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Studies of the Effect of Nicotine on Color Vision in Color Deficient Humans

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STUDIES OF THE EFFECT OF NICOTINE ON COLOR VISION IN COLOR DEFICIENT HUMANS

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ABSTRACT

Previous research showed that oral nicotine use in non-smoking color normal individuals significantly improved color discrimination scores on the F-M 100 and both white and red increment thresholds of the Red Test. Our research sought to determine if similar results could be found when oral nicotine was administered to color deficient individuals.

Ten color deficient individuals were first screened with both the HRR and Nagel Anomaloscope. Next, the F-M 100 and Red Test were performed before and after chewing nicotine gum (four mg). Subjects were introduced to testing procedures in one visit; a second visit, at least 24 hours later, involved nicotine administration with baseline values and data through 30 minutes of use.

While color normal individuals significantly improved on both the F-M 100 and the Red Test with most improvement on the red portion, color deficient individuals significantly improved equally on both white and red portions of the Red Test but showed no reliable improvement on the F-M 100. Baseline color discrimination and age were considered in explaining who might benefit most from nicotine gum but neither showed any correlation. The effect of nicotine gum on Red Test values showed the greatest improvements in the first 10 minutes with gradual improvement the last 20 minutes.

It is possible that color normal individuals gained advantage on the F-M 100 after nicotine gum use because their wavelength opponent systems showed greater improve-

ment than their non-wavelength opponent systems; this is speculated to increase color saturation. Color deficient individuals gained no advantage on the F-M 100 because both wavelength opponent and non-wavelength opponent systems increased equally resulting in no change in color saturation. Therefore, nicotine gum use may not be an effective treatment for the color deficient.

Keywords: F-M 100, Red Test, nicotine, color deficiency

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INTRODUCTION

Smoking has long been known for its association with diseases such as vascular disease, cancer, chronic obstructive lung disease and peptic ulcer disease. Due to nicotine's link with cigarette smoking, it too has been association with these conditions. Nicotine is the major factor behind cigarette addiction but its assumed role in disease development is unclear. In fact, there is a general lack of evidence linking nicotine, per se, with these diseases caused by cigarette smoking. Nicotine is only speculated to play a secondary role in the development of these diseases, while the tobacco smoke is likely the primary culprit (Benowitz, 1986). The strongest case in arguing nicotine as a producer of systemic disease is through its associating with vascular disease because of its well-known effects of increasing blood pressure and heart rate (Benowitz et al, 1988).

With all the negativity surrounding nicotine, its therapeutic properties can be overlooked. Benowitz (1996) explained many of these therapeutic properties. The main role of nicotine as a medication is through nicotine replacement therapy in which nicotine chewing gum and patches replace cigarette smoking to help ease addiction. Nicotine also has a possible role in treating ulcerative colitis, Alzheimer's disease, Parkinson's disease, Tourette's syndrome, sleep apnea, and attention deficit disorder (Benowitz, 1996). Nicotine has also been shown to enhance certain visual processes. Warwick and Eysenck (1963) showed that critical flicker frequency (CFF) threshold was increased after nicotine use. Most recently, while smoking a pack of cigarettes a day has been shown to cause

color vision defects (Bimler and Kirkland, 2004; Erb et al, 1999), Naser et al. (2011) showed nicotine, in isolation (four mg gum), given to non-smokers improved color-vision discrimination (F-M 100) and threshold detection (Red Test).

This research is a continuation of the Naser et al. (2011) study that showed nicotine improves color-vision discrimination and threshold detection. Our research used similar methods but instead of using color normal individuals, we administered nicotine to congenital color deficient subjects. Supporting this research is the idea that nicotinic acetylcholine receptors (nAChRs) are found throughout the nervous system including parts that involve the processing of visual stimuli such as the retina (Liu et al, 2009), lateral geniculate nucleus (LGN), superior colliculus (SC) (Gotti et al, 2005), and visual cortex (V1) (Schröder et al, 1989). These nAChRs are activated by nicotine and could be the explanation as to why nicotine in isolate (not through smoking) affects color-vision.

METHODS

Subjects

Ten color deficient individuals, of several types, were recruited for this study: six deuteranomalous, one protanomalous, and three protanopes. These subjects signed consent forms approved by the IRB that outlined the purpose of the study beforehand. Subjects were required to have had a comprehensive eye exam within the last year to rule out any ocular diseases or disorders. The H.R.R. test was used to confirm color deficiencies.

Subjects were limited to those color deficient individuals that did not smoke, preferably those that had never smoked. Subjects were not accepted if they were current nicotine users of any kind, including nicotine gum or smokeless tobacco. In keeping with the Naser et al. exclusion criterion, those not allowed to participate included individuals with a history of high blood pressure, abnormal heart rhythm, heart palpitation/murmurs, history of strokes, arteriosclerosis, diabetes, asthma, dental work that would prevent the subject from chewing gum, stomach ulcers, overactive thyroid, history of seizures, eye disease, temporomandibular joint disorders, or blood vessel problems. Certain medication also prevented participation because of a possible interaction with nicotine gum including the following: asthma medication, insulin, opioids, Inderal, or bronchodilators.

Testing

Two tests were used to assess nicotine use on color vision disorders: the FM-100 and Red Test.

FM-100: This test uses 85 colored caps (four trays) that vary across the color spectrum but differ only slightly in hue from one cap to the next (Farnsworth, 1943). These caps are placed at random in front of the subjects while being illuminated with a Macbeth lamp just like the H.R.R. test. The preferred illumination for this test is 25 foot candles to simulate an average daylight condition; the Macbeth lamp is a standard method to provide this level of light and color balance (Farnsworth, 1957). A fixed cap in each tray is the starting point and the subjects are asked to place the remaining caps in order so that similar hues are next to each other in the trays.

It has been shown that there may be improvements between the first and second test scores of the F-M 100, but this improvement diminishes on further retests. One study showed a 30% improvement on the second test and little improvement on the third (Farnsworth, 1957). Naser et al. (2011) also found no significant improvement after the second F-M 100 test (but see Hardy et al, 1994; Breton et al, 1988). To avoid this effect in the main experiment, subjects were given a trial run before later tests were done. In all, subjects were asked to perform the F-M 100 three times, once to familiarize themselves with the test, once before the administration of nicotine and once afterwards.

Red Test: The Red Test was used to measure changes in color vision detection threshold. The Red Test (York & Loop, 2008) measures increment threshold of both white and red light. It uses a 30.5 cm x 30.5 cm x 45.7 cm wooden box in which a piece of translucent white plastic is used as one side and is lit from within the box to create a

white background of 9750K (x=.289, y=.277) by multiple light-emitting diodes (LEDs) to a luminance of 150 cd/m^2. Inside the box is a white (7750K) LED that uses the addition of either an empty slide or red filter to project a circular stimuli (2.5 cm/ 2.5° at 57 cm) onto the square plastic screen (27 cm/ 27° at 57 cm). In using the empty slide**,** a white stimulus of 6917K (x=.302, y=.318) is created. This spot of light is changed to a red stimulus (x=.715, y=.286) by placing a Wratten No. 26 filter (dominate wavelength: 620.6 nm, excitation purity: 100%) in front of the LED.

Subjects placed their chins into a chin rest 57 cm from the viewing screen and controlled the intensity of the light source through a potentiometer which adjusted the pulse width of a 1000 Hz square wave flicker. The subject was asked to increase the intensity of the light to the point where it was barely visible (method-of-adjustment). Once the value had been recorded, the intensity is turned down below threshold and the subject performed the test again. The values given by the pulse width modulation controller were in percent (%). To convert these values to cd/m^2, a minolta 1° luminance meter was used to take a series of readings at different % modulations. Figure 1 shows the relationship between these values and gives a best fit line for both the red and white portions of the Red Test. The equation given by the best fit line was used for conversions from % modulation to cd/m^2.

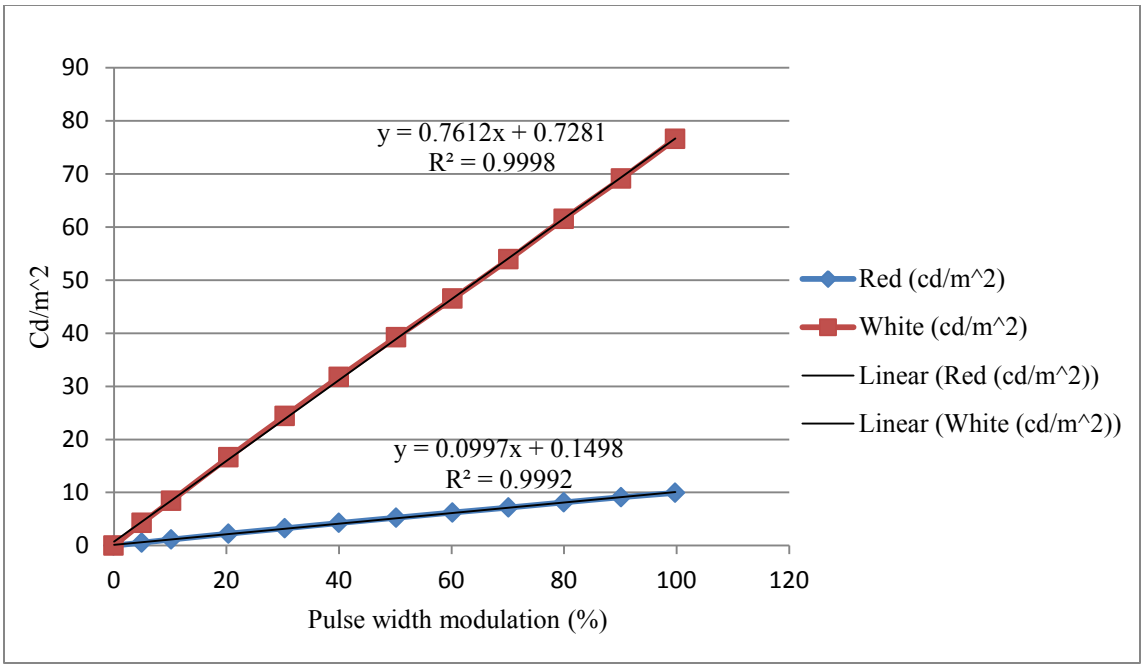

Figure 1. Red Test conversion of pulse width modulation (%) to cd/m^2 with accompanying best fit line. Note the large R^2 values of both the red and white portions of the Red Test.

One complete Red Test included testing both the white and red light five times each. There were a total of five complete Red Tests perform, once following the familiarization with the FM-100, once before nicotine administration and three after nicotine administration. The Red Test has proven to be an effective way to clearly differentiate between color deficient and color normal individuals (York et al, 2008). In our study, it was used to determine if nicotine restores any amount of the loss of sensitivity to long wavelength light found in those with color vision anomalies.

Procedure

The entire experiment required each subject to participate in two visits. The first visit consisted of preliminary items in preparation for the main experiment in the second visit. First, to confirm a color deficiency, a screening was done using an H.R.R. test. Afterwards, we began the informed consent process and checked eligibility through a medical history. During this visit subjects took the F-M 100 test and Red Test to familiarize themselves with the procedure. Five Nagel Anomaloscope settings were also taken to differentiate between dichromats and anomalous trichromats.

The second visit began with baseline measurements of the F-M 100 and the Red Test. After taking these tests, patients were administered nicotine in the form of chewing gum. While chewing the gum, the subjects performed the Red Test at the 10, 20 and 30 minute marks (for a total of five Red Tests including the preliminary test). At 30 minutes the gum was thrown away and the F-M 100 assessment was done again (for a total of three F-M 100 tests including the preliminary testing). A four mg dose of nicotine gum was used because Naser et al. (2011) found four mg to produce a larger effect than two mg. Testing with both the F-M 100 and Red Test was done binocularly in a darkened room without a time limit.

RESULTS

F-M 100 results were quantified as total error scores (TES) which can vary from 0 to a very large number (~1,000). Scoring was assisted by F-M 100 scoring software created by Gretagmacbeth, LLC. All p-values were determined using non-parametric statistical test due to differing population, or types of color deficiencies, between subjects.

The second session pre gum and 30 minutes post gum F-M 100 values were measured and compared by individual (see Figure 2). Of the 10 subjects, 7 improved on their F-M 100 scores while 3 scored worse. Also included in Figure 2 is the average TES of subjects without color deficiencies based on age as determined by Kinnear and Sahraie. (2002).

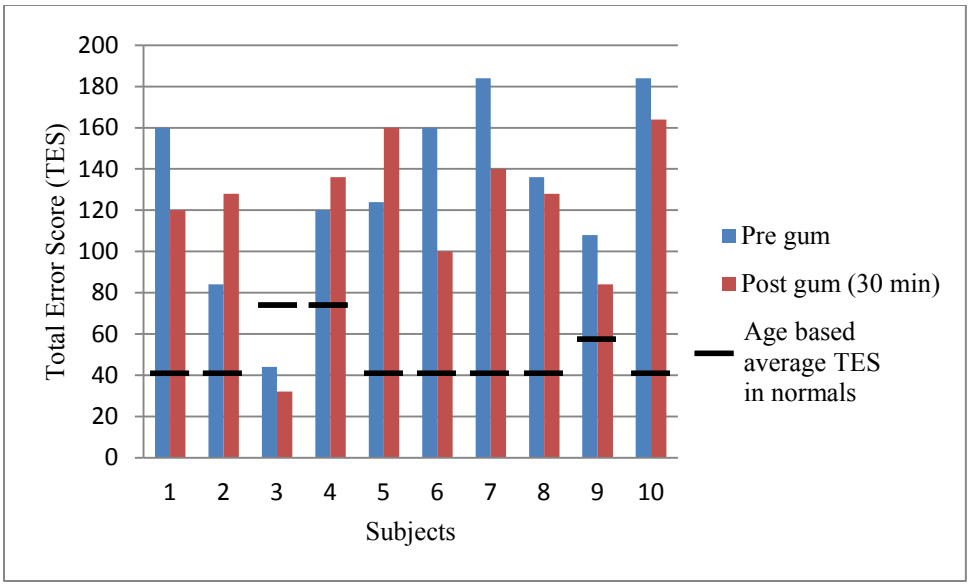

Figure 2. Pre and 30 minute post gum F-M 100 values. Note that a lower TES means better color discrimination.

The pre gum and 30 minute post gum threshold values for the Red Test were averaged and compared. In the white portion of the Red Test 9 out of 10 subjects showed lower (better) thresholds to white light after nicotine use (see Figure 3). All 10 subjects showed better threshold averages in the red portion (see Figure 4).

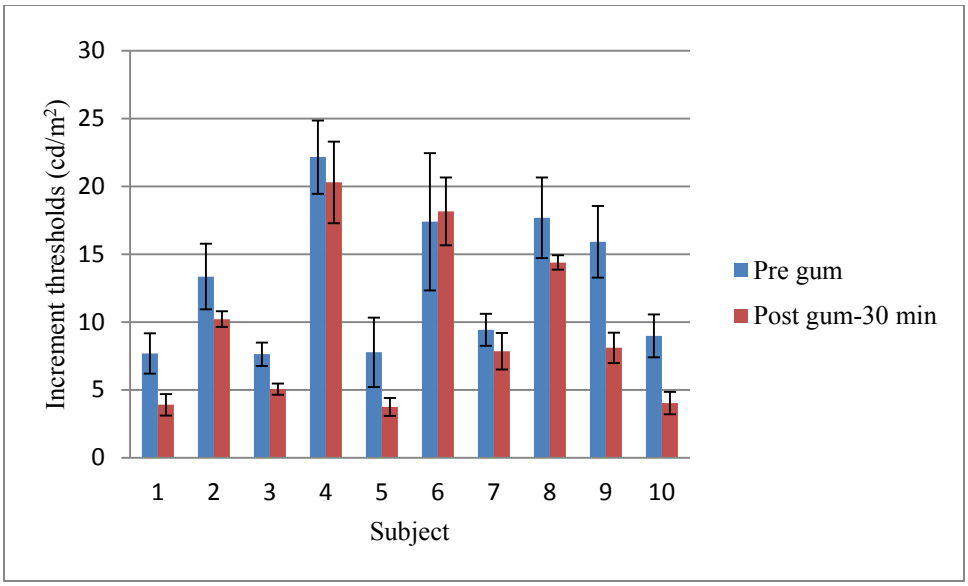

Figure 3. White portion of the Red Test.

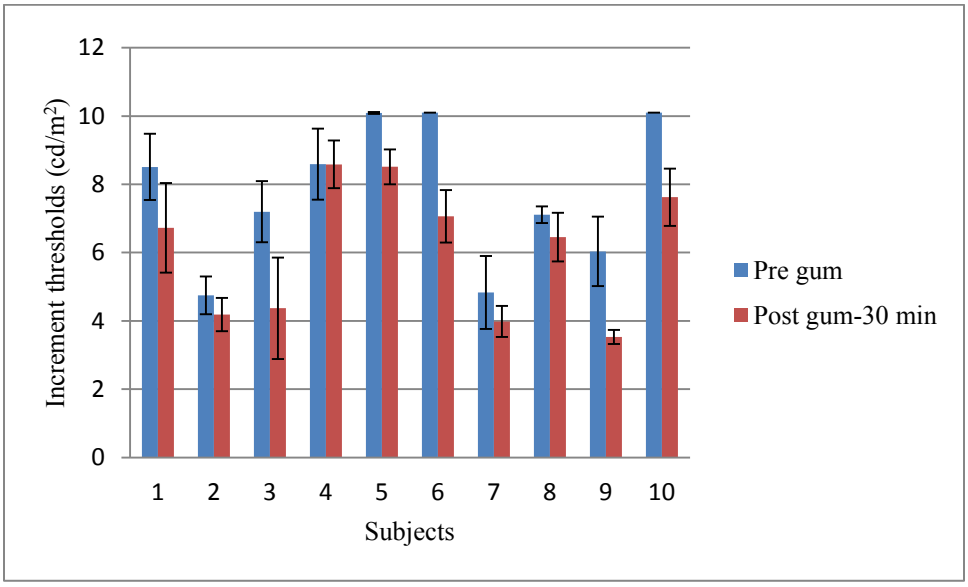

Figure 4. Red portion of the Red Test.

In comparing the improvements between the white and red portions of the Red Test, 7 out of 10 subjects showed greater percent improvements on the white than the red.

There was 9% greater improvement in white thresholds; using a Wilcoxon's signed-rank test (Bradley, 1968), the differences between the two were not significant with $p>.05$. In the white portion, the three protanope subjects had significantly larger percent improvements than the anomalous trichromats ($p<.05$) with a Wilcoxon's rank-sum test (Bradley, 1968). In contrast, significantly larger improvements were not seen on the red portion between the protanopes and anomalous trichromats.

The effect of nicotine, 0 min vs. 30 min, upon both F-M 100 and Red Test scores was evaluated with Wilcoxon's signed-rank test (Bradley, 1968). The experiment found that color deficient individuals reliably improved their sensitivity to white light ($p<.005$) and red light ($p<.005$) after 30 minutes of chewing nicotine gum. On average there was an improvement on the F-M 100 but this improvement was not significant ($p>.05$).

The average thresholds of the Red Test were plotted against the time the nicotine gum was chewed to visualize the trends of decreasing thresholds. These trends can be seen in Figure 5 for the white portion and Figure 6 for the red portion. These graphs show that with both the white and red light, the large majority of improvement in threshold occurs within the first 10 minutes with a continued gradual improvement over the next 20 minutes. To check the reliability of this observed trend, a Friedman's multi-sample test (Bradley, 1968) was performed with data at different times (e.g. 0, 10, 20, 30 min). Both the white and red portions of the Red Test showed reliability with $p<.01$.

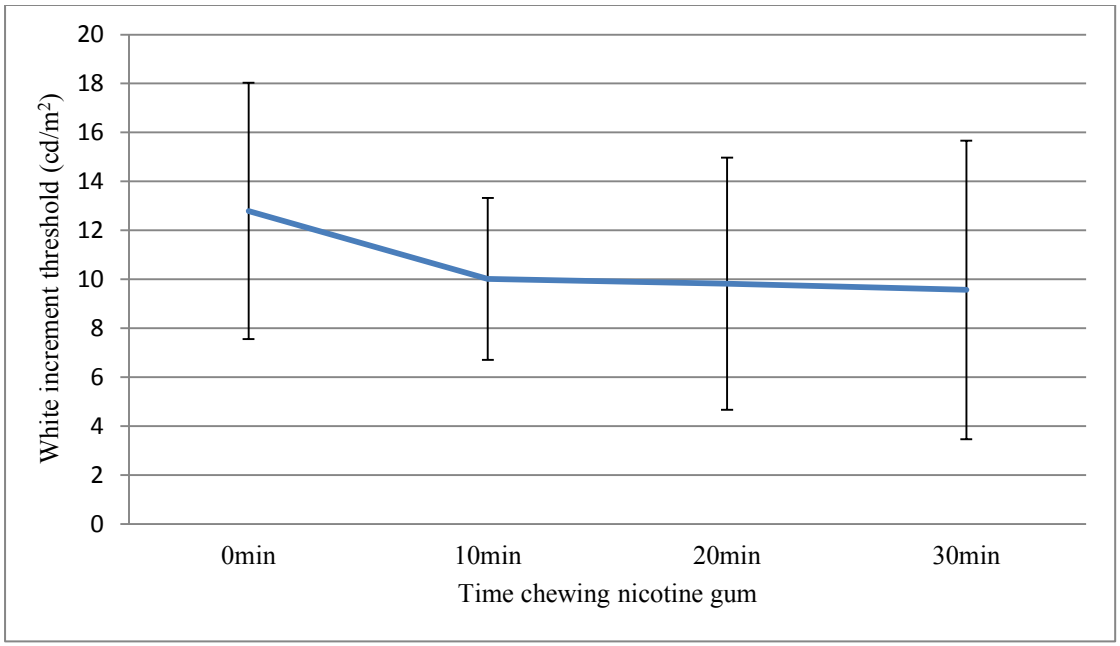

Figure 5. White light thresholds as a function of time as given by the Red Test.

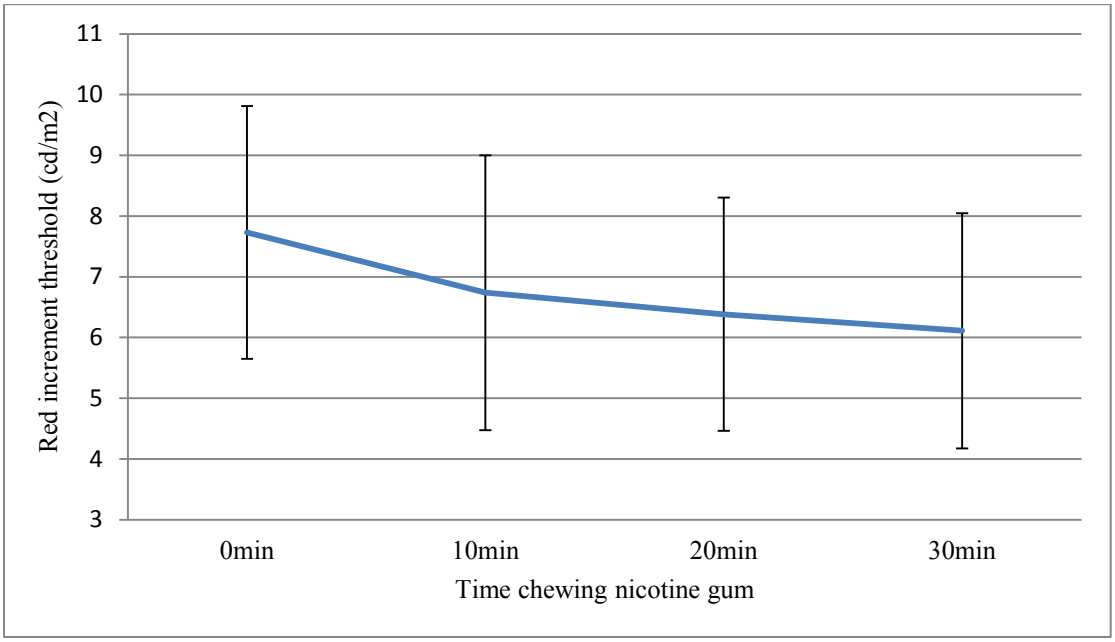

Figure 6. Red light thresholds as a function of time as given by the Red Test.

Correlation between the magnitude of color deficiency, as determined by F-M 100 score, and the extent of the effect from the nicotine gum was investigated. Pre gum TES score was plotted against the pre gum/30 minute post gum fraction for white and red thresholds (see Figures 7 and 8 respectively) and TES values (see Figure 9). These figures have a low R^2 value and $p>.05$, as determined by a Hotelling and Pabst's Test (Bradley, 1968), showing no correlation. With the data collected from this research, including both Red Test values and the TES values, it cannot be concluded that the magnitude of color deficiency has any relationship to the extent of the effect from nicotine.

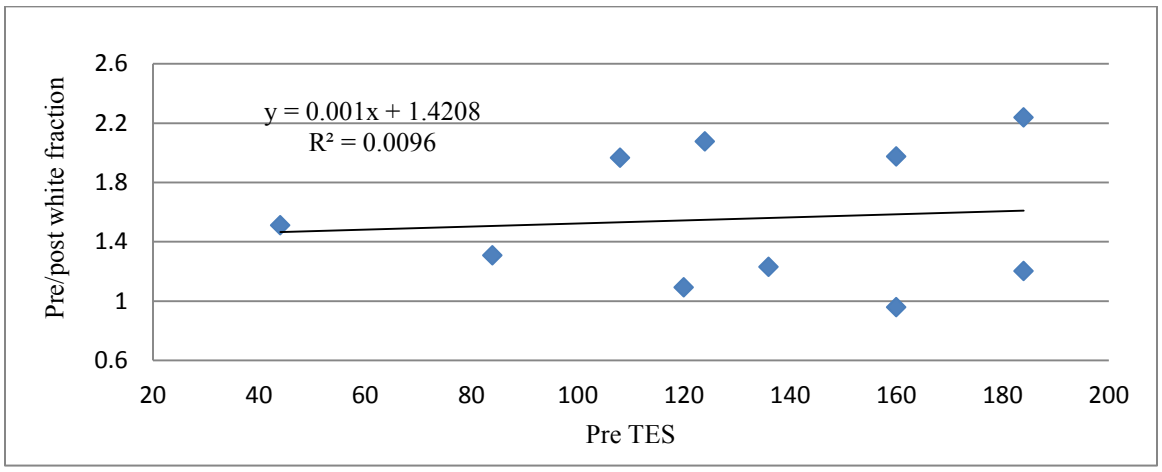

Figure 7. Change in white luminosity thresholds compared to pre gum TES. Changes in thresholds were found by using the fraction given from pre gum thresholds divided by post gum thresholds. Pre gum TES refers to F-M 100 scores pre gum in session 2.

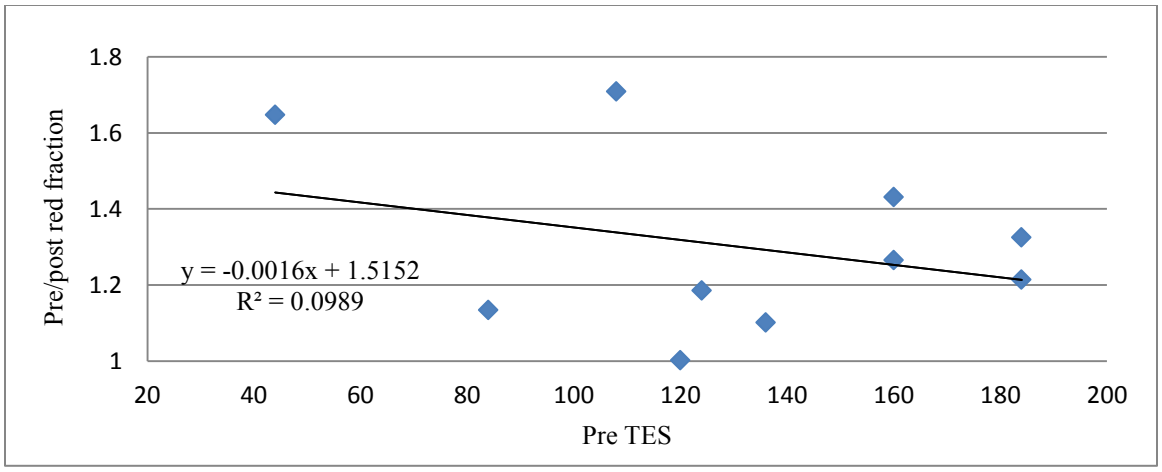

Figure 8. Change in red luminosity thresholds compared to pre gum TES. Changes in thresholds were found by using the fraction given from pre gum thresholds divided by post gum thresholds. Pre gum TES refers to F-M 100 scores pre gum in session 2.

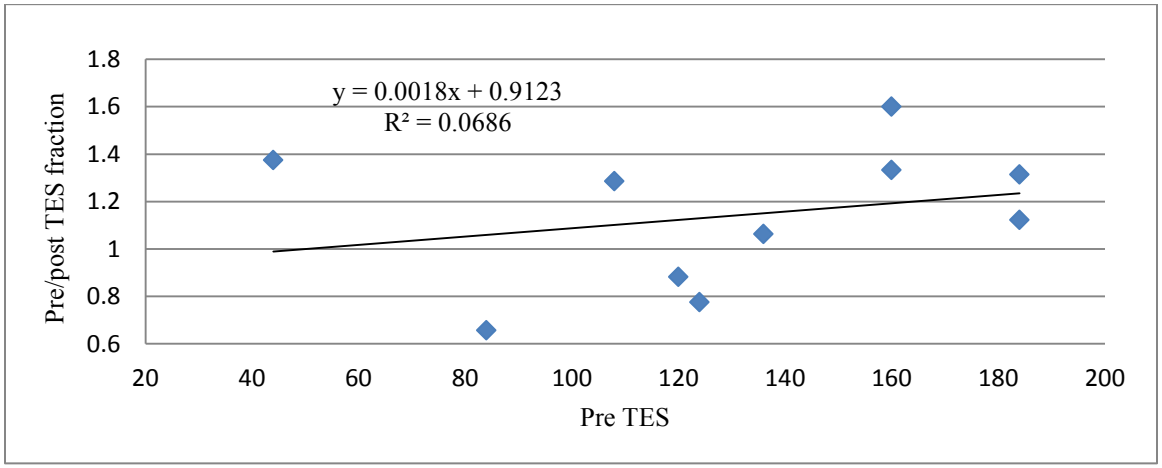

Figure 9. Change in TES compared to pre gum TES. Change in TES were found by using the fraction given from pre gum TES divided by post gum TES. Pre gum TES refers to F-M 100 scores pre gum in session 2.

Age of subject was also considered in explaining the extent of the nicotine effect. Age was plotted against the pre gum/30 minute post gum fraction for white and red threshold (see Figures 10 and 11 respectively) and TES values (see Figure 12). These

figures have a low R^2 value and $p>.05$, as determined by a Hotelling and Pabst's Test (Bradley, 1968), showing age and the varying effects of nicotine are uncorrelated.

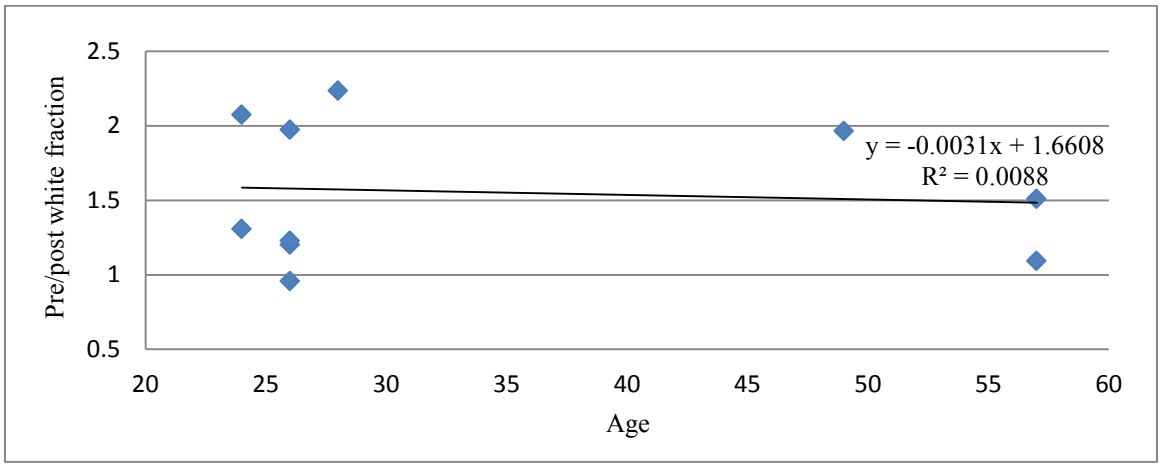

Figure 10. Change in white luminosity thresholds as a function of age.

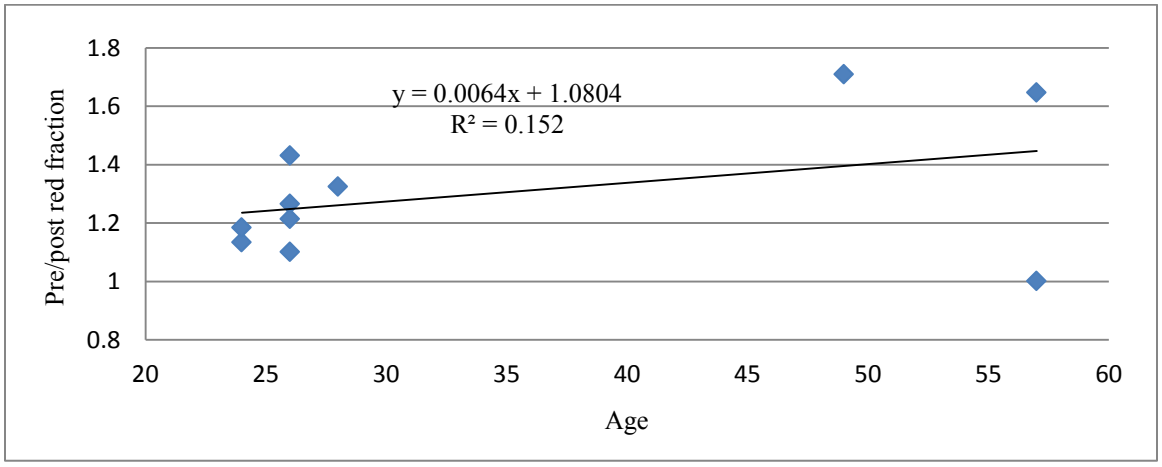

Figure 11. Change in red luminosity thresholds as a function of age.

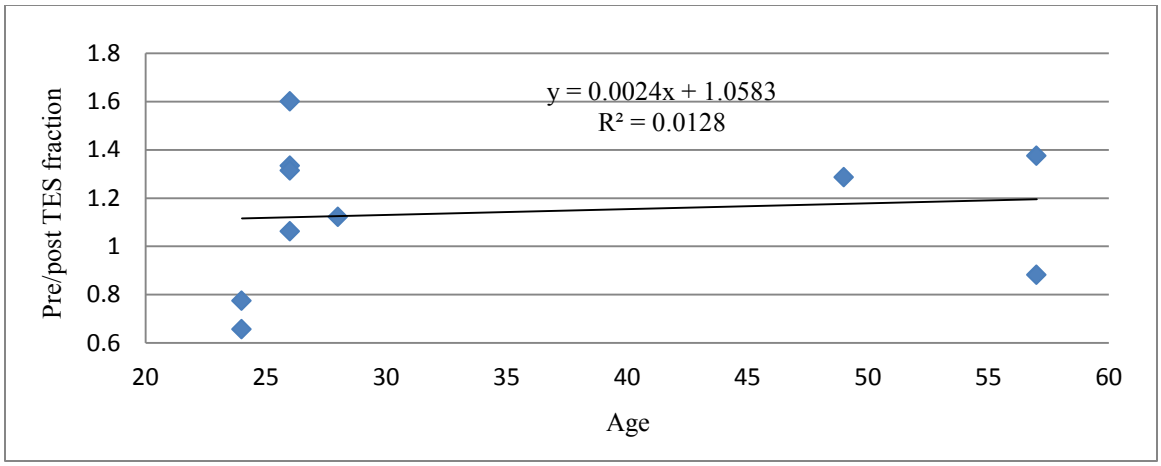

Figure 12. Change in TES as a function of age.

Farnsworth (1957) and Naser et al. (2011) showed that there is a practice effect with the F-M 100. This research showed a 12% improvement between the session 1 and session 2 pre-gum F-M 100 scores (see Figure 13). A Wilcoxon's signed-rank test (Bradley, 1968) showed this small effect was not statistically reliable with $p>.05$.

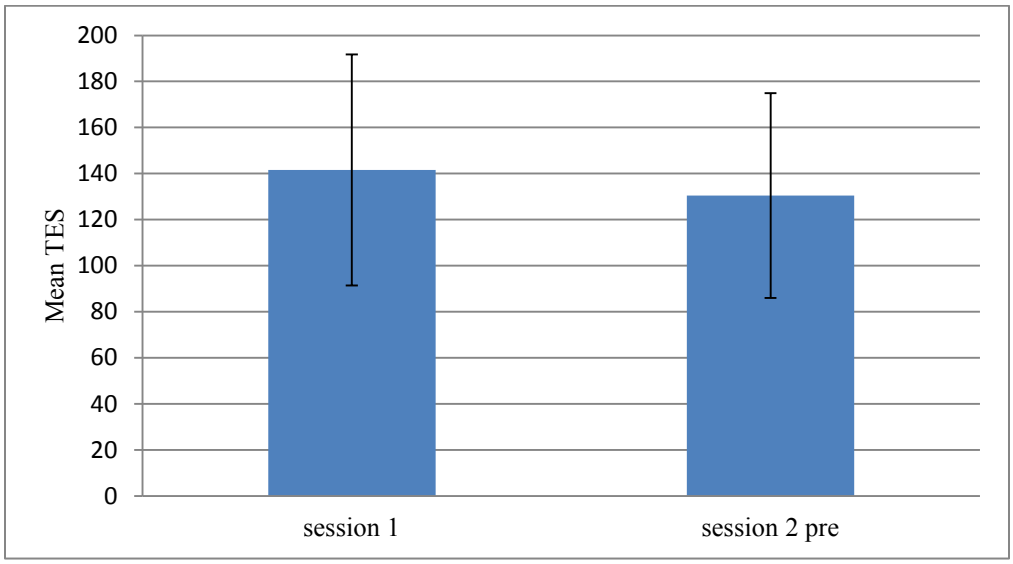

Figure 13. F-M 100 practice effect.

A practice effect for the Red Test showed mixed results. The white thresholds worsened 0.5% while the red thresholds improved by 2% between the first and second sessions (see Figures 14 and 15 respectively). A Wilcoxon's signed-rank test showed both effects were not statistically reliable with $p>.05$.

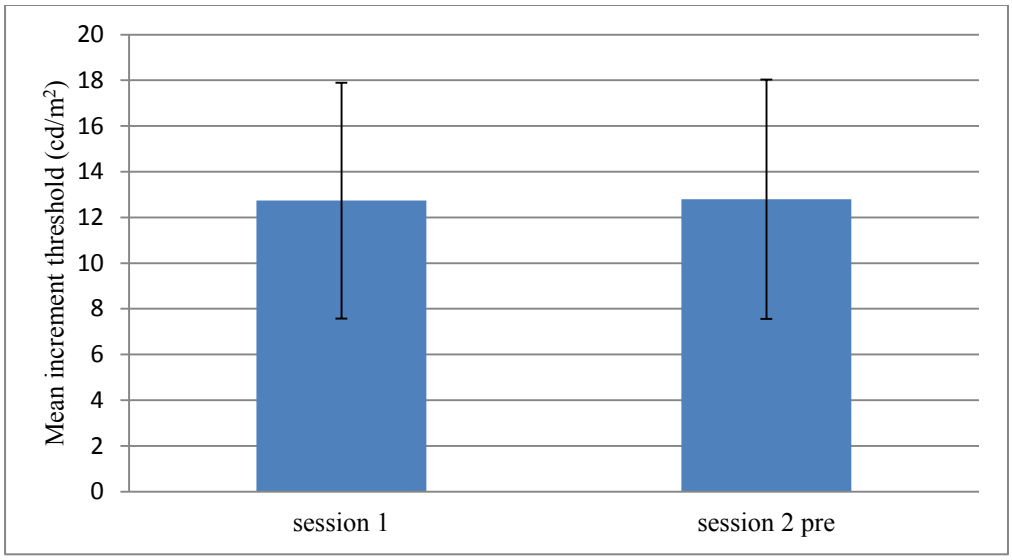

Figure 14. Red Test (white portion) practice effect.

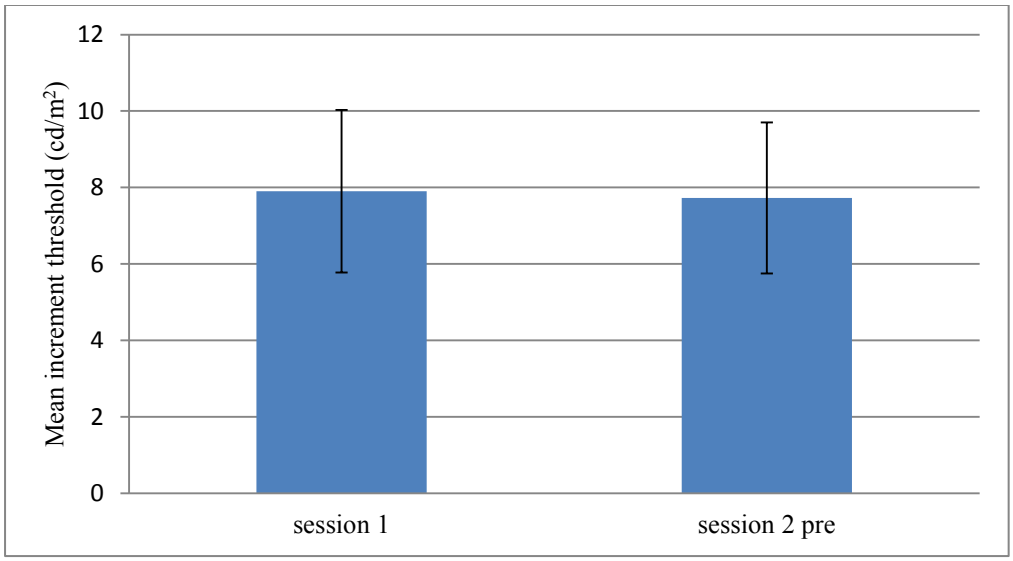

Figure 15. Red Test (red portion) practice effect.

DISCUSSION

Naser et al (2011) found that in color-normal humans, four mg of nicotine gum significantly improved TES on the F-M 100 and improved both white and red light thresholds on the Red Test. We performed a similar experiment to test if the same results would be seen in those that need more help. In total, 10 color deficient subjects were recruited ranging in type including: 6 deuteranomalous, 1 protanomalous, and 3 protanopes.

F-M 100

Our outcome differs from that seen in Naser et al (2011). Statistical analysis showed that our outcome on the F-M 100 was not significant, in comparison with Naser et al (2011), where it showed color-normal individuals significantly improved their TES after 30 minutes of chewing nicotine gum. Our results were mixed with 7 out of 10 subjects improving their TES. Further investigation showed little explanation as to the varying effects of nicotine on the F-M 100 scores. We thought it was possible that the effect of the gum would vary depending on the age of the subject, but this was not the case (see Figure 12). We also investigated if the effect varied depending directly with the subject's baseline color discrimination as scored on the F-M 100. This also proved not to be the case (see Figure 9).

When comparing the subjects TES to the age based average TES, as determined by Kinnear et al. (2002), it is counterintuitive that subject three had a TES below the av-

erage score for color-normal individuals at the same age. Interestingly enough, as noted by Farnsworth (1957), some color defectives show better color discrimination than normals. The F-M 100 scores by subject three underscores that the F-M 100 test is a test of color discrimination and is not designed to test for color defectiveness, although it is used to indicate a type of color deficiency, which, in general, goes along with an abnormally high TES.

Red Test – White Light

In agreement with research done by Naser et al. (2011), our study also showed that four mg of nicotine significantly improved the threshold for white light. It is interesting to note that the three protanopes had the greatest percentage improvements in white light thresholds ($p<.05$). It is also interesting that these protanopes had 3 of the 4 best white thresholds for the pre-gum reading and the 3 best 30 minute post-gum white readings. It is difficult to conclude if this effect was due to their dichromatic state or if it's due specifically to their loss of red receptors (i.e. protanopia) because no data was collected from deuteranopes. Further research that includes subjects from each of the four subtypes of red-green color deficiency is needed to be able to make any conclusions about this effect.

Even with 1 of the 10 subjects showing a minor increase in white light thresholds, the average percentage decrease in thresholds were 9% greater for the white light than the red but did not show reliability ($p>.05$). This differs from results found by Naser et al. (2011) with color-normal individuals, who noted threshold detection improved significantly more with the red stimulus than the white.

Red Test – Red Light

All subjects decreased their red light thresholds after four mg nicotine gum use. These decreases were significant, much like those found in Naser et al. (2011) for color-normals. It is known that colored light is detected at lower thresholds than white light by normals (King-Smith, 1975). Data from each of our subjects showed better red sensitivity than white except for the protanope subjects, which had a greater sensitivity to white light than red. This may also be the case for protanomalous individuals but we were unable to collect sufficient data from this pool of color deficient subjects to make any conclusions.

Explanations

Nicotinic acetylcholine receptors are found throughout the nervous system including multiple areas that process visual stimuli. A possible general explanation as to the effect of nicotine on color perception is its effect at any one or several of these locations.

In both color normal and color defectives, nicotine affects both the color (wavelength opponent) and luminance (non-wavelength opponent) systems. The Red Test intentionally uses a light wavelength that accentuates sensitivity differences between these two systems in both color-defectives and color normal individuals. Naser et al. (2011) chose the Red Test for this reason in an attempt to monitor nicotine's effect on one system in relative isolation from the other. In keeping with the procedure performed by Naser et al. (2011) we also used the Red Test, but it is important to note that those large differences between the wavelength opponent and non-wavelength opponent systems can be greatly reduced in those with a red-green color deficiency (Schwartz,1994).

Naser at al. (2011) found that threshold detection improved more for the red stimulus than the white. They speculated that the wavelength opponent process was more sensitive to the nicotine than the non-wavelength opponent process due to its greater effect on the red light. In our experiment, we found that there was no reliable difference between the effects of nicotine on the white and red increments of the Red Test; 30.2% improvement for white and 21.6% for red. One difference between our experiment and that done by Naser et al. (2011) is that the chromaticity coordinates of the white background was somewhat different; x=0.289, y=0.277 vs. x=0.301, y=0.338 respectively.

It is interesting to find that color-deficient individuals gained greater sensitivity to both white and red light but failed to show reliable improvement on the F-M 100 test. De Valois et al. (1977) proposed that the saturation of a color is determined by response magnitude differences between the wavelength opponent and non-wavelength opponent systems. We speculate that since both systems were improved by equivalent amounts in color deficient individuals, the saturation of color remained constant and the color-deficient gained no advantage when performing the F-M 100 test. In comparison with Naser et al. (2011), threshold for red improved reliably more than threshold for white in color normal individuals, possibly creating a greater saturation of color and an advantage on the F-M 100 test. Therefore, in color defectives, chewing nicotine gum is not a reliable treatment in improvement color discrimination in the color deficient individuals, despite the fact it improves sensitivity to both red and white increments.

We provide two possible explanations as to how the wavelength opponent and non-wavelength opponent system became more sensitive by equal amounts. First, it is possible that while both systems increased in sensitive, the color processing pathway re-

mained more sensitive than the luminance pathway in a proportion similar to pre- nicotine gum values. Second, it's possible that the color-deficient individual's luminance system has greater sensitivity than the color processing system (this is reversed compared to the color-normal individual) and both systems equally increase in sensitivity with the luminance system remaining most sensitive. This possibility is compatible with the fact that color-deficient people, compared to color normal, are insensitive to both middle and long wavelength increments (Schwartz, 1994). At the intensities these color-deficient people do detect, unlike color normal, they may not see the middle and long wavelength increments as colored (Loop et al, 2003).

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APPENDIX

IRB APPROVAL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

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

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

Principal Investigator:	LOOP, MICHAEL S
Co-Investigator(s):	GWYNN, WARREN
Protocol Number:	**F110916019**
Protocol Title:	*Studies of the Effect of Nicotine on Color Vision in Color Deficient Humans*

The IRB reviewed and approved the above named project on 10/23/2013. 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: 10/23/2013
Date IRB Approval Issued: 10/23/13
IRB Approval No Longer Valid On: 10/23/14
Identification Number: IRB00000196

Ferdinand Urthaler, MD

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

Investigators please note:

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

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

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

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

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