The Dopamine Transporter and Risk-Taking Behavior: The Role of Genetics in Addiction

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Abstract

There is always the argument of environment versus biology in attempting to explain human behavior; this study examines the biological component of risk-taking. The Reward Deficiency Syndrome strongly points to a genetic component for risk-taking behaviors and this study sought to analyze the relationship between the dopamine transporter (DAT) and risking-related functioning as assessed with two tasks. In one task, functional magnetic resonance imaging was used to measure brain activity during a monetary feedback task. In the second task, risk-taking behavior was assessed directly using the Balloon Analog Risk Task. Gene sequencing provided allele type to compare to scores on these task, as well as to additional variables (e.g., demographic variables, self-reported substance use behavior). The overarching goal of the study was to examine the relationship between DAT genotype and addiction.

Introduction

When an individual exhibits a deficient reward system, response to natural rewards is affected. Things like food, sex, and similar activities will naturally stimulate the reward pathway in a properly functioning and wired brain. However, some individuals' brains are less responsive to such rewards; as a result they seek that feel good notion elsewhere. The ultimate goal for human existence is to survive and procreate. To accomplish these things, human behavior is driven by the reward process. Reward deficiency syndrome (RDS) represents this behavior at its extreme. RDS is defined as a deficiency of the reward pathway, and according to Blum, Cull, Braverman & Comings (1996), RDS is influential, among other factors, resulting in many personality disorders, and detrimental behaviors such as addictive, impulsive, and compulsive

actions. Dopamine is a major component of the reward pathway and therefore plays a fundamental role in RDS.

DAT is responsible for DA re-uptake from the extracellular space after it has been released. In a way, it is recycling DA. The amount of DA available in the extracellular space after accounting for the job DAT does depends in part on the allele carried. DAT 10 repeat (DAT 10R) and DAT 9 repeat (DAT 9R) correspond to levels in activity of the transporter. DAT 10R is more expressive, it is working more to have the DA recycled out of the extracellular space, and thus results in a decrease in DA there; DAT 9R is the least expressive allele, it is working less to recycle the DA, which results in more extracellular DA present (Bilder et al., 2004; Dreher et al., 2008; Hahn et al., 2011 & Yacubian et al., 2007). Since DA is the chemical with a major involvement in the reward pathway, it is expected that when less of it is in the extracellular space a dysfunction with reward processing can possibly occur.

That deficient processing is believed to manifest as RDS in which case people with it are especially prone to engage in risky behaviors like drug abuse, alcohol use, gambling, risky sexual activity and other things. As a result, their neural response to these activities is far more intense than a natural reward despite the fact that this effect is of shorter duration (Comings & Blum, 2000). The activities they choose to engage in resolves the issue of decreased or lack of response to natural rewards. The deficiency presented from lack of response to healthy rewards results in compensate with the negative behaviors. As the individual receives the chemical response sought the negative behaviors are reinforced. Many components in the brain interact to achieve functions like breathing, moving, comprehension, and a long list of other things. The brain is the fundamental feature of life. As a result many interactions in the brain are involved in the reward system and any number of components could result in the system becoming faulty. This project investigates the relationship between genetics, brain chemistry, and the Reward Deficiency Syndrome theory. In particular, this project seeks to determine how genetics affect the likelihood of risk-taking behavior as a link to addiction.

One variation of risk-taking that scholars widely acknowledge as significant is substance use. Researchers have sought to better understand addiction since every individual responds to substance use differently—some are able to moderate their use; others are more prone to abuse. Understanding which genes contribute to the predisposition of substance abuse may be highly beneficial to many communities. First, it may diversify treatment options. Also, researchers may be able to further examine the link between genetics and other risky behaviors.

Literature Review

Dopamine

Many researchers have studied and supported that dopamine (DA) is a major component involved in the chemical regulation of the reward system (Blum et al., 1996; Bilder et al., 2004; Comings & Blum 2000; Dreher et al., 2008; Hahn et al., 2011 & Yacubian et al., 2007**).** DA is known to people as the happy chemical and plays an important role in the reward process. Although many factors may affect the reward system it is possible that DA imbalance can result in its malfunction. During the experience of reward, cells in the ventral tegmental area (VTA) released DA into the following regions: nucleus accumbens, olfactory tubercle, ventral striatum (VS), the prefrontal cortex (PFC) (Comings & Blum, 2000), striatum, hippocampus, and prefrontal cortex (Dreher et al., 2008). Authors agree that the VS and PFC are especially

important in reward processing. DA levels in those regions of the brain influence many behaviors. Fluctuation in DA supports reward influence on unnaturally rewarding behaviors and hypotheses for why such behaviors occur (Blum et al., 1996; Bilder et al., 2004; Comings & Blum 2000; Dreher et al., 2008; Hahn et al., 2011 & Yacubian et al., 2007).

Since DA availability in the brain is linked to differences in reward processing it is important to understand what may contribute to differences in DA availability. Many studies have found genetic variation in genes involved in DA regulation impact DA levels in the brain. Many studies support the genetic variation from polymorphisms influencing the DA presence in the brain (Bilder et al., 2004; Dreher et al., 2008; Hahn et al., 2011 & Yacubian et al., 2007). RDS posits that several genetic variations involved in dopamine transmission can influence reward processing. Such genetic combinations seem to promote healthy reward processing while other combinations are associated with poorer functioning. Genetic variation influences functioning of the reward pathway. One gene of particular interest is the Dopamine Transporter gene (DAT). Blum et al. (2000) found variation in the allele present correlated to ADHD diagnosis in children. Blum et al. (2000) further found a correlation such that an individual exhibiting any single risky behavior put one at a higher risk of possessing other such behaviors. Although DAT in this study (Blum et al., 2000) was linked to just ADHD and not directly to other behaviors, there is other support for DATs' correlation to other risky behaviors(Moallem et al., 2012 & White et al., 2007).

Brain Activity

Tasks that activate the reward pathway activate a number of brain regions. Specific brain region activity that is consistent across studies depends on both genetics and examination of the reward delivery (Dreher et al., 2008; Hahn et al., 2011 & Yucubian et al., 2007). Throughout their studies activity varied depending on the phase of reward, whether the participants were receiving a reward or anticipating a reward at the time of neural activity examination. Depending on whether there was anticipation for reward or if the reward was actually received, the brain activity fluctuated based on which phase of reward was examined in the study.(Dreher et al., 2008; Hahn et al., 2011 & Yucubian et al., 2007). Brain activity differs between the conditions. Anticipation was correlated with ventral striatum activity while the prefrontal cortex activity was specific to receiving reward (Dreher et al., 2008). That finding was consistent with Yucubian et al., (2007) & Hahn et al., (2011) who both review anticipation of reward and found significant activity in the ventral striatum. The difference in activity is the result of reward manipulation, the reason that there are two significant regions that show separate levels of activity is essentially to the reward pathway involvement of many regions. As the reward type becomes specific it appears that activity too becomes less generalized to multiple regions and more prominent in certain regions. These studies are significant to the current study because examining brain activity in the right regions depends on the timing of reward.

Terms and Tasks

Reward processing has been operationally defined with a number of constructs: impulsivity, reward discounting, novelty seeking, and reward sensitivity. Ultimately they are all a measure of risk taking. From tasks like the guessing paradigm (Yacubian et al., 2007), Balloon Analog Risk Task (Moallem & Ray, 2012; White et al., 2007; Skeel et al., 2008; & Lejuez et al., 2003), and the Bechara Gambling Task (Businelle et al., 2008) there is support for risk taking

correlating with the reward processing system. These tasks correlate with self-reported risk ratings as well as personality tests that measure personality for expected behaviors. The Balloon Analog Risk Task (BART) is well known for having both validity and reliability. It correlates with real life risk taking as the task accurately predicts smokers based on performance when compared to nonsmoking participants (Lejuez et al., 2003). As a result, performance on the BART is a good measure to the likelihood of being an individual who is likely to take part in any number of the risky behaviors discussed.

Substance use as a risky behavior

Risky behavior can be defined in a number of ways, examined through a number of tasks, and include any categorization of the population. A risky behavior of interest is substance abuse, and studies examined cigarette smokers, alcohol drinkers, and individuals who identify as polysubstance users. Indeed, the list of risky behaviors is more extensive and can include sexual behaviors, gambling, stealing and even more behaviors. With substance abuse it is relatively easy to set up in a lab setting and measure the behavior with brain imagining and the tasks mentioned that are good risk taking measures. For example, Moallem et al. (2012) measured impulsivity differences between substance users by comparing task performance between participants who fit into the categories of smokers, heavy drinkers or fitting into a category including both. Their use of substance was the link to impulsivity. That study supported impairments of reward discounting correlating with substance use. This study was not capable of predicting which substances were used or the frequency of different substances used, instead it was an excellent predictor of whether a participant was a cigarette smoker on a non-smoker. Overall it was significant for impulsivity levels being a predictor of potential substance use. White et al. (2007) examined three traits that are directly linked to risk taking: reward sensitivity, impulsivity, and negative affect. They found support for the effect that personality traits had on risk taking as a function of substance dosage. In general there was a positive correlation between stimulant use and impulsivity found in the male participants. Ultimately what these articles hypothesized and found important was the idea that some people exhibit a predisposition for substance use when looking at factors of personality type like a certain degree of impulsivity or risk taking likelihood.

The articles just examined did not look at genes directly but looked at personality through questionnaires. The hypothesis for this paper is that genes influence reward processing which influences behavior. Many of the studies suggest a possible genetic predisposition examination of a few genes to find a possible link to addiction directly (Yacubian et al., 2007), or more generally to reward-seeking behaviors or psychiatric disorder (Dreher et al., 2008; &Hahn et al 20011). It appears certain that specific genes produce specific traits resulting in poorly sought behaviors.

The proposed objective of people indulging in such behaviors is to compensate for their deficient reward system that does not respond appropriately to the natural rewards. People who fit this criterion for RDS will take part in multiple risky behaviors (Comings et al., 2000; Blum et al., 1996). It is therefore important to understand one step at a time; one unnatural rewarding behavior at a time to see what is occurring that results in behaviors in only certain individuals, to understand the predisposition that put them at this disadvantage with risk taking. While some studies examined traits by subjects' performance on tasks that measure impulsivity and risk taking behavior, and others explored the brain activity of participants with specific alleles

present, this study examined both concepts together. In doing so the reference point is the RDS theory, and what results in the variation of behaviors, that is thought to be contributed by genes (Blum et al., 1996; Comings et al., 2000).

One way the variation has been examined and seems of great significance is through substance use. This behavior is important because people are well aware of the harms that substance use can have on the body. Although those risk factors exist many continue using and some people start even with examples of what use does to other people. There is also the factor that some people have more difficulty quitting than others. It is clear that not every individual responds to substance use the same. One difference that has been mentioned is that of genetics. Understanding the pieces of the genetic contribution to the predisposition of substance abuse can do many things for people who suffer. Seemingly of great importance yet to be mentioned is the ability to pin point possible mechanisms of other risky behaviors.

The current study investigated risk taking in individuals who report substance use (specifically, regular cigarette use). The study examines brain activation and multiple task performance similarities between participants. The following hypotheses were made: Hypothesis 1: Participants that present with DAT 10R allele are more likely to show less increase in the caudate nucleus, medial orbital frontal cortex, and ventral striatum region activity while completing the card task than those with DAT 9R. Hypothesis 2: Participants present with DAT10R allele are more likely to make riskier moves on the BART than those with DAT 9R, Hypothesis 3: Overall, poly substance users are expected to show least increase in the caudate nucleus, medial orbital frontal cortex, and ventral striatum brain region activity while completing the card task and also make more riskier moves on the BART.

Method

I. Participants

Fifty-one cigarette smokers participated in the experiment. Participants were solicited through advertisements, which detailed their eligibility. They had to have smoked at least ten cigarettes a day for the past twelve months. Subjects went through an initial screening session to ensure they qualified for the study over the telephone prior to coming in for a one of two in lab screening sessions to ensure qualification. The initial phone screening consisted of standard questionnaires. Prospective subjects were excluded if they self-reported having a mood or anxiety disorder or frequent illicit drug use, suggesting dependence use that was abuse was accepted for the purpose of this study. A standard SLEIC Magnetic Resonance Imagining (MRI) screening safety form was conducted to determine participants' MRI eligibility during the phone screening to ensure the MRI would be a safe. After this initial telephone screening, subjects who retained their eligibility completed two individual in lab sessions.

II. Materials:

The MRI Card Guessing Task

Participants were placed in the MRI to complete a card task utilizing the SLEIC standard operating protocol. Several trials of a card-guessing task adapted from previous research (see Delgado et al., 2000) were completed while in the MRI as brain activity data was collected. For each trial, participants guessed whether the numerical value of a visually displayed “playing

card" was higher or lower than 5. Participants were informed before beginning the task that each card would have a value ranging from 1 to 9, excluding the number 5. Each guess was either correct or incorrect for each trial. After a choice-making period lasting 2,500 ms, a number from 1 to 9 (excluding 5) was presented for 500 ms, followed by feedback (also presented for 500 ms). Such presentation of information informed subjects whether or not their guess was correct. For trials in which a correct guess was made, feedback consisting of a green arrow pointing upward was presented. In contrast, trials in which an incorrect guess was made, feedback consisting of a red arrow pointing downward was presented. Trials concluded with the presentation of a fixation cross for 11.5 s.

Participants were informed that each correct guess led to the addition of $1.00 to the total payment they would receive, while each incorrect guess led to the loss of $0.50 from this total. Participants were unaware that the card values were selected only after the response was made for each trial to ensure an equal number of positive feedback and negative feedback trials. Participants completed a total of 90 interleaved trials (45 of each feedback condition) divided into five runs of 18 trials each (Wilson et al., 2008).

Balloon Analogue Risk Task

With the BART evaluation of real world risk behavior engagement was examined through this task. The computerized task measured the risk-taking tendency. Participants were presented with a small balloon present on the screen and instructed to pump the balloon by clicking a button on the screen. For every click the balloon inflated a small amount and the participant earned money for each successful pump, in which the balloon did not explode. That earned money during the trail was placed into a temporary bank visible on the screen. At any point during the trail the participant could cash out by selecting the button on the screen that was labeled collect. At which time the money in the temporary winnings would be moved to the bank, no longer presented with the risk of losing that money. That trail ended at that point. The next trail began and the participant completed the task in the same manner. Each balloon was expected to pop at some random point between 1 and 128 pumps, with 64 pumps being the average breakpoint. A failure to cash out, that is press collect before the balloon popped resulted in all the temporary earnings being lost for that trail and another trail started after. Risk was measured in this task by the average number of pumps with higher scores being indicative of greater risk-taking likelihood. (Hunt et al., 2005 & Lejuez et al.,2003)

III. Procedure

Session 1

Full completion of Session 1 was about 2.5 hours. Subjects arrived to the lab and informed consent was reviewed and signed. After which, Carbon Monoxide (CO) readings were taken from every participant as a first step. The reading was obtained by requiring participants to first hold their breath for 15 seconds and exhale into the CO monitor. A reading of greater than 10 parts per million qualified participants for continued eligibility. If qualified to continue, subjects completed the Center for Epidemiologic Studies Depression Scale, which screened for current depression state. To remain in the study, participants were required to score below 16 points. Another questionnaire (Mini International Neuropsychiatric Interview) was completed

measuring drug dependence for substances other than nicotine, requiring a score indicating no dependence. At this point, non-eligible individuals were dismissed and compensated for the lab session. Remaining participants completed an Automated Operation-Word memory task to measure working memory and predict cognitive performance (Unsworth, Heitz, Schrock & Engle, 2005). Likewise, Digital Span tasks were completed to take another measurement of working memory.

Participants then provided demographic information and completed several surveys to assess behavior. They completed the Smoking History survey, Fagerstrom Test for Nicotine Dependence (Heatherton, et al., 1991), Nicotine Dependence Syndrome Scale (Shiffman, Waters, & Hickcox, 2004), Relapse Situation Efficacy Questionnaire, Revised Self-Consciousness Scale (Gwaltney, et al., 2001), Self-Control Scale (Tangney, Baumeister, & Boone, 2004) , Self-Consciousness(revised Self-Consciousness Scale; Scheier & Carver, 1985), Positive and Negative Affect Schedule (Watson, Clark, & Tellegen,1988), Barratt's Impulsivity Scale, Sensitivity to Punishment/Reward Questionnaire, and the Balances Inventory of Desirable Responding (Version 6; Paulhus, 1991). Upon completion of these surveys, participants were scheduled for their second session and instructed to avoid smoking or using any other nicotine products for 12 hours before arriving as well as directed to refrain from alcohol and recreational drug use for 24 hours before Session 2.

Session 2

At the start of the second session a second CO reading was completed to ensure that participants had not smoked during the time frame instructed. This second CO reading needed to be less than or equal to half the first reading in order for participants to proceed with the rest of the session. If participants informed the experimenter they had smoked or their CO levels were not in the correct range participants were given one opportunity to reschedule this session. Likewise, the reporting of alcohol and recreational drug use required rescheduling of the second session. After determining compliance with abstinence instructions , the remaining participants provided buccal cell samples. Such samples were collected to provide a source to study a genetic correlation with addiction through examination of the reward process. No deception occurred with the sample collection and this part of the study was completely optional. Participants that gave samples signed an informed consent form. To obtain the sample, subjects used cotton swabs to collect material from the inner lining of their cheeks. The sample was then placed into a vial with a preservative solution, marked with a bar code, which only linked the sample to the participants' subject I.D., and stored. Once the sample was collected, 4 questionnaires were completed: Positive and Negative Affect Scale–State, State Ego Depletion Scale, Smoking Consequences Questionnaire, Questionnaire on Smoking Urges-Brief, measuring for positive/negative affect, self-control, and desire to smoke. After completing the forms, participants rated their current urge rating on a scale 0-100 prior to entering the MRI. All of the steps leading up to the MRI took approximately 30 minutes to complete. The MRI portion of the study consisted of obtaining brain imaging and administering the card task.

Mid-way through the completion of the card task half of the participants were instructed on whether they could (Expected-shift group) or could not (Expected-stable group) smoke upon completion of the MRI task. Random assignment was used to group participants. Participants were grouped this way to examine the expectation of smoking on neural response to monetary gain. During the MRI, prior to the card task, scanning of anatomical imagining and diffusion

tensor (DTI) imaging was retrieved. Participants in the Expected-shift group were instructed after the MRI task to complete the lapse task, a behavioral task modeling smoking lapse behavior (McKee, 2009). Those in the Expected-stable group performed the Balloon Analog Risk task (BART), a computer task measuring risk-taking behavior (Hunt et al, 2005). Participants were instructed to pump the balloon on the screen to earn money with the risk of the balloon popping and losing the money earned during that trail. After the completion of either the Smoking Restraint task or the BART participants were debriefed. Not all of these measures were a focus of the present study. The current study will focus on pumps during the Bart, brain activity during the card task and the genetic sequence from the samples provide.

Results

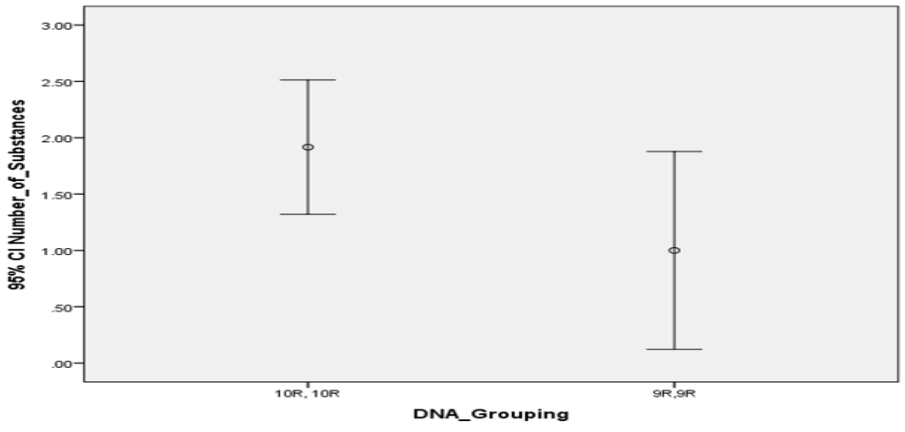
Genotyping

A large percent of the subjects in the experiment presented with at least one 10R allele, specifically 89.5%. That can be further broken down into 51.1% 10R, 10R; 36.2% 9R, 10R; 10.6% 9R, 9R; and 2.1 % with a unique allele type 8R, 10R. For analysis purpose, participants with allele types 10R, 10R were grouped together as Group 1 and participants with genotype 9R, 9R were grouped together as group 2. The 9R, 10R and 8R, 10R genotypes were eliminated from the analysis as done in previous studies since there was a very small number of such participants and as there is very limited data about these genotypes and reasoning behind expected behaviors.

Number of substances used and genotype

Subjects reported number of substances used as a part of a demographic survey. We used an independent samples t-test (2-sided) to see if genotype had an affect on the number of substances participants used. Participants were grouped by genotype (homozygous for 10R or 9R), and number of substances used excluded cigarettes. Using an alpha = 0.05 the t-test revealed no significance: t(27) = 1.401, p = 0.173 (10R: M + SD = 1.9167 +/- 1.41165, 9R: M + SD = 1.0000 +/- 0.70711).This indicated that genotype did not influence the number of substances used. Graph 1 illustrates the averages per genotype of substances used from no substances (zero) up to 5 other substances used within the last 12 month. Even though the difference was not statistically significant we can see that the 10R,10R group averaged a higher number of substances used, which is consistent with our hypothesis.

Graph 1



To examine the effects of other demographic variables on our dependent factors, a t test compared gender and number of substances used. With a t(46)= -1.750 p=.087, d= -0.516 (Female: M + SD = 1.4545+/- 0.96250 Male: M + SD = 2.0769 +/- 1.41204) there was no significance found, suggesting no difference between genders and use reported. Graph 2 illustrates the averages between the genders, which appear to be different. Examining the graph, males reported more substances. A chi-square test was conducted to examine allele frequency between genders as well. Table 1 illustrates the percentages of men and women with 10R alleles or 9R alleles. The analysis shows that $\chi^2(1, N=29)= 0.561$ p = 0.453, suggesting that there is no significance within the distribution of participants. Important to note is that 2 of the cells in the chi-square analysis showed counts of less than 5, because a chi-square test is not considered valid unless there is 80% of the cells with values of at least 5.

Graph 2

Graph 2 illustrates the difference in substances reported between genders

Table 1

Allele Type	Gender	
	Female	Male
10R, 10R	10	14
	34.50%	48.30%
9R, 9R	3	2
	10.30%	6.90%

Table 1 illustrates the percentages gather from a chi-square analysis of allele type occurring in a sample.

Participants were also grouped into categories of drinking pattern and a chi square analysis was conducted to compare drinking patterns between allele types. Participants were

grouped between 3 levels of drinking status: non-drinker, moderate drinker, and heavy drinker. For the purpose of this study, female participants reporting 3 or more drinks in one sitting or more than 7 drinkers in a week were heavy drinkers while the males were classified as heavy drinkers if they consumed more than 4 drinks in one sitting or more than 14 drinkers a week (Benton 2009). With a $\chi^2(2, N=29)= 1.621$, $p = 0.445$, $d = 0.544$ no significance was found between allele types and alcohol drinking frequency. Table 2 illustrates the percentages of allele types that fall into the categories of drinking pattern type.

Table 2

Allele Type	Drinking Pattern			Total
	Non-Drinker	Moderate Drinker	Heavy Drinker	
10R, 10R	1	12	11	24
	3.4%	41.4%	37.9%	82.8%
9R, 9R	1	2	2	5
	3.4%	6.9%	6.9%	17.2%
	2	14	13	29
Total	6.9%	48.3%	44.8%	100.0%

Table 2 shows the number and percentages of participants that were grouped by allele type and drinking behavior.

Behavioral Data: Risky decision-making

Genotype and BART

Participants completed two tasks during the study. Participants were randomly selected to complete the BART as one of the behavioral assessments. Two scores were averaged for each subject, one corresponding to the baseline target pump value and the other to the computer competition target pump value. . A t test analysis compared the baseline target pump between groups, depicting no significance $t(7) = -0.746$, $p= 0.480$, $d = -0.563$ (Type 10R: M+SD= 33.0300+/-13.57624; Type 9R: M+SD = 43.7700 +/- 0), suggesting participants did not differ significantly between pumps targeted. One factor that has an important effect on analysis is sample size. As noted in Table 3, only 1 participant that completed the BART was homozygous for the 9R allele. This greatly reduces statistical power and also limits the validity of any findings. Also was the case when comparing the second score from the BART task, revealing no significant difference in the scores by genotype when participants thought they were competing against the computer for target pump scores. For the group analysis between allele types for this

second score t(7) = 0.118, p = 0.910, d = 0.09 (Type 10R: M+SD= 53.8488 +/- 17.80100; Type 9R 2: 51.6300+/-0). Table 3 illustrates the means between the groups in both conditions.

Table 3

DNA Grouping		N	Mean	Std. Deviation	Std. Error
BART Base Target	10R,10R	8	33.03	13.57624	4.79993
	9R,9R	1	43.77	0	0
BART Computer Competition	10R,10R	8	53.8488	17.801	6.2936
	9R,9R	1	51.63	0	0

fMRI Data

Participants also completed a card task as a measure of affect of reward on brain activity. Specifically for the task, subjects made a response for each trial of the card-guessing task. Responses and reaction times were collected for all trials. Analysis of the caudate nucleus, medial orbital frontal cortex, and the ventral striatum activity was completed for the purpose of the study using peak coordinates, selected based on peak MNI coordinates for activity analysis for the brain regions of interest. Table 4 illustrates the peak coordinates used to examine the brain activity in each region of the brain.

Table 4

Caudate coordinates		MNI		
		x	y	z
Region	Left caudate	-8	14	2
	Right caudate	8	20	2
	Left ventral striatum	-10	10	-4
	Right ventral striatum	12	8	-4
	Medial OFC	0	48	-12

Table 4 shows the MNI coordinates.

Table 5 illustrates the differences in mean scores of brain activity between groups in which the averages for 10R, 10R was less than 9R, 9R as predicted. Table 5 specifically shows a smaller average for Type 10Rin the right caudate while depicting a smaller average also in the left caudate for Type 10R. Looking at the left caudate region, a two-tailed t test for independent samples revealed no significance comparing the activity between, Type 10R and 9R t(23)= -0.250, p= 0.805, d = -0.104 (10R: M+SD= 0.30689840+/- 0.219204970; 9R: 0.33223600 +/- 0.090830558). This suggests the groups did not differ in average increase in brain activity.

Similarly, when comparing participants' activity in the right caudate, no significance was found according to genotype (t(23)= -0.306, p= 0.762, d = -0.127 [10R: M+SD= 0.12612185 +/- 0.150995331; 9R: M+ SD = 14742200 +/- 0.056140239]). When examining the ventral striatum; for the right region no significance was found t(23)= 1.304 p= 0.205 d= 0.544 (10R: M+SD= 0.37410945+/- 0.233488450 9R: M+SD= 0.22914580+/- 0.158715392) left t(23)= 0.016 p= 0.987 d= 0.0066 (10R: M+SD= 0.29586395+/-0.227504175 9R: M+SD= 0.29411260+/- 0.173356512). Similarly when examining the medial orbital frontal cortex, no significance was found t(23)= 1.071 p= 0.295 d= 0.447 (10R: M+SD= -0.05702130 +/- 0.201724510 9R: M+SD= -0.15937820+/- 0.129705602).

Table 5

Group		N	Mean	Std. Deviation
Left Caudate	10R, 10R	20	.30689840	.219204970
	9R,9R	5	.33223600	.090830558
Right Caudate	10R, 10R	20	.12612185	.150995331
	9R,9R	5	.14742200	.056140239
Left VS	10R, 10R	20	.29586395	.227504175
	9R,9R	5	.29411260	.173356512
Right VS	10R, 10R	20	.37410945	.233488450
	9R,9R	5	.22914580	.158715392
Medial OFC	10R, 10R	20	-.05702130	.201724510
	9R,9R	5	-.15937820	.129705602

Overall, poly substance users were expected to have less activity in the caudate nucleus, medial orbital frontal cortex, and ventral striatum, as well as pumping more frequently on the BART than participants not using other substances. This prediction was generally confirmed as significant differences were found in two regions: the left caudate, t(3)= 4.325 p= 0.023 d= 4.994 (0 number of other substances reported: M+SD= 0.60813500 +/- 0.161920382 5 other substances reported M+SD= 0.09750820 +/- 0.109433355and the right ventral striatum, t(3)= 3.638 p= 0.036 d= 4.20 (0 other substances reported M+SD= 0.66755000 +/ 0.225072088 and with 5 other substance M+SD= 0.13002233 +/- 0.118216649. When we analyzed our other brain regions there was no significance found. For the right caudate region t(3)= 1.864 p=0.159 d= 2.152 (0 other substances reported: M+SD= 0.22503650 +/- 0.213466345 and for participants reporting 5 other substances M+SD= 0.00360733 +/- 0.051153754). The left ventral striatum resulted in t(3)= 1.401 p=0.256 d= 1.618 (0 other substances reported: M+SD= 0.49713500 +/- 0.119423264 and for participants with 5 other substances reported M+SD= 0.16713333 +/- 0.304618838). Lastly, we examined the medial orbital frontal cortex and found no significance t(3)= -0.641 p=0.567 d= -0.074. When we examined the scores for the BART, significance was found when participants played the first trial t(1) = 23.054 p= 0.028 d= 46.108 (0 other substances reported M+SD= 43.7700 +/- 0 and when participants reported 5 other substances M+SD= 35.1850 +/- 0.30406). Although there was significance found here, it moves in the direction opposite that of the hypothesis and suggests that participants that reported no substances pump more frequently than those who were classified as polysubstance users in our study. However when participants thought that they were competing against a computer (trial 2), this significant difference disappears, t(1) -1.013 p= 0.496 d= -2.026 (0 other substances:

M+SD= 51.6300+/- 0 and when participants reported 5 other substances: 59.1900 +/- 6.09526). Table 6 illustrates the differences between averages between our genotypes and activity and between our polysubstance users and the pumping frequency on the BART.

Table 6

		t test for Equality of Means		
Number of Substances		t	Mean	Sig. (2-tailed)
BART Base Target	.00	1	43.7700	
	5.00	2	35.1850	.30406
BART Computer Competition Target	.00	1	51.6300	
	5.00	2	59.1900	6.09526
Left Caudate	.00	2	.60813500	.161920382
	5.00	3	.09750820	.109433355
Right Caudate	.00	2	.22503650	.213466345
	5.00	3	.00360733	.051153754
Left VS	.00	2	.49713500	.119423264
	5.00	3	.16713333	.304618838
Right VS	.00	2	.66755000	.225072088
	5.00	3	.13002233	.118216649
Medial OFC	.00	2	-.26624500	.020004051
	5.00	3	-.17199100	.196843077

Discussion

The study examined multiple hypotheses linking risk taking to addiction as a function of DAT allele type.

Hypothesis 1:

Participants that present with DAT 10R allele are more likely to show less caudate nucleus, medial orbitofrontal cortex, and ventral striatum activity while completing the card task than those with DAT 9R.

The study examined the activity in the brain during the receipt of reward during a card task adopted from Delgado et al. (2000). We were specifically interested in the caudate nucleus, which is most responsive to the card task, and we also examined the ventral striatum and medial orbital frontal cortex as they are frequently implicated in reward processing. Table 4 illustrated the coordinates used to capture the most activity region of the brain areas of interest Literature encouraged the examination of the VS and medial OFC as they have either viewed the VS or the PFC. With p values revealing no significance, the mean values of brain activity in the regions examined during the card tasks required noting as they go in the direction of the hypothesis, predicting less activity in with 10R type participants. Specifically the right and left caudate regions have mean values of importance when comparing their directionality of the hypothesis.

That is a result of the caudate region being associated with the most robust activity during the card task. More specifically with the right side expecting more activity than the left side (Wilson et al., 2008) . Finding that it's mean values agree with the hypothesis is valuable and suggest that perhaps future studies could replicate this study using the card task and examining just this brain region. The fact that the average of the medial OFC exhibited decreased activity in participants with both types did not follow our expectations. The literature reported greater activity in the prefrontal cortex when receiving reward for our 9R type participants (Dreher et al., 2008), predicting less activity for participants with 10R. What was actually observed was a decrease in activity. Future literature can examine activity of the medial orbital frontal cortex more extensive as a function of reward.

Hypothesis 2: Participants present with DAT10R allele are more likely to perform poorly on the BART than those with DAT 9R.

As stated above, participants with the 10R allele type are representative of the more expressive gene, which results in more active transport of DA back into the synapse, leaving less DA in the extracellular space (Bilder et al., 2004; Dreher et al., 2008; Hahn et al., 2011 & Yacubian et al., 2007). Although no literature was found to directly measure risk taking with the BART as a function of genotype, prior literature showed the BART accurately distinguished between smokers and non-smokers. In our sample, the BART was unable to predict whether the participants used multiple substances. We were interested in the relationship between the genotype and risk-taking scores; in making this hypothesis that 10Rparticipants would make riskier moves on the BART there is the assumption that there would be some significant difference scores on substance use for these participants to fulfill the compensation idea discussed above. When referring to the analysis in graph 1this supporting the assumption as the means move in the direction of this hypothesis, although no significance was found there was moderate sized effect size. If in fact there was less brain activity than the 9Rtype as predicted in hypothesis 1, we expect the result of less responding to the natural reward of winning money would make participants more likely to partake in a risk-taking task such as the BART. This task is a great measure and accurate indicator of real life risk taking (Moallem & Ray, 2012). Analyzing the data we found no significance. However, when examining the average pumps during the task between genotypes when participants thought they were competing against the computer (refer to table 3), at which the 10R type pumped more frequently on average than the 9R type. Over all this finding seems to mimic more close the risky behavior, gambling, one of the risky behaviors listed early.

Hypothesis 3: Overall polysubstance users are expected to have less activity in the caudate nucleus, medial orbital frontal cortex, and the ventral striatum as well as perform more poorly on the BART by pumping more frequently.

This hypothesis first assumed that our polysubstance participants would be more likely to perform like our 10R genotyped group. This assumption was made based on the idea of there being support for the other 2 hypothesis, if participants with 10R had less brain activity than 9R (hypothesis 1) and they pumped more frequently during the BART (hypothesis 2), then we believed that those participants were less responsive to the natural reward, more responsive to the unnatural reward and thus more inclined to compensating by using more substances. That resulted in the prediction that polysubstance users would behave like the 10R, 10R participants. Although, it has been found that the BART was not a great indicator of polysubstance use

(Lejuez et al., 2003), we found significance for the performance on the task when participants completed the task normally, however the scores were in the opposite direction of the hypothesis. When examining the scores for when participants thought they were competing against the computer, the polysubstance users did in fact pump more frequently on average. That illustrates the similar fining with the 10R participants averages on the BART when they thought they were competing against the computer.

There was also significance found with two regions of the brain (left caudate nucleus and right ventral striatum) in which the brain activity was less in our participants that reported the most number of substances tried in the last 12 months. We also examined 3 additional brain regions, although no p values of significance were found; the left ventral striatum and right caudate nucleus showed brain activity averages that moved in the direction of our hypothesis. In examining the medial orbital frontal cortex scores, illustrated in table 6, noted is an average decrease in brain activity measured during the card task. When examining table 5 it illustrates the mean values of brain activity of participants grouped based on their genotype illustrated there is also an average decrease of brain activity. Much of the literature examined was focused on the caudate nucleus, ventral striatum and the PFC, although there is no direct link to the medial orbitofrontal cortex it was expected to show a similar pattern of activity as the PFC since it lies in that region. While we were interested in examining an increase in activity, less increase was predicted for both the 10R and poly substance users. With the 10R participants there was a smaller negative score meaning the brain activity decreased a lower amount than the 9R (Table 5). Similarly that pattern was found with the participants with more substances reported (refer to table 6). We suspect future work could examine the medial orbitofrontal cortex region.

This study attempted to examine multiple measures and can potentially influence future studies. Perhaps an examination of the relationship between DAT genotype, substance use, and frequency of substances used can be completed. Another study could examine the same tasks with DAT heterogeneous (9R, 10R) subjects who were eliminated from analysis. Specific to the findings with the orbitofrontal cortex, examination of other reward pathway regions require some attention to understand what kind of activity is expected there. In examining the concept of genetics and addiction there are possible benefits linked to establish proper treatment settings for certain types of individual. Understanding if an individual is genetically predisposed to risky behaviors can ultimately establish personalized treatment programs as well propose medication to correct the underlying chemical component examined here and other literature.

Limitations

Over all there was significance noted in the BART scores for both 10R and polysubstance use reported as well as significance found for the polysubstance participants and their brain activation levels. Many of the mean values of the data moved in the direction of the hypothesis but we think our small N may have limited us statistically. There were a limited number of participants and the DAT allele distribution was not symmetrical. This may have impacted our ability to detect this effect. Although we started out with 51 participants, after randomizing the participants who actually volunteered to give DNA and were also not excluded from MRI analysis, the N became 25 for the genotype and brain activity analysis. Also, once participants were randomly assigned to complete the BART or the other task that was not specific to the hypotheses our N for analysis was decreased to a N of just 9. But more importantly as a limitation is the DNA sequencing. Once sequencing of the participants the results for some of the analysis resulted in just one participant with the 9R allele or just one participant with 0

substances reported. These small N values are believed to have limited the analysis of the data. Another limit was that our participants only accurately reported alcohol and while other substance were reported for polysubstance use status we did not have an accurate score of frequency reported. Participants reported how often they drank on a daily or weekly bases, but when comparing other substance use participants were only able to report that they have used other substance over the course of the year but not how often.

Conclusion

According to the National Institute on Drug Abuse, addiction is chronic disease where the individual disregards the health issues that are present as they compulsively seek and abuse drugs. 1 in 8 Americans suffer from either a drug or alcohol problem.7% of Americans are affected by drug abuse and another 2% are affected by a drug addiction. In all of the people associated with drug abuse, 100,000 deaths occur each year in the United States, while tobacco accounts for another 440,000 deaths (Medicine.Net). Despite knowing that all these health risk exist, people continue to experiment with substances, and as a result we witness the struggle for some to quit, while others can successfully try substances and stop. It is certainly significant to continue learning what is so different between individuals that results in such difference in response to drugs. Such an important health topic needs to continue to be studied from the genetic aspect.

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