Research on Neurobehavioral and Physiological Characteristics of Behavioral Addiction

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Section 1: General Introduction

The Current State of Addiction Research

Addiction, in comparison to other mental disorders such as depression, schizophrenia, and autism, has a relatively short history. The recognition that substance abuse is a form of mental illness occurred approximately a century ago. Historically, individuals with addiction were often perceived as morally flawed and lacking in willpower. However, today we understand that addiction is a medical condition that affects the brain and alters behavior (National Institute on Drug Abuse, 2020).

According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013), addiction is defined as a chronic and relapsing disorder characterized by the compulsive seeking and continued use of a substance, despite significant substance-related problems. Excessive consumption of addictive substances directly activates the brain's reward system. Given the functional changes in brain circuits related to reward, stress, and self-control, addiction is considered a brain disorder. These changes can persist for extended periods even after drug use has ceased (Rita Z. Goldstein & Volkow, 2011; National Institute on Drug Abuse, 2020).

In addition to substance use disorders, DSM-5 (American Psychiatric Association, 2013) also recognizes gambling disorder as a form of addiction. This is because, similar to substance abuse, gambling behavior activates the reward system and results in symptoms resembling those seen in substance use disorders. Hence, it is classified as an addictive behavior and treated akin to substance-related disorders (American Psychiatric Association, 2022).

What is Behavioral Addiction?

Similar to gambling disorders, groups of impulse control disorders and obsessive-compulsive disorders have been suggested as possible behavioral disorders that could be considered as addictions, which are referred to as "behavioral addictions" (Grant et al., 2010; Petry et al., 2018; Robbins & Clark, 2015). While there is a growing body of research on conditions like gaming

disorder and internet addiction (S. Kim & Noh, 2019; Moreno-Guerrero et al., 2020; So et al., 2017; Sugaya et al., 2019), evidence for other behavioral disorders being considered as behavioral addictions is not as clear. On the other hand, in clinical practice, conditions such as KM, paraphilia (PP), and shopping addiction are often considered as behavioral addictions. This discrepancy between research and clinical understanding has been highlighted in comprehensive reviews (Petry et al., 2018; Robbins & Clark, 2015). The concept of behavioral addiction is relatively new, and empirical investigations in this area are limited.

To understand the spectrum of behavioral addiction, it is necessary to conduct comparative analyses of various subtypes within this category. While it is suggested that subtypes of impulse control and obsessive-compulsive disorders may potentially be behavioral addictions (Robbins & Clark, 2015), research in these areas is notably limited. There is a distinct paucity of research, both nationally and internationally, on KM and PP.

Impulsivity, a characteristic feature of impulse control disorders, is defined by risky and premature actions, while compulsivity, characteristic of obsessive-compulsive disorders, involves repeating actions despite adverse outcomes. These features are shared by most behavioral addictions, such as gambling addiction and internet addiction, and the role of dopamine in impulsivity-compulsivity disorders is a central focus of the neurobiological basis (Di Chiara & Bassareo, 2007; Grant et al., 2010). Further research is required to comprehensively understand all domains of impulsivity-obsessive-compulsive disorders and their treatments, and it is emphasized that future studies should consider individual differences and genetic factors to enhance the understanding of the complex pathophysiology of behavioral addiction (American Psychiatric Association, 2022).

Traditional addiction research, primarily focused on substance use disorders, has concentrated on elucidating the mechanisms through which substances act on the brain. However, this research comprises two distinct elements: the impact of addictive substances on the nervous system and the physiological responses to environmental cues that trigger drug-seeking behavior (Miyata et al., 2019). While the neurological effects of addictive substances are well-studied, the physiological responses to environmental cues that trigger drug-seeking behavior encompass purely

physiological reactions to environmental stimuli and are analogous to behavioral addiction. In other words, substance use disorders may encompass aspects of behavioral addiction, allowing for a reevaluation of substance use disorders through the lens of behavioral addiction. Therefore, to decipher the essence of addiction, it is essential to focus on physiological reactions triggered by environmental stimuli and conduct a comparative examination of substance use disorders and behavioral addictions.

The Positive Reinforcement Model versus The Negative Reinforcement Model in Addiction

Substance use disorders involves a transition from a state of spontaneous initiation of drug use to a state of compulsive drug use. Different neural circuits are involved in this initial impulsive state and the compulsive state into which one eventually falls. Previous research has shown that the primary neurobiological basis of substance use disorders involves a positive reinforcement in the reward system, primarily driven by cravings (Wise & Robble, 2020) and impulsivity (characterized by poorly thought-out, risky, or inappropriate actions leading to negative outcomes) (Wise & Koob, 2014). However, after compulsive use is established, dopamine is thought to play a less prominent role (Lüscher et al., 2020). Additionally, there are instances, such as in cases of benzodiazepine dependence, where the involvement of pleasure as an emotion is not evident (Lüscher & Ungless, 2006). In situations where substances are abused without directly generating feelings of euphoria, a more compelling explanation can be provided through a negative reinforcement, associated with alleviation of pain (Kosten et al., 1998). Both the positive and negative reinforcement models of addiction have been discussed for decades, and while both are not in conflict, however, how each interacts and contributes to addiction has yet to be settled (Wise & Koob, 2014).

Wise states that the incentive salience provided by positive reinforcement is important for addiction. He explains that addiction can be explained by two types of brain changes: the drug-generated sensitization of anti-reward, and the brain and peripheral changes caused by the memory of the initial euphoric drug experience and the resulting behavioral choice to self-administer. (Wise & Koob, 2014). He argues that the emergence of memories for the latter drug experience is the more

important difference between addicted and non-addicted brains (Wise & Koob, 2014). In other words, it is the memory of the initial euphoric drug experience that may trigger drug craving and compulsive drug seeking. The hypothesis is that intense experiences remembered with rewards are positive reinforcers and shape conditioning learning. This is supported by the fact that in smokers, the primary stimulus for craving is not how long the smoker has not smoked, but when the next cigarette is available (Dar et al., 2010), and in alcoholics, visual stimuli stimulate craving (Ghiţă et al., 2019).

On the other hand, Koob argues that the combination of impaired reward neurotransmitter function and mobilization of the anti-reward system is a powerful source of the negative reinforcement that defines compulsive drug-seeking behavior and addiction. The central concept of the negative reinforcement model suggests that a major driving force behind addiction is the avoidance and escape from negative affects. This model implies that the primary motivator for continued drug use and relapse is not the pursuit of the pleasurable affects of the drug but rather the alleviation or avoidance of negative states such as withdrawal symptoms and emotional distress. Initially, individuals may engage in drug use seeking positive pleasurable affects. However, with continued use, the focus shifts from seeking pleasure to avoiding the discomfort associated with not using the drug. This transition is supported by changes in brain function resulting from chronic drug use. These changes lead to a state where drug use behavior is reinforced as a means to escape or alleviate these negative experiences caused by the absence of the drug (Ahmed & Koob, 2005; Baker et al., 2004). Koob describes addiction as a cycle with three stages: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. Over time, these stages intensify and involve both impulsivity and compulsivity, as well as positive and negative reinforcement. He suggests that these stages interact and intensify, leading to a pathological state of addiction where negative reinforcement becomes dominant (Wise & Koob, 2014).

However, these claims are based specifically on substance use disorder, and it is unclear whether positive and negative reinforcement are similarly involved in behavioral addiction. In the case of behavioral addiction, homeostatic changes due to chronic drug use should not occur, so

negative reinforcement aimed at avoiding eventual withdrawal symptoms, as Koob has pointed out (American Psychiatric Association, 2022; Lüscher & Ungless, 2006), is unlikely to occur. Positive reinforcement is also unlikely to occur as well, given that the drug does not act to directly increase extracellular dopamine levels as it does (American Psychiatric Association, 2022; Lüscher & Ungless, 2006). In this thesis, chapters 2 and 3 were conducted to obtain clues about positive and negative reinforcement in behavioral addiction. In chapter 2, I examined negative affects in behavioral addiction as a cue for negative reinforcement, and in chapter 3, I presented images of conditioned stimuli in order to get evidence of positive reinforcement in KM.

Dopamine Dynamics in Substance Use Disorders

Addictive Substances, such as alcohol, nicotine, cocaine, amphetamines, and cannabinoids, directly or indirectly affect the dopamine system (Lüscher & Ungless, 2006). For instance, cocaine, which directly impacts the dopamine system, acts as an antagonist to the dopamine transporter (DAT), particularly inhibiting dopamine reuptake in the nucleus accumbens, thereby increasing dopamine release (Pifl et al., 1995). Similarly, amphetamines reverse the reuptake mechanism of DAT, causing the release of dopamine from inside the cell (Pifl et al., 1995). Dopamine plays a central role in addiction (American Psychiatric Association, 2022), and it is believed that the breakdown of prediction error is integral to the formation of addiction (Berridge, 2007).

Dopamine neurons are located in the midbrain, primarily in two regions: the substantia nigra pars compacta and the ventral tegmental area (VTA) (Haber, 2014; Haber & Knutson, 2010). Dopamine neurons in the substantia nigra pars compacta project to the dorsal striatum and are involved in motor control (Graybiel et al., 1994). Parkinson's disease, a neurodegenerative disorder, results from the loss of dopamine neurons in this region, leading to impaired motor control (Agid, 1991). On the other hand, dopamine neurons in the VTA project mainly to areas including the hippocampus, amygdala, ventral striatum (including the nucleus accumbens), and prefrontal cortex (Haber, 2014; Haber & Knutson, 2010), where they play roles in cognitive and emotional functions (Le Moal & Simon, 1991). Dopamine, like serotonin and norepinephrine, is a monoamine

neurotransmitter that modulates excitatory neurotransmission via glutamate and inhibitory neurotransmission via gamma-aminobutyric acid (GABA) (Jay, 2003).

The relationship between dopamine and reward was first established by Olds and Milner in 1954 (Olds & Milner, 1954). They implanted electrodes into the medial forebrain bundle, which corresponds to axon fibers of dopamine neurons in the ventral tegmental area, in rats. When rats were allowed to press a lever in an operant chamber and receive electrical stimulation, they repeatedly pressed the lever, suggesting that this brain region was associated with pleasure. In 1957, Carlsson demonstrated that dopamine were produced at the stimulation site and works as neurotransmitter (Carlsson et al., 1957).

In 1986, Schultz discovered that in macaques, the firing of dopamine neurons represented the probability of such rewards (Schultz, 1986). Dopamine neurons have a low spontaneous firing rate when no stimulus is present (4-5 Hz). When unexpected rewards are presented, high-frequency burst firing (20-100 Hz, 2-5 spikes) is induced. When stimuli predicting rewards are presented along with the rewards themselves, burst firing transitions from the rewards to the stimuli predicting the rewards. After burst firing begins in response to reward-predicting stimuli, it is temporarily suppressed if the expected reward is not delivered.

Most midbrain dopamine neurons rapidly activate in response to unexpected food or liquid rewards, coding for the difference between the received and predicted reward value; they show positive responses for better-than-expected rewards, no response for fully predicted rewards, and negative responses for worse-than-expected rewards, similarly reacting to stimuli that predict rewards (Schultz, 2013). Dopamine neuronal activities representing reward prediction error are encoded by dopamine release in regions where dopamine neurons project, including the nucleus accumbens. The prediction of rewards is coded temporally through a transient increase in dopamine release, resulting from burst firing of dopamine neurons responsive to reward prediction, and a temporary decrease in tonic background dopamine levels in the absence of predicted rewards (Anthony A. Grace et al., 2007). The tonic release of dopamine is complementarily regulated by the dopamine transporter (DAT) located on the presynaptic terminals of dopamine axons and the

dopamine D2 receptors, which are autoreceptors situated on the dopamine neurons themselves. DAT is abundant within the synapse and rapidly reuptakes released dopamine back into the synaptic terminal, thereby regulating the amount and duration of release (A. A. Grace, 1991). On the other hand, the amount of dopamine released during phasic neuronal firing is usually higher over a short period, and while some may diffuse away, the primary mechanism for terminating its action is reuptake by DAT (A. A. Grace, 1991). Consequently, the extracellular concentration of dopamine is maintained at a stable level. Autoreceptors regulate dopamine levels by modulating the intensity of their signaling. Increased tonic levels of dopamine enhance autoreceptor signaling, which in turn reduces dopamine synthesis and release, leading to a decrease in phasic dopamine release at synapses. Conversely, reduced tonic dopamine levels weaken autoreceptor signaling, increasing dopamine synthesis and, consequently, enhancing dopamine release at synapses. In brain regions where dopamine neurons project, such as the amygdala and nucleus accumbens, the mechanism of tonic/phasic dopamine release converts reward prediction errors into emotional responses. Specifically, positive reward prediction errors (observed rewards greater than predicted) produce a feeling of pleasure (A. A. Grace, 1991), while negative reward prediction errors (predicted rewards greater than observed) lead to unpleasant feelings (Diederen & Fletcher, 2021).

Research on substance use disorders has shown that repeated excessive dopamine release due to drug use results in homeostatic changes, leading to a decrease in tonic background dopamine levels (Volkow et al., 2017). Consequently, the balance of phasic/tonic dopamine release is disrupted in substance intoxication. Excessive phasic dopamine release is directly linked to intense pleasure and facilitates the memorization and conditioning of behaviors that lead to such intense pleasure. As a result, individuals with substance use disorders exhibit impulsive and compulsive drug-seeking behavior and cravings (A. A. Grace, 2000). On the other hand, negative reward prediction errors, which occur when expected rewards are absent or aversive stimuli are presented, are transmitted via temporary reductions in tonic dopamine levels. In addiction, however, tonic dopamine concentrations are reduced, potentially leading to a weakening of negative reward prediction errors. For example, in Parkinson's disease, the degeneration of dopamine neurons results in a decrease in

the overall amount of dopamine (i.e., a decrease in both phasic dopamine release and tonic dopamine levels) (Agid, 1991). Patients with Parkinson's disease have a higher comorbidity rate of addiction, and it has been suggested that the lack of anhedonia (a decrease in tonic dopamine) rather than positive conditioning (phasic dopamine release) may trigger addiction (van Eimeren et al., 2009). Patients with Parkinson's disease may exhibit changes in reward processing, but the relationship between Parkinson's disease and addiction is complex and involves multiple factors beyond dopamine depletion. The lack of dopamine, and hence potential anhedonia, may contribute to this complexity, but it is not the sole factor.

Tonic and Phasic Dopamine Dynamics in Behavioral Addiction

Until now, it has been unclear why compulsive behaviors repeat without the pharmacological action of substances affecting the central nervous system and why some individuals become addicted while others do not. However, I would like to hypothesize as below, based on the decrease in tonic dopamine levels.

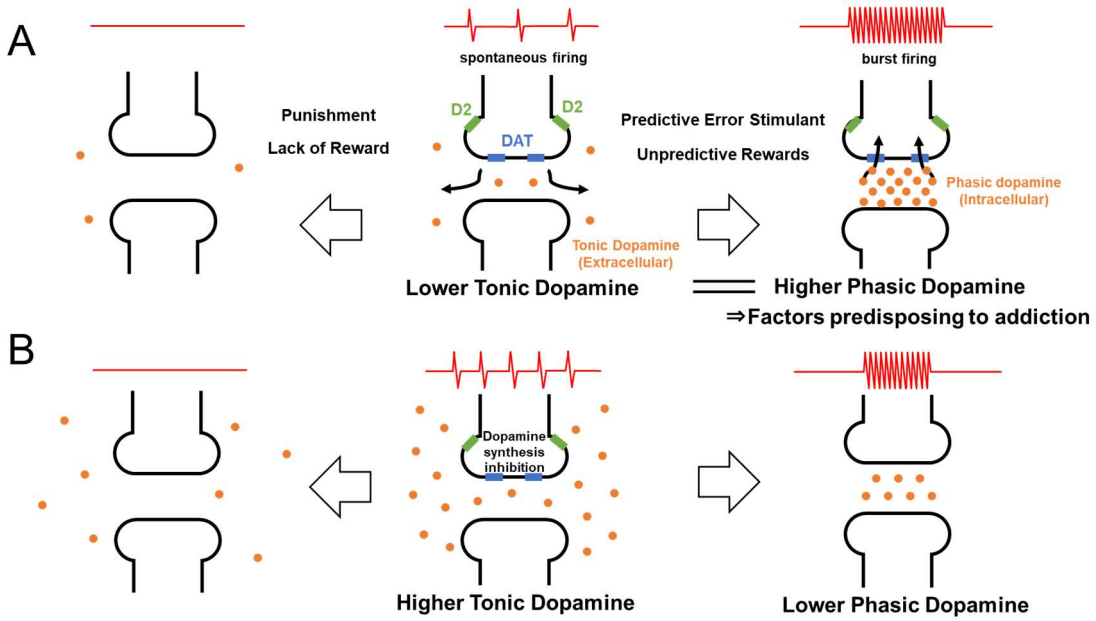
From previous research on substance use disorders, it has been observed that repetitive excessive dopamine release due to drug use leads to homeostasis, resulting in a decrease in tonic background dopamine levels. Conversely, individuals with congenital or other factors leading to low Tonic dopamine levels have relatively large phasic dopamine releases. Consequently, positive affects from rewards become more significant, and they become more susceptible to conditioning. Therefore, those with chronically low tonic dopamine levels may experience heightened phasic dopamine release in response to specific behaviors, leading to conditioning similar to substance use disorder, and repetitive compulsive behaviors may occur without the pharmacological action of substances.

In impulse control disorders like KM and PP, as discussed in the chapter 2, the presence of high negative affects, such as stress and depression, may indicate low tonic dopamine levels. This decrease in tonic dopamine levels can increase phasic dopamine release. It may also reduce the unpleasant sensation of loss, making individuals less susceptible to aversive learning. Generally,

both substance use disorder and behavioral addiction patients tend to experience high stress and depressive states. Stress and depressive states lower tonic dopamine levels, making individuals chronically low in tonic dopamine levels and prone to addiction.

Therefore, individuals with behavioral addiction may exhibit compulsive and impulsive behaviors due to chronically low tonic dopamine levels, leading to enhanced phasic dopamine release and increased susceptibility to conditioning. This may explain why compulsive behaviors repeat in the absence of pharmacological substances, similar to substance use disorders.

Figure 1 Schematic representation of the encoding of reward prediction error by Tonic/Phasic dopamine.



(Adapted from Asaoka, 2020)

Dopamine neurons exhibit spontaneous firing, resulting in the release of low levels of dopamine. In low-level dopamine release, dopamine transporters actively reuptake the released dopamine, maintaining background dopamine concentrations (tonic dopamine). The concentration of tonic dopamine is proportional to the number of dopamine cells that exhibit spontaneous firing (Marinelli & McCutcheon, 2014). When the percentage of cells exhibiting spontaneous firing increases to 70%, the concentration of tonic dopamine increases accordingly. Conversely, if the percentage of cells exhibiting spontaneous firing decreases to 30%, the Tonic dopamine concentration decreases. (A) depicts a state with low tonic dopamine concentration. In such a state, the presentation of stimuli predicting rewards or unexpected reward outcomes leads to the release of intense phasic dopamine, resulting in robust conditioning. Conversely, the temporary cessation of spontaneous firing due to punishment or the absence of expected rewards leads to a minor reduction in Tonic dopamine concentration, making individuals less sensitive to negative rewards (Mangiavacchi et al., 2001). This state is considered to be more prone to addiction.

In contrast, in (B), tonic dopamine concentration is high. Tonic dopamine, through autoreceptors (D2 receptors) located extrasynaptically, suppresses dopamine synthesis to prevent excess dopamine production. Consequently, the release of phasic dopamine in response to positive rewards is regulated, and the reduction in tonic dopamine concentration is more pronounced. Therefore, individuals in this state exhibit heightened sensitivity to negative rewards.

Challenges in Behavioral Addiction Research

Due to the lack of appropriate animal models for behavioral addiction research, there is an urgent need to emphasize the importance of investigating the underlying causal mechanisms of behavioral addiction. Despite its significance, such research has been relatively scarce. Exploring the commonalities between behavioral addiction and substance use disorders is essential for understanding the essence of addiction. To comprehend the spectrum of behavioral addiction, it is necessary to compare the symptoms of various behavioral addictions.

The challenges in behavioral addiction research are as follows:

- Impulse control disorders and obsessive-compulsive disorders have been suggested as subtypes of behavioral addiction (Robbins & Clark, 2015), but research in these areas is significantly limited. Particularly, the scarcity of research on KM and PP is evident both domestically and internationally.
- Since it is unclear whether negative reinforcement is involved in the mechanism of behavioral addiction, it is necessary to quantify negative affect and get clues about negative reinforcement in behavioral addiction.
- To confirm the positive reinforcement model in behavioral addiction, focusing on physiological responses triggered by environmental stimuli is crucial to definitively elucidate the mechanisms of addiction.
- If behavioral addictions are similar to substance use disorders, then monoamines in behavioral addictions need to be measured, as they are thought to be abnormal in the transmission of dopamine and other monoamines. Considering genetic factors should be imperative in future research to enhance the understanding of the complex pathophysiology of behavioral addiction, including impulse-control and obsessive-compulsive aspects.

The primary aim of this study is to investigate behavioral addictions, with a specific focus on KM and PP, which have not been extensively studied, and to elucidate the comprehensive nature of

behavioral addiction by comparing its symptoms with more established disorders like gambling addiction and substance use disorders. Substance use disorders have been widely researched, and their neurobiological foundations are increasingly being understood, thanks in part to the availability of animal models (Sora et al., 2009; Wise & Robble, 2020). By examining behavioral addictions, this study aims to reveal more distinct physiological features of behavioral addiction. In particular, it will focus on negative and positive reinforcement model, and monoamine neurotransmission, including dopamine. Experiments will also include epigenomic research to explore genetic factors.

Section 2: Heightened negative affects associated with neurotic personality in behavioral addiction

Introduction

Behavioral addiction (BA) is a psychiatric condition with an intense desire to repeat an action that is rewarding or alleviating distress, despite negative consequence (Grant & Chamberlain, 2014; Grant et al., 2010; Petry et al., 2018; Robbins & Clark, 2015). BA is hence characterized by impulsive initiation of an action and subsequent development of compulsive seeking of the action (Grant & Chamberlain, 2014; Grant et al., 2010; Petry et al., 2018; Robbins & Clark, 2015). Owing to such characteristics, impulse control disorders, such as KM and compulsive sexual behavior, are thought to meet the criteria of BA, and thereby often considered as this category of disorder (Cuzen & Stein, 2014; Derbyshire & Grant, 2015; Grant, 2006; H. S. Kim et al., 2017). Compulsive sexual behavior is excessive or uncontrolled sexual behaviors or thoughts that are either nonparaphilic or paraphilic (PP), whereas KM is characterized by repetitive, uncontrollable stealing of items for unintended personal use. Although the 5th version of Diagnostics and Statistical Manual of Mental Disorders (DSM-5) has included pathological gambling in the "Substance Related and Addictive Disorder" as a prototypical BA, KM and PP have remained within the category of disruptive, impulse control, and conduct disorders (American Psychiatric Association, 2013; Grant & Chamberlain, 2016), primarily due to insufficient studies on KM and PP. Further investigations are required to establish the concept and definition of BA.

Affective disorders are one of predominant components in a range of psychiatric disorders, and negative affects, such as anxiety, depression, and stress, are subjected for treatment interventions (Derntl et al., 2012; Hägele et al., 2016). Accumulating evidence suggests that alterations of negative affects play important roles in addiction (Baker et al., 2004; Cheetham et al., 2010). In drug addiction, depressive and anxiety disorders are frequently co-morbid (Back & Brady, 2008; G. F. Koob, 1996; Kosten et al., 1998). On the other hand, withdrawal symptoms also facilitate negative emotional arousals, such as anxiety and depression, in drug addicts (G. F. Koob, 1996). Stress plays critical roles in drug addiction. For instance, stress could be a risk factor for not only urges of

symptoms but also subsequent relapse after treatments (George F. Koob et al., 2014; Sinha, 2007). Impulse behavior has been shown to develop in response to stress due to deficits in regulatory control over emotional and motor-related behaviors (George F. Koob et al., 2014; Lemieux & al'Absi, 2016). Drug addicts with co-morbidity of posttraumatic stress disorder exhibit stronger form of impulsive behavior, including aggression (Weiss et al., 2017). Accumulating evidence suggests that BA is similarly associated with negative affects, such as depression and anxiety, independent of symptom types (Akin & İskender, 2011; Blaszczynski & McConaghy, 1989; Starcevic & Khazaal, 2017).

Affects have been demonstrated to have tight relationships with personality (A. A. Augustine & Larsen, 2015). The five-factor model of personality, or the Big Five personality traits, explains that the personality can primarily be divided into five basic dimensions of traits, i.e., extraversion, conscientiousness, agreeableness, neuroticism, and openness (Tupes & Christal, 1992). Subjects with a strong neurotic personality trait exhibit the higher level of negative affects, such as anxiety, stress, and depression, whereas a lower level of neuroticism is associated with better emotional regulations (Barańczuk, 2019; McNiel & Fleeson, 2006). Accordingly, neuroticism has quite often been associated with higher susceptibility of psychiatric disorders (Lahey, 2009; Ormel et al., 2013). Although inconsistent, studies have also shown that individuals with BA exhibit personality traits associated with high anxiety, aggression, and neuroticism (Andreassen et al., 2013; Zilberman et al., 2018). However, affective disorders in BA has remained less explored and less understood than drug addiction to date.

Considering these previous studies demonstrating higher negative affects in addiction in both drug addiction and BA, negative affects may be the important attribute of addiction in general. In this study I investigated whether negative affects were also higher in patients diagnosed with BA, which primarily consisted of KM and PP, than healthy people, and whether associations between negative affects and personality were observed in BA to provide a better appraisal of this psychiatric condition.

Methods

Subjects

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by Japanese Ministry of Health, Labour and Welfare. All experimental procedures were approved by the Human Research Ethics Committee of Kyoto University Primate Research Institute (2019-01), and the Ethics Committee of Kyowa Hospital (Heisei 30-015). Written informed consents were obtained from all participants in advance of experiments. Sixteen hospitalized patients diagnosed with BA (20–72 years old; 56% males and 44% females) who were divided into gambling (n = 1), kleptomania (KM; n = 10) and paraphilia (PP; n = 5) were recruited. As a control (CT) group, 31 healthy adult subjects (18–58 years old; 41% males and 59% females) without a history of psychiatric disorder and smoking in the past 6 months were recruited. Subjects whose full-scale intelligence quotient (FIQ) was estimated below 60 and/or who were unable to understand instructions for the tests were excluded from the study. For 7 of the 31 CT participants, who were students of Kyoto University, homone assay and HAM-A data were not collected, due to lack of access to hospital resources.

Questionnaires

First, I conducted questionnaire survey of negative affects in hospitalized BA patients. Three questionnaire surveys were conducted, of which two were aimed to assess negative affects, and one was for personality traits, respectively, of participants. All questionnaires were in the Likert format and were translated in Japanese from the original English versions. In this study, the Japanese version of questionnaires were used, all of which have been validated with large sample sizes of Japanese population. Negative affects were evaluated using the 21-item version of depression anxiety stress scale (DASS21) and Hamilton anxiety rating scale (HAM-A). DASS21 is a well-established, self-report questionnaire designed to measure symptoms of depression, anxiety, and stress, in both clinical and non-clinical samples of adults (Lovibond & Lovibond, 1995). DASS-21

available at http://www2.psy.unsw.edu.au/dass/Japanese/Japanese.htm was translated by John Naaykens, the certified school psychologist, and tested at multiple higher educational institutes in Japan. HAM-A is a widely used 14-item clinician-administered rating questionnaire to measure severity of anxiety symptoms in adults (Hamilton, 1959). The Japanese version of HAM-A is commercially available, and has been widely utilized in medical institutes, along with one of recent validation studies by Otsubo and colleagues (Otsubo et al. 2005). In this study, the medical attendant who was taking care of recruited BA patients scored this questionnaire for BA patients and a part of CT subjects, who were co-workers of the medical attendant (n = 24). To provide an insight on why negative affects were higher in BA patients, I investigated whether trait personality of subjects might be associated with negative affects using the Japanese version of ten item personality inventory (TIPI-J). TIPI-J is a brief questionnaire measuring Big Five personality traits; extraversion, agreeableness, conscientiousness, neuroticism (emotional instability), and openness (Gosling et al., 2003; Oshio et al., 2012). The questionnaire comprises 10 items, with 2 items each for assessing one personality trait (one item for the positive pole and the other for the negative pole). Participants self-rated how each trait applied to themselves using a seven-point Likert scale. TIPI-J has been developed and validated in the study by Oshio and colleagues (Oshio, Abe, and Cutrone 2012). These have now been described in the revised manuscript. Since affects are often processed unconsciously (Sampson & Davis, 1988), it is possible that explicit, self-referential assessments of negative affects with the questionnaire may not accurately reflect the states of negative affects in participants. Thus, I further evaluated negative affects of BA patients and CT subjects with objective measurements. For BA patients, this questionnaire was also rated by the medical attendant to evaluate a consistency between self- and objective-rating.

Stress Hormone Assay

I further evaluated such self-reported data of negative affects by conducting objective assessments, in which rating of negative affects was conducted by others and blood stress hormone assay with enzyme-linked immunosorbent assay (ELISA) to examine consistency between self-

referential and objective rating of negative affects. Stress can be quantified to some extent by cortisol concentrations (Brown et al., 2016; Hellhammer et al., 2009). Cortisol concentrations in whole blood samples obtained from participants were measured with ELISA to implicitly estimate stress level. Blood samples were collected from BA patients around the noon (11:00–11:30) and from CT subjects in the afternoon (between 15:00 and 17:00) 1 day prior or on the day of other tests and were stored in the freezer at −30°C until the days of processing for ELISA. Sample processing was conducted using a commercially available human ELISA cortisol assay kit from Arbor Assays (catalog no. K003-H1/H5) according to the manual. After processing, ELISA plates were read using iMark microplate Reader (Bio-rad, Hercules, CA).

Data Analysis

Investigators who were not blinded to the experimental conditions collected the data for statistical analyses. All statistical analyses were conducted using Statistica software (StatSoft, Tulsa, OK, USA). A probability value of $p < 0.05$ was considered as statistical significance. Statistical analysis of the data was conducted using parametric tests. When ANOVA was used for analysis, Tukey test was conducted for post-hoc pair wise comparison. Linear correlation and multiple regression analyses were employed to examine correlations between different assessments.

Results

Self-Referential Negative Affects

Explicit, self-referential negative affects, such as anxiety, stress, and depression, were assessed with DASS21 (Lovibond & Lovibond, 1995). Two-way ANOVA with post-hoc pair-wise comparison revealed that all of stress ($F_{1,135}$ = 46.8, $p < 0.001$ in Group; $F_{2,135}$ = 6.89, $p = 0.001$ in Facet; $F_{2,135}$ = 0.572, $p = 0.568$ in interaction; post-hoc Tukey test, $p = 0.010$ in BA vs. CT; Figure 1A and Table 1), anxiety ($p = 0.003$ in BA vs. CT; Figure 1A and Table 1), and depression ($p < 0.001$ in BA vs. CT; Figure 1A and Table 1) scales were higher in BA than CT subjects. When BA patients were separately analyzed into KM and PP patients, although an overall trend of difference was observed between KM and PP patients, there was no statistically significant difference in any scales ($F_{1,39}$ = 3.76, $p = 0.060$ in group; $F_{2,39}$ = 1.14, $p = 0.330$ in facet; $F_{2,39}$ = 0.340, $p = 0.714$ in interaction; Figure 1B). These results suggest that BA patients were recognizing higher negative affects than CT subjects.

Objective Rating of Negative Affects and Correlation With Self-Assessments

HAM-A (Hamilton, 1959) was conducted by the medical attendant who was taking care of BA patients during hospitalization. HAM-A was also conducted by the same medial attendant for a part of CT subjects who were co-workers of the medical attendant (n = 24). HAM-A data were acquired for only these 24 subjects out of whole CT subjects (n=31). Consistent with the self-rating in DASS21, higher anxiety was rated for BA patients than CT subjects in HAM-A (unpaired t-test, t_{38} = 5.27, $p < 0.001$; Figure 2A and Table 1). Although KM patients tended to receive higher scores in this questionnaire than PP patients, this did not reach statistical significance (t_{13} = 1.91, $p = 0.078$; Figure 2B).

In CT subjects, positive correlations between HAM-A scores and each of stress ($r = 0.437$, $p = 0.033$; Figure 2C) and anxiety ($r = 0.418$, $p = 0.042$; Figure 2C), but not depression, scales in DASS21 were observed. In contrast, in BA patients, there was a positive correlation between HAM-

A scores and depression (r = 0.594, p = 0.015; Figure 2C), but not other, scales in DASS21. These results suggest that anxiety was higher in BA patients than that in CT subjects in the objective assessment. Note that, however, the self-referential negative affect scores were well correlated ($r \geq 0.3$) with the objective-rating scores for the CT subjects, but the correlation was found only in the Depression score for the BA subjects.

Stress Hormone Assay and Correlation With Self-Assessments

Basal blood cortisol concentrations in BA patients were significantly higher than those in CT subjects ($t_{38} = 2.46$, p = 0.019; Figure 3A and Table 1). Cortisol concentrations were not different between KM and PP patients (Figure 3B). Cortisol concentrations of BA patients were positively correlated with stress (r = 0.586, p = 0.017; Figure 3C) and anxiety (r = 0.806, $p < 0.001$; Figure 3C), but not depression, scales in DASS21, whereas none of these scales in DASS21 was correlated with cortisol concentrations in CT subjects (Figure 3C). In BA patients, a pattern of correlations between DASS21 scores and stress hormones was maintained, albeit significantly weaker, with a statistically significant correlation observed only for anxiety (r = 0.576, p = 0.025; Figure 3C). This pattern persisted even when both including and excluding an outlier that fell within the mean ± 3 standard deviation range. These results suggest that, consistent with self-assessments, basal blood cortisol concentrations supported higher stress level in BA patients than CT subject. Moreover, correlations between self-referential negative affects and stress hormone concentrations were observed in BA patients but not in CT subjects.

Personality Traits and Associations With Negative Affects

In TIPI-J, BA patients self-rated their own personality traits significantly different from those of CT subjects ($F_{1,225} = 0.016$, p = 0.901 in group; $F_{4,225} = 4.80$, p = 0.001 in trait; $F_{4,225} = 5.38$, $p < 0.001$ in interaction; Figure 4A and Table 1). Post-hoc pair-wise comparisons revealed that neuroticism was marginally significantly higher in BA patients than CT subjects (p = 0.080; Figure 4A and Table 1). No statistically significant difference in any personality traits was observed

between KM and PP patients (Figure 4B). Correlations (Pearson's r) of each personality trait and assessments of negative affects are illustrated in Figure 4C. In this analysis, positive correlations were observed between neuroticism and negative affects both in BA patients and CT subjects, with the correlations slightly stronger in BA patients than CT subjects. Moreover, negative correlations were observed between negative affects and two personality traits, agreeableness and openness, in BA patients, but not in CT subjects. However, these negative correlations seems not accurately reflect the associations between these personality traits and negative affects, given that BA patients rated themselves significantly higher agreeableness and openness than those in objective personality evaluation of BA patients given by the medical attendant (two-way ANOVA with repeated measures; $F_{4,75} = 4.44$, $p = 0.003$ in trait; $F_{1,75} = 8.80$, $p = 0.004$ in group; $F_{4,75} = 4.39$, $p = 0.003$ in interaction; post-hoc Tukey test, $p = 0.015$ and $p = 0.035$ in self- vs. objective-rating for agreeableness and openness, respectively; Figure 4D). Such over-rating of their own personality traits and consequent emergence of negative correlations with negative affects are reminiscent of cognitive bias in self-recognition. Collectively, these results suggest that higher neurotic personality trait may account for higher negative affects in BA patients.

Discussion

In this study, I have shown that negative affects, such as stress, anxiety, and depression are higher in BA patients than CT subjects, which are relatively consistent between self-referential and objective assessments. Moreover, such heightened negative affects in BA patients are associated with the stronger neurotic personality.

There are several major limitations in the current study. The most crucial limitation is a sample size. In this study, only 16 BA patients were recruited for assessments, including several association analyses. In addition, BA patients in this study were heterogeneous, divided primarily into KM and PP. Although, in all measurements, negative affects tended to be lower in PP patients than KM patients, none of them reached statistically significant difference, which is most likely due to especially the small sample size in PP patients (n = 5). Comparisons between KM and PP patients with a larger sample size will be required for the future study to further characterize whether there is difference of negative affects, depending on symptom types. I have recently shown using the genome-wide methylation analysis that DNA methylation status is different between KM and PP patients (Section 4).

Previous studies have reported that negative affects are heightened in BA, such as pathological gambling and Internet addiction (Akin & İskender, 2011; Blaszczynski & McConaghy, 1989; Starcevic & Khazaal, 2017). Thus, my study extends these previous studies by demonstrating that heightened negative affects are also observed in other BA, such as KM and PP. Collectively with heightened negative affects in drug addiction (Baker et al., 2004; Cheetham et al., 2010), such heightened negative affects are critical and mutual characteristics involved in addiction.

There is also a novel finding in the current study, which is different from the previous studies investigating negative affects in behavioral addiction. Assessments of negative affects in previous studies are primarily based on self-reports (Akin & İskender, 2011; Blaszczynski & McConaghy, 1989; Starcevic & Khazaal, 2017), whereas I examined negative affects with both self-referential and objective assessments in this study. Consequently, correlations were found between

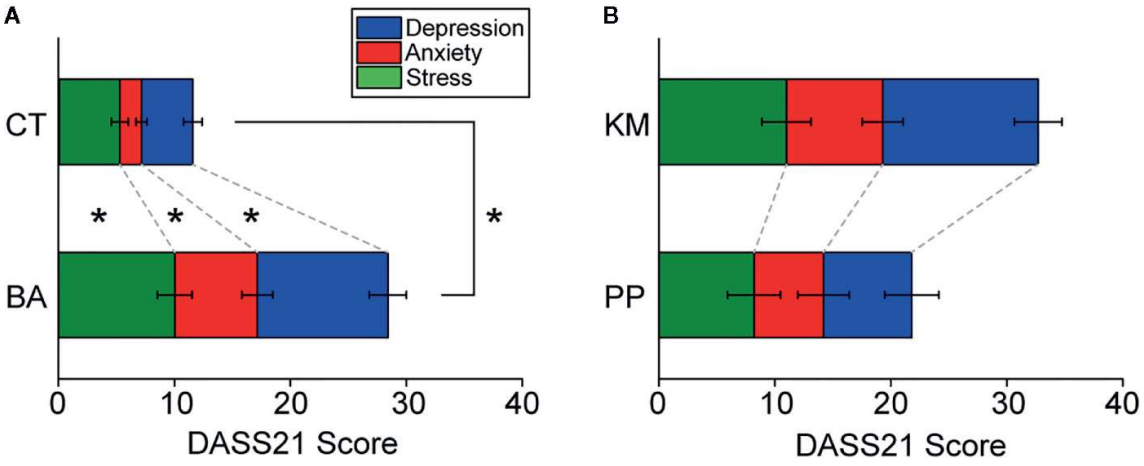
negative affects and some of personality traits, such as openness and agreeableness, in BA patients, but not CT subjects. Moreover, the objective evaluations of personality traits for BA patients were substantially different from self-rating in openness and agreeableness. On the other hand, the self-assessment and objective evaluation of BA for Neuroticism, conscientiousness, and extraversion were consistent. The BA patients overestimated some aspects of their own personality traits. This overestimation might be related to their negative affects. Such associations are reminiscent of Dunning-Kruger effects, the cognitive bias on self-recognition, that poor self-recognition and low cognitive ability lead people to overestimate their own capabilities (Kruger & Dunning, 1999). Cognitive bias is under the stringent relationship with affects, and emotional states distort cognitions and decision-making (Harding et al., 2004; Kircanski et al., 2012). Therefore, heightened negative affects may augment cognitive bias including the bias on self-recognition in BA. Associations of personality traits with BA have been examined in the previous studies, but the findings are inconsistent (Andreassen et al., 2013; Zilberman et al., 2018). Such inconsistency may partly be explained by this self-recognition bias.

In this study, positive correlations were observed overall between subjective assessment of negative affects with DASS21 and objective assessments of anxiety with HAM-A and with a stress hormone, cortisol in both BA patients and CT subjects; however, there were some exception in CT subjects. In the CT subjects, all the three scales of DASS 21 had correlation with HAM-A score, whereas only the Depression score was correlated with HAM-A score in BA patients. One of reasons for such limited correlations may be an involvement of cognitive bias, such as Dunning-Kruger effects (Kruger & Dunning, 1999). Another reason would be that DASS21 and HAM-A assess quite different aspects of anxiety. There was no significant correlation between HAM-A scores and cortisol level in BA. Thus, HAM-A assesses anxiety more heavily based on somatic aspects, such as respiratory, cardiovascular, and gastrointestinal symptoms (Hamilton, 1959), whereas DASS21 contains only some somatic assessment items (Lovibond & Lovibond, 1995). For cortisol assay, blood sampling was conducted different time windows of the day between BA patients (around the noon) and CT subjects (afternoon). Previous studies have shown daily fluctuations of salivary and

serum cortisol concentrations, which are higher in the morning or soon after awaking, and rapidly decline before noon, and then keep slightly decreasing towards the evening (Ljubijankić et al., 2008; Ross et al., 2014). Higher cortisol concentrations in BA patients than CT subjects may partly be due to such different timing of sampling between them; however, this does not explain better correlations with negative affects assessment with DASS21 in BA patients than CT subjects. Overall, the correlations between DASS21 and HAM-A were more accurate in CT subjects than BA patients, while those between DASS21 and cortisol assay were better in BA patients than CT subjects, but the exact reason for these specific patterns remains unclear..

In conclusion, this study has shown heightened negative affects, such as stress, anxiety, and depression in BA patients, which is consistent with heightened negative affects in drug addiction. Such heightened negative affects are also associated with the stronger neurotic personality trait, suggesting that the neurotic personality may be one of risk factors for BA. A further study with a larger sample size of BA patients will be required to confirm whether these findings can ideally still be retained, to further clarify the characteristics of the subcategories of the BA (i.e., KM and PP).

Figure 1



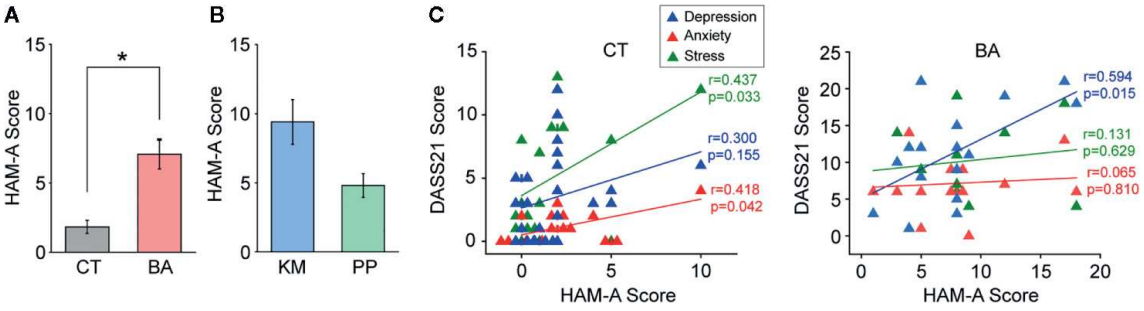
Self-assessment of negative affects with 21-item version of depression anxiety stress scale (DASS21). (A) A stacked bar graph showing scores in stress, anxiety, and depression scales of DASS21, respectively, in behavioral addiction (BA) patients and control (CT) subjects. Error bars indicate s.e.m. *$p < 0.05$. (B) A bar graph similar to (A) but showing those in kleptomania (KM) and paraphilia (PP) patients.

Table 1. **A summary of results for DASS21, HAM-A, cortisol assay, and TIPI-J in BA patients and CT subjects.**

		BA	CT
DASS21	*Stress*	10.0 ± 1.49*	5.23 ± 0.73
	Anxiety	7.13 ± 1.34*	1.87 ± 0.47
	Depression	11.3 ± 1.59*	4.42 ± 0.81
	Total	28.4 ± 3.89*	11.5 ± 1.69
HAM-A		7.69 ± 1.17*	1.83 ± 0.47
Cortisol (pg/mL)		408 ± 52.2*	278 ± 25.9
TIPI-J (self-rating)	*Extraversion*	7.31 ± 0.60	9.32 ± 0.45
	Agreeableness	9.19 ± 0.79	10.8 ± 0.36
	Conscientiousness	8.19 ± 0.60	6.94 ± 0.46
	Neuroticism	9.88 ± 0.80	7.45 ± 0.38
	Openness	8.44 ± 0.83	8.29 ± 0.53
TIPI-J (other-rating)	*Extraversion*	8.06 ± 0.67	—
	Agreeableness	6.06 ± 0.38	—
	Conscientiousness	7.63 ± 0.88	—
	Neuroticism	10.1 ± 0.71	—
	Openness	5.56 ± 0.36	—

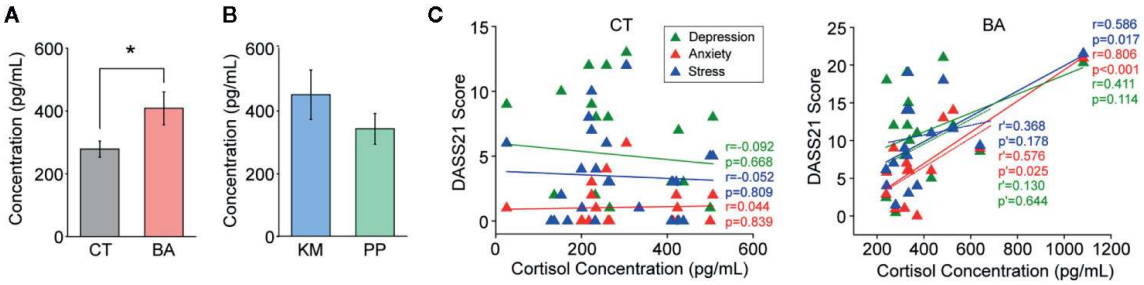
*Statistically significant difference compared to CT ($p < 0.05$).
DASS21, 21-item version of Depression Anxiety Stress Scale; HAM-A, Hamilton Anxiety Rating Scale; TIPI-J, Japanese version of Ten Item Personality Inventory; BA, Behavioral addiction; CT, Control; —, Not tested.

Figure 2



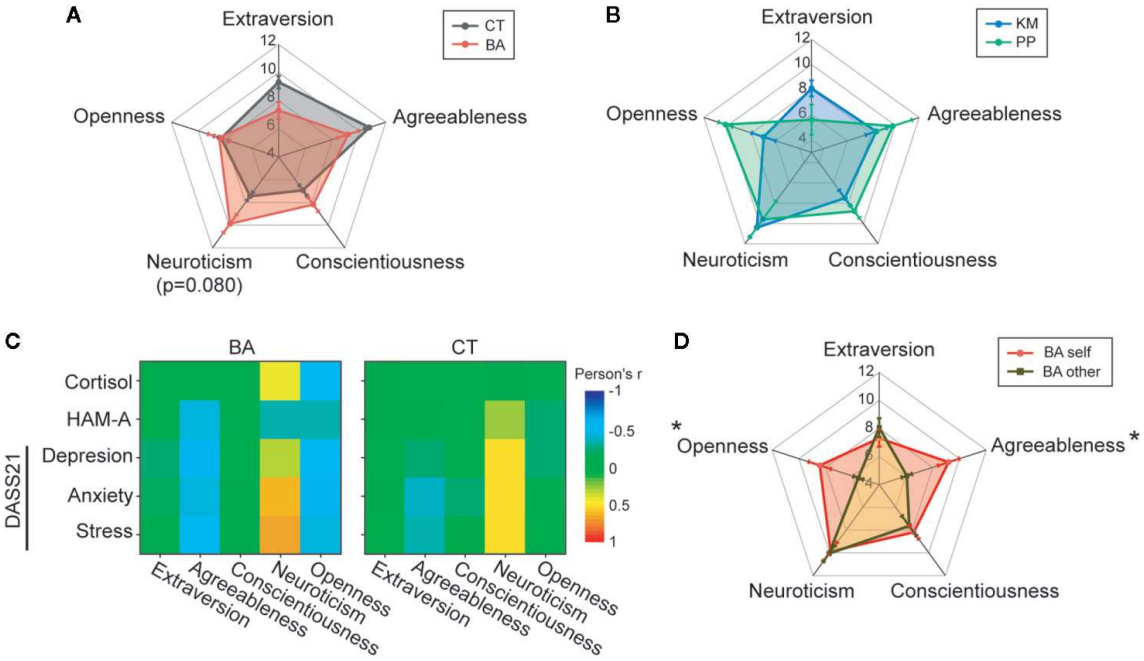
Objective assessment of anxiety with Hamilton anxiety rating scale (HAM-A) and correlations with self-assessment of negative affects. (A) A bar graph showing HAM-A scores in between behavioral addiction (BA) patients and control (CT) subjects. Error bars indicate s.e.m. *p < 0.05. (B) A graph similar to (A) but showing those in kleptomania (KM) and paraphilia (PP) patients. (C) Graphs showing correlations between HAM-A and 21-item version of depression anxiety stress scale (DASS21) scores in CT subjects (left) and BA patients (right). Each line indicates a linear correlation for stress, anxiety, and depression scales of DASS21, respectively.

Figure 3



Blood cortisol concentrations and correlations with self-assessment of negative affects. (A) A bar graph showing cortisol concentrations in blood samples from behavioral addiction (BA) patients and control (CT) subjects. Error bars indicate s.e.m. *$p < 0.05$. (B) A graph similar to (A) but showing those in kleptomania (KM) and paraphilia (PP) patients. (C) Graphs showing correlations between cortisol concentrations and 21-item version of depression anxiety stress scale (DASS21) scores in CT subjects (left) and BA patients (right). Dashed lines with r' and p' indicate person's r and p-values, respectively, excluding the outlier.

Figure 4



Personality traits and correlations with negative affects. (A, B) Rader charts comparing big five personality traits between behavioral addiction (BA) patients and control (CT) subjects (A) and between kleptomania (KM) and paraphilia (PP) patients (B). Error bars indicate s.e.m. $p = 0.080$ in BA vs. CT at neuroticism. (C) A graph showing color-coded correlations (Pearson's r) between each personality trait and negative affect assessments in BA patients and CT subjects. (D) A rader chart illustrating self-rating shown in (A) and objective rating made by others. *$p < 0.05$ in BA self vs. other in each trait.

Section 3: Distinct Situational Cue Processing in Individuals with Kleptomania

Introduction

Diagnostic manuals of psychiatric disorders such as the DSM-5 (American Psychiatric Association, 2013) and International Classification of Diseases, 11th Revision (World Health Organization, 2018) define substance use disorder (drug addiction) as "a chronic, relapsing disorder characterized by compulsive drug seeking and use despite adverse consequences" (National Institute on Drug Abuse, 2020). Compulsive seeking of drugs in addiction could be explained by emotional problems, such as craving to pleasures by taking drugs and aversion to withdrawals by lack of drugs (G. F. Koob, 1996). However, a greater emphasis has been placed on the mechanisms of drug addiction such that drug addiction is envisioned by a maladaptive incentive learning process (Berridge & Robinson, 2016; Di Chiara & Bassareo, 2007; Everitt & Robbins, 2016).

In addition to drugs, people can be addicted to specific patterns of behavior, that is, behavioral addiction (Grant et al., 2010). The concept of behavioral addiction was initially proposed in the 1960s with respect to compulsive, pathological gambling (Abrams et al., 1964), which is now formally categorized as an addictive disorder in diagnostic manuals. Furthermore, addiction to technology, such as the internet and gaming, has recently been recognized and included in the International Classification of Diseases, 11th Revision (World Health Organization, 2018). Although behavioral and drug addiction are suggested to share several core features (Griffiths, 2005), the concept of behavioral addiction is still debatable, and its definition has not yet been established to date, primarily due to insufficient investigations (Petry et al., 2018).

There is insufficient evidence to establish a clear definition of behavioral addiction; some psychiatric conditions currently categorized as "disruptive, impulse control and conduct disorders (referring as impulse control disorder hereafter)" in the diagnostic manuals have also been suggested to meet the criteria of addiction. Therefore, the term "behavioral addiction" is often used to describe

a variety of symptoms (Grant et al., 2010). Kleptomania (KM) is one such psychiatric disorder categorized as an impulse control disorder and is suggested to meet the criteria for addiction. A problematic behavior, such as shoplifting, could be associated with not only KM but also other psychiatric disorders, such as borderline personality disorder (BPD). However, in BPD, such antisocial behavior could be driven primarily by negative affects, such as depressive moods, whereas in KM, uncontrolled behavior could be driven primarily by positive affects, such as cravings and elation. Thus, although problematic behavior could be similar between KM and BPD, the processes behind these disorders are clearly different (or even opposite). In particular, the experiences of craving and elation related to engaging in problematic behavior among patients with KM are consistent with those of addiction. I have previously shown that some affective and physiological features observed in KM patients overlap with those reported in individuals with drug addiction (Asaoka et al., 2020, 2021). However, insufficient studies still make it difficult to comprehensively understand KM as a behavioral addiction, and further investigation is required.

In this study, I investigated how situational cues associated with their problematic behaviors were processed in KM patients to further evaluate KM as a behavioral addiction. In particular, behavioral responses with situational cues associated with KM symptoms were examined with eye-tracking analysis for gazing patterns.

Methods

Participants

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Japanese Ministry of Health, Labor and Welfare. All experimental procedures were approved by the Human Research Ethics Committee of Kyoto University Primate Research Institute (2019-01), and the Ethics Committee of Kyowa Hospital (Heisei 30-015).

During this study, a total of 22 patients were diagnosed with KM and hospitalized in Kyowa Hospital (Obu, Aichi) in Japan. These patients were diagnosed with KM as an impulse control disorder (for Item 312.32 [F63.2] in DSM-5) due to repetitive, uncontrolled shoplifting of sales of goods and foods in stores; diagnosis was by a psychiatrist who has been working in psychiatric hospitals for over 20 years and who is an expert in addiction treatments. The patients were screened to identify for those who exhibited a clear and strong craving to engage in their problematic behaviors and who had no comorbid psychiatric disorders or minor conditions, if any. With this screening, 11 patients with KM agreed to participate in this study (4 males, 7 females; age = 36.7 ± 4.86 years old), of which 7 patients had no comorbidity and 4 had comorbidities with attention deficit/hyperactivity disorder (1 patient), gender dysphoria (1 patient), and eating disorders (2 patients). None of the participants were diagnosed with BPD. Healthy control individuals without smoking and psychiatric histories (CT; n = 27; 11 males, 16 females; age = 37.9 ± 2.02 [mean ± SEM] years old) were also recruited. Upon enrolling in the study, written informed consent was obtained from all participants in advance of the experiments.

A short form of the Japanese version of the Wechsler Adult Intelligence Scale-III, consisting of the information test in the Verbal Comprehension Index and the picture completion test in the Perceptual Organization Index, was administered to estimate the full-scale intelligence quotient (eFIQ) of participants (Kobayashi et al., 1993).

Image Presentation with Eye-Tracking

For image presentations, the following 6 static images (Figure 1a) were presented in pseudorandom order through the LCD monitor: a grocery store with (MKT+) and without (MKT) a person as situational cues, images of outside scenery with (OUT+) and without (OUT) a person, and sales products, particularly foods and stationaries. Each image was presented for 15 seconds along with a 5-second interval between images, during which a fixation point was presented in the center of the motor (Figure 1b).

While images were presented, gazing patterns specifically, the number of fixations, the average duration of fixations, the average dispersion of fixations, the number of eye blinks, and changes expressed as coefficient of variation (CV) of pupil diameters over time during gazing were recorded using a wearable eye-tracker (Pupil Core, Pupil Labs GmbH, Berlin, Germany). The area of interest (AOI) was also set for later offline eye-tracking analysis around the person as a square of the same size within the MKT+ and OUT+ images (Figure 1c).

In previous eye tracking research, it's believed that fixations, or temporary pauses in eye movement, reflect attention rather than the eye movements themselves. Human gaze involves fixations, rapid movements between fixations called saccades, and slower movements like smooth pursuit. Within fixations, there are subtle movements such as tremor, microsaccade, drift, and glissade, which are thought to reflect minute muscle movements but cannot be directly recorded in eye tracking. Eye tracking data is obtained from videos of the eye and face direction, detecting gaze, pupil size, and blinking. In my study, I used a dispersion-based method to detect fixations and did not separate subcategories of fixation for analysis (https://docs.pupil-labs.com/core/terminology/#fixations). The parameters for fixation, pupil size, and blinking are not equivalent or independent but are all obtained in eye tracking.

Data Analysis

Investigators who were not blinded to the experimental conditions conducted data collection and statistical analyses. Statistical analysis was conducted using JASP (JASP Team, 2022,

JASP Version 0.16.3 [Computer Software]) and OrignPro (OriginLab Corporation). After removing outliers, I utilized Bayesian over frequentist methods for statistical analysis, as the sample size in the current study was small, and thus, Bayesian methods were superior to frequentist methods as the former approach would not lose power and would retain precision in analysis (van de Schoot et al., 2014).

Although the eFIQ was lower in BA patients than in CT subjects ($KM = 79.3 \pm 4.88$, $CT = 100.8 \pm 2.77$; $BF_{10} = 88.3$, $error\% = 7.04e^{-7}$), this factor was not considered in statistical analyses, as it is unlikely that the eFIQ exerted significant effects on the measurements evaluated in this study, such as gazing patterns to image.

Results

Gazing Pattern on Image

To determine whether KM patients exhibited different behavioral responses to a situational cue compared with CT subjects, eye-tracking analysis was conducted to examine their gazing patterns on image presentations. Bayesian independent sample t test for group comparisons revealed no difference between KM patients and CT participants in the number, duration, and dispersion of fixations, the number of eye blinks, and the CV of pupil size for each image (Table 1). In particular, the duration of fixations made in the AOI of the MKT+ image tended to be shorter, whereas the duration and dispersion of fixations made on the OUT image tended to be longer and smaller, respectively, in KM patients than in CT participants (Table 1). However, there was weak, anecdotal evidence supporting the alternate hypothesis instead of the null hypothesis with respect to the duration of fixations made in the AOI of the MKT+ image (BF10 = 2.41, error% = 0.007). For the OUT image, there was also weak evidence supporting the alternate hypothesis for the duration of fixations (BF10 = 4.51, error% = 0.001) and for the dispersion of fixations (BF10 = 1.93, error% = 0.018).

Although only subtle, if any, differences were observed between KM patients and CT participants with any of single measurements alone, it is possible that the combination of these measurements—that is, the pattern of gazing—could be different between groups. Thus, each data point of fixation duration, dispersion, and number, CV of pupil size, and the number of eye blinks were normalized, and principal component analysis (PCA) was conducted to examine correlations of gazing patterns between images. This analysis unveiled that correlations of gazing patterns between MKT and the remaining images were substantially lower than those between other images among KM patients (Figure 2c–d), whereas this difference was not observed among CT participants (Figure 2a–b). Therefore, the correlations between MKT and other images in KM patients were weaker than the correlations observed among CT participants (BF_{10} = 8.27, error% = 0.003; Figure 2e). These results suggest that gazing patterns consisting of fixation duration, dispersion, and number, CV of

pupil size, and the number of eye blinks made on a situational cue are different between KM patients and CT participants.

Discussion

In this study I found that KM patients exhibit distinct behavioral responses to images with situational cues associated with their symptoms, that is, compulsive and impulsive stealing. In particular, when the image of a situational cue (scene of supermarket) included a social signal (without a person), fixations on such a social signal might be shorter in KM patients than in CT participants.

My current study demonstrated that gazing patterns to such a cue were at least distinct from responses to other cues in patients with KM. Although patients with KM who participated in this study exhibited craving related to their problematic behavior, it remains unclear whether the findings may be related to cue-induced craving, such as those observed in substance use disorder (Berridge & Robinson, 2016; Di Chiara & Bassareo, 2007; Everitt & Robbins, 2016) and behavioral addiction, such as gambling disorder (Limbrick-Oldfield et al., 2017), gaming disorder (Ko et al., 2009), problematic internet use (Niu et al., 2016), and pathological buying (Trotzke et al., 2014). Craving can be assessed with subjective self-reported measures (R. Z. Goldstein et al., 2010) and objective physiological signal measures, such as heart rate and skin conductance (Laberg & Ellertsen, 1987; Ooteman et al., 2006; Witteman et al., 2015), although such physiological responses in cue-induced craving are often controversial (Ooteman et al., 2006; Reid et al., 2006). Whether the current findings are associated with craving will be addressed in my future study. Another major limitation of the current study is the small sample size, which makes the findings rather . Thus, current findings need to be validated with a larger sample size.

In some specific conditions, such as under the effects of psychostimulants, the association between eye blink rate and striatal D2 receptor availability has been demonstrated in healthy humans (Demiral et al., 2022). Moreover, an association of eye blink rate has been reported with symptom severity in patients with gambling disorder and the amount of addictive substance consumption in healthy individuals (Mathar et al., 2018). In addition to eye blink rate, pupil diameter has also been suggested to reflect noradrenaline function (Larsen & Waters, 2018; Preuschoff et al., 2011);

however, this association is not yet clear, as a recent study has demonstrated that dorsal raphe serotonin (5-HT) neuron activation also increases pupil size in rodents (Cazettes et al., 2021), suggesting that neither noradrenaline nor 5-HT alone but multiple facets of monoamine transmission may be involved in the regulation of pupil sizes. Several primate studies have suggested an association between spontaneous eye blink rate and striatal dopamine D2 receptor availability, with higher availability increasing eye blink rate (Groman et al., 2014). This is highly controversial, however, as another study has also shown that the association of eye blink rate with D1 receptor availability is even stronger than that with D2 receptor availability (Jutkiewicz & Bergman, 2004). Most human studies are actually unable to replicate such DA-eye blink association (Dang et al., 2017; Sescousse et al., 2018; Trutti et al., 2019). In the current study, the number of eye blinks and changes in pupil sizes were measured in an attempt to indirectly assess alterations in monoamine transmission. There was no difference among KM patients or among CT participants. 5-HT is also involved in the control of gazing to social cues in primates (Weinberg-Wolf et al., 2022). I observed moderate evidence of differences in fixations in the AOI (the person in the image) of the MKT+ image. Thus, KM patients tended to exhibit context-dependent gazing patterns to the social cues in the images that were different from those of CT participants, suggesting that if there is any, altered 5-HT transmission may be present in KM patients.

In conclusion, my study, despite the nature due to the small sample size, provides evidence that impulse control disorders such as KM may involve altered processing of situational cues. Whether this finding is also applicable to other impulse control disorders, such as paraphilia, remains unclear and needs further investigation.

Figure 1

A design of image presentation. (a) Six images presented to participants, which are photos of the market with (MKT+) and without (MKT) a person, outside sceneries with (OUT+) and without (OUT) a person, and commodities, such as stationaries (STNY) and foods (FOD). (b) A schematic diagram of the image presentation procedure. Participants were indicated as "We will now show you 12 images of supermarkets and streets. Each image will last 15 seconds. Look at each image at your will. Please be careful not to move your head during this time. Before the images appear, a screen with a black background and a white circle in the center will appear for 5 seconds. During this time, keep staring at the white circle in the center of the screen as long as you can". (c) Images of MKT+ and OUT+ illustrating the area of interest (AOI) as yellow boxes.

Table 1. Descriptive Statistics and Bayesian t Test for Eye-Tracking Data

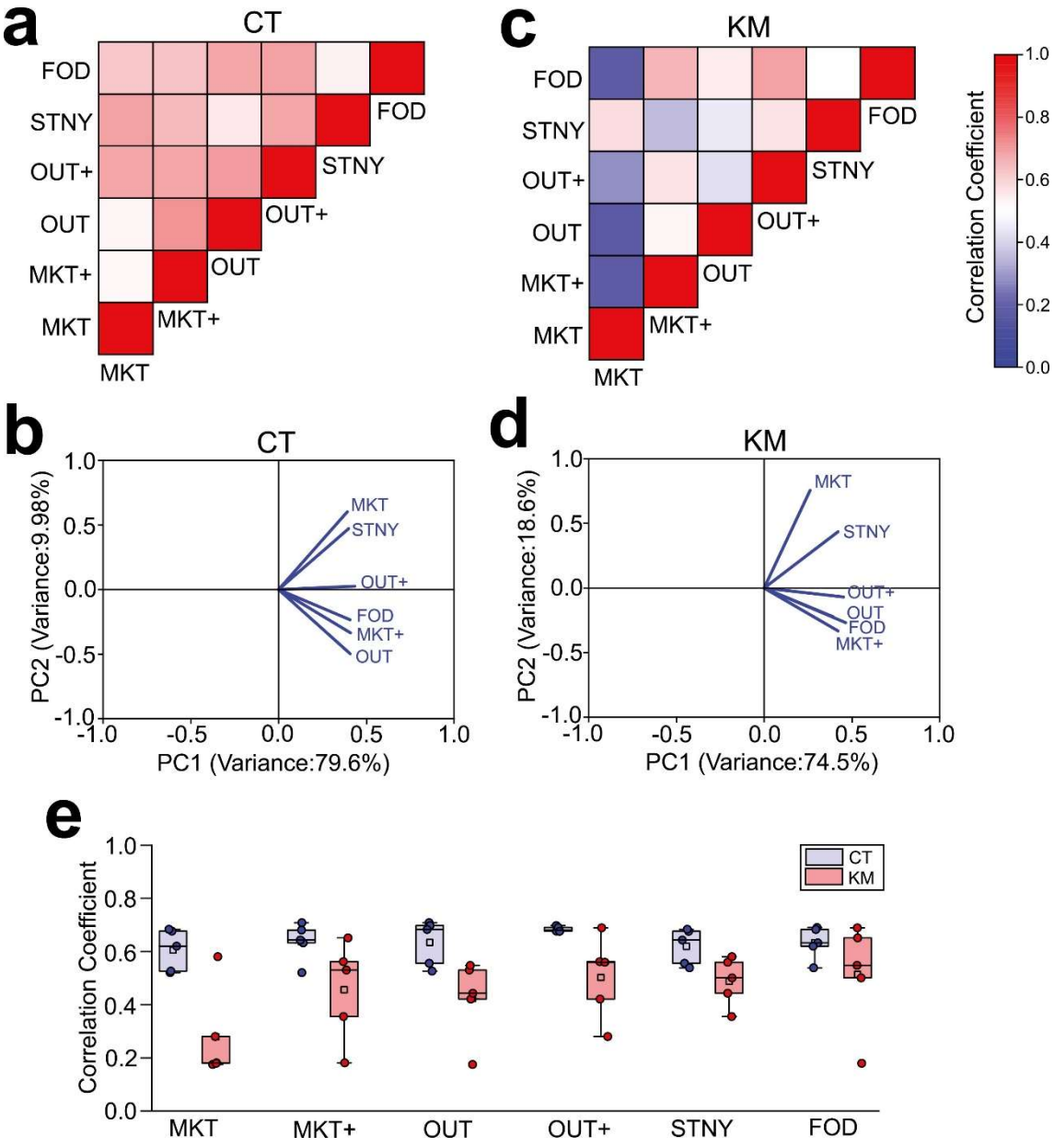
Image	Measure	Participant				BF_{10}	Error%
		KM		CT			
		Mean	SEM	Mean	SEM		
MKT+	Fix Dur	156	3.27	163.8	3.305	0.506	0.0004
	Fix Disp	0.76	0.041	0.785	0.022	0.912	0.001
	Fix N	24.82	7.447	26.96	4.933	0.639	0.0007
	CV Pupil	0.272	0.09	0.253	0.049	0.644	0.0007
	Blink N	1.455	0.578	2.444	0.451	0.64	0.0007
MKT+ AOI	Fix Dur	107.8	93.4	167.7	23	2.409	0.007
	Fix Disp	0.735	0.031	0.769	0.102	0.505	0.0009
	Fix N	3.636	1.889	10.85	2.684	0.93	0.004
OUT+	Fix Dur	181.5	10.725	154.7	4.965	0.662	0.0007
	Fix Disp	0.701	0.035	0.777	0.022	0.871	0.001
	Fix N	18.46	7.685	28.78	4.827	1.4	0.0009
	CV Pupil	0.282	0.088	0.188	0.032	0.493	0.0003
	Blink N	1.727	0.764	2.481	0.507	0.652	0.0008
OUT+ AOI	Fix Dur	152.7	31.98	164.6	14.65	0.509	0.0004
	Fix Disp	0.742	0.057	0.741	0.028	0.783	0.001
	Fix N	3.273	1.854	6.333	1.382	0.492	0.0003
MKT	Fix Dur	172.5	5.79	161.2	5.147	0.538	0.0005
	Fix Disp	0.769	0.024	0.764	0.028	0.811	0.001
	Fix N	26.55	8.391	29.33	5.32	0.575	0.0006
	CV Pupil	0.507	0.191	0.381	0.107	0.494	0.0003
	Blink N	1.636	0.527	2.407	0.484	0.642	0.0007
OUT	Fix Dur	230.7	22.41	164.8	4.153	4.514	0.001
	Fix Disp	0.611	0.046	0.736	0.025	1.927	0.018
	Fix N	23.27	7.295	29.7	4.846	0.645	0.0007
	CV Pupil	0.346	0.15	0.515	0.122	0.497	0.0003
	Blink N	3	0.953	2.593	0.451	0.553	0.0005
STNY	Fix Dur	155.1	6.327	158.6	3.118	0.494	0.004
	Fix Disp	0.752	0.052	0.814	0.017	0.945	0.008
	Fix N	22.36	6.815	28.85	4.509	0.582	0.0004
	CV Pupil	0.368	0.132	0.271	0.067	0.573	0.0003
	Blink N	1.091	0.343	1.704	0.452	0.695	0.0005
FOD	Fix Dur	156.7	7.683	153.8	4.276	1.067	0.0003
	Fix Disp	0.702	0.074	0.783	0.022	1.192	0.001
	Fix N	23.09	7.88	24.04	4.955	0.528	0.0006
	CV Pupil	0.291	0.114	0.345	0.089	0.492	0.0005
	Blink N	1.545	0.679	1.963	0.458	0.566	0.019

Abbreviations: Blink N, number of blinks; CT, control; CV pupil, coefficient of variation of pupil diameter changes; Fix Disp, dispersion of fixations; Fix Dur, duration (ms) of fixations; Fix N, number of fixations; KM, kleptomania; SEM, standard error of mean. This study employs hypothesis testing within the framework of Bayesian methods, utilizing the Bayes Factor (BF) as a metric. The BF is an index that represents the relative evidence between two hypotheses, such as the null hypothesis (H0) and the alternative hypothesis (H1). The BF10 value indicates the evidence for the alternative hypothesis H1 compared to the null hypothesis H0, calculated as BF10 = P(Data|H1) / P(Data|H0), where P(Data|H1) and P(Data|H0) are the likelihoods of the data under the alternative and null hypotheses, respectively. In Bayesian statistics, the uncertainty of parameter estimates, represented as %error, is also crucial. A smaller %error indicates less uncertainty in the estimate, suggesting greater reliability of the results. The strength of evidence is assessed based on the value of BF:

- BF10 < 1: More evidence for the null hypothesis H0, which is better supported than the alternative hypothesis H1.
- 1 < BF10 < 3: Weak evidence for the alternative hypothesis H1.
- 3 < BF10 < 10: Substantial evidence for the alternative hypothesis H1.
- BF10 > 10: Strong evidence for the alternative hypothesis H1.

The Bayes Factor (BF10) is used to evaluate the relative evidence between two hypotheses in Bayesian hypothesis testing, while the %error provides a measure of the uncertainty in parameter estimates, with smaller values indicating more reliable results.

Figure 2



Correlations of gazing patterns to image presentations. A correlation matrix with color-coded correlation coefficients (a) and component loading plot (b) of gazing patterns between images in control (CT) participants. (c, d) A correlation matrix and component loading plot similar to (a) and (b) but showing those of kleptomania (KM) patients. (e) A box plot for correlation coefficients between one of the images and the rest of the others.

Section 4: Monoamine and genome-wide DNA methylation investigation in behavioral addiction.

Introduction

Behavioral addiction (BA) is a psychiatric condition characterized by repeated, impulsive and compulsive seeking of specific behavioral processes, even though consequent negative outcomes (Grant et al., 2006, 2010; Robbins & Clark, 2015). In the current diagnostic manuals, such as the DSM-5 (American Psychiatric Association, 2013) and ICD-11 (World Health Organization, 2018), pathological gambling and Internet and gaming disorders are officially categorized into this psychiatric disorder. However, in many preclinical studies and the realms of clinical setting, impulse control disorders, such as kleptomania (KM), paraphilic (PP) and nonparaphilic sexual disorder, are also thought to meet the criteria of BA, and are, therefore, quite often considered BA (Grant & Chamberlain, 2016).

BA and drug addictions are known to be comorbid with several psychiatric disorders, such as schizophrenia (SCZ) and autism spectrum disorder (ASD). It has long been known that drug addiction is highly prevalent in SCZ (Awad, 2016; Chambers et al., 2001). Moreover, BA such as pathological gambling is also higher in SCZ patients (Bergamini et al., 2018; Desai & Potenza, 2009). Studies have shown associations of drug addiction and BA with ASD, with a higher prevalence of drug addiction and BA, such as gaming disorder, in individuals with ASD (Butwicka et al., 2017; Engelhardt et al., 2017; So et al., 2017; Wijngaarden-Cremers et al., 2014). In particular, a recent study suggests that common molecules and pathways may be involved between drug addiction and ASD (Rothwell, 2016).

Since BA is still a conceptually new disorder, it has remained largely unclear to what extent BA may share its biological mechanisms with those of drug addiction. One of the central mechanisms involved in drug addiction is indeed monoamine transmission, particularly dopamine (DA) (Nutt et al., 2015; Wise & Robble, 2020), and less suggested for norepinephrine (NE) (Sofuoglu & Sewell, 2009) and serotonin (5-HT) (Müller & Homberg, 2015), given that most, if not all, addictive substances stimulate the DA system. Animal model studies have demonstrated that

some addictive drugs, such as amphetamine and cocaine, have been shown to decrease the basal, and tonic DA levels in the striatum (Gerrits et al., 2002; Parsons et al., 1991), which, in turn, could increase phasic DA release upon taking these drugs (Anthony A. Grace et al., 2007). Nevertheless, only a few studies have examined whether monoamine concentrations may be altered in peripheral blood samples in addicted patients. A study has reported that plasma DA and one of the metabolites of norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), were increased with the duration of heroin and cocaine use in patients (Macedo et al., 1995), whereas another study has shown decreased plasma levels of the DA metabolite homovanillic acid (HVA) in individuals with alcohol addiction depending on the genetic backgrounds of catechol-o-methyltransferase (COMT) (Köhnke et al., 2003). In relation to BA, an association of blood DA level and Internet addiction in adolescents has been demonstrated (Liu & Luo, 2015).

Accumulating evidence suggests that epigenetic processes also play pivotal roles in drug addiction, whereas this has not yet been examined in BA. Exposures to addictive substances could alter gene expression in a tissue-specific manner in the brain through epigenetic processes (Renthal & Nestler, 2008); alternatively, epigenetic changes could occur early in development, predisposing an individual to increased vulnerability to addiction (Nielsen et al., 2012). Indeed, similar processes are thought to take place in BA, which is essentially initiated by environmental stimuli in adulthood or during development. Recent studies with genome-wide DNA methylation assays have unveiled alterations in methylation patterns in drug addiction patients compared with healthy subjects (Cecil et al., 2016; Gatta et al., 2021). DNA methylation is an epigenetic process that regulates gene expression, and as such, it was thought that CpG sites on promoter regions play crucial roles in inhibiting gene expression (Doerfler et al., 1988). However, more recent studies have unveiled that CpG sites are also substantially located in other regions, including gene bodies, and the methylation of such CpGs in gene bodies is also involved in the regulation of gene expression (Jjingo et al., 2012; P. A. Jones, 2012; Yang et al., 2014).

Collectively, in this study, I investigated whether monoamine concentrations were altered in BA patients, and whether such alterations were associated with epigenetic processes, such as DNA

methylation. To address this issue, blood samples were obtained from BA patients and control (CT) subjects, and a high-performance liquid chromatography (HPLC) assay was conducted to measure monoamine concentrations. Plasma monoamines and their metabolites have been found to be related to some extent to those of the central nervous system (D. S. Goldstein et al., 1983; David S. Goldstein et al., 2003; David S. Goldstein & Holmes, 2008). Moreover, a genome-wide DNA methylation assay with an Infinium Human Methylation EPIC Bead Chip array was conducted to compare the methylation status of DNA between BA patients and CT subjects, particularly whether DNA methylation on any genes associated with monoamine transmission was altered.

Methods

Subjects

This study was conducted in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Japanese Ministry of Health, Labour and Welfare. All experimental procedures were approved by the Human Research Ethics Committee of Kyoto University Primate Research Institute (2019-01), and the Ethics Committee of Kyowa Hospital (Heisei 30-015). Upon enrolling in the study, written informed consent was obtained from all participants in advance of the experiments. As a control (CT) group, blood samples were obtained from 24 healthy subjects without a history of smoking or psychiatric disorders (41.0 ± 7.43 years old; 9 males, 15 females). For the behavioral addiction (BA) group, blood samples were collected from hospitalized patients diagnosed with BA (n = 16; 36.9 ± 14.7 years old; 9 males, 7 females), which consisted of symptoms of gambling (n = 1), kleptomania (KM; n = 10) and paraphilia (PP; n = 5).

Blood sampling of approximately 3–5 mL from each participant was conducted in an EDTA-coated tube. Sampling in BA patients was conducted between 11:00 and 12:00, and in CT subjects between 16:00 and 17:00. Samples were frozen immediately after sampling and stored at − 30 °C in the freezer until processing for HPLC and DNA extraction.

HPLC

High-performance liquid chromatography (HPLC) was conducted to investigate whole blood monoamine concentrations. For this assay, first, blood samples were centrifuged at 10,000 rpm for 5 min. Then, 490 μL from the surface of blood samples was placed into another 1.5 mL tube, and 10 μL of 10 pg/μL isoproterenol (ISO) as an internal standard was added to the samples, followed by 100 μL of 0.5 mol/L perchloric acid to remove all proteins. The samples were centrifuged again at 2,000 rpm for 15 min, and 10 μL each of supernatant from these samples was applied for HPLC.

Using a similar procedure described in a previous study (Lee et al., 2018), HPLC was carried out using an HTEC-500 HPLC electrochemical detection system (Eicom, Tokyo, Japan) with the EICOMPAK SC-5ODS column and CA-ODS precolumn. The mobile phase consisting of 0.1 M acetic acid-citric acid buffer (pH 3.5) and methanol (83:17, v:v), along with 190 mg/L sodium 1-octanesulfonate and 5 mg/L EDTA-2Na, was used. The mobile phase flow rate was 500 μL/min, and the working electrode was set at + 750 mV against the Ag/AgCl reference electrode.

The standard with the known concentration of target substances (DA, 5-HT, NE, Epi, MHPG, NM, DOPAC, HVA, 3-MT, and 5-HIAA) and ISO were used to quantify and identify the peaks on the chromatographs. The quantification of target substances in a sample was based on the following formula: $(ISO_{std}/TA_{std}) \times (TA_{spl}/ISO_{spl}) \times A \times (1/B)$, where ISO_{std} and TA_{std} are the areas under the peaks of ISO and a target substance in the standard, whereas ISO_{spl} and TA_{spl} are the areas under the peaks of ISO and a target substance in a sample; A is the amount of ISO added to the sample; and B is the volume of the blood sample used.

Genome-wide DNA methylation assay

Blood samples obtained from 10 CT, 5 KM, and 5 PP subjects were used for methylation assay. In CT and KM groups, 10 and 5 samples were selected from 24 CT and 10 KM subjects, respectively, based on characteristics of symptoms, for which the attending physician described as most typical, along with matching for age and sex. Genomic DNA was extracted from whole blood samples with the ISOSPIN Blood and Plasma DNA Kit (Nippon Gene, cat. #312-08131) according to the protocol provided by the manufacturer. DNA quality was checked by the Picogreen (Promega, cat. #E2670) method using a synergy HTX Multi-Mode Reader, whereas the purity of DNA was assessed by NanoDrop spectrometry. In addition, DNA conditions were further assessed by gel electrophoresis. After these quality controls of extracted genomic DNA, 500 ng of DNA in each sample was bisulfite converted with the EZ DNA Methylation Kit (Zymo Research, cat. #D5001). Then, a genome-wide DNA methylation assay was conducted with an Illumina Infinium MethylationEPIC BeadChip kit (Illumina, cat. #WG-317-1001) (Moran et al., 2016), and the array

was scanned with an Illumina iScan scanner.

Array data were processed and analyzed using Illumina GenomeStudio v2011.1 (Methylatioin Module v1.9.0) and R 3.6.0 (https://www.r-project.org). Each methylation data point is represented by fluorescent signals from the M (methylated) and U (unmethylated) alleles. Background intensity computed from a set of negative controls was subtracted from each analytical data point. Thus, after image analysis and the extraction of raw data for 865,918 CpGs, preprocessing and quality checks were conducted to reduce systematic bias to avoid statistically erroneous conclusions through the correction, filtering, transformation, and normalization of data. Background correction and dye bias equalization were conducted with library (lumi) in R. Filtering was conducted for the detected CpGs by a detection p-value, excluding CpGs with detection p-value ≥ 0.05 in more than 25% of all samples, or p-value = NA in at least one sample. This filtering resulted in 845,978 CpGs to be analyzed. Then, beta mixture quantile (BMIQ) normalization (Teschendorff et al., 2013) was conducted to reduce assay bias using BMIQ in R, followed by data transformation to calculate $\beta\text{-value} = (\max(M, 0))/(|U| + |M| + 100)$, which is the ratio of fluorescent signal intensity of the methylated probe and the overall intensity (sum of methylated and unmethylated probe intensities). The β-value reflects the methylation level of each CpG site. A β-value of 0–1 was reported to signify the percentage of methylation, from 0 to 100%, respectively, for each CpG site. In addition, the M-value, which is the log2 ratio of the intensities of methylated probe versus unmethylated probe was also calculated for statistical analysis (Du et al., 2010). Using β- and M-values, the Δmean (the difference between the average β-values fir the BA patients and the CT subjects), odds ratio (by transforming Δmean into M-value and measuring the ratio between unmethylated intensity and methylated intensity for the BA patients versus that of the CT subjects), and fold change (the ratio of methylation rates between the BA patients and the CT subjects) were also calculated for each CpG site.

Data analysis

Comparisons between the BA patients and the CT subjects and between the KM group and

the PP group in HPLC and genome-wide methylation assays were conducted with unpaired t tests. A probability value of $p < 0.05$ was considered to indicate statistical significance. Using the data of hyper- and hypomethylated CpG sites in BA patients compared to CT subjects from genome-wide methylation assays, gene network analysis was also conducted using the databases available on the internet. Whether hyper- and hypomethylated CpG sites in blood samples of BA patients were correlated with methylation status in brain tissues was analyzed with IMAGE-CpG (https://han-lab.org/methylation/default/imageCpG) (Braun et al., 2019), gene network analysis was conducted with GeneMANIA (https://genemania.org/) (Warde-Farley et al., 2010), tissue specific protein–protein interaction analysis and gene–disease association analysis were conducted with NetworkAnalyst (https://www.networkanalyst.ca/) (Zhou et al., 2019), and more detailed gene–disease analysis was conducted with DisGeNET (https://www.disgenet.org/) (Piñero et al., 2017), following the respective manuals at each website.

Results

Subjects

Blood samples were obtained from 24 healthy CT adults and from hospitalized patients who were diagnosed with BA (n = 16), who were further divided into patients with symptoms of gambling (n = 1), kleptomania (KM; n = 10) and paraphilia (PP; n = 5). All samples of 24 CT subjects and 16 BA patients were processed for blood monoamine assay with HPLC, whereas the genome-wide DNA methylation assay was conducted with samples from 10 CT subjects and 5 each from KM and PP patients, totaling 10 BA patients. Ten CT and 5 KM samples for the methylation assay were selected from the entire subjects based on characteristics of symptoms, for which the attending physician described as most typical, along with the consideration of matching for age and sex.

Blood monoamine concentrations

HPLC assays were conducted to measure the blood concentrations of DA, 5-HT, NE, and epinephrine (Epi) and their metabolites, MHPG, normetanephrine (NM), 3,4-dihydroxyphenylacetic acid (DOPAC), HVA, 3-methoxytyramine (3-MT), and 5-hydroxyindole acetic acid (5-HIAA). MHPG was significantly lower (unpaired t test, $t_{38} = -2.30$, $p = 0.027$), whereas HVA ($t_{38} = 4.08$, $p < 0.001$), 3-MT ($t_{38} = 2.33$, $p = 0.025$), and 5-HT ($t_{38} = 2.22$, $p = 0.032$) were significantly higher, in blood samples from BA patients than in those from CT subjects (Fig. 1a). Moreover, the ratio of one DA metabolite, HVA, to DA was significantly higher in BA patients than in CT subjects ($t_{38} = 2.80$, $p = 0.008$), whereas the ratio of the 5-HT metabolite 5-HIAA, to 5-HT was not different between BA patients and CT subjects (Fig. 1b). Monoamine concentrations of BA patients were further analyzed separately for KM and PP patients. However, none of the measurements were significantly different between KM and PP patients (Fig. 1c, d). These results suggest that increased DA turnover may be involved in BA regardless of its symptom types.

Genome-wide DNA methylation assay

Genome-wide DNA methylation in DNA extracted from blood samples of BA patients and CT subjects was examined with an Infinium HumanMethylationEPIC BeadChip array. The ratio of methylated probe intensity to the overall intensity (β-value) of 845,978 CpG sites after data preprocessing and quality checks was distributed more below 0.1 or above 0.9 (Fig. 2a), suggesting that many CpG sites were either unmethylated or fully methylated in both the BA patients and CT subjects. Among these CpGs, 106 hypomethylated ($\Delta\text{mean} \leq -0.2$, where $\Delta\text{mean} = \text{mean(average of } \beta \text{ in BA patients)} - \text{mean(average of } \beta \text{ in CT subjects)}$, and $p < 0.05$ in unpaired t test) and 80 hypermethylated ($\Delta\text{mean} \geq 0.2$) CpGs, with odds ratios varying from 0.015 to 58.6 and fold changes varying from -8.51 to 11.4, respectively, were identified in the BA patients compared to the CT subjects (Fig. 2b,d,e). However, these methylation differences were modest at most, and none of them was significant with multiple testing correction using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995). Many of these 186 CpG sites were located in the gene bodies and intergenic regions (Fig. 2c). A heat map of hierarchical clustering on distance similarity for samples and CpGs (Euclidean distance, complete linkage), using the M-value (the log2 ratio of the intensities of methylated probe versus unmethylated probe) of significant data, shows distinct methylation patterns between the BA patients and the CT subjects (Fig. 2f).

Functional associations and relevance to other psychiatric disorders

Given that the methylation status of DNA in peripheral blood tissues is not necessarily identical to that in brain tissues (Braun et al., 2019), I further analyzed whether there was correspondence in methylation level between the blood and brain tissues for 186 identified CpGs with hyper- and hypomethylation, using the database at IMAGE-CpG (Braun et al., 2019). With this analysis, 64 CpGs with hyper- and hypomethylation were identified, and subsequent analyses were conducted with the genes identified where those 64 CpGs are present.

First, because previous studies have reported epigenetic changes in monoamines in drug addiction, I aimed to hypothesize that similar changes exist in behavioral addiction. To test this

hypothesis, I performed gene network analysis using the database at GeneMANIA (Warde-Farley et al., 2010), which allows me to examine how genes interact or are related to each other.. This analysis unveiled extensive associations among the identified genes (coexpression, 91.3%; physical interactions, 5.92%; genetic interactions, 1.46%; pathway, 0.96%; shared protein domains, 0.33%; Fig. 3a), along with significant convergence (Q-value < 0.1) identifying 118 gene ontologies, which were primarily associated with membrane trafficking, such as transport vesicle and endocytic vesicle membrane, and immune functions, such as MHC class II receptor activity and cellular response to interferon-gamma. On the other hand, regions with altered gene expression in this study did not include monoamine-related gene regions.

While GeneMANIA is suitable for predicting the function of specific genes, it is not suitable for detailed biological analysis, hence the NetworkAnalyst database (Zhou et al., 2019) was used for network analysis of each brain region associated with behavioral addiction and its association with other psychiatric disorders..

The nucleus accumbens, frontal cortex, amygdala, substantia nigra, hippocampus, and ventral tegmental area are among the brain regions primarily involved in drug addiction. Focus on these regions, an example of tissue specific protein–protein interactions in the nucleus accumbens is illustrated in Fig. 3b, which demonstrates CUL3 (Cullin 3: a member of the cullin family of proteins) as the largest node, along with several other nodes, such as ACTN1 (Alpha-actinin-1: a member of the actinin family of actin-binding proteins), DTX2 (Deltex 2: a member of the Deltex family) and TGFBR1 (Transforming Growth Factor Beta Receptor 1: a member of the serine/threonine kinase family of receotors). Similar network structures with these genes as nodes also emerged for the frontal cortex, hippocampus, amygdala, and substantia nigra.

Gene–disease associations were further evaluated with network analysis (Fig. 3c). This analysis provided associations of the identified genes with psychiatric disorders, such as ASD (degree of convergence = 2; betweenness = 1,388) and SCZ (degree of convergence = 2; betweenness = 31.51). In addition, more detailed gene–disease associations were also examined using the database at DisGeNET (Piñero et al., 2017), with which 37 out of 64 genes were identified

for associations with diseases, including psychiatric disorders. Among such psychiatric disorders, the strongest associations with BA were observed for intellectual disability (9 genes; CTNND2, GNAL, VSX2, TGFBR1, CUL3, FBXO8, GALNS, ABAT, GLIS3), ASD (8 genes; HLA-DRB1, HLA-DQB1, CTNND2, IRS2, HLA-DRB1, ABAT, CTNND2, PCSK6) and SCZ (8 genes; HLA-DRB1, HLA-DQB1, GSTT1, GNAL, CTNND2, CUL3, HLA-DQA1, GLIS3). In addition, SEMA6A (Semaphorin 6A: member of the semaphoring family, which is a large group of proteins known for their role in axon guidance and neuronal development), GSTT1 (Glutathione S-Transferase Theta 1: an enzyme that belongs to the glutathione S-transferase family), and SCARB1 (Scavenger Receptor Class B Member 1: a protein that functions as a receptor for high-density lipoprotein cholesterol), which are included in the identified 64 genes with hyper- and hypomethylation, were associated with drug addiction (alcohol, amphetamine, cannabis, cocaine, marijuana, and phencyclidine).

These results suggest that the genes in which hyper- and hypomethylation were observed in the BA patients may be associated with biological functions such as membrane trafficking, which may affect synaptic transmission in the brain, and the immune system. Moreover, some of these genes associated with BA may overlap with candidate genes of other psychiatric disorders, such as SCZ and ASD. In particular, SEMA6A, GSTT1, and SCARB1 could be candidate genes that may be mutually involved in BA and drug addiction.

DNA methylation differences between KM and PP

Since the BA patients manifested heterogeneous symptoms, I analyzed genome-wide DNA methylation data separately for patients with KM and PP. When the KM and PP groups were compared, 88 hypo- and 99 hypermethylated CpG sites were identified in the KM group compared to the PP group (Fig. 4a). A heat map of hierarchical clustering also showed distinct methylation patterns between the KM group and the PP group (Fig. 4b). These results suggest that methylation patterns may be different between KM and PP, although differences in other measurements, such as blood monoamine concentrations, are less clear between them.

Receiver operating characteristic analyses of monoamines and methylation

One of the important implications of the data is that DNA methylation and monoamines might be developed into biomarkers. The fact that it is possible to observe DNA methylation and monoamine concentration changes in blood suggests feasibility. Thus, I conducted receiver operator characteristic (ROC) analyses (Zweig & Campbell, 1993) to examine the sensitivity and specificity of these markers.

Although the reliability of analyses was limited by the small sample sizes, ROC analyses revealed that blood concentrations of HVA, 5-HT, and MHPG, which were significantly lower or higher in BA patients than in CT subjects, successfully distinguished BA patients from CT subjects (the area under the curve (AUC) significantly larger than 0.5 with asymptotic p-value < 0.05), but other monoamines did not (Fig. 5a,b). A similar ROC analysis was also conducted with 186 hyper- or hypomethylated CpG sites. AUCs with asymptotic p-values < 0.05 were observed in 95 out of these 186 hyper- or hypomethylation sites (Fig. 5c).

These results suggest that some blood monoamine concentrations and blood cell DNA methylation status could distinguish BA and CT, and thereby might be used as biomarkers of BA.

Discussion

In this study, I have shown that increased DA turnover and DNA methylation differences are involved in BA. Such DNA methylation differences appear to occur in genes that play roles in cell membrane trafficking and the immune system and are associated with other psychiatric disorders, such as intellectual disability, ASD, SCZ, and drug addiction. Moreover, although DA turnover was not different, DNA methylation was clearly distinct between the symptoms of KM and PP. In particular, none of participants who were subjected to investigation in either the BA patients or the CT subjects had any specific chronic diseases or were on medication, excluding the possibility of such effects on alterations in blood monoamine concentrations and DNA methylation.

Although I did not conduct such post-hoc validation by a different sequencing method, reproducibility between samples using the M value with 1,000 randomly selected CpGs was very high (Pearson's correlation coefficient of 0.98 or higher in all paired cases). Moreover, a recent study by Noble and colleagues demonstrated that the detection of methylation level changes with the Illumina Infinium Methylation EPIC BeadChip and bisulfite-based amplicon sequencing (BSAS) in subjects with cannabis and tobacco users versus nonusers is highly correlated (adjusted $R2 = 0.8878$ and 0.8683 in BSAS vs. EPIC and vice versa, respectively) (Noble et al., 2021). Such correlations are found to be even more accurate at modest levels of methylation changes than robust ones. However, there are several limitations in this study, among which the most obvious and important one is the small sample size, with $n = 10$ for BA patients, and $n = 5$ for control subjects. In addition, BA patients were further divided into KM ($n = 5$) and PP ($n = 5$) patients. Thus, the results reported in this study must be interpreted cautiously under such limitations. This is particularly important, since all 186 CpGs with statistically significant hyper- or hypomethylation identified in BA patients compared to those of controls were not robust, and none of them reached statistically significant differences with multiple testing correction using the Benjamini–Hochberg procedure. Nevertheless, it is still reasonable that functional analysis is performed on a larger set of genes including only nominally significant sites to explore whether important functional interrelationships exist. Further

analysis with a larger sample number is crucial and may still potentially yield a different picture of epigenetic alterations in BA. Another limitation is that no validation was conducted on the results by a different method, such as pyrosequencing or targeted sequencing, to confirm hyper- and hypomethylation in BA patients. Indeed, since this study included only a small number of samples, with only modestly significant sites, the validation of such significant sites is important.

In this study, I examined blood monoamine concentrations, given previous studies showing correlations between blood and brain monoamine levels. For instance, in human subjects, plasma concentrations of the DA metabolite, HVA, were found to be increased with stress (Sumiyoshi et al., 1998) and physical activity (Kendler et al., 1983), which is consistent with an increase in DA release by stress (Pruessner et al., 2004) and physical activity (Wang et al., 2000) in the striatum. The 2-deoxyglucose (2DG)-induced increase in DA in the striatum has also been indirectly correlated with increased plasma HVA in human subjects (Adler et al., 2000; Breier et al., 1993). In animal studies, the stimulation of the nigrostriatal pathway increases, whereas lesions of the pathway decrease plasma DA metabolites (Bacopoulos et al., 1979). The effects of D2 antagonists and apomorphine on the brain have also been demonstrated to alter plasma HVA concentrations (Kendler et al., 1982). In my study, a higher concentration of HVA, but not of DA itself, was observed, along with a higher HVA/DA ratio, in BA patients than in CT subjects. Collectively, these observations suggest that DA release may be increased in the brains of BA patients. In relation to this finding, other studies have shown an association between increased blood DA and HVA concentrations and Internet addiction(Liu & Luo, 2015) and alcoholism (Köhnke et al., 2003), although in Internet addiction, DA is elevated, and whether its metabolites are increased remains unknown. In alcoholism, a genetic background of COMT appears to play a significant role in causing such increased plasma HVA concentrations. In addition to DA, a decrease in one NE metabolite, MHPG, and an increase in 5-HT, but not its metabolite, were observed in BA patients. Although both NE (Sofuoglu & Sewell, 2009) and 5-HT (Müller & Homberg, 2015) transmission have been suggested in drug addiction, how such lower MHPG and higher 5-HT levels may be involved in BA remains less clear.

Given that epigenetic processes have been suggested in the mechanisms of drug addiction (Lax & Szyf, 2018; Nestler & Lüscher, 2019), an analysis of genome-wide DNA methylation was conducted to examine whether underlying epigenetic differences might exist between BA patients and CT subjects. This analysis unveiled 186 hyper- or hypomethylated CpG sites in BA patients compared to CT subjects, most of which were located on gene bodies and intergenic regions but not in promoter regions. Although such methylation on gene bodies has been relatively unexplored, accumulating evidence suggests that methylation in gene bodies is also crucial in the regulation of gene expression (Jjingo et al., 2012; P. A. Jones, 2012; Yang et al., 2014). Approximately one third of CpG sites were found to be correlated with the methylation status of DNA in brain tissues with the IMAGE-CpG database, many of which were, in fact, on the gene bodies. Thus, we conducted gene network analysis to examine the biological functions of the genes in which hyper- or hypomethylated CpGs are present. No direct association was found for monoamine transmission. Instead, this network analysis revealed that genes with hyper- and hypomethylation in BA are involved in cell membrane trafficking and the immune system. Indeed, cell membrane trafficking is the major machinery of synaptic release (G. J. Augustine et al., 1999; Holtzman, 1992). Accumulating evidence also suggests that cell membrane trafficking plays important roles in neurodevelopment (Winkle & Gupton, 2016). The identified immune pathways or genes include major histocompatibility complex (MHC)-class II, T cells, and Interferon-gamma (IFNγ). Studies have shown that MHC-class I is involved in neuronal functions, such as synaptic release, axonal regeneration, synaptic plasticity and neurodevelopment (Cebrián et al., 2014; McAllister, 2014). Moreover, MHC-class I is expressed in dopamine neurons, which, in turn, suppress relapse to reward seeking (Murakami et al., 2018). In contrast, the expression of MHC-class II in the central nervous system is found only in glial cells, such that its roles are more limited than MHC-class I (Perlmutter et al., 1992). Recent studies have demonstrated that T cells yield substantial influence on cognitive functions and the underlying neural activities (Kipnis et al., 2012). In particular, consistent with my finding, a study has now shown that the T cell DNA methylation profile is different in individuals with drug addiction than that in healthy subjects (Lax et al., 2018). IFNγ is a cytokine whose role in

neuronal functions and behaviors such as social interactions has also been reported (Filiano et al., 2016; Monteiro et al., 2017).

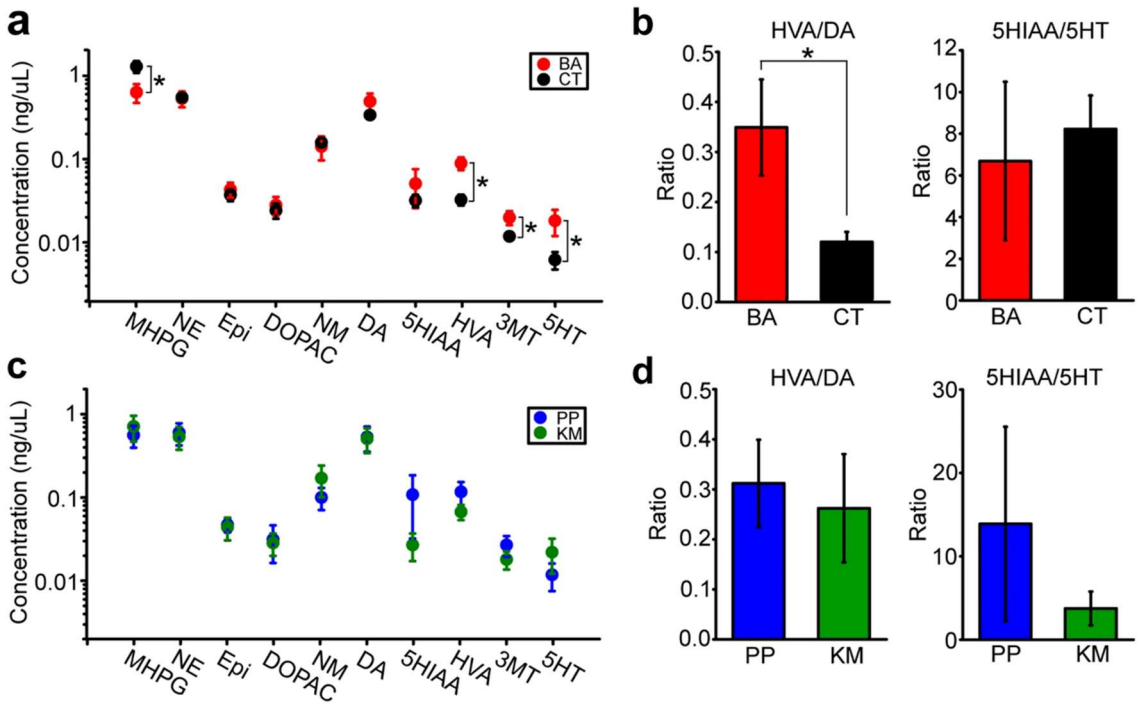
Tissue specific protein–protein interaction analysis was conducted with the online database based on the genes identified with hyper- and hypomethylation. A similar protein–protein interaction network emerged regardless of brain areas, including the frontal cortex, basal ganglia, nucleus accumbens, hippocampus, amygdala, and substantia nigra. Hubs of proteins in this network included CUL3 (BA-CT fold change = 3.41), ACTN1 (BA-CT Fold Change = 6.20), DTX2 (BA-CT Fold Change = -1.54), and TGFBR1 (BA-CT Fold Change = 4.27). CUL3 was the largest node in the network, whose deficiency has been shown to cause social deficits and heightened anxiety (Dong et al., 2020). ACTN1 is a postsynaptic scaffolding protein that interacts with two other postsynaptic scaffolding proteins, SHANK3 and HOMER3 (Sakai et al., 2011). The Homer family of proteins, including HOMER3, is suggested to moderate neuroplasticity associated with drug addiction (Szumlinski et al., 2008). In addition, the downregulation of DTX expression with chronic intermittent ethanol exposure (Melendez et al., 2012), and the upregulation of TGFBR1 with withdrawal from cocaine self-administration in the nucleus accumbens (Gancarz-Kausch et al., 2013) have been reported in rodent studies.

It is of particular interest to note that gene–disease association analysis also unveiled associations of the gene with hyper- and hypomethylation in BA patients with intellectual disability, SCZ, and ASD. Indeed, deficits in synaptic transmission and neurodevelopment are suggested in intellectual disability, SCZ, and ASD (Berdenis van Berlekom et al., 2020; E. J. H. Jones et al., 2014; Owen et al., 2011; Zoghbi & Bear, 2012). In addition, some of the genes, such as SEMA6A (BA-CT Fold Change = 5.34), GSTT1 (BA-CT Fold Change = 5.04), and SCARB1 (BA-CT Fold Change = -1.95) are also associated with addiction to an assortment of drugs, including alcohol, amphetamine, cannabis, cocaine, marijuana, and phencyclidine, and, therefore, could be candidate genes that may be mutually involved in BA and drug addiction.

In conclusion, my study suggests that peripheral blood DNA methylation status and monoamine concentrations may be altered in BA. The genes identified with hyper- or

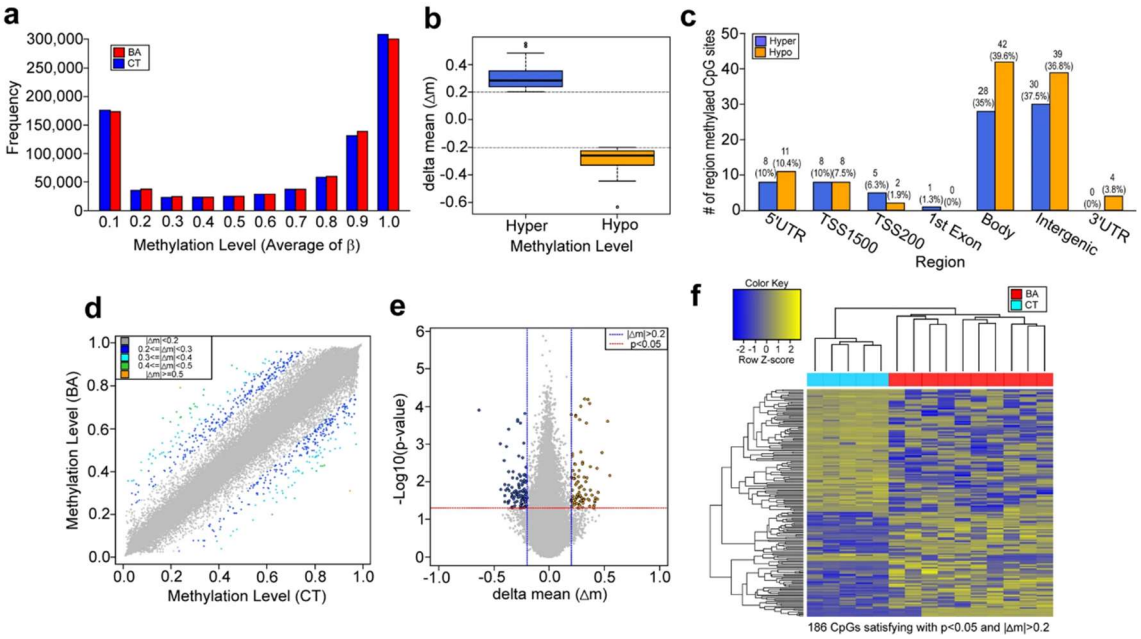
hypomethylation in BA may play roles in cell membrane trafficking and immune functions. Moreover, some of the genes identified with altered methylation in BA appear to include candidate genes for drug addiction, and other psychiatric disorders, such as intellectual disability, SCZ and ASD.

Figure 1



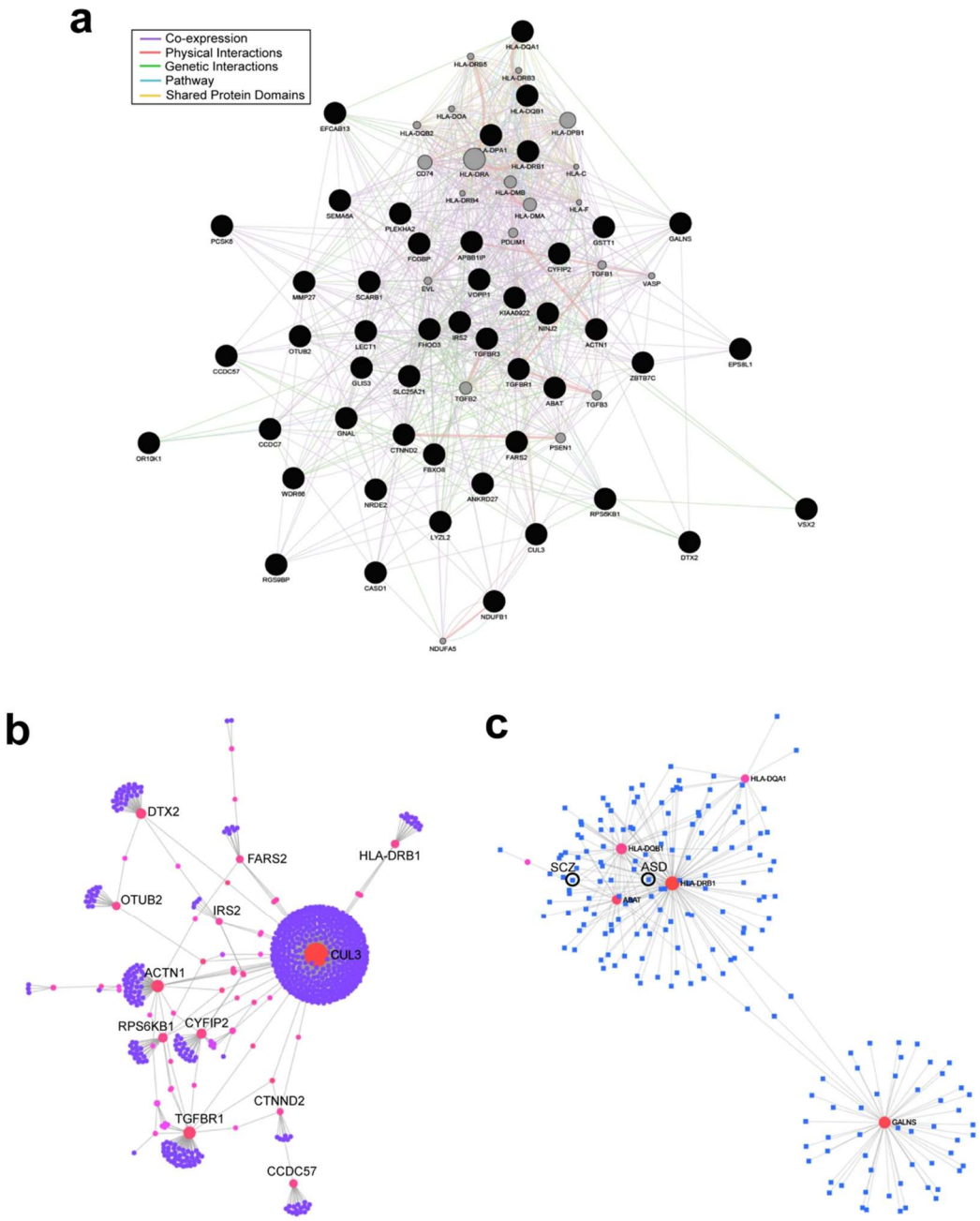
Blood monoamine concentrations in BA patients and CT subjects. (a) A graph showing comparisons of monoamine concentrations between BA patients and CT subjects. *DA* dopamine, *5-HT* serotonin, *NE* norepinephrine, *Epi* epinephrine, *MHPG* 3-methoxy-4-hydroxyphenylglycol, *NM* normetanephrine, *DOPAC* 3,4-dihydroxyphenylacetic acid, *HVA* homovanillic acid, *3-MT* 3-methoxytyramine, *5-HIAA* 5-hydroxyindole acetic acid. Error bars indicate the s.e.m. *$p < 0.05$. (b) Bar graphs showing the ratio of one of the DA metabolites, HVA and DA (left) and that of the 5-HT metabolite, 5-HIAA and 5-HT (right), in BA patients and CT subjects. (c,d) Graphs similar to (a) and (b) but showing comparisons between KM and PP patients.

Figure 2



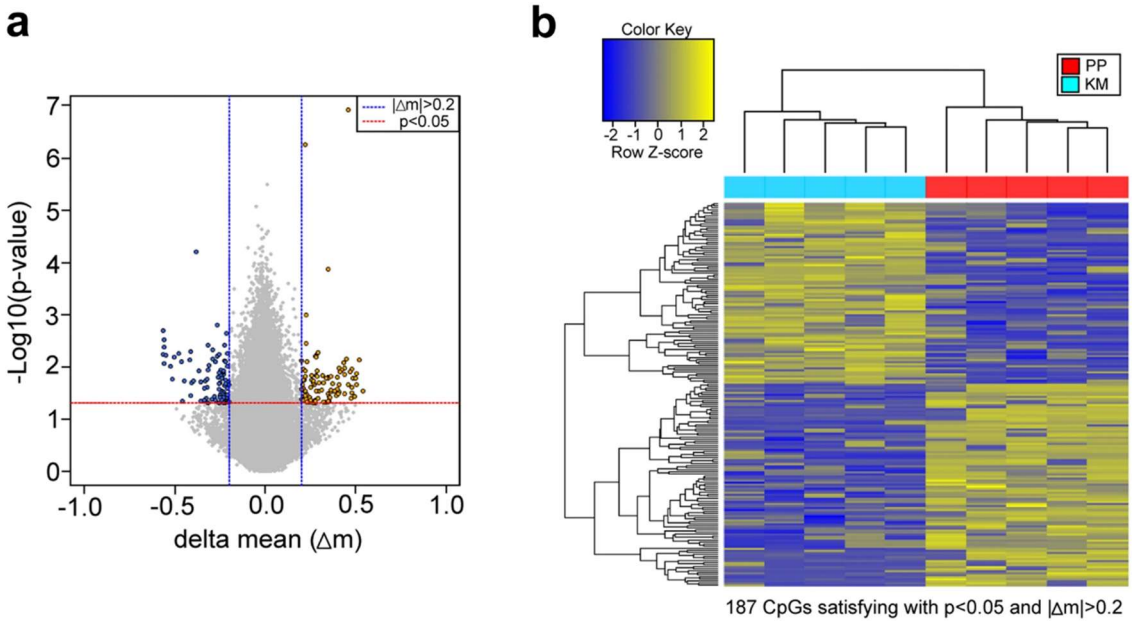
Genome-wide DNA methylation assay in BA patients and CT subjects. (a) A histogram showing the distribution of methylation status on CpGs in BA patients and CT subjects. (b) A box plot showing the levels of hyper- and hypomethylated CpGs in BA patients compared to CT subjects. The dashed line indicates the cut-off ($|\Delta\text{mean}| > 0.2$) for hyper- and hypo-methylation. (c) A bar graph showing the distribution of functional locations for hyper- and hypomethylated CpGs. (d) A scatter plot showing methylation status of the BA patients versus the CT subjects, with the color coding for the level of hypo- and hypermethylation comparing between the BA patients and the CT subjects. (e) A volcano plot for Δmean versus p-value in t test, with the color illustrating the hyper- and hypomethylation that fill the criteria of $|\Delta\text{mean}| \geq 0.2$ and $p < 0.05$. (f) A heat map showing hierarchical clustering on distance similarity for samples and CpGs between the BA patients and the CT subjects.

Figure 3



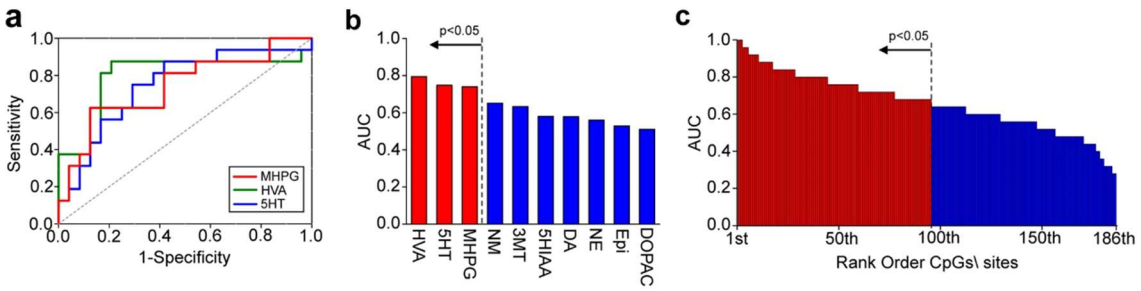
Gene network analyses for the genes with hyper- and hypomethylation in BA patients. (a) A diagram illustrating gene network analysis in which interactions of the genes (coexpression, physical interactions, genetic interactions, pathway, and shared protein domains) are shown. (b) A diagram similar to (a) but showing network analysis for protein–protein interactions in the nucleus accumbens constructed with the genes with identified hyper- and hypomethylation. (c) A network diagram similar to (a) but showing network analysis for gene–disease associations.

Figure 4



Comparisons of genome-wide DNA methylation assays between KM and PP patients. (a) A volcano plot for Δmean versus p-value in the t test, showing hyper- and hypomethylation in KM patients compared to PP patients. (b) A heat map showing hierarchical clustering on distance similarity for samples and CpGs between the KM group and the PP group.

Figure 5



ROC analyses of monoamines and DNA methylation. (a) A graph showing ROC curves of MHPG, HVA, and 5-HT, which are significantly higher or lower in BA patients than in CT subjects. (b) A bar graph showing the AUCs of all monoamines, with red and blue bars representing monoamines whose AUCs are higher or lower than the asymptotic p-value of 0.05, respectively. (c) A graph similar to (b) but showing AUCs of 186 hyper- or hypomethylated CpGs in BA patients. These 186 CpGs are ranked from the 1st to 186th based on the values of AUCs.

Section 5: General Discussion

Aims of this thesis

This thesis aims to investigate behavioral addiction (BA), particularly focusing on under-researched areas such as kleptomania (KM) and paraphilia (PP) and compare their symptoms with more established disorders like gambling addiction and substance use disorders. The challenges in this field include the lack of appropriate animal models, the need to understand the role of negative reinforcement, the importance of investigating positive reinforcement models, and the measurement of monoamines to explore similarities with substance use disorders. The study also aims to examine the neurobiological foundations of behavioral addiction, including aspects of impulse control, obsessive-compulsive disorders, and genetic factors, by comparing them with substance use disorders and conducting preliminary epigenomic research.

Summary of Results

In the chapter 2, the relationship between negative affects such as anxiety, stress, and depression were examined in patients with BA diagnosed with mainly KM or PP, and CT subjects. It was revealed that BA patients have significantly higher levels of stress, anxiety, and depression compared to the CT subjects. However, there were no significant differences observed between KM and PP patients. The objective assessment of anxiety using the HAM-A scale also showed that BA patients had higher anxiety levels than the CT subjects, which was consistent with their self-assessment using the DASS21 scale. Although kleptomania tended to have higher anxiety levels compared to paraphilia patients, this difference was not statistically significant. There was inconsistency between self-reported negative affects and objective assessments. In the CT subjects, a positive correlation was observed between stress and anxiety, while in BA patients, a positive correlation was found only between depression scores. The baseline levels of cortisol, a stress hormone, in the serum, were higher in BA patients compared to the CT subjects and showed a positive correlation with stress and anxiety scores. This suggests a strong association between self-reported negative affects and cortisol levels in BA patients. In the assessment of personality traits,

BA patients exhibited self-assessments that differed from the CT subjects, with slightly higher neuroticism scores. However, there were no significant differences in personality traits between KM and PP patients. Neuroticism showed a positive correlation with negative affects in both BA and CT subjects. BA patients tended to overrate agreeableness and openness. I revealed that BA patients are more likely to experience higher levels of negative affects and exhibit higher neurotic personality traits compared to CT subjects. The correlation between self-reported assessments and objective assessments of negative affect, as well as cortisol levels, in BA patients suggests a complex interplay of psychological and physiological factors. The focus of the chapter 2 was on understanding negative affects, such as stress, anxiety, and depression, in BA patients compared to CT subjects. As a result, these negative affects were significantly higher in BA patients, which is consistent with prior research on other addictions. This study also differed from previous research by using both self-report and objective methods to assess negative affects. Consequently, negative correlations were observed between these affects and specific personality traits, particularly openness and agreeableness, in BA patients but not in CT subjects. BA patients' self-assessment of nature traits of their characteristics significantly differed from objective assessments. These negative correlations suggest that patients may overestimate certain personality aspects. Additionally, this study found a positive correlation between self-reported negative affects (DASS21) and objective assessments of anxiety (HAM-A) and stress hormone (ELISA). However, these correlations were not perfectly consistent. For instance, the correlation between the DASS21 scale and HAM-A was stronger in CT subjects than in BA patients, but the correlation with cortisol measurements was stronger in BA patients. This study proposes that such discrepancies may be due to cognitive biases or differences in the aspects of anxiety assessed by DASS21 and HAM-A. On the other hands, the other characteristic factors, neuroticism, In the hormone analysis, differences in cortisol sampling times between BA patients and CT subjects may have influenced the observed correlation patterns. Overall, I found that BA patients had higher levels of stress, anxiety, depression, and cortisol compared to CT subjects. BA patients also exhibited higher neuroticism and tended to overrate certain personality traits, though further study with larger sample size is needed in the future.

In the chapter 3, I investigated differences in eye movements between KM patients and CT subjects using eye tracker. Eye tracking analysis revealed no significant differences between KM and CT subjects in terms of fixation count, duration, dispersion, blinks, or pupil size. KM patients exhibited weaker correlations in gaze patterns between Kleptomania-related images (MKT images) and other images. These results suggest that KM patients exhibit different gaze patterns to situational cue images which induces addictive, shoplifting behavior compared to CT subjects, indicating distinct behavioral processing. I examined how individuals with KM react behaviorally and neurologically to images containing situational cues related to impulsive symptoms (shoplifting). As a result, KM patients were found to have shorter fixation times on social signals within images, displaying distinct gaze patterns, pupil size, and blink rates. The presence of social signals within images significantly influenced how KM patients processed cues, although it is still unclear whether these findings directly relate to cue-induced cravings, as observed in various addictive disorders. While this study attempted to draw parallels with craving seen in substance use and behavioral addictions, the precise relationship will need further investigation. This study might provide evidence that impulse control disorders like kleptomania may involve distinct processing of situational cues, but further research is needed to confirm these findings and explore their applicability to other impulse control disorders.

In the chapter 4, I analyzed blood samples from 16 individuals with BA, including KM and PP, to study monoamine concentrations and DNA methylation patterns. High-performance liquid chromatography (HPLC) measurements of blood monoamine concentrations revealed that BA patients had lower MHPG levels but higher HVA, 3-MT, and 5-HT levels compared to CT subjects, with no significant differences observed between KM and PP patients. Genome-wide DNA methylation assays identified numerous CpG sites that were methylated in the BA patients, with 106 sites showing low methylation and 80 sites showing high methylation in the BA patients compared to the CT subjects, albeit with moderate significance. Methylation changes were associated with other psychiatric disorders, and gene network analysis indicated their involvement primarily in membrane transport, immune function, and associations with conditions like autism, schizophrenia,

and substance abuse. A comparison between KM and PP patients revealed differing methylation patterns in 88 low-methylation and 99 high-methylation CpG sites, suggesting differences between these BA subtypes. Furthermore, specific blood monoamine concentrations and DNA methylation patterns were shown to potentially distinguish BA patients from CT subjects, suggesting their potential as biomarkers for behavioral addictions. These findings may contribute to a better understanding of the biological basis of behavioral addictions and the development of biomarkers. I revealed an increase in dopamine (DA) turnover and distinct DNA methylation patterns in individuals with BA. These methylation changes were found in genes related to membrane transport, immune function, and were associated with intellectual disabilities, autism spectrum disorder (ASD), schizophrenia (SCZ), and substance abuse, among other psychiatric disorders. Although DA turnover was similar across various BA symptoms, significant differences in DNA methylation were observed between KM and PP. Importantly, the study excluded potential influences of chronic illnesses and medication on blood monoamine levels and DNA methylation. However, this study is constrained by its small sample size, and the statistical significance after multiple testing corrections is not firmly established. Therefore, caution is required when interpreting the results, and further research with larger sample sizes and additional validation methods is necessary. Despite these limitations, this study's methylation analysis demonstrated high reproducibility, providing some reliability to these findings. Blood monoamine concentrations were also investigated due to established correlations between blood monoamine levels and brain monoamine concentrations. BA patients exhibited higher concentrations of HVA, a DA metabolite. This finding aligns with previous research that associated elevated blood DA and HVA levels with internet addiction and alcohol addiction. Similarly, fluctuations in NE and 5-HT levels were observed in BA patients, but the exact roles of these neurotransmitters in BA remain unclear. Moreover, this study examined genome-wide DNA methylation, identifying 186 CpG sites with altered methylation in BA patients. Methylation is believed to regulate gene expression and is correlated with DNA methylation in brain tissues. While direct associations with monoamine transmission were not observed in gene network analysis, involvement in membrane transport and immune system function was suggested. Tissue-specific

protein-protein interaction analysis revealed consistent networks spanning various brain regions, with key proteins like CUL3, ACTN1, DTX2, and TGFBR1 emerging as central nodes. These proteins are involved in social behavior, synaptic scaffolding, and neuroplasticity and have been observed to change in response to substance exposure and withdrawal in animal experiments. Furthermore, gene-disease association analysis identified genes with both increased and decreased methylation in BA patients, which were associated with intellectual disabilities, SCZ, ASD, and substance abuse. These associations suggest that some genes may be common between BA and substance use disorder, as well as other psychiatric disorders.

In conclusion, in the chapter 2, I provided evidence of elevated negative affects consistent with those observed in substance use disorder, suggesting a potential role for neuroticism as a risk factor for behavioral addiction. In the chapter 3, I offered evidence that impulse control disorders like kleptomania may involve distinct processing of situational cues. In the chapter 4, I showed that BA is associated with increased DA turnover and unique DNA methylation patterns, suggesting a potential biological basis for these disorders. These findings provide important insights into the underlying mechanisms of behavioral addiction and its potential overlap with substance use disorder.

Negative and Positive Reinforcement in Behavioral Addiction

The negative reinforcement model in substance use disorders explains why, especially in withdrawal symptoms after continued drug intake, people continue to engage in drug-taking behaviors to alleviate symptoms (Wise & Koob, 2014). In behavioral addiction, on the other hand, there are no withdrawal symptoms, so negative reinforcement similar to that in the context of substance use disorders is unlikely to occur. However, as confirmed in the chapter 2, negative affect in behavioral addiction is clearly high, and indeed kleptomania and sexual preference disorder patients are known to testify that they did so "to relieve stress" (Grant, 2002). This was also seen in the patients in this study. Thus, negative reinforcement may be involved in behavioral addiction as well.

However, unlike substance use disorders, it is unclear whether negative reinforcement is present only

in the later stages of addiction. In behavioral addiction, negative reinforcement may be involved from the beginning, i.e., the goal is to avoid negative emotions from the beginning of the behavior, and this may become habitual.

In order to distinguish behavioral addiction from substance use disorders in terms of the presence or absence of negative reinforcement in the early stages of addiction, it is necessary to follow the person/animal who becomes addicted and observe the early stages of behavioral addiction. However, it may be difficult to confirm the presence or absence of negative reinforcement in the early stages of behavioral addiction because it is difficult to establish animal models in behavioral addiction, and it is also difficult to intervene in humans to make them addicted.

Positive reinforcement is also known to be deeply involved in substance use disorders. Positive reinforcement with the release of phasic dopamine, which occurs when a drug is ingested, increases the incentive salience, and right caudate nucleus may be activated by game-related visual stimuli, leading to the urge and craving for games.

In the chapter 3, kleptomania patients also showed different gaze patterns. This may be associated with positive reinforcement and Incentive salience in kleptomania patients. On the other hand, the study in the chapter 3 did not investigate craving directly and did not include functional neuroimaging studies. In the future, functional brain imaging methods such as fMRI and PET that can investigate the basal ganglia and simultaneously quantify craving through questionnaires, etc., will support the Incentive salience hypothesis of positive reinforcement in behavioral addiction. In other words, in behavioral addiction, as in substance use disorders, there is the emergence of "memories of behavioral experiences," and positive reinforcement accompanied by the release of phasic dopamine is thought to produce craving.

In general, this research might explain both negative and positive reinforcement mechanisms in behavioral addiction. However, it is unclear whether these mechanisms interact in behavioral addiction as well as in substance use disorders. In this regard, I suspect that, unlike substance use disorders, negative and positive reinforcement may occur simultaneously. That is, negative reinforcement is avoidance of pain, but avoidance of pain does not lead to a normal state

but may also produce reward. This mechanism could explain the hypothesis from tonic and phasic dopamine dynamics and may also explain why some people become addicted to substances or behaviors and others do not.

Future Directions of Experimental Designs

This study has significant practical limitations in terms of sample size and experimental designs, and there are several ways in which it deviates from an ideal experiment. First, as mentioned above, the sample size is small compared to that of a typical human psychology experiment, and there are concerns about lack of statistical power. This can be addressed by increasing the sample size in the future. Second, in the case of a human experiment on psychiatric disorders such as this study, it is not possible to prove whether the factors that differ between patients with the disorder and healthy subjects actually play a role in causing the disorder. For example, in the case of this study, the increase in negative affect found in the theft patients does not really prove that it contributes to the theft. Many of the KM and PP patients in this study were awaiting trial after being arrested by the police, and many were in addiction treatment to gain an advantage in that trial. Case-control experiments such as this one are useful for exploratory research, but they cannot prove causality. Several experimental designs and methods can be used to address this problem. One is to conduct an intervention experiment, and another is to conduct a prospective cohort study. If the findings of the present study were developed into an intervention experiment, it is possible to identify a causal role for addiction if pharmacological intervention or noninvasive transcranial stimulation improved the symptoms of addiction. Such designs are currently being investigated in drug addiction and obsessive-compulsive disorder. Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) can provide stimulation deeper in the brain than TMS and tDCS. The development of technologies such as transcranial ultrasound stimulation (TUS or tFUS), which can provide stimulation deeper into TMS and tDCS, is expected to lead to a deeper understanding of addiction.

In addition, prospective cohort studies may be useful for conditions with a relatively large

number of patients, such as gambling addiction, but are difficult to conduct for conditions with a small number of patients per population, such as KM and PP patients.

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