERG (n=5) responses were obtained after ten minutes of light adaptation. Nicotine and placebo gums were administered for a total of 30 minutes and were discarded immediately before testing. Amplitude and latency values are shown in Table 1. No significant differences were seen with either dosage of nicotine on a-wave amplitudes or a- and b-wave implicit times. B-wave amplitudes were fit to the Naka-Rushton equation, and Rmax and K values are shown in Table 2. No significant changes were seen with the individual Rmax and K values. However, repeated measures ANOVA on the normalized b-wave amplitude responses revealed a significant effect of condition F (1.20, 33.57) =6.09, p=0.01. Post-hoc pairwise comparisons indicated significant increases in the b-wave amplitudes under the 4mg nicotine condition only
Repeated measures ANOVA did not indicate any significant effect of condition on summed OP amplitudes and implicit times.

Discussion

Cigarette smoking causes a number of physiological changes in humans that can directly and indirectly affect the retina. For example, smoking is known to change cardiovascular responses which in turn can affect retinal responses via altered blood flow. There are numerous additives (~ 600) in cigarettes, some of which have been shown to alter electrophysiological measures of brain activity (e.g., menthol and propylene glycol). While it is reasonable to assume that the combination of chemicals from tobacco smoke affects the retina, it is all but impossible to isolate the effects of specific compounds. This study was designed to examine how nicotine in isolation, administered as gum, affects the human retina using ERG measurements. The key findings of this study are summarized in Table 3.

Under dark- and light-adapted conditions, we observed changes in strength of the response as measured by b-wave amplitudes. The dark-adapted b-wave amplitude decreased with both dosages of nicotine. Previous studies have shown changes in the dark-adapted ERG with cigarette smoking and acetylcholine, a nicotinic agonist. Dmitrieva et al. (2007) studied the expression of α7 nicotinic acetylcholine receptors (α7nAChRs) in rabbit retina. Their data showed α7nAChR expression in a population of cone ON-bipolar cells, glycinergic and GABAergic amacrine cells, and ganglion cells. No expression was seen in rod bipolar cells or AII amacrine cells which comprise the major rod pathway. However, the underlying mechanism for the changes we observed
in the dark-adapted b-wave amplitudes could be attributed to the rod pathway that feeds into calbindin-positive cone ON-bipolar cells\textsuperscript{28}. Another possible underlying mechanism for the changes we observed in the dark-adapted b-wave amplitude is feedback mechanisms from amacrine cells onto rod and/or cone bipolar cells are. Studies of rabbit retina have shown that nicotine and nicotinic agonists increase the release of dopamine and change the response properties of ganglion cells that express nicotinic receptors.\textsuperscript{12,29}

Light-adapted ERGs were measured on two different occasions. In the first experiment, light-adapted testing began approximately one hour after the initiation of nicotine exposure. In the second experiment testing began thirty minutes after the initiation of nicotine exposure. The results from these two experiments revealed opposite changes with the 4mg dose. In the first experiment, the b-wave amplitudes were significantly decreased, whereas in the second experiment, the b-wave amplitudes increased under the 4mg condition. The 2mg experiment showed little or no changes in either case. This discrepancy in our findings could be attributed to a couple of factors: 1) Based on our knowledge of maximal nicotine concentration, we believe the peak concentration of nicotine had declined in Experiment #1, measured one hour post nicotine intake compared to thirty minutes post nicotine in Experiment #2; 2) Potency, efficacy, and desensitization rate vary for different subtypes of nAChRs which could explain our findings.\textsuperscript{30} Nonetheless, these data indicate that nicotine alters retinal function through the cone pathway, which is similar to that reported by Jurklies and Gundogan. In rabbit retina, \(\alpha_7\) nicotine acetylcholine receptors (\(\alpha_7\nAChRs\)) have been shown to be expressed on retinal neurons and processes in several types of neurons including a class of cone bipolar cells.\textsuperscript{28} Nicotinic receptor expression in non-human
primate retina also suggests that nicotine may affect the cone pathway. The observed changes from Experiment #1 were unexpected and are inconsistent with previous findings, however, they are suggestive of desensitization and/or the recovery of desensitization of nicotinic receptors. Published data indicate that nAChRs are expressed primarily in the inner retina, specifically amacrine and ganglion cells. Our analysis of the OPs derived from Experiment #2 indicates very little to no change with summed OP amplitudes and latencies. Individual peak analysis did reveal changes in dark- and light-adapted conditions. Pharmacological studies indicate differing sensitivities of early and late OP peaks to dopamine, GABA, and glycine, with OPs diminishing in the presence of these neurotransmitter antagonists. Our results showed nicotine increased peak amplitudes of OP2 in dark-adapted conditions and OP2, 3 and 5 in light-adapted conditions (data not shown). These data would suggest an increase in inhibitory neurotransmitter release with nicotine based on the above mentioned pharmacological studies.

The results from this study show that nicotine changes the response properties of the retina, via nAChRs, in a naïve visual system that has no previous direct exposure to nicotine. What is unknown is exactly how nicotine and nAChRs interact to allow for the changes observed. We can hypothesize possible mechanisms based on the knowledge of prior studies investigating the effects of nicotine or nicotinic agonists on the retina of other species. Neal and colleagues investigated the role of nicotinic agonists on the activation of GABAergic amacrine cells in rabbit retina. Application of nicotine and/or epibatidine yielded an increase in the release of dopamine indirectly through GABAergic amacrine cells. They concluded that nicotine stimulates the release of GABA and
indirectly stimulates the release of dopamine via inhibitory neurotransmission via GABA.\textsuperscript{12} nAChRs have been identified on amacrine cells and their processes in various species; in rabbit retina, nAChRs were identified specifically on GABAergic amacrine cells and terminals of ON-cone bipolar cells.\textsuperscript{8, 10, 28} It is possible that nicotine could initiate a process of disinhibition by increasing the release of glutamate from the cone bipolar terminals causing a positive feedback on the second order neurons by increasing the release of GABA leading to an increase in dopamine. Since dopaminergic amacrine cells interact with AII amacrines, an inhibitory feedback mechanism could be responsible for the changes observed in our dark-adapted conditions.

A limitation of this study is that we have no quantification of nicotine levels for our subjects. Ideally, we would be able to measure blood serum nicotine levels to quantify the amount of nicotine being absorbed through the gum. Without this information, the following three issues remain and cannot be evaluated against our response measures: First, we cannot definitively identify when nicotine concentrations reached their maximum. However, based on Russell and colleagues’ (1976) investigation into blood nicotine levels in cigarette smoking and nicotine gum, we can estimate when nicotine might reach the maximum level in our studies. Their study revealed maximum blood plasma nicotine levels thirty minutes after consumption of 4mg nicotine gum which was comparable to that of smoking one cigarette.\textsuperscript{24} Secondly, we have no information about the latency between nicotine ingestion and the point at which nicotine reaches levels sufficient to affect nAChRs. One study measured blood flow at the papilla and showed a decrease after cigarette smoking, however there is no other information related to this factor.\textsuperscript{35} Thirdly, nicotine metabolism and uptake will vary across
individuals based on their body mass index (BMI) and other physiological factors. We cannot quantitatively account for these individual differences and a better understanding of the concentration of nicotine and its time course for individual participants would enhance the interpretation of our data.

Nevertheless, the results from this study show that nicotine, itself, affects the functional properties of retinal neurons. Additional research is required into the expression of nAChRs in the retina of both non-human primates and humans to better understand how nicotine alters visual processing. We plan to use psychophysical measures, e.g., contrast sensitivity, to explore the effects of nicotine at a behavioral level. Beneficial effects of nicotine have been observed in relieving symptoms and treatment of Parkinson’s disease (PD). Janson and colleagues (1993) have shown that nicotine acts as a neuroprotector in dopaminergic neurons in the brain of rats with PD. In relation to this study, Gottlob (1987) showed decreased b-wave amplitudes in both dark- and light-adapted conditions in patients with PD, which is indicative of a disturbance in the inner retina possibly, with the dopaminergic system. Eventually, the information from our current study may lead to research into the role of nicotine in ocular diseases, such as AMD and glaucoma.
References


Figure Legends

**Figure 1:** Individual ERG responses for both scotopic and photopic intensity ranges.  
*Left:* Dark-adapted series with responses ranging over a 4.9 log unit range.  *Middle:* Light-adapted series with a 2.1 log unit response range.  *Right:* Representative ERG trace depicting measurements: A- a-wave amplitude from baseline to the tip of the first negative inflection; B- b-wave amplitude from the a-wave to the tip of the first positive peak; IT<sub>A</sub>- a-wave implicit time from time zero to A; IT<sub>B</sub>- b-wave implicit time from time zero to B.

**Figure 2:** Individual ERG responses for placebo, 2mg nicotine gum, and 4mg nicotine gum under both dark- and light-adapted conditions. Experiment 1: *Top Left:* Dark-adapted waveforms measured at +0.45 log scotopic trolands (td).  *Top Right:* ISCEV dark-adapted OP waveform measured at +2.28 log scotopic trolands (td).  Experiment 2:  *Bottom Left:* Light-adapted waveforms measured at +2.16 log photopic trolands (td).  *Bottom Right:* ISCEV light-adapted OP waveform measured at +2.28 log photopic trolands (td) against a 30 cd/m<sup>2</sup> background.

**Figure 3:** Dark-adapted ERG values for placebo and nicotine conditions.  ERGs were measured after 30 minutes of dark adaptation.  Amplitudes are plotted against the log retinal illuminance measured by scotopic trolands (td).  *Top:* Individual b-wave amplitude responses curve fitted to the Naka Rushton equation for placebo and 2mg nicotine.  *Middle:* Normalized responses for b-wave amplitudes under 2mg nicotine condition (n=8), and *Bottom:* Normalized responses for b-wave amplitudes for 4mg nicotine (n=9).  Significant amplitude decreases were seen with both 2mg and 4mg of nicotine (p<0.01)  *Error bars= ±SEM*
**Figure 4:** Light-adapted ERG values for placebo and nicotine conditions. Amplitudes are plotted against log photopic trolands (td). *Top Left:* Individual b-wave amplitude responses for placebo and 4mg nicotine from Experiment #2. Amplitude responses were increased with nicotine. Experiment #1: *Top Right:* Normalized responses for b-wave amplitudes under 2mg nicotine (n=8), and *Bottom Left:* Normalized responses for b-wave amplitudes for 4mg nicotine (n=10; p=0.01). Experiment #2: *Bottom Right:* Normalized responses for placebo and nicotine conditions (n=5; p≤0.01). *Error bars= ±SEM*
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<td>35.2(1.6)</td>
<td>15.0(0.3)</td>
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<td></td>
<td>37.5(1.8)</td>
<td>37.5(1.8)</td>
<td>37.5(1.8)</td>
<td>15.0(0.3)</td>
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<td></td>
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<tr>
<td>b-wave amplitude (µV)</td>
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<td></td>
</tr>
<tr>
<td>Placebo/2mg</td>
<td>317.45(36.9)</td>
<td>302.48(36.9)</td>
<td>413.37(35.9)</td>
<td>140.48(16.5)</td>
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<td>Placebo/4mg</td>
<td>319.66(47.1)</td>
<td>321.90(32.1)</td>
<td>461.11(40.5)</td>
<td>145.33(17.9)</td>
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<td>338.09(61.9)</td>
<td>326.29(42.0)</td>
<td>374.39(33.0)</td>
<td>147.75(12.5)</td>
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<tr>
<td></td>
<td>299.62(33.1)</td>
<td>328.93(40.5)</td>
<td>459.96(73.6)</td>
<td>159.04(13.6)</td>
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<tr>
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<tr>
<td>b-wave latency (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo/2mg</td>
<td>93.8(4.0)</td>
<td>86.6(2.9)</td>
<td>46.3(1.3)</td>
<td>29.4(0.2)</td>
</tr>
<tr>
<td>Placebo/4mg</td>
<td>92.4(3.4)</td>
<td>87.4(2.4)</td>
<td>52.4(1.9)</td>
<td>29.8(0.2)</td>
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<td></td>
<td>95.5(5.1)</td>
<td>94.9(4.8)</td>
<td>48.8(0.6)</td>
<td>29.4(0.2)</td>
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<td></td>
<td>86.8(3.0)</td>
<td>88.2(2.6)</td>
<td>51.6(1.3)</td>
<td>30.2(0.4)</td>
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<td>ISCEV Parameters:</td>
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<tr>
<td>Experiment #1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject numbers were different for 2mg and 4mg nicotine conditions. Placebo/2mg= placebo condition for 2mg nicotine (n=8). Placebo/4mg= placebo condition for 4mg condition (n=9)</td>
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<td></td>
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<tr>
<td>Experiment #2:</td>
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<tr>
<td>Subject numbers were equal across nicotine conditions (n=5). Values in (…) indicate the standard error of the mean (SEM).</td>
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Table 2: B-wave amplitude Naka Rushton fit parameters for dark- and light-adapted conditions.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Rmax Mean (SD)</th>
<th>K Mean (SD)</th>
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<tbody>
<tr>
<td><strong>Experiment #1 Dark-adapted</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo/2mg</td>
<td>397.95 ± 84.99</td>
<td>0.44 ± 0.09</td>
</tr>
<tr>
<td>2mg</td>
<td>394.80 ± 75.34</td>
<td>0.71 ± 0.71</td>
</tr>
<tr>
<td>Placebo/4mg</td>
<td>429.72 ± 126.04</td>
<td>0.42 ± 0.07</td>
</tr>
<tr>
<td>4mg</td>
<td>424.26 ± 110.18</td>
<td>0.52 ± 0.20</td>
</tr>
</tbody>
</table>

| **Experiment #1 Light-adapted** |                |             |
| Placebo/2mg         | 133.75 ± 31.46 | 30.78 ± 9.88|
| 2mg                 | 135.62 ± 31.61 | 33.63 ± 10.48|
| Placebo/4mg         | 143.96 ± 42.33 | 29.83 ± 9.08|
| 4mg                 | 132.30 ± 38.14 | 36.67 ± 12.76|

| **Experiment #2 Light-adapted** |                |             |
| Placebo               | 138.6±31.3     | 27.2±8.6    |
| 2mg                   | 136.1±37.7     | 26.4±2.2    |
| 4mg                   | 155.7±30.2     | 27.8±7.2    |

SD: standard deviation
Table 3: Overview of ERG changes with nicotine.

<table>
<thead>
<tr>
<th>ERG Components</th>
<th>Results</th>
</tr>
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<td><strong>Dark-adapted:</strong></td>
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<tr>
<td>a-wave</td>
<td>no changes</td>
</tr>
<tr>
<td>b-wave</td>
<td>decreased amplitudes with 2mg and 4mg nicotine</td>
</tr>
<tr>
<td><strong>Light-adapted:</strong></td>
<td></td>
</tr>
<tr>
<td>a-wave</td>
<td>no changes</td>
</tr>
<tr>
<td>b-wave</td>
<td>increased amplitudes with 4mg nicotine</td>
</tr>
</tbody>
</table>
figure 1: Individual ERG responses for both p.k and intensity ranges. Left: Dark-adapted series with responses ranging over a 4.9 log unit range. Middle: Light-adapted series with a 2.1 log unit response range. Right: Representative ERG trace depicting measurements: A- a-wave amplitude from baseline to the tip of the first negative inflection; B- b-wave amplitude from the a-wave to the tip of the first positive peak; ITA- a-wave implicit time from time zero to A; ITb- b-wave implicit time from time zero to B.
Figure 2: Individual ERG responses for placebo, 2mg nicotine gum, and 4mg nicotine gum under both dark- and light-adapted conditions. Experiment 1: Top Left: Dark-adapted waveforms measured at +0.45 log scotopic trolands (td). Top Right: ISCEV dark-adapted OP waveform measured at +2.28 log scotopic trolands (td). Experiment 2: Bottom Left: Light-adapted waveforms measured at +2.16 log photopic trolands (td). Bottom Right: ISCEV light-adapted OP waveform measured at +2.28 log photopic trolands (td) against a 30 cd/m² background.
Figure 3: Dark-adapted ERG values for placebo and nicotine conditions. ERGs were measured after 30 minutes of dark adaptation. Amplitudes are plotted against the log retinal illuminance measured by scotopic trolands (td). Top: Individual b-wave amplitude responses curve fitted to the Naka Rushton equation for placebo and 2mg nicotine. Middle: Normalized responses for b-wave amplitudes under 2mg nicotine condition (n=8), and Bottom: Normalized responses for b-wave amplitudes for 4mg nicotine (n=9). Significant amplitude decreases were seen with both 2mg and 4mg of nicotine (p≤0.01) Error bars= ±SEM
Figure 4: Light-adapted ERG values for placebo and nicotine conditions. Amplitudes are plotted against log photopic trolands (td). *Top Left:* Individual b-wave amplitude responses for placebo and 4mg nicotine from Experiment #2. Amplitude responses were increased with nicotine. Experiment #1: *Top Right:* Normalized responses for b-wave amplitudes under 2mg nicotine (n=8), and *Bottom Left:* Normalized responses for b-wave amplitudes for 4mg nicotine (n=10; p=0.01). Experiment #2: *Bottom Right:* Normalized responses for placebo and nicotine conditions (n=5; p≤0.01). *Error bars = ±SEM*
THE EFFECTS OF NICOTINE ON SINE-WAVE FLICKER ERGS: APPLICATION OF DFT AND T² CIRC

by

S.B. VARGHESE, N. NASER, T. THAN, C. STRANG, K.T. KEYSER, AND E.E. HARTMANN

In preparation for Visual Neuroscience

Format adapted for dissertation
Abstract

Nicotinic acetylcholine receptors (nAChRs) are expressed in neurons of non-human primate retina, primarily in the cone pathway. Prior studies from our lab have shown significant changes in light-adapted electroretinograms (ERG) responses with nicotine in humans. The aim of this study was to further investigate the retinal level and/or pathways on which nicotine impacts. Sine-wave flicker ERG responses were obtained from eleven healthy adult non-smokers. Participants were tested before (baseline) and after chewing nicotine gum on each of two visits. The baseline test was administered first, and participants were randomly assigned to either 2mg or 4mg nicotine gum on the first visit. Participants received the other dosage of nicotine on the second visit. ERG responses were collected using the Espion ERG system, adhering to ISCEV equipment guidelines. Responses were recorded from one eye for each participant using a Burian-Allen lens electrode. ERGs were measured using a 540nm stimulus at a mean luminance of 30 cd/m². The stimulus was a full-field sinusoidally flickering light. A total of 15 recordings were obtained at frequencies between 24 and 82Hz. While the magnitudes of the first (F1) and second (F2) harmonic fourier components of the response waveforms for baseline and nicotine conditions decreased with increasing rates, analysis showed a further significant decrease in magnitude of F1 with 2mg nicotine (p=0.002) and no significant changes with 4mg nicotine (p=0.24) compared to baseline. Linear regression was used to determine critical flicker fusion (CFF) thresholds. CFF threshold data revealed a decreased threshold with 2mg nicotine (p=0.02). Our results are consistent with evidence that nicotine affects retinal responses, primarily at the level
of bipolar and amacrine cells. It is likely that nicotine activates nAChRs expressed by amacrine cells resulting in increased inhibitory feedback onto cone bipolar cells.

Introduction

Nicotinic acetylcholine receptors (nAChRs) are pentameric receptors comprised of subunits α2-α6 and β2-β4 in α/β combinations, or of subunits α7-α9 in homomeric forms and are members of the family of ligand-gated ion channels. (McGehee, 1999; Clementi et al., 2000; Dani, 2001). The subunit composition of nAChRs has been shown to determine their pharmacological and functional properties, including agonist/antagonist affinity, channel open time, and desensitization rate (Russell et al., 1976).

nAChRs have been detected in cells of the retina, lateral geniculate nucleus (LGN), superior colliculus, and primary visual cortex (V1) in various species (Hutchins & Hollyfield, 1985; Keyser et al., 1988; Keyser et al., 2000; Dani, 2001; Marritt et al., 2005; Disney et al., 2007; Gotti et al., 2007; Liu et al., 2009). In the retina of mice, chick and rabbit, nAChRs have been identified in populations of bipolar, amacrine, and ganglion cells and in processes throughout the inner plexiform layer (IPL)(Keyser et al., 2000; Cox et al., 2008; Liu et al., 2009). Ligand binding studies in human retina revealed muscarinic and nicotinic binding sites in the IPL, however it was not entirely clear which cell types were involved (Hutchins & Hollyfield, 1985). A recent immunohistochemical study of the retina of non-human primates also showed receptor expression in amacrine, bipolar, and ganglion cells (Liu et al., 2009).
Prior clinical research involving nicotine and visual electrophysiology has been largely limited to cigarette smoking studies. Junemann and Damaske (1968) compared ERG responses from individuals who were non-smokers and smokers who had briefly abstained from smoking. Their results revealed that cigarette smoking led to a decrease in the b-wave amplitude of the dark-adapted ERG (Jü nemann & Damaske, 1968).

Gundogan et al. (2006) evaluated the effects of cigarette smoking using pattern ERGs (pERG), which measures contributions from inner retinal cells, retinal ganglion cells, and optic nerve head, and multifocal ERGs (mfERGs) (Gundogan et al., 2006; Gundogan et al., 2007). Their results revealed changes in the pERG (Gundogan et al., 2006), (Holder, 2006), and in the mfERG in cigarette smokers compared to non-smokers (Gundogan et al., 2007).

Electrophysiological measures of temporal processing at the retinal level can be obtained using flickering stimuli. Specifically, electroretinogram (ERG) recordings obtained under full-field stimulus conditions using sinusoidally modulated light can be used to evaluate the temporal limits of retinal neuron and pathway responses. Retinal contributions to the photopic flicker ERG consists of cones, bipolar and amacrine cells (Odom et al., 1992; Bush & Sieving, 1996; Kondo & Sieving, 2001; Viswanathan et al., 2002). Previous studies have shown different retinal influences on the fundamental (F1) and secondary (F2) harmonics of the photopic flicker ERG dependent on the frequency of the temporal stimuli (Bush & Sieving, 1996). However, the underlying conclusion speculates that bipolar cells and inner retinal cells, i.e. amacrine cells, have important contributions on both F1 and F2 components (Viswanathan et al., 2002).
Previous research in our lab on the effects of nicotine on retinal function as assessed by full-field ERGs in dark- and light-adapted conditions indicated nicotine has a greater impact on the light-adapted ERG compared to the dark-adapted ERG (Varghese et al., 2011). We observed significant increases in the light-adapted b-wave amplitudes with a higher (4mg) dose of nicotine in comparison to a lower (2mg) dose of nicotine. These findings are consistent with the expression of nicotinic acetylcholine receptors (nAChRs) in retinal neurons of several species including rabbit and non-human primate (Dmitrieva et al., 2007; Liu et al., 2009).

To our knowledge there are no reports describing the effect of nicotine on psychophysical measures of temporal processing and/or flicker ERGs. However, Barlow and Baer (1967) showed that the critical flicker fusion (CFF) thresholds (the acuity limit of temporal processing), obtained psychophysically, increased in both heavy and light smokers immediately after smoking (Barlow & Baer, 1967). A subsequent study investigated the combined effects of cigarette smoking and alcohol consumption. The results revealed an increased CFF threshold with cigarette smoking alone (Leigh, 1982). However, CFF thresholds decreased under the combined effect of smoking and alcohol.

Cigarette smoking can cause a number of physiological changes in humans that can directly and indirectly affect the retina. There are numerous additives (~ 600) (Rabinoff et al., 2007) in cigarettes, some of which have been shown to alter electrophysiological measures of brain activity (e.g., menthol and propylene glycol). (Rabinoff et al., 2007) While it is reasonable to assume that the combination of chemicals from tobacco smoke affects the retina, it is all but impossible to isolate the effects of specific compounds. The reported changes in the above mentioned studies cannot be unequivocally attributed to
any one compound including nicotine. Based on prior research, the purpose of this study was to extend our investigation of the effects of nicotine on temporal processing in the visual system using flicker ERGs.

Materials and methods

Participants

ERGs were measured from eleven visually normal participants ranging in age from 22-30 years (mean ± SD= 26.4± 2.6) after informed written consent was obtained. Each participant was provided with a comprehensive eye exam including a visual field (24-2 SITA, standard) and color vision test (Hardy, Rand, and Rittler; H.R.R., 4th edition; binocular) to rule out any ocular abnormalities, retinal diseases, or color vision defects. The experimental protocol adhered to the tenets of the declaration of Helsinki and was approved by the UAB Institutional Review Board for Human Use.

ERGs were recorded from one eye of each participant. Eyedrops were used to dilate the pupil (1% tropicamide; Alcon Laboratories, Inc., Fort Worth, Texas, USA) and to anesthetize the cornea anesthetized (0.5% proparacaine hydrochloride; Bausch and Lomb Incorporated, Tampa, Florida, USA). A bipolar Burian-Allen contact lens electrode (Hansen Ophthalmic Development Lab, Coralville, Iowa, USA) wetted with Celluvisc (Allergan, Inc., Irvine, California, USA) was used to record the ERG; an EKG skin electrode placed on the opposite mastoid process served as the ground lead. The fellow eye was patched and recording proceeded after 10 minutes of light adaptation to 30 cd/m².
**Flicker ERGs**

Full field stimuli were produced using an array of light-emitting diodes (LEDs) presented in an Espion Color Dome (Diagnosys, Lowell, Massachusetts, USA). The background luminance was 30 cd/m² at 560 nm (Espion “Green”: CIE coordinates: x: 0.108, y: 0.647, z: 0.770). Sinusoidal flicker stimuli were presented at the following temporal frequencies in Hertz (Hz): 24, 28, 32, 36, 40, 44, 48, 52, 56, 64, 68, 72, 76, 80, and 82. The ERG was band-pass filtered (0-100 Hz), sampled at 5000 Hz, averaged across 30 sweeps and stored.

**Nicotine administration**

In each session, ERGs were recorded before and after the administration of nicotine in the form of nicotine gum (GlaxoSmithKline, Moon Township, Pennsylvania, USA) with a dosage of 2 mg or 4 mg. A single piece of 4 mg nicotine gum has been shown to yield blood nicotine levels similar to smoking one cigarette (Russell *et al.*, 1976). Participants were tested in two separate visits that were at least one week apart. Participants were randomly assigned to receive either 2 mg or 4 mg nicotine gum on the first visit and received the other dosage level at the second visit. Participants were masked to the nicotine level; the experimenter was also masked during both data collection and initial analysis. After a baseline series of ERGs were obtained, the Burian-Allen electrode was removed, and the participant chewed the nicotine gum. After 30 min the gum was discarded, and the ERG session was repeated.

**Data analysis**

**Fourier analysis**
The data obtained were exported to MATLAB (MathWorks, Natick, Massachusetts, USA) for Discrete Fourier Transform (DFT) analysis. The extracted data from Fourier analysis is comprised of complex numbers (real plus imaginary) as seen in the following equation:

$$z = r \cdot e^{i\theta}$$  \hspace{1cm} [Equ. 1]

where \( r \) = amplitude or size of the response and \( \theta \) = phase or timing of the peak of the response to a stimulus cycle. Equ.1 can be written differently in terms of sine and cosine components:

$$z = x + yi$$  \hspace{1cm} [Equ. 2]

where \( x \) = cosine component, \( y \) = sine component, and \( i \) = imaginary component. Sine and cosine components of the flicker data were extracted and used to calculate the magnitude and phase of the fundamental (F1) and secondary (F2) harmonics of the Fourier transform.

\( T^2 \) circ analysis-

The \( T^2 \) circ statistic is a multivariate statistic that is an enhancement on the Hotelling \( T^2 \) statistic. The \( T^2 \) statistic ignores specific components of Fourier analysis. For example, the \( T^2 \) statistic overlooks the relationship between the real and imaginary parts of Fourier components. The \( T^2 \) circ statistic was designed for analysis of variability of Fourier components, and to determine the confidence limits and the significance of differences between groups (Victor & Mast, 1991). The \( T^2 \) circ statistic is shown to be a sensitive detector of signal from background noise in electrophysiological testing (Zemon et al., 1997). This statistic was applied to the data to evaluate the reliability of the measures (Victor & Mast, 1991; Zemon et al., 1997).
Statistical analysis-

Baseline vs. baseline and baseline vs. nicotine comparisons were made using a paired student’s t-test to determine statistical significance with the initial p value set at 0.05 (Microsoft Excel, 2010).

CFF thresholds

CFF thresholds were calculated from F1 magnitude data over the frequency range of 40Hz to 68Hz. Linear regression was used to determine the exact threshold. The initial magnitude for threshold was set at 4uV. This was determined by averaging all participant’s baseline F1 magnitude and 95% CIs at 68Hz. The mean magnitude was found plus the mean 95% CI, and the mean 95% CI was added to the mean F1 magnitude to determine the initial magnitude. Sign tests (B statistic) were used to analyze CFF thresholds with the initial p value set at 0.05 (Microsoft Excel, 2010).

Results

Individual Flicker ERG Responses

A complete data set was obtained from 11 participants. Figure 1 presents a representative data set from one participant. Consistent with prior studies (Kondo & Sieving, 2001), response amplitudes increased as flicker increased from 24 Hz to 40 Hz, and then steadily decreased with increasing frequency. Figure 2A,B compares raw 40 Hz flicker ERG waveforms from a representative participant under each testing condition. In comparison to baseline, flicker ERG amplitude decreased for both nicotine doses. This decrease was not frequency specific, as it was observed throughout the frequency range
examined (Fig. 2C,D). Eight out of the ten participants tested showed decreases in amplitudes with 2 mg nicotine compared to seven out of ten with 4 mg nicotine.

**Fourier and $T^2$ circ Analysis**

Fourier analysis was applied to the raw flicker ERG data to obtain the sine and cosine components for $T^2$ circ analysis. $T^2$ circ was applied to the data to calculate mean F1 and F2 magnitudes and the 95% confidence intervals (CI) to evaluate the reliability of the signal. If the 95% CI did not cross zero, the signal is by definition reliable and out of the noise. Figure 3 shows results from DFT and $T^2$ circ analyses for magnitude and phase for F1 from two subjects. For subject, ID 10 (Fig. 3 top panels), reliable F1 magnitudes were obtained up to 80Hz, at which point the data became unreliable as indicated by the 95% CIs. A similar pattern was observed for 8 other participants. The only exception was subject, ID 11 (Fig. 3 bottom panels), whose baseline data were not different than noise. This subjects data were excluded from further data analysis. Data points for each individual participant for both baseline and nicotine measures for F1 and F2 can be seen in Appendix A and B. Individual data points for the remaining 10 participants were eliminated from analysis if the 95% CI was not out of the noise. There were no changes in the phase of the responses between baseline and nicotine conditions (Figure 4), for 2mg comparison.

**Effects of Nicotine on the F1 component**

Figure 4 compares average F1 magnitudes and phase data for the 2 mg and 4 mg sessions. Comparisons of F1 magnitude between conditions and sessions were conducted
using paired student’s t-test. Baseline measurements were significantly different between sessions \((t=-7.31, p\leq0.0001)\). Therefore, all further comparisons were made using data collected within a single session. F1 magnitudes decreased with both dosages of nicotine. This decrease was statistically significant for 2mg nicotine \((t=-5.61, p=0.0002)\) where seven out of ten participants showed a decrease in response magnitudes for all frequencies tested, but not 4mg \((4mg: t=-1.24, p=0.24)\) where only five participants had decreases for all frequencies (Figure 4). No significant changes were seen with F1 phase for either dose \((2mg: t=-0.76, p=0.46; 4mg: t=0.52, p=0.62)\).

**Effects of Nicotine on CFF**

CFF was calculated using linear regression from 40 to 68 Hz. Mean CFF for both baselines and nicotine conditions (2mg and 4mg) are presented in Table 1. Figure 5 shows the linear regression models for baseline and nicotine conditions. Mean CFF was significantly decreased for 2 mg nicotine compared to baseline \((B \text{ statistic}= 1.00, p=0.02)\). No significant differences were seen with 4 mg nicotine \((B \text{ statistic}= 3.00, p=0.34)\).

**Discussion**

The results from this study reveal a dose-dependent effect of nicotine on photopic flicker ERG responses in non-smokers. Two mg nicotine gum decreased F1 magnitudes and had no effect on phase or timing of the response compared to baseline and 4 mg nicotine. Retinal CFF measures showed decreased CFF with ingestion of 2mg nicotine and no significant changes with 4mg nicotine. These data indicate nicotine has a greater
impact on bipolar and amacrine cell response properties. Based on previous findings from our lab, our hypothesis was nicotine would have an impact on both F1 and F2 components, primarily on the response magnitudes and not phase. It was anticipated that the change in these magnitudes would increase with 4 mg nicotine gum based on results from our prior study, i.e. 4 mg nicotine gum increased the cone-driven b-wave amplitude.

Previous studies have documented ERG changes associated with cigarette smoking (Jünemann & Damaske, 1968; Gundogan et al., 2007). Junemann and Damaske (1968) reported that cigarette smoking decreased the amplitude of the dark-adapted (rod-driven) ERG. Gundogan et al. (2007) found that changes in multifocal ERGs, recorded under light-adapted conditions, were larger in cigarette smokers (Gundogan et al., 2007). To separate the effect of nicotine from the many other components of cigarette smoke (Rabinoff et al., 2007), our lab examined whether nicotine gum would alter the human ERG and found, in non-smokers, that nicotine decreased the dark-adapted ERG and increased the light-adapted ERG (Varghese et al., 2011).

Effects of nicotine on F2 magnitudes

The F2 magnitudes obtained from the flicker ERG showed an overall decrease with 2mg nicotine and 4mg nicotine (data not shown) in one subject. Seven out of the ten subjects had data points that were determined reliable for F2 magnitudes; however, the data were highly variable and not consistent. Of the data points that were reliable for baseline and nicotine conditions, 2mg nicotine gum appeared to have a greater effect on F2 magnitudes than 4mg nicotine. Because the majority of the data were unreliable, there are some explanations that could attest for these findings: 1. Kondo and Sieving (Kondo
& Sieving, 2002) showed in their study of flicker ERGs in primates that sine-wave flicker stimuli yielded smaller 32Hz flicker ERG F1 and F2 magnitudes compared to square-wave and pulsed flickered stimuli. 2. Our application of $T^2$-circ analysis allowed us to determine the reliability of each individual data point. Overall, small F2 magnitudes from sine-wave flicker stimuli were not reliably above the underlying ERG noise level. While we started with 11 subjects, one participant’s data set was completely excluded and the F2 data from 5 of the remaining 10 were not useable for the F2 analysis, determined by the 95%CI, for F2 magnitude analysis (see Appendix B). Of that data, the magnitude of the F2 responses of one participant were significantly altered by nicotine administration, suggesting that a more reliable signal to noise ratio would have allowed the observed trends in the responses of the other participants to reach significance.

**Effects of nicotine on CFF**

Our data showed comparable findings in CFF measures in comparison to previous studies in psychophysical CFF threshold measurements in cigarette smokers. Previous studies showed increased CFF thresholds in cigarette smokers immediately after smoking (Barlow & Baer, 1967; Leigh, 1982). In this current study, the data showed a decreased CFF with 2 mg nicotine, implying nicotine decreased the retina’s capacity to discriminate changes to flickering light in non-smokers. While decreases in CFF were seen with 4 mg nicotine, the data were not statistically significant. Though these data are consistent with previous psychophysical findings in smokers, there are several important factors to identify: 1. Prior studies determined CFF thresholds by use of psychophysical measurements, determined throughout the whole visual system, where our methods
determined CFF by extracting thresholds from flicker ERG responses, determined at the retinal level. It is possible that nicotine in non-smokers could show an overall decreased threshold in psychophysical CFF; 2. Chronic exposure to nicotine has been documented to cause an upregulation of nAChRs in the brain (Govind et al., 2009). An addictive property of nicotine, the more exposure to nicotine one has, the greater the upregulation of nAChRs, thereby, requiring more nicotine to activate the receptors, which, in turn, changes the affinity and response properties of the nAChRs (Govind et al., 2009). It is reasonable to speculate that chronic exposure to nicotine could attribute to the increased CFF thresholds seen in studies with cigarette smokers (Barlow & Baer, 1967; Leigh, 1982); 3. Cigarette smoke has been shown to have numerous additives/chemicals/toxins that could directly/indirectly affect retinal and/or visual responses. Several additives found in cigarette smoke (i.e., menthol and propylene glycol) have been shown to have an effect on electrophysiological responses of brain activity (Rabinoff et al., 2007). The results from cigarette smokers seen in previous studies could be attributed to the effects of cigarette smoke.

Effects of nicotine on F1 magnitudes

Our findings demonstrated changes in retinal responses to flickering stimuli assessed using flicker ERGs after participants chewed nicotine gum. F1 response magnitudes derived from DFT of flicker ERG data showed decreases with the lower dosage of nicotine gum (2mg). The changes seen in the F1 magnitudes with 2mg nicotine gum are consistent with nAChR expression in retina. Specific locations of nAChR expression in the retina have been reported in the proximal retina including
bipolar cells, amacrine cells, ganglion cells and processes within sublamina a and b of the inner plexiform layer (IPL) (Hutchins & Hollyfield, 1985; Dmitrieva et al., 2007; Liu et al., 2009). Dmitrieva et al. (Dmitrieva et al., 2007) studied the expression of $\alpha_7$ nicotinic acetylcholine receptors ($\alpha_7$nAChRs) in rabbit retina. Their data revealed $\alpha_7$nAChR expression on a population of cone ON-bipolar cells, glycinergic and GABAergic amacrine cells, and ganglion cells (Dmitrieva et al., 2007). Liu et al. (2009) data showed nAChR expression throughout the retina of non-human primates with the majority of the nAChR expression in the inner retina.

Based on previous findings with nicotine and full-field ERGs from our lab, we anticipated a greater change in the F1 magnitude with the 4mg nicotine dose. However, there was no change between baseline and 4mg nicotine on F1 magnitudes, suggesting the possibility of receptor desensitization at the higher dose. nAChRs vary in subunit composition, for example $\alpha_7$-nAChRs (homomeric) desensitize rapidly, while $\beta_2$-containing nAChRs (heteromeric) desensitize more slowly. The different combinations nAChR subunits determine their pharmacological and functional properties such as agonist/antagonist affinity and desensitization rate (Lindstrom et al, 1999). nAChRs have been shown to be expressed in retinal neurons of various species i.e. rabbit, non-human primate and human (Hutchins & Hollyfield, 1985; Dmitrieva et al., 2007; Liu et al., 2009) Liu et al. (2009) examined the distribution of nAChR subunits in the non-human primate retina. They identified the expression patterns of various nAChR subunits ($\alpha$3-4, 6-7 and $\beta$2 and 4) throughout the retina, with a higher concentration of subunits in the inner retina (Liu et al., 2009) These data are consistent with the interpretation that the
lack of change in responses with 4mg nicotine could be due to the desensitization of specific nAChR subtypes within the inner retina.

Overall conclusions

Several studies have determined the retinal contributions to the sine-wave flicker ERG. Kondo and Sieving (2001) investigated the photoreceptor and postreceptor contributions to the photopic sine-wave flicker ERG by isolating components with glutamate analogs (DL-2-amino-4-phosphonobutyric acid (APB) and cis-2,3-piperidinedicarboxylic acid (PDA)) and testing at temporal frequencies ranging from 6 Hz to 64 Hz. Their results revealed that after application of APB (ON- pathway blocker to second order neurons), responses to flickering stimuli increased, and after application of PDA (OFF- pathway blocker to second order neurons) flicker responses decreased (Kondo & Sieving, 2001). They concluded that activity in second or third order neurons contributed more to the photopic sine-wave flicker ERG at higher temporal frequencies in comparison to photoreceptor contributions to temporal frequencies less than or equal to 10 Hz (Kondo & Sieving, 2001). A subsequent study by Kondo and Sieving (2002) using sine-wave, square-wave, and pulsed flickering stimuli showed after application of APB and PDA, responses to flickering stimuli, regardless of type of stimuli, decreased indicating photoreceptor input was minimal (Kondo & Sieving, 2002). Viswanathan et al. (2002) examined inner-retinal contributions to the photopic sinusoidal flicker ERG in macaque retina by applying APB, PDA, tetrodotoxin (TTX, blocks Na⁺ dependent spiking activity of retinal ganglion cells and amacrine cells), N-methyl-D-aspartate
(NMDA, suppresses all spiking activity from retinal ganglion cells, amacrine cells, and interplexiform cells), and 6-cyano-nitroquinoxaline-2,3-dione (CNQX, blocks retinal bipolar and horizontal cell activity). Their results showed F1 peak magnitudes were shifted to lower temporal frequencies and highly attenuated F2 magnitudes (Viswanathan et al., 2002). They concluded that the F2 component of the photopic flicker ERG relies more on inner than outer retinal contributions. They also concurred with Kondo and Sieving (Kondo & Sieving, 2001, 2002) that the inner retina contributes to the F1 component as well (Viswanathan et al., 2002).

We can speculate that the decreases observed in our F1 magnitudes with 2mg nicotine gum were the result of nicotine acting on the nAChRs within the inner retina. As mentioned previously, nAChRs are expressed primarily in the proximal retina of non-human primates, specifically on amacrine cells and ganglion cells (Liu et al., 2009). Prior studies have shown that nicotine and/or nicotinic agonists affect the retina in other species. Neal et al. (2001) investigated the effects of nicotinic agonists on the activation of GABAergic amacrine cells in rabbit retina. Their results revealed an increase in the release of dopamine via inhibitory neurotransmission of GABA after application of nicotine and/or epibatidine (Neal et al., 2001). It is possible that nicotine could activate nAChRs expressed by GABAergic amacrine cells causing an increase in release of inhibitory neurotransmitters, i.e. GABA, thereby increasing inhibitory feedback onto the cone bipolar cells. This would be consistent with our observed decreases in F1 magnitude with nicotine. It is also reasonable to speculate that the decreases seen in the F2 magnitude with nicotine could be indicative of nicotine’s interaction with the amacrine cells expressing nAChRs. The results from this current study coincide with the
changes we observed with oscillatory potentials (OPs) individual peak amplitudes from our previous work (Varghese et al., 2011). Individual OP peak amplitudes for OP2, -3, and -5 increased with nicotine, which is indicative of increased inhibitory neurotransmitter release (Ogden, 1973; Wachtmeister & Dowling, 1978; Wachtmeister, 1980, 1998; Varghese et al., 2011).

One limitation of this study is that no specific measures of serum nicotine levels were conducted. Ideally, knowing blood nicotine levels would allow us to correlate the specific nicotine levels with the magnitude of the observed changes. Nonetheless, based on the findings of Russell et al.’s study, we can estimate the time point at which nicotine would reach its maximum level (Russell et al., 1976). Their study investigated the time point at which nicotine reaches its maximum concentration in blood serum levels with comparing 2mg and 4mg nicotine gum and smoking one cigarette. They showed that 4mg nicotine gum had comparable nicotine concentration levels to that of smoking one cigarette and the nicotine concentration reached its peak around 30 minutes. Our experimental design was based on these findings and all testing was completed within a 30 minute time frame after nicotine intake. Metabolism and uptake of nicotine is dependent upon body mass index and other physiological factors. Therefore, blood serum nicotine levels would provide more definitive information for interpreting our results.

The results from this study show that nicotine affects retinal responses to sinusoidal flickering stimuli with a primary focus of change with bipolar and amacrine cells. Additional research is needed to further our understanding of expression of nAChRs in human retina as well as non-human primate retina. Future directions include
measurement of changes in inner retinal responses using pERG to determine what effects nicotine has on human ganglion cell responses. Nicotine has been shown to have beneficial effects in alleviating symptoms of Parkinson’s Disease (Janson & Møller, 1993; Fagerström et al., 1994). It is hoped that the results from this study and future studies involving nicotine will lead to investigations with nicotine and/or nicotinic agonists being a potential therapeutic in diseases such as PD as well as ocular diseases including but not limited to AMD and glaucoma.
Acknowledgements:

We would like to thank Dr. Neal Peachey for his critical feedback and assistance on preparation and revisions on this manuscript.

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Reference List


Figure 1: Representative baseline ERG recordings from one subject across the frequency range examined. Each trace is an average of 30 trials.
Figure 2: Individual participant data.  
A, B: 40 Hz ERGs for baseline and 2 mg (A) and 4 mg (B) nicotine conditions. In both sessions, nicotine increased ERG amplitude.  
C: Comparison of F1 magnitude data for baseline and 2 mg nicotine. F1 magnitudes decreased significantly with 2mg nicotine (p≤0.0001).  
D: F2 magnitude data derived from DFT for baseline and 2mg nicotine. F2 magnitudes were significantly decreased with 2mg nicotine (p≤0.0001) compared to baseline. Error bars are 95% CI using T^2_circ analysis.
Figure 3: Individual participant data for the F1 magnitude and phase for baseline and 2mg nicotine from Fourier analysis and $T^2$ circ analysis. Error bars indicate 95% confidence interval (CI). Top Left: If the 95% CI bars do not cross the x-axis, data points are out of the noise and were used for final analysis. Bottom Left: This individual’s data were excluded from final analysis because most data points for baseline and nicotine were in the noise. Both Top and Bottom Right panels show individual data for F1 phase with little to no change between baseline and 2mg nicotine conditions.
Figure 4: Mean F1 magnitudes and phase for baseline and nicotine (2mg and 4mg). Fundamental magnitude plotted against temporal frequency ±SEM. 

Top Left: Mean F1 magnitude for baseline and 2mg nicotine. Magnitudes were significantly decreased under nicotine condition (p value = 0.0002). 

Bottom Left: Mean F1 magnitude for baseline and 4mg nicotine. Changes in magnitude seen with 4mg nicotine were not significant (p value = 0.24). 

Top Right: Mean F1 phase for baseline and 2mg nicotine. Bottom Right: Mean F1 phase for baseline and 4mg nicotine. No statistically significant changes were seen with phase for both 2mg and 4mg nicotine.
Figure 5: CFF measures obtained from flicker ERG F1 magnitude data (n=10).

Thresholds were determined using linear regression of F1 magnitudes from 40Hz to 68Hz. Magnitude threshold was calculated to be 4uV determined by the upper 95% CI for baseline condition at 68Hz. Top panel: Linear regression analysis for CFF for baseline and 2mg nicotine. R² value was 0.94 for baseline and 0.93 for 2mg nicotine. Bottom panel: Linear regression analysis for CFF for baseline and 4mg nicotine. R² value was 0.94 for baseline and 0.92 for 2mg nicotine.
Table 1: CFF measures derived from linear regression of F1 flicker ERG magnitudes.

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P-values were obtained using sign tests. SEM= standard error of mean.
**Appendix A: T^2 circ analysis of F1 magnitude data points in and out of the noise.**

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O- data points out of noise; X- data points in noise
ID- participant’s ID number
ID 11 in *italics*- participant excluded based on data points in noise
### Appendix B: T² circ analysis of F2 magnitude data points in and out of the noise.

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ID- participant’s ID number
ID 11 in italics- participant excluded based on data points in noise
EFFECTS OF NICOTINE ON SPATIAL AND TEMPORAL CONTRAST SENSITIVITY IN NON-SMOKERS

by

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Abstract

Nicotinic acetylcholine receptors have been identified in multiple locations throughout the visual pathway: retina, lateral geniculate nucleus, and visual cortex (V1). The purpose of this study was to evaluate the effects of nicotine on spatial and temporal contrast sensitivity (sCSF and tCSF) in non-smokers. Twelve healthy, visually normal participants were enrolled in this study. We determined both spatial and temporal contrast sensitivity using a psychophysical method of adjustment procedure. Seven spatial frequencies were tested ranging from 0.5 to 25 cycles per degree; six temporal frequencies were evaluated over the range of 1 to 40 Hertz. Contrast thresholds were measured twice during each testing session: before and after nicotine. Baseline measurements were obtained prior to administration of nicotine in gum form. After baseline measurements were complete, nicotine was administered in gum form (2mg and 4mg). Participants chewed the nicotine gum for 30 minutes, threw the gum away, and repeated the contrast threshold measurements. Data were normalized to peak sensitivities for both spatial and temporal contrast thresholds. Repeated measures ANOVA revealed a significant effect of nicotine on both spatial and temporal CSF. Pairwise comparisons showed 2mg nicotine significantly increased normalized sensitivities in both sCSF and tCSF, while 4mg nicotine decreased sCSF sensitivities and had no effect on tCSF. These results suggest that 2mg nicotine has a greater impact on visual function compared to 4mg nicotine. These data are consistent with previous findings evaluating the effects of nicotine on gain modulation in V1 of macaque cortex. We can conclude from these findings that nicotine, in low doses, can increase the visual system’s sensitivity to contrast.
Introduction

Nicotinic acetylcholine receptors (nAChRs) are members of the ligand-gated ion channel family. Neuronal nAChRs have a pentameric structure and can be heteromeric or homomeric, based on the subunit composition. The heteromeric form of neuronal nAChRs is comprised of subunits $\alpha_2$-$\alpha_6$ and $\beta_2$-$\beta_4$ in an $\alpha/\beta$ composition; the homomeric form is comprised of $\alpha_7$ and $\alpha_9$ subunits (Clementi, Fornasari, & Gotti, 2000; Dani, 2001; McGehee, 1999). The subunit composition of nAChRs has been shown to determine their pharmacological and functional properties, including agonist/antagonist affinity, channel open time, and desensitization rate. For example, the $\alpha_7$ nAChR is highly permeable to calcium and has a higher desensitization rate with agonist binding compared to other subtypes of nAChRs. Acetylcholine (ACh), an excitatory neurotransmitter, is the primary agonist that binds to nAChRs. Nicotine, the addictive stimulant found in tobacco, can also bind to these receptors initiating a response similar to ACh binding.

nAChRs are widely distributed throughout the central nervous system (CNS) and have been identified within the visual system in various species, including the retina, the lateral geniculate nucleus (LGN), and visual cortex (V1) (Dani, 2001; Disney, Aoki, & Hawken, 2007; Dmitrieva, Strang, & Keyser, 2007; Gotti et al., 2007; Hutchins & Hollyfield, 1985; Keyser, Hughes, Whiting, Lindstrom, & Karten, 1988; Keyser et al., 2000; Liu et al., 2009; Marritt et al., 2005). nAChR subunits have been identified in retinal layers including cone outer segments, ON-cone bipolar cells, GABAergic amacrine cells and processes, and ganglion cells (Dmitrieva et al., 2007; Hutchins & Hollyfield, 1985; Liu et al., 2009). Prior studies have shown that nicotine and/or
nicotinic agonists affect the retina of other species. Neal et al. (Neal, Cunningham, & Matthews, 2001) investigated the effects of nicotinic agonists on the activation of GABAergic amacrine cells in rabbit retina. Their results revealed an increase in the release of dopamine via inhibitory neurotransmission via GABA after application of nicotine and/or epibatidine, (Neal et al., 2001).

nAChRs have been shown to be expressed in the LGN and play a role in visual development (Gotti et al., 2005; Grubb & Thompson, 2004). In nAChR β2/- subunit knockout mice, retinal ganglion cells (rGCs) lack spontaneous calcium driven activity; and, in addition, abnormal segregation of ON and OFF cells in the LGN is seen (Gotti et al., 2005; Grubb & Thompson, 2004). ACh has been shown to affect dorsal LGN (dLGN) response properties by increasing spontaneous and visually evoked firing in these cells (Grubb & Thompson, 2004). nAChRs have been identified in striate visual cortex V1 in macaque, primarily found at the thalmo cortical synapse in GABAergic neurons feeding from the parvocellular (PC) pathway (Disney et al., 2007). Nicotine acts on nAChRs in V1 by increasing the gain of the neurons, thus, enhancing the detection of visual stimuli (Disney et al., 2007).

Previous studies investigating the effects of nAChR agonist/antagonist in the visual system are limited. Smith and Baker-Short (1993) used pharmacological separation of nAChRs and muscarinic acetylcholine receptors (mAChRs) using nicotine, an nAChR agonist, and scopolamine, an mAChR antagonist to evaluate the mechanisms that contribute to human contrast sensitivity (CSF) (Smith & Baker-Short, 1993). Their results showed increased contrast thresholds for both spatial and temporal stimuli under the influence of scopolamine. In contrast, nicotine (1.5mg by buccal absorption)
increased thresholds at low spatial frequencies (< 4 cycles/degree) and decreased
thresholds at higher spatial frequencies. Nicotine had an opposite effect on temporal
CSF: thresholds decreased at lower temporal frequencies and increased with higher
frequencies (Smith & Baker-Short, 1993).

The aim of this study was to investigate the effects of nicotine, administered as
gum (2mg and 4mg), on spatial and temporal CSF in non-smokers. In Smith and Baker-
Short’s study, the participants recruited were “social” smokers. The design of our study
allowed us to test the effects of nicotine on a naïve visual system in non-smokers, who
had no history of tobacco use. Prior research in our lab has investigated the effects of
nicotine on the human electroretinogram (ERG) (Varghese, Reid, Hartmann, & Keyser,
2011). Our findings with nicotine, alone, were similar to previous reports on ERGs and
cigarette smoking (Gundogan, Erdurman, Durukan, Sobaci, & Bayraktar, 2007; Jurklies,
We anticipate that our results with non-smokers will be comparable to the earlier studies
with smokers (Smith & Baker-Short, 1993).

Methods

Participants

Twelve visually normal adult (7 females and 5 males) ranging in age from 23-30 years
(mean ± S.D.: 27.25 ±2.05 ) participated in this study. Each participant received a
comprehensive eye exam to rule out any ocular abnormalities. Testing included a visual
field (24-2 SITA, standard) and color vision test (Hardy, Rand, and Rittler; H.R.R., 4th
edition; binocular). Written informed consent was obtained after the study procedures
were explained, including warning of possible side effects. The experimental protocol adhered to the tenets of the declaration of Helsinki and was approved by the UAB Institutional Review Board for Human Use.

*Stimuli and Procedure*

Stimuli were generated using a commercially available system (ViSaGe system; Cambridge Research Systems, Ltd., Kent, UK) and the Visual Psychophysics Engine (VPE) software. Stimuli were presented on a cathode ray tube computer monitor (IBM Corporation, Armonk, NY) with a mean luminance of 30 cd/m$^2$ and a refresh rate of 100Hz. Spatial stimuli were vertical sine wave gratings; temporal stimuli consisted of a uniform field presented at a various rate of flicker. The size of the image, for both spatial and temporal stimuli, was 30cm x 40cm and was presented 250cm away from the observer, which subtended a visual angle of 7-8 degrees.

Contrast thresholds were measured using the method of adjustment procedure, with 10 trials for each stimulus condition. Spatial stimuli were measured at seven different spatial frequencies (0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 24.0 cycles/degree [cpd]). Temporal stimuli were measured across six temporal frequencies (0.5, 1.0, 3.0, 8.0, 25.0, and 40.0 Hertz [Hz]) on a circular uniform field. Spatial and temporal frequencies were presented at random during each testing block, i.e. spatial contrasts were interleaved throughout the spatial testing block. A warning tone sounded when the stimulus was presented. Participants adjusted the contrast by pressing the “up” or “down” keys on a wireless keyboard until the gratings were undetectable. Participants were allowed to titrate above and below their threshold for each stimulus before specifying threshold.
Once the participant was satisfied with that contrast, the “spacebar” was pressed to record the threshold. Each subject performed a practice test, baseline test, and experimental test.

**Nicotine Administration**

Two dosages (2mg and 4mg) of nicotine gum (GlaxoSmithKline, Moon Township, PA, USA) were used in this study. A single piece of four mg nicotine gum has been shown to yield blood nicotine levels similar to smoking one cigarette (Russell, Feyerabend, & Cole, 1976). Subjects were tested at two separate visits that were at least one week apart. Each testing session included a practice session, a baseline condition followed by a nicotine condition. Nicotine gum dosage was randomly assigned on the first visit; the other dosage of gum was administered on the second visit. On each visit, the participant would undergo a practice session first followed by a baseline testing session. Nicotine gum was administered immediately following the baseline session. Gum was administered 30 minutes prior to the experimental testing and was discarded when testing started. The participant was masked to the nicotine level; the experimenter was also masked during both data collection and initial analysis.

**Data analysis**

The data obtained were exported to Microsoft Excel for analysis. Each participant’s data were initially inspected for outliers. Outliers for each spatial and temporal frequency tested that were at least ±2SD were eliminated. No more than two points either above or below the mean were removed for each tested frequency. Baseline data for each testing session was compared for statistical significance. Data for both spatial and temporal contrast sensitivity were normalized to the peak sensitivity for each individual subject. Within subjects comparisons among baseline and both nicotine
conditions were made using repeated measures ANOVA (SPSS, Inc., Chicago, IL). Post-hoc pairwise comparisons were made using paired student’s t-test.

**Results**

*Individual sCSF and tCSF responses*

Spatial and temporal CSF responses were obtained from twelve participants. Figure 1 shows sCSF and tCSF data from a representative participant. The sCSF baseline curve shows sensitivity increased up to peak sensitivity at around 1 cpd, and decreased for the remaining spatial frequencies tested. Under the 2mg nicotine condition, sensitivities increased at all spatial frequencies tested except 1cpd; the peak sensitivity shifted to 2cpd. Four mg nicotine revealed an overall decrease in spatial sensitivities with a peak at 4cpd. The baseline tCSF curve shows an increase in temporal sensitivities with increasing temporal frequency up to a peak sensitivity at 8Hz. tCSF sensitivities increased under the 2mg nicotine condition across all temporal frequencies with peak sensitivity remaining at 8Hz. Temporal sensitivities did not change for this particular participant under the 4mg condition.

*Effects of nicotine on spatial contrast sensitivity*

Spatial contrast sensitivities for baseline measurements from two testing sessions were analyzed and were compared for each participant. There were no significant differences between baseline (p=0.22). Participant’s nicotine data were normalized to the baseline peak sensitivity which was 4 cpd. Normalized sCSF curves for baseline and nicotine conditions are shown in Figure 2. Repeated measures ANOVA indicated a significant effect of nicotine on sCSF for nicotine compared to baseline ($F_{1,6}=22.18$, $p=0.001$).
Post-hoc pairwise comparisons revealed a significant increase in sensitivity with 2mg nicotine ($t=4.76, P=0.003$) and a significant decrease with 4mg nicotine ($t=4.52, P=0.004$).

**Effects of nicotine on temporal contrast sensitivity**

Baseline sensitivity data for both testing visits were compared and analyzed for statistical significance and were not statistically different ($p=0.18$). tCSF data were normalized to the baseline peak sensitivity (8 Hz). Mean normalized tCSF data for baseline and nicotine conditions are shown in Figure 3. Repeated measures ANOVA indicated a significant effect of condition on tCSF sensitivities ($F_{2,10}=7.07, P=0.012$). Pairwise comparisons revealed significantly increased tCSF sensitivities under 2mg nicotine condition ($t=-2.60, P=0.05$), but no significant change in tCSF sensitivities under 4mg nicotine from baseline ($t=1.48, P=0.20$).

**Discussion**

We investigated the effects of 2mg and 4mg nicotine, administered as gum, on both spatial and temporal contrast sensitivity in individuals who were non-smokers. The results showed different effects of nicotine on psychophysical measures of spatial versus temporal contrast sensitivity. Sensitivity for both spatial and temporal CSF increased under the 2mg nicotine condition, in comparison, sCSF sensitivity decreased with 4mg nicotine and there was no effect of 4mg nicotine on tCSF. Smith and Baker-Short’s (1993) study investigated the effects of nicotine and scopolamine on spatial and temporal contrast sensitivity in social smokers. Their results showed decreased sensitivities at high spatial frequencies, and, in contrast, increased sensitivities at low spatial frequencies.
under nicotine conditions (1.5mg by buccal absorption) (Smith & Baker-Short, 1993). In comparison to our results, the sCSF data revealed 2mg nicotine increased sensitivities across all spatial frequencies tested, and 4mg nicotine decreased sensitivities for all spatial frequencies (Figure 2). The greatest impact of nicotine on sCSF appears to be in the middle spatial frequency range (2-8cpd). For tCSF, our data showed increased sensitivities across all temporal frequencies with the greatest impact at 8Hz and 25Hz under 2mg nicotine conditions, and little to no effect on tCSF sensitivity under the 4mg condition (Figure 3).

The discrepancies in both results could be attributed to the difference in testing stimuli, experimental procedure, and participant recruitment. It is important to note that in Smith and Baker-Short’s study, the subject population consisted of “social smokers.” It is known that cigarette smoke contains numerous chemicals and toxins that could directly/indirectly affect visual responses and/or vision. It has been shown that certain additives (e.g., menthol and propylene glycol) can alter electrophysiological measures in brain activity (Rabinoff, Caskey, Rissling, & Park, 2007). In addition, nicotinic receptor upregulation has been well documented in relation to chronic nicotine exposure (Govind, Vezina, & Green, 2009). It is reasonable to speculate that the results from Smith and Baker-Short’s study could be attributed to its subject’s prior exposure to nicotine and/or long term effects of cigarette smoking in general. In this study, nicotine introduced to a naïve visual system that had no prior exposure to nicotine. Thereby, the results indicate a dose-dependent response to nicotine, itself, in acute administration. Nonetheless, it is clear that nicotine does impact visual responses to contrast sensitivity measurements.
The results from this study revealed dose-dependent effects on both spatial and temporal contrast sensitivity, which could be attributed to nAChR subunit composition within the visual pathway from the retina through LGN and V1. Disney et al. (2007) investigated nAChR expression, including different nAChR subunits, and the way in which nicotine affects neuronal responses in V1 cortex of macaque (Disney et al., 2007). Their results revealed that the nAChR β2 subunit was highly expressed on presynaptic terminals of thalamocortical synapses of V1 neurons, specifically in layer 4c (Disney et al., 2007). These findings are indicative of cholinergic modulation on excitatory and inhibitory neurons receiving thalamic input. They also showed that nicotine (0.25M) increased the gain of these β2-containing nAChRs only in layer 4c of V1 cortex (Disney et al., 2007). Disney et al. (2007) concluded that nicotine had an excitatory effect on inhibitory GABAergic neurons. It is possible that the response properties of nAChRs in V1 can explain the changes observed with nicotine on spatial and temporal contrast sensitivity. β2 containing nAChRs show rapid membrane depolarization with nicotine and also a rapid recovery (Disney et al., 2007). When ACh and/or nicotine act upon these neurons, the probability of vesicular release is increased. It is reasonable to infer that 2mg nicotine acts on β2 nAChRs on GABAergic neurons in V1, increasing the gain in these cells by increasing vesicular neurotransmitter release, therefore, increasing spatial and temporal contrast sensitivity under 2mg nicotine.

In contrast, we found decreased sCSF sensitivities and no changes in tCSF under 4mg nicotine. nAChR subunit composition is related to the pharmacological and functional properties of nAChRs (Lindstrom, 2000). β2 containing nAChRs have response properties that consist of rapid membrane depolarization, faster recovery, and a
high affinity to receptor agonists, such as, nicotine (Disney et al., 2007). In comparison, the homomeric α7-nAChR has been shown to have the opposite properties of the β2-containing nAChR: α7-nAChRs show a rapid desensitization and have a lower sensitivity to ACh (Nelson & Lindstrom, 1999). It is possible that 4mg nicotine is sufficient enough to desensitize the α7-nAChRs, thereby, leading to a decrease in gain in V1 cortex.

Prior research in our lab has investigated the effects of nicotine on retinal responses measured by both full-field flash and flicker ERGs. Our results have shown that nicotine changes retinal responses in individuals who are non-smokers. In our full-field flash ERG measurements, both dosages of nicotine affected dark- and light-adapted ERG responses (Varghese et al., 2011). Our laboratory observed decreased dark-adapted b-wave amplitudes under 2mg and 4mg nicotine conditions, and an increase in light-adapted b-wave amplitudes under 4mg nicotine (Varghese et al., 2011). These findings suggest that nicotine affects retinal bipolar cell responses and inner retina functioning, including amacrine cells. We have also found that 2mg nicotine affects flicker ERG responses and retinal CFF. Specifically, 2mg nicotine decreased response magnitudes, as well as, decreased CFF (data not yet published). Our results suggest that 2mg nicotine has a greater impact on visual function compared to 4mg nicotine. In both flicker ERG and s-and tCSF data, 2mg nicotine increased visual sensitivity, implying that nicotine could enhance vision as a whole.

One limitation of our study is that we have no specific measures of the blood nicotine levels for our participants. Ideally, knowing blood nicotine levels would allow us to correlate the specific nicotine levels with the observed changes in contrast sensitivity. Nonetheless, based on the findings of Russell et al.’s study we can estimate
the time point at which nicotine would reach its maximum level (Russell et al., 1976). Their study investigated the time point at which nicotine reaches its maximum concentration in blood serum levels with comparing 2mg and 4mg nicotine gum and smoking one cigarette. They showed that 4mg nicotine gum had comparable nicotine concentration levels to that of smoking one cigarette and the nicotine concentration reached its peak around 30 minutes. Our experimental design was based on these findings and all testing was completed within a 30 minute time frame after nicotine intake. Metabolism and uptake of nicotine is dependent upon body mass index and other physiological factors. Therefore, blood nicotine levels would provide more definitive information for interpreting our results.

We can infer from the results of this study, that nicotine, in gum form, affects s- and tCSF in non-smokers in a dose-dependent manner. The increase in sCSF and tCSF with 2mg nicotine indicates an increase in the visual system’s ability to detect changes in contrast. In other words, acute administration of nicotine can improve vision. Implications of these findings can be related to visual diseases/disorders that can be potentially blinding. Age-related macular degeneration (AMD) and glaucoma are both common visual diseases that can be classified with contrast sensitivity losses (Bose, Piltz, & Breton, 1995; Monés & Rubin, 2005). Contrast sensitivity losses have also been seen in patients with Parkinson’s Disease (PD) (Price, Feldman, Adelberg, & Kayne, 1992). It is reasonable to assume that nicotine and/or a nicotinic agonist could be used as a possible therapeutic to aide in vision improvement in patients with AMD, glaucoma, and PD.
Reference List


Figure 1: Individual responses for baseline, 2mg nicotine, and 4mg nicotine for spatial and temporal contrast sensitivity. Top: Responses from a representative participant for sCSF. Data is plotted as log sensitivity versus log spatial frequency (cpd). Two mg nicotine gum increased sCSF sensitivity compared to baseline; in contrast, 4mg nicotine decreased sensitivity. Bottom: Individual responses for tCSF. Data is plotted as log sensitivity versus log temporal frequency (Hz). Two mg nicotine increased sensitivity to temporal stimuli, whereas, 4mg nicotine had little to no effect compared to baseline.
Figure 2: Mean normalized sCSF data for baseline and nicotine conditions (2mg and 4mg). Sensitivity is plotted over log spatial frequency (cpd). Sensitivities significantly increased (p=0.003) with 2mg nicotine compared to baseline. Four mg nicotine decreased significantly decreased contrast sensitivities (p=0.004). Error bars: ±SEM.
Figure 3: Mean normalized tCSF data for baseline and nicotine (2mg and 4mg) conditions. Normalized sensitivity is plotted against log temporal frequency (Hz). Sensitivities were significantly increased with 2mg nicotine compared to baseline (p=0.05). Little to no changes in sensitivities were seen with 4mg nicotine (p=0.20). Error bars: ±SEM.
CONCLUSIONS

Global Summary

The overall purpose of these studies was to determine the effects of nicotine on human visual function, specifically on retinal function, temporal vision and spatial vision. The specific aims were designed to test the effects of nicotine, administered as gum, on naïve study participants, that is, individuals who were non-smokers. In addition, the specific aims were designed to test the effects of nicotine in a dosage dependent manner, i.e. two dosages (2mg and 4mg) of nicotine gum were administered. Previous research with humans primarily focused on assessing visual function in individuals who were cigarette smokers. These studies have shown that individuals who smoke have decreased response amplitudes measured by electroretinograms (ERGs) and increased sensitivities in spatial and temporal vision measured by spatial and temporal contrast sensitivity function (sCSF and tCSF) (Barlow & Baer, 1967; Gundogan, Durukan, Mumcuoglu, Sobaci, & Bayraktar, 2006; Gundogan, Erdurman, Durukan, Sobaci, & Bayraktar, 2007; Jünemann & Damaske, 1968; Smith & Baker-Short, 1993). However, tobacco/cigarette smoke has numerous chemicals and additives that could be directly or indirectly correlated with previous findings. Several additives found in cigarettes (menthol and propylene glycol) have been shown to affect electrophysiological measures of brain activity (Rabinoff, Caskey, Rissling, & Park, 2007). Our experiments were designed to reveal the effects of nicotine on a naïve visual system, with each specific aim designed to assess different levels of visual functioning.
The purpose of specific aim 1 (SA1) was to evaluate the effects of nicotine on retinal responses measured by ERG recording. Visual processing is initiated in the retina and previous research has shown that nicotinic acetylcholine receptors (nAChRs) are expressed in several different types of neurons in the retina of various species (Dmitrieva, Strang, & Keyser, 2007; Hutchins & Hollyfield, 1985; Keyser, Hughes, Whiting, Lindstrom, & Karten, 1988; Keyser et al., 2000; Liu et al., 2009). Based on these observations, we hypothesized that nicotine, ingested in the form of gum, would affect the light-adapted retinal pathway based on evidence of nAChR expression in cone pathways of rabbit and non-human primate retina (Dmitrieva et al., 2007; Liu et al., 2009). Data from SA1 confirmed the hypothesis that nicotine does have an impact on retinal function. Specifically, both dosages of nicotine decreased b-wave response amplitude in dark-adapted full-field ERGs; however, the timing of the responses remained unchanged. B-wave response amplitudes are thought to arise from bipolar cell activity for both dark- and light-adapted ERGs (Frishman, 2006). The dark-adapted ERG data are consistent with previous findings from Junemann and Damaske (1968) who investigated the effects of cigarette smoking on the dark-adapted ERG. Data from the light-adapted ERGs revealed an increase in the b-wave amplitude only with 4mg nicotine but the timing of the response was unchanged. These data were similar to the findings of Jurklies et al. (1996) and Gundogan et al. (2007) studies, who showed increased light-adapted b-wave amplitudes in response to nicotine exposure (Jurklies, Kaelin-Lang, & Niemeyer, 1996) and cigarette smoking (Gundogan et al., 2007). Our findings indicate that nicotine has an impact on retinal information processing presumably by activating
nAChRs in the human retina, which is consistent with nAChR expression in the retina, primarily in the cone pathway and within the inner retina (Liu et al., 2009).

The purpose of specific aim 2 (SA2) was to extend the findings from SA1 by investigating the impact of nicotine ingestion on temporal vision measured at the retinal level using flicker ERGs. Previous studies, using psychophysical measures, have shown that cigarette smoking decreases sensitivities to flickering stimuli (Barlow & Baer, 1967). I hypothesized that nicotine would have a similar effect on temporal sensitivities measured by retinal responses. Data from SA2 showed that 2mg nicotine ingestion had a greater impact on temporal vision compared to 4mg nicotine ingestion. The F1 magnitudes from the flicker ERG revealed an overall decrease after 2mg nicotine ingestion across all temporal frequencies measured (24Hz-82Hz). In addition, retinal CFF determined by linear regression from the F1 magnitudes, showed 2mg nicotine caused a decrease in CFF. In comparison, CFF under 4mg nicotine did not change. I hypothesized that nicotine would increase CFF. However, the data showed the opposite effect by decreasing the retinal sensitivity to flickering stimuli. These data are consistent with nAChR expression in neurons in the retina of various species (Dmitrieva et al., 2007; Keyser et al., 2000; Liu et al., 2009) and the results suggest that nicotine has an impact on inner retinal cells such as amacrine cells, and their processes. In theory, nicotine could exert effects on GABAergic amacrine cells by increasing the release of inhibitory neurotransmitters, GABA and dopamine, thereby leading to an increase on inhibitory feedback/feedforward onto cells in the ON- and OFF-pathways.

The purpose of specific aim 3 (SA3) was to measure the effects of nicotine on spatial vision. Smith and Baker-Short (1993) in their study evaluating drug effects on
spatial and temporal contrast sensitivity in “social” smokers, showed that nicotine differentially affected spatial contrast sensitivity by increasing sensitivities at low spatial frequencies and decreasing sensitivities at high spatial frequencies (Smith & Baker-Short, 1993). They also showed that nicotine had the opposite effect on temporal contrast sensitivities: decreased sensitivity at low temporal frequencies and increased at high temporal frequencies. I hypothesized that nicotine would have a similar effect in non-smokers. Data from SA3 showed a dosage dependent effect of nicotine on spatial and temporal contrast sensitivity. The data revealed increased sensitivity to spatial contrast with 2mg nicotine, while a decrease in sensitivity was observed with 4mg nicotine. These results both confirmed and disproved my hypothesis. Smith and Baker-Short (1993) showed increased contrast sensitivities to low spatial frequencies and decreased sensitivities at high frequencies with 1.5 mg nicotine. Compared to the data from SA3, 2mg nicotine increased sensitivities at all spatial frequencies tested, with the greatest effect seen in the middle spatial frequencies (2-8 cycles per degree). The results from the temporal contrast sensitivity with 2mg nicotine ingestion were similar: sensitivities were increased across all temporal frequencies tested, while the 4mg nicotine revealed no significant changes. As with spatial contrast sensitivity, the hypothesis was confirmed and disproved. A previous study (Smith and Baker-Short, 1993) showed that temporal contrast sensitivities were decreased at low temporal frequencies and increased at high temporal frequencies with nicotine. The temporal contrast sensitivity data were similar to the results from SA2 with both showing increased sensitivities with 2mg nicotine. These findings are consistent with nAChR expression in visual cortex of primates (Disney, Aoki, & Hawken, 2007). Disney et al. (2007) showed β2-containing nAChR expression
in visual cortex, and also reported that nicotine increased the gain of cells containing $\beta_2$ nAChRs. Disney et al. (2007) concluded that nicotine can enhance vision by increasing the gain in those cells.

**Future Directions**

The results from these studies revealed that nicotine, itself, does impact visual processing and function at both retinal and cortical levels. One limitation of these studies is that we do not have specific measures of nicotine levels in the participants tested. The experimental testing was conducted within 30 minutes of nicotine intake. This was based on Russell et al. (1976) study showing that blood nicotine levels after smoking one cigarette were comparable to that after chewing 4mg nicotine gum; 2mg nicotine gum levels were lower after intake. Their study also showed that nicotine concentrations reached a peak after 30 minutes of nicotine ingestion (Russell, Feyerabend, & Cole, 1976). It would be beneficial in future studies to measure blood nicotine levels to enhance the interpretation of future findings.

These data could have implications extending beyond the realm of vision research. Parkinson’s disease (PD) is a neurodegenerative disease that acts on the dopaminergic system within the central nervous system. Fagerstrom et al. (1994) have shown that nicotine plays a role in alleviating the symptoms in patients with PD (Fagerström, Pomerleau, Giordani, & Stelson, 1994). In addition, Janson and Møller (1993) revealed that nicotine acts as a neuroprotector on dopaminergic neurons in the brain of rats with PD (Janson & Møller, 1993). Vision loss has been noted in patients with PD, specifically, both dark and light-adapted b-wave amplitudes are decreased with
PD (Gottlob, Schneider, Heider, & Skrandies, 1987). These changes are indicative of the impact of PD on dopaminergic neurons in the retina. Our data have shown that nicotine can change the functional properties of retinal neurons and can enhance vision. It is possible future studies could show that nicotine may be used therapeutically, not only for PD, but also in visually-related disorders and diseases such as age-related macular degeneration and glaucoma.
GENERAL LIST OF REFERENCES


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

UAB's Institutional Review Board for Human Use (IRB) has an approved Federally Assessed with the Office for Human Research Protections (OHRP). The Assurance number is FWA 00005960 and it expires on January 24, 2017. The UAB IRB also is compliant with 21 CFR Parts 50 and 46.

Principal Investigator: VARGHESI, STEFANIE BRYANT
Co-Investigator(s):
Protocol Number: F070209601
Protocol Title: Effects of Needle on Processing in the Human Atrial Vagal Nervous: ERG and CSE

The IRB reviewed and approved the above named project on 12/19/2012. 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: 12/19/2012.
Date IRB Approved: 12/21/12.
Identification Number: IRB0000254

Investigations 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.

Ferdinand Uthaler, M.D.
Chairman of the Institutional Review Board for Human Use (IRB)