Proceedings of The National Conference
On Undergraduate Research (NCUR) 2018
University of Central Oklahoma
Edmond, Oklahoma
April 5-7, 2018

Factors involved in Reward Dependence and Marijuana Use in College Students

Ogechukwu Otiji and Erica Giron
School of Science and Technology
Georgia Gwinnett College
1000 University Center Lane
Lawrenceville, Georgia 30043 USA

Faculty Advisors: Dr. Jennell Talley, Dr. Matthew T. Schmolesky and Dr. Jill Penn

Abstract

Personality traits defined by Cloninger include persistence, novelty seeking, harm avoidance, and reward dependence. Specifically, reward dependence (RD) is described as a dependence and attachment to the approval of peers and society, while harm avoidance (HA) is defined as the tendency to respond intensely to signals of aversive stimuli and to learn passively to avoid punishment, novelty, and frustrating non-reward. *Cannabis,* popularly known as marijuana, is the most widely used semi-illicit substance in the United States. Used for medical and recreational purposes, long term consumption of marijuana can lead to impaired motor skills as well as a decrease in short-term memory. The purpose of this study was to investigate the correlation between RD, HA and marijuana use in college students. Two hundred and one Georgia Gwinnett College students participated in this study by completing the Temperament and Character Inventory. Additionally, we collected DNA via buccal swabs for genotyping. The data suggest no significant difference in RD scores between males and females ($n=200$, $p>0.05$). There was no significant correlation between RD and the frequency of students' marijuana use ($r=0.07$, $p>0.05$) or between participants' perceived risk of marijuana use and their RD score ($r=0.08$, $p>0.05$). Analysis of the frequency of marijuana use and perceived risk resulted in a negative correlation ($r=-0.39$, $p<0.01$), as expected. No significant correlation was noted between participants' age and either their RD score ($r=0.033$, $p>0.05$) or their frequency of marijuana use ($r=0.07$, $p>0.05$). Interestingly, a one-way ANOVA suggests that there is no significant difference between the RD score for individuals based solely on their religiosity ($p=0.11$). Because the serotonin pathway has been implicated in reward dependency, the relationship between reward dependence, harm avoidance, marijuana use, and the STin2 polymorphism of the serotonin transporter gene (*SLC6A4*) were investigated.

Keywords: STIN2 polymorphism, Marijuana, Harm avoidance.

1. Introduction

Personality traits are influenced by genetic factors. These traits predispose individuals to some personality disorders and mental illness[1]. In a 1993 paper Cloninger and his coauthors identified three personality traits using the Tridimensional Personality Questionnaire (TPQ)[4]. Later the process of identifying personality traits was modified by Cloninger, Svrakic and Przybeck, resulting in a new inventory called the Temperament and Character Inventory (TCI)[4]. It measures four temperament traits (novelty seeking, harm avoidance, reward dependency and persistence) as well as three-character traits (self-directedness, cooperativeness and self-transcendence)[2]. The TCI is also related to Zuckerman's alternative five–factor personality theory which aimed at explaining human personality traits using five factors (impulsive sensation seeking, neuroticism-anxiety, aggression-hostility, sociability and activity). In a 2015 study, Zuckerman and Cloninger worked together to determine the relationship between both personality models[44]. In

this study they found a high correlation between Zuckerman's impulsive sensation seeking scale and Cloninger's novelty seeking scale[44].

Among the measured temperaments are reward dependence (RD), and harm avoidance (HA). RD is described as a dependence and an attachment to the approval of peers and society[3], and also as a tendency of dependence, sensibility, being friendly and lively[4]. RD has historically been associated with certain neurotransmitters of the brain such as norepinephrine and serotonin[4,5]. Both personality traits have been linked to mental and personality disorders including depression and anxiety[1]. The average RD score recorded by Cloninger was 15.5± 4.4[4]. Individuals with higher than average RD are generally more social, extroverted, and tender-hearted[2], but are also more prone to addictive behaviors that help with achieving or continuing a pleasant mental state. Interestingly, adolescents with disorders such as nonsuicidal self-injury disorder were reported to have a much higher reward dependency score[6]. In support of this observation, one study investigating fifty patients with binge-purge type eating disorders scored higher for reward dependency[7]. Alternatively, individuals who score lower for RD are described as more practical, socially insensitive and cold[4].

HA can be defined as the tendency to respond intensely to signals of aversive stimuli, to learn passively to avoid punishment and, novelty, and to fear uncertainty[4]. Within the HA scale, there are 3 sub-scales: HA1 (anticipatory worry vs. uninhabited optimism), HA2(fear of uncertainty) and HA3(shyness with strangers)[9]. HA has a heritability estimate of 42% to 57% and it is known to be highly stable throughout an individual's life[1]. The average HA score reported by Cloninger was 12.6±6.8[4]. Individuals with elevated HA scores are at a greater risk for depression, eating disorders, and obsessive-compulsive disorder (OCD)[1,5]. In healthy individuals, elevated HA scores are correlated with an increased risk to develop a mood or anxiety disorder[8]. Although there are various negative attributes associated with high HA scores, advantages include cautiousness and careful planning in hazardous situations[9].

One focus of behavioral research is how personality traits affect an individual's predisposition for engaging in unhealthy habits such as addiction. *Cannabis*, popularly known as marijuana, is the most widely used semi-illicit substance in the United States[10]. Marijuana is used for medical and recreational purposes even though long-term consumption can lead to impaired motor skills, depression and a decrease in short-term memory[11]. Despite its risks, marijuana is legal for recreational purposes in nine states in the U.S and legal for medicinal purposes in twenty, and a significant number of Americans believe it should be legal in all states due to potential of economic benefits[30]. A 2012 study found that 18.7% of young adults (18-25) reported using marijuana, while only 7.2% of youth (12-17), and 5.3% of adults reported marijuana use[10]. Our research team has shown a correlation between how individuals view marijuana risk and their frequency of marijuana use, suggesting that people who do not regularly use marijuana view it as a risky substance[25].

Understanding if one's temperament affects their likelihood of engaging in specific risk behaviors is one aspect of behavior research. Identifying correlations between people's genotype and risk-taking behaviors also provides a biological connection to behavioral research. Interestingly, the HA trait is linked to serotonin[29]. Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter produced in discrete brain regions and released throughout the brain. It has been linked to mood stabilization, regulation of carbohydrate cravings, appropriate digestion, sleep cycle, behavior, muscle contraction, and pain control[12]. A low concentration of serotonin in the body is associated with a decrease in immune system function, elevated anxiety, sleep troubles as well as depression whereas high concentrations of serotonin may lead to serotonin syndrome which causes agitation, and confusion among other symptoms[15]. Nerve cells are responsible for transporting serotonin which is stored in the synaptic vesicles of the pre-synaptic neurons. Serotonin is released into the synaptic cleft when an action potential reaches the axon terminal and the membrane is depolarized. The serotonin transporter protein (5-HTT), regulates the amount of serotonin in the synaptic cleft by facilitating its re-uptake from the synapse[13]. Re-uptake terminates the synaptic activity of serotonin[14]. Serotonin is produced in serotonergic neurons, many of which are in the raphe nuclei in the brainstem; their axons radiates throughout the brain allowing serotonin to be released in diverse brain regions[41]. Serotonin also impacts the hypothalamus where it regulates cognition, attention and sleep[31]. It is essential to the development and function of the hypothalamic-pituitary-adrenal (HPA)[31], which is an important stress response system in the brain. An imbalance of the brain serotonin system contributes to many developmental disorders as well as neuropsychiatric disorders including aggression, depression, schizophrenia, substance abuse and drug addiction[31,32].

The *SLC6A4* gene encodes the serotonin transporter and is known to possesses several polymorphisms*;* it is found on chromosome 17 and its protein product is responsible for clearing serotonin from the synapse by transporting it back to the presynaptic neuron[16]. The 5-HTTLPR (5-HydroTryptamine Transporter Gene-Linked Polymorphic Region) is a 44 base pair insertion/deletion in the promoter region of the *SLC6A4* gene[13]. The short (S) variant (14 repeats) results in lower serotonin uptake than the 16-repeat long (L) variant because the deletion lowers the level of gene expression[33]. The S/S genotype is associated with a higher rate of depression than the L/L genotype[17]. Additionally, the S/S genotype is correlated with increased marijuana use[11].

STin2 is another polymorphism that occurs in the second intron of *SLC6A4* and is also composed of a variable number of tandem repeats (VNTR)[18]. There are three major alleles, STin2.9, STin2.10 and STin2.12, corresponding to 9, 10 or 12 repeat units of the 17-bp repeat sequence[17]. STin2.10 and STin2.12 are high frequency alleles, while STin2.9 is a low frequency allele[35]. STin2.12 is linked to depression as well as a poor response to antidepressants[33], obsessive compulsive disorder (OCD) and schizophrenia[17,19,34]. *In vitro* studies revealed that the STin2 VNTR can act as a transcriptional regulator of the *SLC6A4* gene[19]; studies done on the hindbrain of transgenic mice revealed a greater transcriptional activity in STin2.12[16]. No previous study has shown a direct connection between this polymorphism and marijuana use; among other aims the current research study focused on the correlation between these variables.

In our study, we assessed the relationships between temperament, genotype, and frequency of engaging in marijuana use with the demographics of age, sex, and race. Multiple studies support the claim that females score higher than males for reward dependence on the Temperament and Character Inventory (TCI)[20,36]. Additionally, women tend to score higher in HA as well, suggesting that sex influences temperament[21]. Carriers of the STin2.10 allele score lower for reward dependence (TCI), this supports the notion that while sex has an influence, alleles of genes involved in neurotransmitter pathways also impact temperment[22]. Men with the STin2.12 polymorphism were more likely to exhibit signs of obsessive-compulsive disorder, one of the known disorders associated with a higher reward dependence scores[18]. In a study by Bjørnebekk and colleagues a negative correlation between the age of participants and reward dependency was found[20], whereas no correlation was found between HA and age[37]. According to one of the studies in 2015, marijuana use in African American young adults was higher than their Caucasian counterparts[23]. According to a recent study, cumulative lifestyle use of marijuana was associated with the male gender[38], and young adults between the ages of 11-18 are at a higher risk of using marijuana[39].

The purpose of this research study was to investigate the correlations between RD and HA personality traits and marijuana use as well as the STin2 polymorphism and other demographic data. Based on previous research, we hypothesized that:

1) No correlation would be found between personality traits and age.
2) Females would have a higher RD and HA score than males.
3) There would be a correlation between reward dependence and marijuana use.
4) There would be a correlation between harm avoidance and marijuana use.
5) There would be a difference in marijuana use between races.
6) Individuals with the STin2.12/12 genotype would use marijuana more frequently than individuals with other genotypes.

2. Methods

2.1.Participants

Two hundred and one participants were volunteers from Georgia Gwinnett College's Biology and Psychology department. The participants were 138 females, 62 male and 1 participant who identified as "other," they were incentivized using extra credit and ranged in age from 18 to 65 years old (average 22.65, SD 6.85).

2.2 Procedures

Participants signed up for DNA collection sessions through the SONA system. Each session was started with the reading of a script which contained the instructions for data collection, as well as instructions on how to complete the questionnaire associated with the study. Participants were informed of their anonymity and were asked to sign a consent form before providing DNA samples. First participants rinsed their mouth thoroughly with water. Using a Catch-all Buccal Swab (Epicenter), a swab of the inner cheek was done approximately 20 times on each side. The cotton swabs containing the DNA samples were then air dried for approximately 15 minutes, and then stored at -20^{o}C. A DNA extraction protocol was performed to extract DNA from the cotton swabs and further analysis was carried out on the samples. After the DNA collection sessions, participants were instructed to take questionnaires using the online SONA system at a time of their choosing within 48 hours. All procedures and materials were approved by the GGC IRB committee.

2.3 Measurements

2.3.1. the temperament and character inventory (TCI)

Version 9 of Cloninger's TCI was administered to evaluate temperament and character traits. The TCI is comprised of 240 "True" or "False" questions developed by Cloninger. They measure seven specific traits: 1, harm avoidance (HA); 2, reward dependence (RD); 3, novelty seeking (NS); 4, persistence (P); 5, self-directedness (SD); 6, cooperativeness (CO), and 7, self-transcendence (ST). For this specific study, the numerical values for HA and RD were evaluated. The answers to the questions converted to numerical values that provided a scale of measurement, higher values were assigned higher on the personality scale and lower values were lower on the personality scale. This survey was completed using the SONA computer system.

2.3.2. physical risk frequency inventory (PRFI)

The PRFI is a 28-question survey aimed at determining the frequency at which participants partake in different physically risky behaviors. Behaviors assessed on this survey include reckless driving, extreme sports, casual sex, heavy alcohol drinking and marijuana use among other things. For this study frequency of marijuana was the only aspect of interest from this survey. The survey prompted participants to indicate "0" if they have never participated in the behavior, indicated "1" if they had tried marijuana 1-3 times in their life total, or indicate 2 through 7 to indicate how many times on average per year they participated in the behavior if they do (see Table 1).

Table 1. The table below represents the meaning of the numerical values used in the assessment of the physical risk frequency and the physical risk assessment of Marijuana use.

Value	**Values for Physical Risk Frequency**	**Values for Physical Risk Assessment**
0	Never used Marijuana	Activity poses no risk
1	Tried Marijuana 1-3 times total	Activity poses little risk
2	Tried Marijuana 1-10 days per year	Activity poses moderate risk
3	Tried Marijuana 11-20 days per year	Activity poses moderate risk
4	Tried Marijuana 21-30 days per year	Activity poses moderate risk
5	Tried Marijuana 30-40 days per year	Activity poses extreme risk
6	Tried Marijuana 41-50 days per year	Activity poses extreme risk
7	Tried Marijuana more than 51 days	N/A

2.3.3. *physical risk assessment inventory (PRAI)*

The objective of this 28-question questionnaire was to measure how risky participants perceive the aforementioned risky behaviors to be. The questionnaire was measured from a range of 0-6 with 0 being no risk associated with behavior, 1- little risk, 2-4 being moderate and 6 being extreme risk, participants were prompted to assign a numerical value to each risky behavior.

2.3.4. *sociodemographic*

This survey obtained data on a participant's age, gender, nationality, race/ethnicity, marital status, student status (grade level), and religious activity.

2.4. Genotyping

DNA obtained through the buccal swab method was extracted using a rapid DNA extraction protocol (Epicenter). The swabs were dipped and rolled in QuickExtract DNA extraction solution at least five times. The solution was then vortexed for 10 seconds and incubated at 65^0C for a minute, vortexed again for 15 seconds, and was incubated at 98^{o}C for two minutes. The mix was vortexed a final time for 15 seconds and then stored at -20^{o}C.

The extract was then further analyzed by a polymerase chain reaction (PCR), each contained 1X Go Taq Buffer, 2µM of deoxynucleotide (dNTP), 1µl of 20 mM STin2 forward and reverse primers, 1µl of GoTAQ enzyme, 31µl of Ultra-pure water and 4µl of DNA. The sequence of the forward primer used in this study was 5'-FAM-TGGATTTCCTTCTCTCAGTGATTGG-3', the forward primer had a fluorescence amitide (FAM) probe, the sequence of the reverse primer used is 5'-TCATGTTCCTAGTCTTACGCCAGTG-3'. The samples were denatured in a thermocycler at 94^0C for 5 minutes and then underwent 40 cycles of: 1 min at 94^0C, 1 min at 60^{o}C, and 1 min at 72^{o}C and a final 20-minute elongation at 72^{o}C. A fragment length analysis was performed on the products of the PCR reaction to determine the length of the STin2 polymorphism.

2.5 Statistical analysis

The data was analyzed using SPSS. Correlations between the personality traits, risky behavior and other variables were measured using Pearson's correlation coefficients. One-way ANOVAs and T-tests were carried out to assess average differences for key variables.

3. Data

3.1. Relationships between temperaments, gender, and age

We surveyed 201 individuals (138 female, 62 males, 1 other); the individual who stated "other" was removed from the gender analysis. A significant difference was found between the reward dependence scores between males and females ($p<0.05$; Table 2). On average males scored lower than females (Table 2). There was a significant difference in harm avoidance scores between male and female participants ($p<0.01$). Females on average scored higher on the harm avoidance scale than males (Table 2) this is in accordance with previous research [26]. Of the 201 participants, the average age of the participants was 22.65±6.85, with a range being 18-65 years (Table 2).

Table 2. comparison of mean between personality traits and gender and the age breakdown of participants

	Overall Statistics			**Females**			**Males**			**p-values for sex**
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	
Age	22.65	6.85	195	23.33	7.99	135	21.10	2.58	60	N/A
RD	20.36	4.15	200	20.84	3.90	138	19.34	4.53	62	0.02
HA	14.62	7.65	200	17.04	7.27	138	12.19	7.154	62	$p < 0.01$

3.2. Correlation between risky behavior, and temperaments and age

3.2.1. correlation between frequency of marijuana use, temperaments and age

The correlation between the age of participants and their reward dependence score was not significant (r-value: 0.03; $p>0.05$; Table 3), whereas the correlation between harm avoidance and participants age was (r-value: -0.15; $p<0.05$; Table 3), contrary to previous research[37,40]. There was no correlation between reward dependence scores of participants and how frequently they reported using marijuana (r-value:0.07; $p>0.05$; Table 3) in our study, nor was

there a correlation between an individual's harm avoidance score and their frequency to use marijuana (r-value: -0.02; p>0.05; Table 3). No correlation was observed between participants' reward dependence score and their age (r-value: -0.07; p>0.05; Table 3).

3.2.2 correlation between risk assessment of marijuana use and temperament

As aforementioned, risk assessment measures how risky participants view the behavior, in this case marijuana use, this is measured on a scale of 0-6 shown in Table 1. No significant correlation was observed between reward dependence and their risk assessment score (r-value:0.08; p>0.05; Table 3). Furthermore, no correlation was found between harm avoidance and each participant's risk assessment score (r-value:0.04; p>0.05; Table 3).

Table 3. correlation between temperament, frequency of marijuana use and risk assessment of marijuana use

Correlation Tested	**r-value**	**p-value**
Reward dependence and age	0.03	0.68
Harm avoidance and age	-0.15	0.03
Reward dependence and frequency of marijuana use	0.07	0.32
Harm avoidance and frequency of marijuana use	-0.02	0.69
Frequency of marijuana use and age	-0.07	0.68
Reward dependence and risk assessment of marijuana use	0.08	0.27
Harm avoidance and risk of assessment of marijuana use	0.04	0.55

3.3 Comparison by Race

3.3.1. comparison of means between temperament, race and marijuana use

Table 4. comparison of means between temperament, race, frequency and risk assessment of marijuana use.

	Asian (n=36)	**Black or African American (n=48)**	**Hispanic or Latino (n=34)**	**White or Caucasian (n=61)**	**Biracial/ Multiracial (n=20)**	**p-value**
Average Reward Dependence	19.69	20.79	20.62	20.61	19	0.41
Frequency of Marijuana use	0.50	1.75	1.41	1.02	2.10	0.02
Physical Risk Assessment	3.56	2.54	2.5	2.34	1.25	0.001

A one-way ANOVA was conducted to investigate the average reward dependence score by race of the participants. There was no significant difference seen in RD score by race (p>0.05; Table 4). However, a one-way ANOVA did reveal a significant difference in marijuana use between different races (p<0.05). Post hoc Tukey tests revealed the difference to be due to students who identified as Asian having a significantly lower average of marijuana use compared to their counterparts (Table 4).

A one-way ANOVA revealed a p-value of 0.001 (Table 4), again supporting a significant difference in how different races view marijuana use. Post hoc turkey tests revealed the difference was due to students who identified as Asian having a significantly higher average physical risk assessment of marijuana use compared to other races.

4. Conclusions

The purpose of this study was to investigate the relationship between reward dependence, harm avoidance, marijuana use and the STin2 polymorphism of the *SLC6A4* gene in college students. It was hypothesized that females would score higher on the RD and HA scale based on previous research which showed this pattern[26,27]. Our study found a significant difference between the average RD score for female and male participants. This finding supported our hypothesis and was in line with previous research which states that males on average score lower than females on the RD scale[26]. There was a difference in HA scores for males and females, on average, females had a higher HA score than males. This finding supports our hypothesis as well as previous research studies that show women tend to be more cautious in participating in events presenting harm[26, 27]. This finding is not surprising since women tend to be naturally more cautious in certain situations whereas men tend to have a more adventurous disposition[27].

We hypothesized that there would be no correlation between age and reward dependence or harm avoidance. Our hypothesis was supported for reward dependence and age as there was no correlations between these variables in our study. Alternatively, a negative correlation between harm avoidance and participants' age was observed. This means an individual's RD score does not change with age, while an individual's HA score decreases as they get older (in our study). This finding is not in agreement with a 2013 study done by Josefsson and colleagues which found no change in participants' harm avoidant score over time, while a decrease in reward dependency was seen as participants grew older[43]. Our findings could have been influenced by the demographics of our participants. Our study participants consisted of mostly college-aged students, while previous studies investigating the correlation of age and personality traits focused on a more representative demography.

We hypothesized there would be a correlation between RD and marijuana use as well as HA and marijuana use. No correlation was found between participants' RD score and their frequency of marijuana use nor their risk assessment (how risky they believe using marijuana is) and RD score. This result is supported by previous research that was also unable to link marijuana use to RD[28]. Similarly, no correlation was found between HA scores in this study and marijuana use or risk assessment scores for participants. This is in line with a study which stated that although marijuana use leads to anxiolytic effects, which can lead to depression and depression is typically associated with HA, no study has been able to confidently provide a correlation between these two variables[28]. Likewise, no correlation was found between the use of marijuana and age, this is contradictory to previous findings that indicates there is a positive correlation between age and marijuana use[10]. Again this could be skewed due to the limited number of individuals over a large age range.

We also investigated the relationships between reward dependence, marijuana use and race. No significant difference was found between reward dependence scores and race of participants. When the frequency of marijuana use was evaluated by race, a one-way ANOVA test revealed a difference in the average scores between the races. A post hoc Turkey was then carried out which revealed a significant difference between students who identify as Asian compared to other races. This was supported by previous research which states that non-white participants were more likely to view regular marijuana use as risky; though the study did not specifically look at Asians[42]. This makes sense in our data set since those identifying as Asians had the highest risk assessment score meaning that on average they believed there is a high risk associated with marijuana use. Thus, it is not surprising that they have a lower frequency of marijuana use.

We hypothesized marijuana use would be greater in individuals with the STin2.12 homozygous genotype due to its increased transcriptional rate of *SLC6A4*[16]. At the time of this publication we genotyped 26 out of 201 individuals, thus our sample size is too small to adequately analyze. Very preliminarily, we have identified individuals homozygous for the STin2.12 allele and those with the heterozygous genotype Stin2.10/12. To date, we have not detected a difference between these genotypes (STin2.12 and STin2. 10/12) and the frequency of marijuana use and risk assessment, nor a difference between the genotypes and HA or RD, but this is expected given the small size of our genotyped sample.

Limitations to this study include the sample size used (201 students participated in this study), which limits the statistical power to observe important but relatively weak associations. Another aspect is the innate errors associated with using surveys in studies, as participants are not always truthful in answering questions. Though it is challenging to rectify this, the survey does contain a question asking if they are being honest, that can be used to exclude students who are not truly reading the survey. The participants were incentivized and were recruited from GGC's Biology and Psychology department, which is not necessarily representative of Georgia Gwinnett College's population; recruitment from other departments is currently underway. The ratio of male to female participants in the study is disproportional and this limits the accuracy of the study. Finally, only 26 DNA samples were genotyped for this particular allele and this is not representative of the data we have collected thus far; further genotyping is underway.

Lastly, we are currently exploring other polymorphisms in the serotonin transporter gene (5-HTTLPR) and are working towards investigating other neurotransmitter system effects on personality traits (e.g. dopamine). We will combine data from the LPR genotypes and STin2 to determine if there is any combinatorial effect of these polymorphisms on behavior. We are currently surveying more individuals to increase our sample size and continuing genotyping of both the LPR and STin2 polymorphisms of all participants. We would also like to increase male participation in our study. To conclude, the findings of this study are important in educating college aged adults and the general public. They help in understanding the role of genetics on personality traits and its impact on lifestyle choices made by young adults.

5. Acknowledgments

This research project would like to thank Georgia Gwinnett College, the Vice President of Academic and Student Affairs, SEED grant, and the School of Liberal Arts for funding. We thank Maryann Aniekwe and Carmen Roland for their help with data analysis.

6. References

1. Bey, K., Lennertz, L., Kaufmann, C., Grützmann, R., Klawohn, J., Riesel, A., & Wagner, M. (2017). Harm Avoidance and Childhood Adversities in Patients with Obsessive-Compulsive Disorder and Unaffected First-Degree Relatives: Evidence for A Diathesis-Stress Model. *European Neuropsychopharmacology*, 135(4), 328-338.
2. Bajraktarov, S., Novotni, A., Arsova, S., Gudeva-Nikovska, D., & Vujovik, V. (2017). Character and Temperament Dimensions in Subjects with Depressive Disorder: Impact of the Affective State on Their Expression. *Open Access Macedonian Journal of Medical Sciences*, 5(1), 64-67.
3. Ebstein, R. P. (2006). The Molecular Genetic Architecture of Human Personality: Beyond Self-report Questionnaires. *Molecular Psychiatry*, 11(5), 427-445.
4. Cloninger, R., Svrakic, D., & Przybeck, T. (1993). A Psychobiological Model of Temperament and Character. *Archives of General Psychiatry* 50(12), 975-990.
5. Mochcovitch, M., Nardi, A., & Cardoso, A. (2013). Temperament and Character Dimensions and Their Relationship to Major Depression and Panic Disorder. *Revista Brasileira De Psiquiatria*, 35(1), 106.
6. Tschan, T., Peter-Ruf, C., Schmid, M., & In-Albon, T. (2017). Temperament and Character Traits in Female Adolescents with Nonsuicidal Self-Injury Disorder with and Without Comorbid Borderline Personality Disorder. *Child and Adolescent Psychiatry and Mental Health*, 11, 4.
7. Gat-Lazer, S., Geva, R., Gur, E., & Stein, D. (2017). Reward Dependence and Harm Avoidance Among Patients with Binge-Purge Type Eating Disorders. *European Eating Disorders Review*, 25(3), 205-213.
8. Schuerbeek, P. V., Baeken, C., & Mey, J. D. (2016). The Heterogeneity in Retrieved Relations between the Personality Trait 'Harm Avoidance' and Gray Matter Volumes Due to Variations in the VBM and ROI Labeling Processing Settings. *Plos One*, 11(4), e0153865.
9. Petrosini, L., Cutuli, D., Picerni, E., & Laricchiuta, D. (2016). Viewing the Personality Traits Through a Cerebellar Lens: A Focus on the Constructs of Novelty Seeking, Harm Avoidance, and Alexithymia. *The Cerebellum*, 16(1), 178-190.
10. Brackenbury, L. M., Ladd, B. O., & Anderson, K. G. (2015). Marijuana Use/Cessation Expectancies and Marijuana Use in College Students. *The American Journal of Drug and Alcohol Abuse*, 42(1), 25-31.
11. Otten, R., & Engels, R. C. (2011). Testing Bidirectional Effects between Cannabis Use and Depressive Symptoms: Moderation by the Serotonin Transporter Gene. *Addiction Biology*, 18(5), 826-835.
12. Lesch, K. (1998). Serotonin Transporter: From Allelic Variation in Functional Expression to Gene Knock-out. *European Neuropsychopharmacology*, 8, 118-119.
13. Goldman, N., Glei, D. A., Lin, Y. H., & Weinstein, M. (2010). The Serotonin Transporter Polymorphism (5-HTTLPR): Allelic Variation and Links with Depressive Symptoms. *Depression and Anxiety*, 27(3), 260-290.
14. Vleugels, R., Verlinden, H., & Broeck. J. (2015). Serotonin, Serotonin Receptors and Their Actions in Insects. *Neurotransmitter*, 2, e314.
15. Ayano, G. (2016). Common Neurotransmitters: Criteria for Neurotransmitters, Key Locations, Classifications and Functions. *Advances in Psychology and Neuroscience*, 1(1), 1-5.

16. Schürks, M., Rist, P. M., & Kurth, T. (2010). STin2 VNTR Polymorphism in the Serotonin Transporter Gene and Migraine: Pooled and Meta-Analyses. *The Journal of Headache and Pain*, 11(4), 317-326.
17. Pizzo de Castro, M. R., Maes, M., Guembarovski, R. L., Ariza, C. B., Reiche, E. M., Vargas, H. O., Vargas, M. M., de Melo, L. G., Dodd, S., Berk, M., Watanabe, M. A., …Nunes, S. O. (2014). SLC6A4 STin2 VNTR Genetic Polymorphism is Associated with Tobacco Use Disorder, but Not with Successful Smoking Cessation or Smoking Characteristics: A Case Control Study. *BMC Genetics*, 15, 78.
18. Lei, X., Chen, C., He, Q., Chen, C., Moyzis, R.K., Xue, G., … Dong, Q. (2012). Sex Determines Which Section of the SLC6A4 Gene Is Linked to Obsessive–Compulsive Symptoms in Normal Chinese College Students. *Journal of Psychiatric Research*, 46(9), 1153-1160.
19. Fan, J. B., & Sklar, P. (2004). Meta-Analysis Reveals Association between Serotonin Transporter Gene STin2 VNTR Polymorphism and Schizophrenia. *Molecular Psychiatry*, 10, 928-938.
20. Bjørnebekk, A., Westlye, L. T., Fjell, A. M., Grydeland, H., & Walhovd, K. B. (2011). Social Reward Dependence and Brain White Matter Microstructure. *Cerebral Cortex (New York, N.Y. : 1991)*, 22(11), 2672-2679.
21. Marek, P., & Klose, J. (2001). Personality Disorders Diagnostics Through the C.R.Cloninger`s Theory. *Psychiatrie*, 5(4), 226-231.
22. Kazantseva, A. V., Gaysina, D. A., Faskhutdinova, G.G., Noskova, T., Malykh, S. B., & Khusnutdinova, E.K. (2008). Polymorphisms of the Serotonin Transporter Gene (5-HTTLPR, A/G SNP in 5-HTTLPR, and STin2 VNTR) and Their Relation to Personality Traits in Healthy Individuals from Russia. *Psychiatric Genetics*, 18(4), 167-176.
23. Buckner, J. D., Shah, S. M., Dean, K. E., & Zvolensky, M. J. (2015). Cannabis Use Frequency and Use-Related Impairment Among African-American and White users: The Impact of Cannabis Use Motives. *Ethnicity & Health*, 21(3), 318-331.
24. Nguyen, N.N., & Newhill, C. E. (2016). The Role of Religiosity as a Protective Factor Against Marijuana Use Among African American, White, Asian, and Hispanic Adolescents. *Journal of Substance Use*, 21(5), 547-552.
25. Ali, Z., & Roland, C. (2017) Is Marijuana Use Linked to Your Genes? *Proceeding of the National Conference on Undergraduate Research*, 31, 691-701.
26. Delvecchio, G., Garzitto, M., Fagnani, C., Fornasari, L., Stazi, M.A., Picardi, A., … Brambilla, P.(2016). Normative Data and Effects of Age and Gender on Temperament and Character Dimensions across the Lifespan in an Italian Population: A Cross-sectional Validation Study. *Journal of Affective Disorders*, 204, 83-91.
27. Gagnon, K. T., Cashdan, E. A., Stefanucci, J. K., & Creem-Regehr, S. H. (2015). Sex Differences in Exploration Behavior and the Relationship to Harm Avoidance. *Human Nature*, 27(1), 82-97.
28. Grunberg, V. A., Cordova, K. A., Bidwell, L. C., & Ito, T. A. (2015). Can Marijuana Make It Better? Prospective Effects of Marijuana and Temperament on Risk for Anxiety and Depression. *Psychology of Addictive Behaviors: Journal of the Society of Psychologists in Addictive Behaviors*, 29(3), 590-602.
29. Nahman-Averbuch, H., Yarnitsky, D., Sprecher, E., Granovsky, Y., & Granot, M. (2014). Relationship between Personality Traits and Endogenous Analgesia: The Role of Harm Avoidance. *Pain Practice*, 16(1), 38-45.
30. Caspar, S., & Joukov, A. (2018). The Implications of Marijuana Legalization on the Prevalence and Severity of Schizophrenia. *Health Matrix*, 28(1), 175-200.
31. Brummelte, S., Mc Glanaghy, E., Bonnin, A., & Oberlander, T. F. (2016). Developmental Changes in Serotonin Signaling: Implications for Early Brain Function, Behavior and Adaptation. *Neuroscience*, 342, 212-231.
32. Popova, N. K., Kulikov, A. V., Kondaurova, E. M, Tsybko, A. S., Kulikova, E. A., Krasnov, I. B., …Naumenko, V. S. (2014). Risk Neurogenes for Long-Term Spaceflight: Dopamine and Serotonin Brain System. *Molecular Neurobiology*, 51(3), 1443-1451.
33. Kumar, S., Ranjan, P., Mittal, B., & Ghoshal, U. (2012). Serotonin Transporter Gene (SLC6A4) Polymorphism in Patients with Irritable Bowel Syndrome and Healthy Controls. *Journal of Gastrointestinal and Liver Disease*, 21(1), 31-38.
34. Gomes, C. K., Vieira-Fonseca, T., Melo-Felippe, F. B., Andrade J. B., Fontenelle, L. F & Kohlrausch, F. B. (2018). Association Analysis of SLC6A4 and HTR2A Genes with Obsessive-Compulsive Disorder: Influence of the STin2 Polymorphism. *Comprehensive Psychiatry*, 82, 1-6.
35. Schürks, M., Rist, P. M., & Kurth, T. (2010). STin2 VNTR Polymorphism in the Serotonin Transporter Gene and Migraine: Pooled and Meta-Analyses. *The Journal of Headache and Pain*, 11(4), 317-326.
36. Plieger, T., Felten, A., Melchers, M., Markett, S., Montag, C., & Reuter, M. (2018). Association between a Functional Polymorphism on the Dopamine-β-hydroxylase Gene and Reward Dependence in Two Independent Samples. *Personality and Individual Differences*, 121, 218-222.
37. Wilson, R., Buchman, A., Arnold, S., Shah, R., Tang, Y., & Bennett, D. (2006). Harm Avoidance and Disability in Old Age. *Experimental Aging Research*, 32(3), 243-261.

38. Reis, J. P., Auer, R., Bancks, M. P., Goff, D. C., Lewis, C. E., Pletcher, M. J., Rana, J. S., Shikany, J. M., … Sidney, S. (2017). Cumulative Lifetime Marijuana Use and Incident Cardiovascular Disease in Middle Age: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *American Journal of Public Health*, 107(4), 601-606.

39. King, K. A., Vidourek, R. A., & Merianos, A. L. (2015). The Association between Parenting Behaviors and Marijuana Use Based on Adolescent Age. *Drugs: Education, Prevention and Policy*, 22(4), 334-343.

40. Westlye, L. T., Bjørnebekk, A., Grydeland, H., Fjell, A. M., & Walhovd, K. B. (2011). Linking an Anxiety-Related Personality Trait to Brain White Matter Microstructure. *Archives of General Psychiatry*, 68(4), 369-377.

41. Smit, M., Vállez García, D., de Jong, B. M., Zoons, E., Booij, J., Dierckx, R. A., Willemsen, A. T., de Vries, E. F., Bartels, A. L., … Tijssen, M. A. (2018). Relationships between Serotonin Transporter Binding in the Raphe Nuclei, Basal Ganglia, and Hippocampus with Clinical Symptoms in Cervical Dystonia: A [^{11}C]DASB Positron Emission Tomography Study. *Frontiers in Neurology*, 9, 88.

42. Wu, L. T., Zhu, H., & Swartz, M. S. (2016). Trends in Cannabis Use Disorders Among Racial/Ethnic Population Groups in the United States. *Drug and Alcohol Dependence*, 165, 181-190.

43. Josefsson, K., Jokela, M., Cloninger, C. R., Hintsanen, M., Salo, J., Hintsa, T.… Keltikangas-Järvinen, L.(2013). Maturity and Change in Personality: Developmental Trends of Temperament and Character in Adulthood. *Development and Psychopathology*, 25(03), 713-727.

44. Zuckerman, M., & Cloninger, C. R. (1996). Relationships between Cloninger's, Zuckerman's, and Eysenck's Dimensions of Personality. *Personality and Individual Differences*, 21(2), 283-285.