UNIVERZA NA PRIMORSKEM
FAKULTETA ZA MATEMATIKO, NARAVOSLOVJE IN INFORMACIJSKE TEHNOLOGIJE

MASTER'S THESIS
(MAGISTRSKO DELO)

EFFECTS OF AMPHETAMINE-INDUCED SENSITIZATION ON STRESS RESPONSE AND BRAIN ACTIVITY
(UČINKI Z AMFETAMINI INDUCIRANE SENZITIZACIJE NA STRESNI ODZIV IN MOŽGANSKO AKTIVNOST)

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Effects of Amphetamine-induced sensitization on stress response and brain activity

(Učinki z amfetamini inducirane senzitizacije na stresni odziv in možgansko aktivnost)

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Ključne besede: senzitizacija, amfetamini, stres, shizofrenija, fMRI, MIST

Izvleček: Senzitizacijo dopaminergičnega (DA) sistema lahko opredelimo kot okrepljen odziv na večkraten vnos substanc, ki izzovejo sproščanje dopamina, ali občasno izpostavljenost stresnim dogodkom. Nekatere teorije navajajo, da je senzitizacija DA sistema eden od glavnih dejavnikov pri razvoju psihotičnih simptomov pri shizofreniji. Shizofrenija je kompleksna duševna motnja, ki jo povzročajo genetski in okoljski dejavniki ter njihove interakcije. Študije kažejo, da stres v zgodnjem življenjskem obdobju in/ali zloraba drog povečujeta tveganje za razvoj psihoze. Cilj magistrske naloge je bil izvesti prvo MR-študijo navzkrižne senzitizacije med uporabo amfetaminov in stresom pri ljudeh. Še posebej so nas zanimali učinki senzitizacije na mehanizme stresnega odziva in možgansko aktivnost. Devet zdravih moških prostovoljcev je prejelo tri odmerke bodisi d-amfetamina (amfetamin skupina n = 8) bodisi placeba (skupina placebo n = 1), čemur je sledil dan v fMRI skenerju, kjer so prostovoljci reševali Montreal Imaging Stress Test. Odkrili smo šibko pozitivne učinke amfetaminske senzitizacije na subjektivno zaznavanje droge, kot tudi na fiziološki odziv udeležencev. Dokazali smo tudi povezavo med stresnim odzivom in povečano subjektivno zaznavo droge po senzitizaciji, in nevronsko aktivacijo v ventralnem striatumu, centru ki je povezan z akutnim stresom. Rezultati so pokazali, da stres in amfetamini potencirajo drug drugega, pri čemer vključujejo in potekajo preko specifičnih skupnih nevronskih poteh.

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Abstract: Sensitization of the dopaminergic system can be described as an amplified response to repeated administration of dopamine releasing agents or intermittent stress exposure. Some theories suggest that sensitization of the dopaminergic system is a major cause of the development of psychotic symptoms in schizophrenia. Schizophrenia is a complex mental disorder caused by genetic and environmental factors and their interactions. Early life stress and drug abuse seem to increase the risk of developing psychosis. The aim of the present master's thesis was to conduct a first amphetamine-stress cross-sensitization MR study in humans. We were specifically interested in the effects of sensitization on stress response mechanisms and brain activity. Nine healthy male volunteers received d-Amphetamine (amphetamine group n = 8) or placebo (placebo group n = 1) for three days, followed by an fMRI session in which they performed the Montreal Imaging Stress Test. We found weak positive effects of amphetamine sensitization on overall subjective drug experience as well as on physiological measures. We found a correlation between increased subjective drug experience after sensitization and neural activation in the ventral striatum during the stress task. Although preliminary, our results suggest that stress and amphetamine cross-sensitize and involve specific neural patterns. This study suggests some promising avenues to better understand the etiology of schizophrenia through the dopamine sensitization hypothesis.

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5-HT – serotonin
ACTH – adrenocorticotropic hormone
AMPH – amphetamine
CAT – catecholaminergic system
CNS – central nervous system
CORT – cortisol
CRH – corticotropin-releasing hormone
CrICI – credible intervals
DA – dopamine
DAT – dopaminergic transporter
dBP – diastolic blood pressure
DEQ – drug effects questionnaire
ELISA - enzyme-linked immunosorbent assays
fMRI – functional magnetic resonance
FWE – family-wise error
FWHM – full-width at half-maximum
GC – glucocorticoids
GLM – general linear model
GRs – glucocorticoid receptors
HC – hippocampus
HC – hippocampusHPA - hypothalamic pituitary adrenocortical
HR – heart rate
MAO – monoamine oxidase
MCMC – Markov-Chain Monte Carlo
MDA - 3,4-methylenedioxyamphetamine
MDE - 3,4-methylenedioxy-N-ethylamphetamine
MDMA - 3,4-methylenedioxymethamphetamine
MIST – Montreal imaging stress task
MNI – Montreal Neurological Institute
mPFC – medial prefrontal cortex
MR – magnetic resonance
MRs – mineralocorticoid receptors
NA – noradrenaline
NAcc – nucleus accumbens
NUTS – No-U-Turn
PFC – prefrontal cortex
ROI – region of interest
SAM - sympathetic adrenomedullary system

sBP – systolic blood pressure
THC - Δ9-tetrahydrocannabinol
VTA – ventral tegmental area

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1 INTRODUCTION

1.1 THE CONCEPT OF SENSITIZATION

Drugs of abuse, including amphetamines, cocaine, opiates, nicotine, Δ9-tetrahydrocannabinol (THC), and alcohol, represent a diverse group of compounds that markedly differ in their neurochemical and behavioral actions (Robinson and Berridge 1993; Weidenauer et al. 2016). It is well known that repeated drug administration leads to tolerance, that is, diminished response to drugs, but in some circumstances behavioral drug effects may simultaneously increase. The latter phenomenon is called sensitization (Mayer and Quenzer 2005; Robinson and Berridge 1993). In a pharmacological context, sensitization is defined as an amplified response to a repeated administration of a substance (Mayer and Quenzer 2005; Weidenauer et al. 2016). More specifically, sensitization denotes a non-associative learning process in which repeated exposure to a stimulus leads to a progressive amplification in the behavioral and neurochemical response (Weidenauer et al., 2016). For instance, studies in animals have shown that repeated intermittent administration of D-amphetamine produces a progressive increase in locomotor activity and stereotyped behaviour (Robinson and Becker 1986; Robinson and Berridge 1993). Furthermore, studies in humans have shown that repeated exposure to low-dose amphetamine progressively increases alertness, euphoria and attention but also elevates stress response (Strakowski, Sax, Rosenberg, DelBello, Adler 2001; Booij et al. 2006). It is well known that physiological effects of acute amphetamine administration include elevated blood pressure and heart rate (Meyer & Quenzer 2005; Urman-Yotam & Ostacher 2014), although concerning amphetamine sensitization, studies did not report large effect (Boileau et al., 2006; O'Daly, Joyce, Stephan, Murray, & Shergill, 2011).

Drugs of abuse have a common mechanism of action. They directly or indirectly increase the brain extracellular dopamine (DA) levels immediately after drug administration. Yet, a release of dopamine is also a central process in the neurochemistry of behavioral learning, where dopamine release and specific patterns of activity in dopaminergic neurons are detected during conditioned learning paradigms. Animal models show that learning rate in conditioning paradigms can be influenced by manipulating brain DA transmission. Higher levels of extracellular DA are associated with an increased response to the conditioned stimulus, and lower levels are associated with decreased conditioned response. These findings represent the core of the dopamine theory of addiction. Furthermore, there is substantial evidence that sensitization to psychostimulants is associated with a progressive increase in the amount of DA released in response to a given dose of the drug. Explained in the term of "drug learning", also used when referring to psychostimulant sensitization, release of brain DA is a neurochemical mechanism common to behavioral learning and sensitization to psychostimulant drugs (Weidenauer et al. 2016).

Behavioral sensitization to amphetamine-like psychostimulants is a consequence of intermittent administration of amphetamine (AMPH); it is defined as an augmentation in the behavioral effect of a psychostimulant upon re-administration. The underlying mechanisms of intermittent AMPH intake lead to long-lasting changes in behaviour. It is believed that altered dopamine neurotransmission plays a critical role in reinforcing addictive behaviour, as well as in behavioral stimulant effects (Robinson and Berridge 1993; Boileau et al. 2006; Pierce and Kalivas 1997). It has been proposed that behavioral sensitization involves modifications in the midbrain dopamine system (Boileau et al. 2006). Intermittent exposure to AMPH repeatedly stimulates dopamine receptors in ventral tegmental area (VTA), which triggers a cascade of molecular events and changes in neuronal plasticity that, in turn, enhance dopamine release (Boileau et al. 2006). The enduring changes on D1 receptors in VTA contribute to increase glutamate and decrease in GABA transmission (Venzina 1996; Pierce and Kalivas 1997). These changes promote the increased firing frequency and/or burst activity of mesoaccumbens dopamine neurons. The changes in presynaptic and postsynaptic dopamine transmission in the nucleus accumbens (NAcc) and striatum contributes to the expression of behavioral sensitization to psychostimulants. These changes include augmented extracellular dopamine in NAcc and striatum, reduced density of dopamine transporters, significantly reduced affinity of binding sites of dopamine transporter and enhanced D1 receptor sensitivity (Pierce and Kalivas 1997).

Altered dopamine neurotransmission may also be linked to the genesis of psychotic symptoms in schizophrenia (Boileau 2006; Peleg-Raibstein, Knuesel, Feldon 2008). There are also evident cases of non-psychotic individuals, who have repeatedly used AMPH and developed a psychosis that resembles paranoid schizophrenia (Robinson and Berridge, 1993).

Stress is also well known to importantly contribute to the development and exacerbation of neuropsychiatric disorders (Booij et al. 2006). For instance, prenatal infections and nutrition, early life stressors and urbanicity, which interact with multiple genes induces persistent sensitization in DA and glutaminergic systems and induce enhanced reactivity to mild stressors, which can lead to the relapse and/or development of schizophrenia over the sensitization of stress response (Yuii, Suzuki, Kurachi, 2007).

1.2 AMPHETAMINES

Amphetamine (AMPH) is the parent compound of a family of synthetic psychostimulants, which also includes MDMA (3,4-methylenedioxymethamphetamine), MDA (3,4-methylenedioxyamphetamine) and MDE (3,4-methylenedioxy-N-ethylamphetamine). AMPH exists in two chemical forms, L-amphetamine and D-amphetamine. As a central nervous system (CNS) stimulant, it is used to treat narcolepsy, obesity, and attention deficit

hyperactivity disorder (ADHD) (Meyer & Quenzer 2005, Bramness et al. 2012, Seiden, Sabol, Ricaurte, 1993).

1.2.1 Effects

As a psychostimulant, AMPH can prolong wakefulness, increase attention and feeling of energy, decrease fatigue and produce euphoric effects in humans, and often lead to its habitual use or abuse. In some cases, it also causes empathogenic (producing experiences of emotional communion), and hallucinogenic effects (Bramness et al. 2012, Carvalho et al., 2012). Behavioral effects of AMPH in humans have close parallels to those in animals. In both humans and animals, low dose of AMPH increases stereotypic (unnecessary repetitive movement) behaviour and locomotor activity, whereas higher doses produce species-specific stereotypies (Seiden et al. 1993). Physiological effects include elevated body temperature and heart rate, increased blood pressure, pupil dilation and the lack of appetite (Meyer & Quenzer 2005; Urman-Yotam & Ostacher 2014). Adverse reactions include anxiety, aggression, irritability, confusion, panic attacks and impulsivity (Bramness et al. 2012, Urman-Yotam & Ostacher 2014).

1.2.2 Pharmacodynamics

AMPH is indirect agonist of the catecholaminergic (CAT) system and is structurally related to the monoamine neurotransmitters, which accounts for its potent effects on the monoamine system (Meyer & Quenzer, 2005). AMPH stimulates alpha- and beta- adrenergic receptors in the body and brain, and increase the activity of DA, noradrenaline (NA) and serotonin (5-HT), however we will primarily focus on DA activity (Urman-Yotam & Ostacher 2014). AMPH increases DA activity over transporter-mediated DA release into synapse, inhibition of DA reuptake and finally, over the inhibition of monoamine oxidase (MAO) activity (Seiden et al. 1993).

First, AMPH is recognized by dopaminergic transporter (DAT) and enters the DA neuron through DAT, or by diffusion across the cell membrane directly (Carvalho et al. 2012, Meyer & Quenzer 2005). Inside the cell, AMPH interacts with the vesicular monoamine transporter 2 (VMAT2) and enters the synaptic vesicle, which causes the release of DA from the synaptic vesicle into cytosol, and thereby depletes vesicular neurotransmitter storage (Bramness et al. 2012, Carvalho et al., 2012). Once in the cytoplasm, DA exits the neuron, which is mediated by DAT, and it releases into the extracellular fluid or synaptic cleft (Carvalho et al. 2012, Meyer & Quenzer 2005, Seiden et. al 2013). AMPH is a substrate for the DAT that competitively inhibits DA reuptake, which therefore increases concentration of DA within the synaptic cleft (Calipari, Ferris, Jones 2013; Bramness et al. 2012; Kuczenski & Segal 2002). Finally, AMPH is known for its MAO inhibitory properties, where it inhibits

the metabolism of monoamines and therefore increases the cytosolic DA content (Carvalho et al. 2012, Seiden et. al 2013).

1.2.3 Amphetamine and dopaminergic system

Once the DA is within the synaptic cleft, it binds to the two major subclasses of DA receptors; D1 and D2. Post-synaptic D1-like and D2-like (which is also pre-synaptic and therefore autoreceptor) receptors are involved in the regulation of cognition, affect, neuroendocrine secretion, motor activity and several neurological disorders such as schizophrenia (Jaber, Robinson, Missale, Cargon, 1996). DA receptors have the highest density in the brain areas, involved in three major groups of DA circuit: Nigrostriatal pathway involves DA cell bodies in the substantia nigra and projects to the striatum (Seiden et. al 2013). Nigrostriatal pathway plays a critical role in motor activity, and therefore in AMPH induced stereotypy and locomotor behaviour (Creese & Iversen, 1974). Mesolimbic pathway originates from the ventral tegmental area (VTA) and projects to the ventral striatum, nucleus accumbens (NAcc), amygdala and hippocampus (Cole, Konradi, Douglas, Hymann, 1995; Seiden et. al 2013). Mesolimbic pathway is also known as the »reward pathway«, which accounts for the reinforcing behaviour, and euphoric and addictive properties of AMPH (Bramness et al. 2012; Cole et al. 1995). D2 receptors in the mesolimbic pathway also play an important role in psychotic symptoms of schizophrenia and AMPH induced psychosis (Peleg-Raibstein et al. 2009; Snyder 1976). The last major group of DA cell bodies lies in the VTA and projects to the prefrontal cortex (PFC), and is essential for cognitive control, motivation and emotional response. Excessive DA and glutamate in the cortex may overwhelm GABAnergic interneurons, causing dysregulation of the signals, which may relate to the psychotic symptoms caused by AMPH intoxication (Hsieh, Stein, Howells, 2014). Some DA cell bodies are located in the arcuate nucleus of the hypothalamus and send forth short axons which descend to give off terminals in the median eminence. This DA tract plays a role in regulating the secretion and synthesis of the trophic hormones of the pituitary gland (Snyder, 1972).

1.2.4 Amphetamine psychosis

AMPH is considered to be psychotomimetic. In patients diagnosed with schizophrenia, AMPH can rapidly exacerbate psychotic symptoms at even small doses, and if a patient is in remission, it can elicit psychosis, that can be clinically indistinguishable from acute paranoid schizophrenia (Robinson & Becker 1986; Snyder 1974). Nevertheless, psychotic symptoms after AMPH intake are also observed in the non-psychiatric individuals (Bramness et al., 2012). In the non-psychiatric individuals, *toxic psychosis* can occur after a large single dose of AMPH and is characterized by confusion and disorientation (Seiden et al., 1993). On the other hand, perhaps the most dramatic effect of AMPH has been described in those, who

chronically used the drug (Robinson & Becker 1986). *Repeated-use psychosis*, which is considered to resemble schizophrenia, can occur in chronic AMPH users after continuous high-dose use (500-1000 mg/day), or after lower-dose use (20-80mg/day, or 0.3-1.2 mg/kg) and is characterized by increased motor activity, repetitive and compulsive behavior, lack of insight, suspicion, disorganization of thoughts, social withdrawal, delusions and paranoia (Seiden et al., 1993; Bramness et al., 2012). Link between the AMPH intake and psychotic symptoms has been described in many studies, where the escalating doses of AMPH were applied until the induction of psychotic symptoms in healthy volunteers (Ellinwood, Sudilovsky, Nelson, 1973). Almost undistinguishable differences between AMPH induced psychosis and schizophrenia led to the hypothesis that AMPH psychosis might serve as a model for paranoid schizophrenia (Snyder, 1972).

1.3 SCHIZOPHRENIA

Schizophrenia is a complex, chronic mental health disorder characterized by various positive and negative symptoms (Mizrahi et al. 2012; Patel, Cherian Gohil, Atkinson, 2014). Positive symptoms include delusions, hallucinations, thought disorder and motor symptoms, while negative include poverty of speech, apathy, social withdrawal, and impaired cognitive ability. The presence of pronounced positive symptoms refers to an acute psychotic episode, and generally, positive symptoms are reduced with antipsychotics. Negative symptoms frequently occur in-between the acute psychotic episodes and represent a major therapeutic challenge in the treatment of schizophrenia (APA, 2013). The fact that the positive symptoms are reduced in response to antipsychotic treatment, that usually entails blocking of $D_{2/3}$ dopamine receptors, is one of the reasons why psychosis has been associated with a hyper-dopaminergic activity (Weidenauer et al., 2016).

Symptoms of schizophrenia usually occur during the late teenage years and early twenties, whereby most people are diagnosed in their late teens to early 30s. On the other hand, symptoms of late onset schizophrenia occur at age of 40 or later, which is relatively rare in comparison to early onset schizophrenia (Mayer & Quenzer 2005; Howard, Castle, Wessely, Murray, 1993).

The current evidence implies that the various phenotypes of the illness arise from several complex factors, including genetic susceptibility and environmental influences, which importantly contribute to the development of psychotic illness (Collip, Myin-Germeys, Van Os, 2008; Howes & Kaupur 2009; Patel et al. 2014; Mizrahi et al. 2012; van Winkel, Stefanis, Myin-Germeys, 2008). Genetic factors play an important role in the causation of schizophrenia (Tsuang, 2000). For example, in case of monozygotic twins, the risk for psychosis development of a twin sibling with psychosis is 48%, and in case of second-degree relatives, the risk is 4% (Patel et al., 2014). However, since there is around a 48% concordance rate for schizophrenia among monozygotic twins, there should be substantial epigenetic or environmental factors, which affect expression of the genes, that contribute to

the development of the illness. Some environmental and social factors clearly contribute to the development of schizophrenia; (1) Exposure to psychosocial stress including childhood abuse (trauma), urbanicity, migration, unemployment, social isolation, and (2) drug exposure (Howes & Kapur 2009; Patel et al., 2014; Mizrahi et al. 2012). There is a suggestion, that development of schizophrenia begins in utero; obstetric complications like bleeding during pregnancy, infections and excess stress levels during second trimester (a key stage in neurodevelopment), have been linked to an increased risk of offspring developing schizophrenia (Howes & Kapur 2009; Patel et al., 2014). Despite numerous studies on etiology of schizophrenia, the precise cause, and interaction between molecular genetic measures and psychosocial stressors is still unknown. However, according to current interpretations, it seems that all known risk factors converge in a common final pathway, where environmental exposures may induce, in interaction with (epi)genetic factors, changes in DA neurotransmission that causes psychotic symptoms in schizophrenia. These environmental changes with (epi)genetic factors may induce psychological or physiological alterations that can be traced to a final common pathway of altered DA neurotransmission, broadly referred to as sensitization (Laurelle 2000; Howes & Kapur 2009; Collip et al. 2008; van Winkel et al. 2008; Weidenauer et al. 2016).

1.3.1 Dopamine hypothesis

While genetic and environmental etiological factors influence neurodevelopmental and neuroplastic processes, which contribute to predispose people to the disease, the actual onset and course of the illness is predominantly described with neurobiological basis of DA hypothesis, which, despite its limitations, remains the preeminent neurochemical theory of schizophrenia (Davis, Kahn, Ko, Davison, 1991; Grace 1991; Lieberman, Sheitman, Kinon, 1997). DA hypothesis presumes, that certain DA pathways are overactive in schizophrenia, or specifically, that psychotic symptoms develop because of hyperactivity in the DA neurotransmission, which runs over DA cell bodies, located in the VTA of the midbrain, to their terminal fields in NAcc and limbic cortex (Lieberman et al. 1997; Seeman 1987). Evidence for DA hypothesis is derived indirectly from pharmacological background; (1) drugs that decrease DA activity are considered to be antipsychotic, where the therapeutic dose of antipsychotic drug is proportional to their binding affinity for the D_2 receptor and (2) drugs that promote DA activity (e.g., AMPH), may be psychotomimetic (Meltzer & Stahl 1976; Creese & Iversen. 1974; Lieberman 1997). Although the DA hypothesis presumes that DA activity is higher than normal in schizophrenia, there is data indicating that it also involves low prefrontal activity and therefore decreased DA. It is suggested, that deafferentation of frontal cortical neurons is linked to hypodopaminergia in mesocortial and hyperdopaminergia in mesolimbic dopamine neurons (Davis et al. 1991; Pycock, Kerwin, Carter, 1980). Hypodopaminergic state in frontal cortical terminal fields of the mesocortical DA neurons, whose cell bodies are located in VTA, has been hypothesized to be the basis of

the negative symptoms, while hyperactivity of mesolimbic (VTA, NAcc, limbic cortex) DA neurons, are believed to cause positive symptoms (Lieberman et al., 1997). It has been proposed that the mechanism, which regulates DA release in subcortical regions, runs by two independent mechanisms: *phasic*, impulse dependent DA release resulting from VTA neuron firing and *tonic*, sustained release regulated by PFC afferents. Lower activity in PFC results in decreased tonic inhibition of phasic DA release, which results in more phasic DA release from VTA (Grace, 1991).

1.3.2 Schizophrenia and endogenous sensitization

As mentioned previously, and reviewed by Lieberman and colleagues (1987), a number of studies have provided evidence that schizophrenic patients, as a group, display increased behavioral sensitivity to the psychotogenic effects of acute psychostimulant administration. In some cases, patients with schizophrenia develop emergence or worsening of psychotic symptoms after acute psychostimulant exposure at doses that do not induce psychosis in healthy subjects. While this enhanced response is observed in patients, previously never exposed to psychostimulants, this sensitized state is termed *endogenous*, in contrast to the substance induced sensitized state observed in subjects with a history of substance-induced psychosis.

Laurelle (2000) summarized key features of exaggerated behavioral response in patients with schizophrenia which emerged over the years.

1. In schizophrenic patients, the clinical response to acute psychostimulant challenge is highly heterogeneous. In approximately 40% of patients it comes to a worsening of positive psychotic symptoms, 40% show no changes and 20% show diminishing of positive symptoms following acute psychostimulant administration. Negative symptoms decrease or cause no changes.
2. General behavioral activation (i.e., euphoria, restlessness, talkativeness) is unrelated to the psychotogenic reaction induced by the psychostimulant challenge. Thus, the psychotogenic reaction is more than a simple behavioral activation that would make the psychotic processes more obvious. When a psychotogenic reaction occurs, the psychotic response is comparable to the »spontaneous« psychosis presented by the subject during the active episode of the subject's illness.
3. The psychotic response is state dependent. Patients who responded with a psychotic reaction to a psychostimulant challenge during an acute episode, didn’t show psychotic reaction when in remission. Vulnerability to psychostimulant-induced psychosis is also associated with a higher rate of relapse followed by neuroleptic discontinuation. Therefore, psychostimulant challenge has been proposed as a predictor of relapse. So, the tendency to developing psychotic reaction to a psychostimulant challenge might unmask an active phase of the illness, not readily identifiable by the clinical symptomatology in the absence of a challenge.

Support for the endogenous sensitization validity also includes recent neuroimaging reports, which demonstrate enhanced striatal dopamine release induced by an acute AMPH challenge in first-episode schizophrenia patients relative to healthy controls. It is further linked to an overexpression of mesolimbic dopamine D_2 receptors in the patients. This evidence has led to the *endogenous sensitization* hypothesis of schizophrenia, which postulates that a sensitized DA system is intrinsic to the disease and is responsible for the genesis of psychotic symptoms (Peleg-Raibstein, Yee, Feldon, Hauser, 2009).

1.4 STRESS

Besides the substance use disorder, psychological stress is well known to importantly contribute to the development and exacerbation of neuropsychiatric disorders including anxiety, depression and schizophrenia (Booij et al. 2016; Lodge & Grace 2011). For instance, prenatal infections and nutrition, early life stressors, childhood trauma and urbanicity, are more common in people with schizophrenia than in the general population (Yuii, Suzuki & Kurachi, 2007). The major explanatory model for the impact of psychosocial factors on schizophrenia is the *neural diathesis-stress model*, which incorporates endogenous vulnerability and psychosocial stress over neural mechanisms and developmental processes in etiology (Walker, Mittal, Tessner, 2008; Walker & Diforio, 1997). The process, which is potentially involved, is sensitization; that is, following repeated exposure to stressors and/or psychostimulant drugs, some effects can become progressively greater (Booij et al. 2006). It has been proposed that exaggerated responses to stress are key in the etiology of psychosis in vulnerable individuals (Soliman et al., 2008).

A certain situation is perceived as stressful if the environmental demand of a particular event exceeds the natural regulatory capacity of an organism available. Those situations include unpredictability and uncontrollability (Koolhaas et al., 2011). In the short term, stress produces adaptive changes that help respond to the stressor, while in the long term, however, it produces changes that might be maladaptive. When a person's self or body is exposed to harm or threat, the result is a cluster of physiological changes generally referred to as the stress response. Either psychological or physical stressor, it produces the same core pattern of physiological changes. Most frequently implicated in mental health is however, chronic psychological stress (Pinel & Barnes, 2018). Although psychological stress is included in most etiologic models of schizophrenia, and the association has been documented since the 1950s, however, biological mechanisms by which stress actually affect schizophrenia are still not entirely clear (Corcoran et al., 2003; Walker et al., 2000).

1.4.1 Mechanisms

The biological effects of stress are mediated by two key player systems, the hypothalamic pituitary adrenocortical (HPA) axis and the sympathetic adrenomedullary (SAM) system. SAM has a fast noradrenergic component corresponding to hyper vigilance and alertness, while activation of a slower HPA system provides and mobilize energy (oxygen and nutrients) to active organs and tissues to tackle the present stress-provoking event (Koolhaas et al., 2011). Both of them are tightly connected with the dopaminergic system, but we will primarily focus on HPA, since it has been linked with a range of mental disorders, including psychoses.

1.4.1.1 HPA axis

The HPA axis is a major part of the neuroendocrine system, mediating the stress response in mammals. It involves three chemical messengers: corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and glucocorticoids (GC). In response to stress, cells in the periventricular nucleus of hypothalamus release CRH, which stimulates the pituitary to release ACTH. In turn, ACTH stimulates the adrenal cortex to secrete glucocorticoids, specifically cortisol (CORT) in primates and corticosterone in rats (Corcoran et al. 2003, Walker et al. 2008). The adrenal glands have an essential role in stress response providing both the catecholamines to activate the organism to actively cope with the stressor and the corticosteroids to counteract the primary stress reaction and let the organism return to homeostasis (Gispen-de Wied, 2000). Cortisol acts on the brain through binding to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), which are located on cell bodies, including neurons. Glucocorticoid receptors are located in various regions throughout the brain, including the pituitary, the hypothalamus and have a particularly high density in the hippocampus (HC). Binding to these receptors in the hippocampus triggers a negative feedback system that dampens hypothalamic release of CRH and ACTH, thus modulating HPA activity. HC has also been implicated in an assessment of stressor intensity. In primates, cortisol acts to synchronize other components of the stress response and alter the excitability of neuronal networks. (Corcoran et al. 2003; Dedovic et al.; Walker et al. 2008). It has been proposed that prolonged activation of the HPA axis, which is the consequence of chronic stress, with persistent hypersecretion of glucocorticoids can have adverse effects on changes in regional brain volumes and also neuronal structure and function. These changes in neurobiological pathways leading from stress to brain dysfunction may also have a potential role in psychosis (Pruessner, Cullen, Aas, Walker, 2017). Brain regions that are most sensitive to the detrimental effects of stress exposure include HC, PFC and amygdala (Arnstein 2009; Vyas, Mitra, Shankaranarayana Rao, Chattarji, 2002; Mizoguchi, Yuzurihara, Isige, Sasaki, Chui, Tabira, 2000). A growing body of evidence has shown the vulnerability of HC to degenerative changes caused by chronic

stress, resulting in structural and functional hippocampal damage. Chronic stress induces dendritic atrophy and debranching in pyramidal neurons of the HC, neurotoxicity and decreasing neurogenesis. These alterations contribute to subsequent cognitive functions, such as learning and memory, and also provide a potential explanation for the hippocampal shrinkage associated with many psychopathologies, including schizophrenia (Joo Kim, Pellman, Kim, 2015; Lodge, Grace 2011; Vyas et al. 2000). In PFC, exposure to chronic stress leads to extensive functional and architectural alterations. Structural changes include loss of dendritic material; dendrite length, branching and spine density, which are associated with marked PFC dysfunction in attentional set shifting and working memory (Arnstein, 2009). Relationship between the PFC and the hippocampus is also disrupted by chronic stress, which causes rigidity in memory consolidation. In contrast to the PFC and the hippocampus, chronic stress in amygdala results in expansion of dendritic arborization and hypertrophy. Based on the comprehended knowledge about chronic stress on essential brain regions that mediate stress response it is clear, that chronic stress weakens the structures that provide negative feedback (PFC, hippocampus) and strengthen the structures that promote the stress response (Arnstein, 2009; Vyas et al.2002; Mizoguchi et al. 2000).

1.4.2 Habituation and sensitization

In normal human subjects, cortisol release is linked with acute exposure to stressors across the life span. For example, a brief maternal separation is associated with an increase in cortisol release in human infants, and adults' cortisol release is heightened in response to a variety of stressful experiences, including the anticipation of public speaking and examinations. Habituation to a stressor is manifested in diminished biological and behavioral responses with repeated exposure, but under certain conditions, there appears to be a sensitization effect of exposure to stressors. For example, when neonatal rats were exposed to stressors of sufficient magnitude, not only immediate behavioral changes and increased release of corticosterone occured, but it also produced augmentation of subsequent behavioral and biological responses to the stressor, which suggest hyperreactivity of HPA axis (Walker et al. 1997). The responsivity of the HPA axis can be probed through challenge, either pharmacological or by inducing stressor. For example, dexamethasone, a steroid, normally provides negative feedback to the HPA axis, leading to a suppression of cortisol secretion. An abnormal dexamethasone test, a test used to assess adrenal gland function by measuring changes in cortisol levels, shows failure in cortisol suppression, which indexes the deficit in negative feedback and thus hyperresponsiveness of HPA axis (Corcoran et al., 2003). There are several factors contributing to determining whether the GC response shows habituation or sensitization; stressors of greater intensity and lower controllability are more likely to produce sensitization in rodents, however in human infants, stressor intensity and individual differences in neonatal health status appear to influence the likelihood of sensitization. Furthermore, chronic stress and persisting elevation of GC also

lead to permanent changes in HPA axis, where the most notable is impaired negative feedback system that serves to dampen HPA activation (Walker et al. 1997).

1.4.3 Stress reactivity in psychosis

In schizophrenia, stress has been predominantly described in terms of the impact of »life events« and expressed emotions (Gispen-de Wied, 2000). A few studies suggest that sensitization, as previously mentioned, might be an underlying mechanism connecting stress and psychosis. Sensitization may be hypothesized to represent underlying vulnerability of stress reactivity pathways characterized by increased emotional and psychotic reactions to stress. The process of increased emotional and psychotic reactions, or behavioral stress sensitization, occur when previous exposures to severe or enduring stressors result in increased responses to small stress events of daily life (Myin-Germeys, Van Os, Schwartz, 2001; Myin-Germeys, Van Os, 2007; Myin-Germeys, Delespaul, Van Os, 2005). For example, childhood trauma has been suggested to increase the sensitivity to minor stress in daily life (Gispen-de Wied 2000; Glaser, van Os, Portegijs, Myin-Germeys, 2006). Furthermore, there is evidence that exposure to excessive levels of high expressed emotion or stressful life events can precipitate to episodes of schizophrenia (Yeap & Thakore, 2005). This sensitivity to stress in schizophrenic patients has been conceptualized in the *neural diathesis-stress mode*l where schizophrenia is described as the result of complex interaction between biological and psychological factors. Genetical determination and stressful life events all contribute to an individual's vulnerability to develop a psychosis under stressful circumstances (Gispen-de Wied, 2000). In order to understand sensitization and association between stress and psychosis, it is important to involve a plausible hypothesis of biological mechanisms, where dysfunction of HPA axis and DA system seem to be a reasonable candidate system.

1.4.4 HPA dysfunction in schizophrenia

A *neural diathesis-stress model* was already mentioned in the previous section, but will be here explained in more detail. It is a model of schizophrenia by Walker and Diforio (1997), which suggests that HPA axis may be responsible for triggering a cascade of events resulting in neural circuit dysfunction, including alterations in DA signaling. The model is based on evidence that HPA axis hormones, especially cortisol, affect brain and behavior. This suggests a link between HPA activity and psychosis.

Schizophrenia is known to be associated with elevated cortisol, and the administration of corticosteroids have been observed to induce psychotic symptoms (Walker & Diforio 1997, Walker et al. 2000). Manifestation of HPA dysregulation in schizophrenic patients can be seen by means of increased baseline cortisol and ACTH hormone levels, increased cortisol response after pharmacologic challenge, and also through abnormalities in GCs (Corcoran et

al. 2003; Walker & Diforio 1997; Yeap & Thakore, 2005). Furthermore, many studies reported the reduction in the hippocampus volume, which plays a key role in dampening HPA activity. Although hippocampal volume is partly genetically determined, environmental contribution seems to be greater (Corcoran et al., 2003; Lodge & Grace, 2011). Synergistic relation between activation of HPA axis and DA circuits is also suggested, where evidence shows that glucocorticoid secretion may increase DA activity, especially in the mesolimbic system (Mizrahi et al. 2012; van-Winkel et al., 2008). Last, etiological factors of schizophrenia including prenatal exposure to maternal stress or glucocorticoid administration, drugs of abuse and early, prolonged and severe childhood trauma, may also contribute to HPA dysregulation (van-Winkel et al., 2008). Stimulants such as amphetamines, which are associated with increased risk for psychosis, also increase cortisol secretion in humans (van-Winkel et al., 2008).

1.4.5 HPA axis and dopamine

To understand the neural mechanisms involving HPA activity on the expression of vulnerability for psychosis, it is essential to examine the interaction between glucocorticoids and the DA system, which is most often postulated to subserve psychotic symptoms.

Walker & Diforio (1997) reviewed studies indicating the association between activation of the HPA axis and DA neurotransmission and its link to the presence of psychotic symptoms and point out several important issues. First, stress exposure elevates cortisol and DA release. Many studies showed that exposure to stress increases secretion of cortisol and DA, demonstrated in the animal as well as human studies. There is also evidence of a causal effect of HPA activation on DA release, demonstrated in corticosterone administration to animals, which resulted in heightened DA metabolism in NAcc and the caudate, suggesting that cortisol release triggers subcortical DA activity. Augmented rate of DA-mediated locomotion was also observed.

Second, there is a relation between the magnitude of cortisol release and DA activity. Studies show that cortisol release and DA activity are related in normal human subjects and also in patients with schizophrenia and affective disorder, with a correlation of 0.50 for schizophrenia patients; and 0.64 for healthy participants between the magnitude of the increases in cortisol and DA release following challenge.

Third, both DA administration and stress can produce sensitization. Administration of DA agonists, such as AMPH and methamphetamine, augments sensitivity to subsequent DA agonism. In rats, sensitization manifest in behavioral patterns, such as increased stereotypies, locomotor activity and responsivity to novel environments but it also involves (1) augmented DA release, increased DA receptor sensitivity and density; (2) association with augmented responses to stress; and (3) blocking what by DA antagonists. Furthermore, it has been noted that stress produces similar sensitization effects as stimulants, where both prenatal and postnatal exposure to stress can enhance the behavioral response of rats to DA

agonists. Booij et al. (2006) showed that repeated exposure to D-amphetamine increases DA responses to stress in humans and also that it produces cross-sensitization. For example, before the D-amphetamine regimen, exposure to a stress task increased behavioral and physiological indices of stress, but following the D-amphetamine regimen, the stress-induced cortisol responses were even higher. This finding supports a cross-sensitization of amphetamine and stress in humans where both stress and d-amphetamine activate HPA axis, resulting in increased cortisol levels. Furthermore, an animal study showed that postnatal exposure to stress produces an augmented behavioral response to self-administrated DA agonists. This suggests that vulnerability to develop AMPH self-administration may be influenced by stressful experiences, and that previous contact with the drug may enhance a predisposition to AMPH-taking behavior (Piazza, Demeniere, LeMoal & Simon, 1990).

Fourth, HPA activation augments DA synthesis and receptors. Iuvone, Morasco & Dunn (1977) demonstrated in the animal study, that administration of corticosterone significantly increases the rate of whole-brain DA synthesis. Furthermore, there is a suggestion that activation of the HPA axis can alter DA receptors. Studies in the review of Walker and Diforio (1997) showed that prenatal stress exposure produced changes in elevated D2 receptors and decreased D3 receptors in NAcc. D2 receptors seem to be involved in behavioral sensitization to stimulants, where rats have been behaviorally sensitized to DA agonists and showed an elevation in D2 but not D1 receptors. Furthermore, Henry and colleagues (1995) suggested that changes in DA receptors are the consequence of the long-lasting alterations following stress exposure. Specifically, the stress of sufficient magnitude permanently alters the modulation of the HPA axis, such that corticosterone release is augmented and hippocampal glucocorticoid receptors are changed. Long-lasting hypersecretion of corticosterone seems to enhance DA receptors density and also augments DA release.

Lastly, DA can enhance HPA activation. Evidence for this can be found in studies, where it has been shown, that DA antagonists reduce cortisol release in schizophrenic patients. Also, depletion of DA through the lesion of the VTA results in a decrease in both baseline and stress-induced corticosterone in rats. As mentioned previously, in humans, DA agonists produce a significant increase in cortisol release (Booij et al. 2006; Mokrani, Duval, Croq, Bailey, Macher, 1995). Furthermore, neonatal lesions of the VTA have an impact on the DA system, where it may alter the normal hormonal response to stress. This implies that the dopaminergic system may have a direct influence on the HPA axis (Howes et al. 2017; Pani et al. 2000).

1.4.6 Stress and sensitization

The underlying mechanism of stress sensitization involves neurochemical sensitization of the mesolimbic dopamine system; the repeated exposure to sensitizing life stressors (or DA drugs) progresses into increased stress-associated neurochemical activation, mainly HPA

hormones and DA (Mizrahi et al. 2012). Evidence suggests that glucocorticoid secretion may increase dopamine activity, especially in the mesolimbic system. Studies show that patients with schizophrenia manifest HPA dysregulation, such as increased baseline cortisol levels. During the active periods of the illness, it is proposed that the dopaminergic system is hyperresponsive to environmental stimuli and exposure to even moderate levels of stress may produce excessive DA release, precipitating illness in vulnerable individuals and relapse (Mizrahi et al. 2012; van-Winkel, 2008). For instance, animal studies show that acute psychological and/or physical stress lead to cortical dopamine release and thereby lowers striatal DA release (Pycock et al., 1980). Furthermore, animals being repeatedly exposed to stressors exhibit reduced cortical baseline DA activity which can lead to sensitization of the mesostriatal system (Hollon, Burgeno, Phillips, 2015). In human studies, where subjects were exposed to the early life stress, augmented secretion of DA in the striatum and a decreased PFC dopamine activity was observed in response to acute stress (Nagano-Saito et al., 2013; Pruessner, Champagne, Meaney, & Dagher, 2004). As mentioned previously, there is also evidence that repeated exposure to stressors is interchangeable with repeated administration of psychostimulants (Antelman et al., 1980; Kalivas, Richardson-Carlson, & Van Orden, 1986). There is also evidence which supports the idea that schizophrenia results from an endogenous sensitization, where patients with schizophrenia show an increased DA response to stress (Mizrahi et al., 2012). Taken together, these findings offer a potential explanation for aetiology of schizophrenia, where repeated exposure to stressful events in early childhood may lead to altered DA transmission in early adulthood (Lieberman et al., 1997), yet the brain mechanisms that fail to regulate stress responses are not entirely clear.

1.4.7 Psychosocial stress and brain activity

While the neurochemical processes of the stress are well understood, the neuroimaging studies have only begun to investigate the neural correlates of the stress response. A lack of appropriate protocols to induce and measure stress in a functional imaging environment made direct assessment of changes in brain activity difficult.

In healthy humans, the stress response seems to be regulated by several cortical and subcortical regions, being activated or deactivated in response to stress (Dedovic et al., 2009; Pruessner et al., 2008). Animal studies showed that the limbic system as well as cortical areas play an important role in the regulation of the stress response. Rodent studies show that hippocampus (HC) and medial prefrontal cortex (mPFC) provide a tonic inhibitory input to the HPA axis (Ledoux & Daw 2018). In rats and primates, mPFC is high in glucocorticoid receptors density, which supports the notion that mPFC, as well as HC, with its distinct functions in higher order processing and its various ascending and descending projections play a crucial role in HPA axis regulation (Kern et al. 2008).

A study of Pruessener and colleagues (2008) in humans showed that a stressful arithmetic challenge with negative evaluation feedback led to deactivation across a network of limbic system structures, including HC, amygdala, insula, hypothalamus, ventral striatum and also orbitofrontal cortex. Furthermore, a degree of deactivation in the HC was associated with the release of cortisol, where participants with a higher cortisol response also deactivated the HC, thus releasing the tonic break of the HC in the HPA axis (Pruessner et al., 2008). In another study, event-related Montreal Imaging Stress task (event MIST) was used to investigate neural correlates of psychosocial stress, combining challenging mental arithmetic with negative social evaluative feedback. Participants who responded to negative social evaluation showed reduction of brain activity in mPFC and HC, which was largely lacking in non-responders (Dedovic et al., 2009). A positron emission tomography (PET) study of Kern and colleagues (2008) showed, that increased glucose metabolic rate in the mPFC areas is inversely associated with psychosocial stress-induced salivary cortisol concentrations in healthy subjects which, again, suggests that the mPFC is engaged as a part of regulatory circuitry to modulate the response to a stressful stimulus.

A review article of Dedovic (2009) summarized results of the studies investigating effects of psychological stress on neural activity. Various studies reported increased neural activation in ventral striatum (caudate nucleus and putamen), thalamus and anterior cingulate cortex based on the neuroimaging stress task applied. On the contrary, decreased brain activity was reported in the hippocampus (Dedovic, D'Aguiar, Pruessner, 2009).

To date, there seems to be only a single study that systematically probed the functional brain correlates of acute stress in patients with schizophrenia using functional magnetic resonance (fMRI). The results showed an enhanced activation of HC during the stress-task, as well as enhanced activation of HC and left-amygdala during post-stress periods in healthy controls but not in schizophrenia patients (Castro et al., 2015).

In summary, stress-vulnerability and dopamine sensitization hypothesis provide a possible explanation for some key features of psychotic disorders. Findings reviewed so far in this master thesis suggest that sensitization to psychostimulants and stress are underlined by overlapping neurochemical mechanisms and thereby cross-sensitize. Importantly, elevated dopaminergic stress response has also been found in schizophrenia patients, which goes in line with the concept of endogenous sensitization in schizophrenia, where exposure to early life stress might lead to altered dopamine release in early adulthood. Dopamine cross-sensitization between stress and psychostimulants is a well described phenomenon in animal studies and also demonstrated in humans, which provides a potential explanation of schizophrenia aetiology. Although, up until now, no studies have ever looked at the brain activity in context of cross-sensitization. At this point, we want to expand our knowledge and look at the activity on the neural level to try to determine, which brain mechanisms (fail to) regulate stress response in sensitization and therefore provide an explanation for a broader understanding of schizophrenia aetiology.

We investigated the cross-sensitization between amphetamines and stress response in humans. We performed the first cross-sensitization MR study in humans. To understand the effects of sensitization on the regulatory mechanisms and to classify which brain networks fail to regulate stress response in sensitization, we will compare the brain activity during the stress challenge between the two treatment groups, i.e. d-Amphetamine sensitized versus control (placebo) group.

Our goal is to test whether sensitized participants respond stronger to a mild psychosocial stressor. We will specifically look into the difference in the brain activity between the treatment and placebo group to classify which brain networks fail to regulate the stress response in sensitization. We are also interested in cortisol response differences between two groups after a stress challenge.

To test, to which degree, or even, participants were sensitized, we will obtain the data from heart rate, systolic and diastolic blood pressure, and the drug effects questionnaire (DEQ).

We expect some effects of acute amphetamine administration and sensitization on elevated heart rate and blood pressure in amphetamine challenged and / or sensitized participants.

We hypothesize that the sensitized group will show a higher increase in cortisol levels following the stressor.

We expect augmented brain activity in ventral striatum (caudate, putamen) and decreased neural activity in the HC in comparison between control condition and stress condition.

Furthermore, we hypothesize that the sensitized group will show a larger deactivation in the hippocampus.

The work of this master thesis will contribute to the better comprehension of possible underlying mechanisms of onset and/or relapse in schizophrenia. Furthermore, we will aim to elucidate interconnectivity and interchangeability between stress and drugs of abuse, especially amphetamine, and their possible impact on dopamine transmission which contributes to neuropsychiatric disorders.

2 METHODS

d-Amphetamine administration

For sensitization, we followed Boileau's (2006) dosing scheme. d-Amphetamine was administered orally (0.4 mg/kg body weight) for 3 consecutive days within approximately one week (on days 1, 3 and 6) at the Department of Psychiatry and Psychotherapy in the form of Attentin®, 5mg capsules. Volunteers in the placebo group received Mannitol instead. Four hours after drug-application, at the earliest, subjects were discharged from the study site. Approximately 14 to 21 days after the third day of drug (A3) administration, on the last day of the study (A4), participants in both groups underwent an amphetamine challenge based on the same dosing scheme and procedure as used for sensitization. We monitored

heart rate, systolic and diastolic blood pressure at predose (between -5 and -1 min), 30 min and 60 min after d-Amphetamine administration. Appropriate medication was available in the unlikely event of an excessive increase in blood pressure (180 mmHg systolic).

Assessment of Salivary Cortisol

We collected saliva samples in Salivette® tubes to determine salivary cortisol levels. Two samples of saliva were taken before the administration of d-Amphetamine and six samples after the administration of d-Amphetamine. Salivary cortisol was measured with standard enzyme-linked immunosorbent assays (ELISA) by an external institution.

Functional Magnetic Resonance Imaging

Participants spent approximately 60 mins in the scanner which was located at the University Dental Clinic Vienna at the Neuroimaging Center of the University of Vienna, where the fMRI sessions were conducted. We acquired functional neuroimaging data using a 3 Tesla Magnetom Skyra MRI systems (Siemens Medical, Erlangen, Germany), each equipped with a 32-channel head coil and a high-performance gradient system with the following parameters: echo time (TE)/repetition time (TR) = 34/704 ms, flip angle = 50°, interleaved acquisition, 32 axial slices coplanar to the line connecting anterior and posterior commissures, field of view = 210 mm, matrix size = 96×96, voxel size = 2.2×2.2×3.5 mm. Structural images were acquired using a magnetization-prepared rapid gradient-echo sequence (TE/TR = 2.29/2300 ms, 176 sagittal slices, voxel size = 0.9×0.9×0.9 mm, flip angle = 8°, field of view = 240 mm).

2.1 PARTICIPANTS

This work was part of a larger study. The sample size was smaller than we had planned, due to the COVID-19 situation. In total, we collected complete data sets from 9 participants. We only tested healthy male participants between the ages of 21 and 30, because we were interested in amphetamine sensitization primarily due to its similarity to the hypersensitive dopaminergic state seen in the first episode schizophrenic patients. We, therefore, restricted our sample to the age range in which schizophrenia is most likely to manifest for the first time. Since the period between the first AMPH intake and the post-sensitization testing phase was approximately 3 weeks, females were excluded from the study, since part of their period cycle could be an unwanted source of variance. Participants were recruited from an existing participant pool. All volunteers had to be right-handed native German speakers, which was confirmed with Flinders Handedness survey (Nicholls, Thomas, Loetscher, & Grimshaw, 2013). They also underwent a general physical examination and neuropsychological assessment:

Inclusion criteria:

- 18-35 years old males in good general health based on anamnesis status and physical examination
- No psychiatric conditions as determined by the Mini-International Neuropsychiatric Interview (M.I.N.I. PLUS) (Sheehan et al., 1998)
- No abnormalities in laboratory screening including thyroid urinalysis, blood cell count, serum electrolytes, liver and kidney function.
- No clinically relevant findings in electrocardiogram ECG
- No clinically relevant findings in blood pressure and pulse (vital signs)
- No regular use of illegal drugs or alcohol abuse based on declared anamnesis and confirmed by urine drug screening
- No history of repeated AMPH or other stimulant drug use

Exclusion criteria:

- Evidence of present psychiatric or neurological illness according to M.I.N.I.-Plus (any personal or first-degree relative history of schizophrenia, bipolar disorder, attention-deficit/hyperactivity disorder, and substance dependence)
- Recreational use of psychostimulant drugs in the past two years; lifetime use of psychostimulants more than five times in the lifespan
- Medically significant biochemical or haematological abnormality on screening laboratory studies
- Clinically relevant abnormalities in the ECG
- History of myocardial infarction or angina pectoris
- Presence of ferromagnetic metal in the body or heart pacemaker
- Claustrophobia
- Any history of arterial hypertension or paroxysmal hypertensive states
- Established diagnosis of advanced arteriosclerosis
- Established diagnosis of hyperthyroidism
- History of hypersensitivity to sympathomimetics
- History of head trauma resulting in loss of consciousness that required medical intervention
- Lifetime history of substance dependence (except nicotine)
- Suicidal ideation or likelihood of a suicide or homicide attempt
- MR scanner incompatibility

After admission to the study, participants gave informed consent. Their participation was compensated with a flat fee of €340. From completing all tasks of the overall study, they could gain up to about €110.

2.2 MATERIALS

The drug effects questionnaire (DEQ) (Morean et al. 2013):

The DEQ (Morean et al. 2013) is widely used in studies to assess the acute subjective experience of the administered drug, in our case amphetamine. The aim of the questionnaire is to examine subjects' subjective experience of the effects of a drug within four different categories. It assesses the extent to which participants feel the AMPH (feel), feel high from AMPH (high), like the effect of AMPH (like) and want more from the drug (*more*). The principle of a Likert scale is used, requiring participants to tick their level of agreement with the statement on a line with two extremes, where 1 is not at all and 10 is extreme.

Montreal Imaging Stress Task (MIST) (Tomova et al. 2017):

In the scanner, participants performed an adapted version of the MIST developed by Dedic et al. (2005) and adapted by Tomova et al. (2017). MIST involves a mental arithmetic challenge under time pressure, along with a social evaluative threat. The evaluative threat is induced by a failure to achieve the minimum performance in the arithmetic tasks, followed by consistent negative feedback given by the experimenter that if they do not improve their performance, it will not be possible to use their data. Participants also receive real-time feedback on a computer screen that their performance appears to be below the group average. Moreover, one half of the screen shows a live video-feed of an experimenter watching them and taking notes as they complete the task. In the control condition, participants complete the mental arithmetic task, but without time pressure, evaluation of their performance, or observation by the experimenters.

MINI-International Neuropsychiatric Interview (MINI-Plus):

The M.I.N.I.-Plus (Sheehan et al., 1998) is a structured diagnostic interview developed by psychiatrists and clinicians in the United States and Europe for DSM-IV and ICD-10 psychiatric disorders. It was developed to meet the need for a brief but concise structured psychiatric interview for clinical trials and epidemiological studies with a completion time of approximately 20 minutes.

2.3 PROCEDURE

This thesis was a part of a larger study, so the number of tasks and visit days was greater than mentioned in this paper. We only described parts of the study that were relevant for our thesis.

We performed a randomised, blind, placebo-controlled, parallel-group design. Brain activity was investigated using functional MR imaging (fMRI). Amphetamine sensitization was used as a treatment to temporarily increase striatal sensitivity. Subjects were randomly assigned to either a treatment (n = 8) or a placebo group (n = 1), where the treatment group received three doses of amphetamine within 6 days, and the placebo group received placebo pills instead. Approximately 2-3 weeks after sensitization (first administration of AMPH or placebo) subjects from both groups received amphetamine and were tested again in a short fMRI session where they performed an arithmetic task in the scanner under tight time pressure, followed by negative evaluative feedback. The study was conducted at the Psychiatric Clinic of the Medial University of Vienna and the Dental Clinic of the Dental Medical University of Vienna.

First day of the study (A1) was the time participants received their first dose of amphetamine / placebo. They also received it on the second (A2) and third (A3) days of the study. On the fourth day (M1) of the study, approximately 2 weeks after the third day (A3), participants underwent a scanning session during which participants completed a MIST task in the MR scanner. On the fifth day of the study (A4), participants from both groups received doses of amphetamine.

For sensitization and testing sessions, participants were scheduled to come to the psychiatric clinic between 9 and 10 AM to keep hormonal levels comparable. Study day M1 was a testing session; study days A1, A2, A3 were sensitization sessions, while A4 was a post-sensitization session.

During the sensitization sessions (A1, A2 and A3), participants received the respective dose of d-amphetamine or placebo at about 15 minutes upon arrival. Baseline heart rate and (systolic and diastolic) blood pressure were measured as well as baseline saliva samples were collected in Salivette cortisol tubes for later salivary cortisol analysis once 10 minutes before drug administration and at intervals of approximately 30 minutes immediately after drug administration, i.e. 0, 30, 60 and 90 minutes after administration. Also starting immediately after drug administration, participants filled out a Drug Effects Questionnaire (DEQ) questionnaire at the same intervals. If participants did not show any abnormal signs, they were dismissed for the day about 90 minutes after drug administration. Testing session (M1) included an additional scanning session in the MR scanner. On post-sensitization session (A4) participants from both groups received the respective dose of d-amphetamine and underwent all procedures mentioned in A1, A2 and A3, with additional tasks that aren’t relevant for the purpose of this study.

At the drug testing sessions, participants were asked to come soberly to the clinic. Upon arrival, participants underwent a urine drug test. Participants were further asked to report any alcohol consumption within the last 24 hours. Participants whose drug tests were

positive were excluded from further participation and participants who consumed alcohol within the last 24h were rescheduled. Eligible participants then filled out an MR-compatibility questionnaire before drug administration.

Potential risks/inconveniences to volunteers

Subjects could decide to withdraw from the study at any time. If adverse events would have appeared, the subjects would stay under medical supervision unless the physician believes that all adverse events have resolved or can be followed up by outpatient procedures.

Acknowledgement / approval of the study

The investigator (or a designated Clinical Research Organisation (CRO)) will submit this protocol and any related document provided to the subject (such as subject information used to obtain informed consent) to an Ethics Committee (EC) or Institutional Review Board (IRB). Approval from the committee was obtained before starting the study.

Insurance

Insurance coverage for the whole study period is provided according to the "Rahmenvertrag" of the Medical University of Vienna. Details on the existing patients' insurance are given in the patient information sheet.

Ethics and Good Clinical Practice (GCP)

The study was performed following the Declaration of Helsinki (1964), including current revisions, the Austrian Drug Law (Arzneimittelgesetz, AMG, 2004) and the GCP guidelines of the European Commission. Approval from the ethics committee of Medical University of Vienna (Votum EK Nr. 1313-2019) was obtained before starting the study. All subjects participating in this particular study were insured through the Department of Clinical Pharmacology in accordance with §38 of the Austrian Medicines Act.

3 ANALYSIS

This section is devoted to a more detailed description of the methods and data analysis. We have covered the general overview at Bayesian Multilevel Modelling, which we used to

analyze physiological and subjective measures of amphetamine sensitization. We analyzed both physiological and subjective measures in R[1] and fMRI data in MATLAB[2].

3.1 BAYESIAN MULTILEVEL MODELLING

When choosing the right method for our data analysis, we decided to use Bayesian Multilevel Modeling (MLMs) since we were dealing with repeated measurements and unequal sample sizes. The multilevel strategy is especially useful when dealing with repeated measurements or unequal sample sizes. Bayesian Multilevel Models can be described in terms of hierarchical regression analysis, because the parameters of one regression model are themselves modeled as outcomes of another regression model. (Nalborczyk, Batailler, Loevenbruck, Vilain, & Bürkner, 2017). We built our Bayesian multilevel model using the brms package in R (Bürkner, 2017). The brms package allows the specification of multilevel models, which are fitted using the probabilistic programming language Stan. Stan implements Markov-Chain Monte Carlo (MCMC) and the No-U-Turn Sampler (NUTS), which techniques draw samples from the posterior distribution rather than computing or approximating the posterior distribution directly (Bürkner 2017, McElarth 2015). The effects of the predictor in question, as derived from the sample estimates obtained for the model, include the posterior distribution of the mean and standard deviation, as well as the two-sided 95% credible interval CI of the mean (Nalborczyk et al. 2017). 95% CI represents the 0.95 probability that this credible interval includes the population value of the specific estimate, based on the data, the model, and its priors. The width of the CI also expresses the certainty of the model estimate, with a relatively narrow CI indicating more certainty and a relatively wide CI indicating more uncertainty.
From the examination of effect sizes, we obtained Cohen's d criterion, a standardized effect size that expresses the difference between two groups in terms of their pooled standard deviation. The outcome or index is referred to as δt, which is the estimated difference between group means divided by the square root of the sum of all variance components (Nalborczyk et al. 2017). δt is reported in the same way as the effects of each predictor from the previous paragraph. The percentage of the posterior distribution of each estimate above (positive) or below 0 (negative), gives us information about positive or negative probability effects.

3.1.1 Assessment of sensitization

We were interested in whether sensitization (session A1, A2, A3 compared to A4) and acute amphetamine administration after sensitization (amphetamine group compared to placebo group) influenced both the physiological and subjective experience of the administered drug.

[1] Version 4.0.3 (2020), R Core Team, `https://www.R-project.org`
[2] Version 9.3.0.713579 (R2017b), The MathWorks Inc, Natick, Massachusetts, US

We collected DEQ questionnaire data for the purpose of assessing subjective drug effects and heart rate (HR), systolic and diastolic blood pressure (sBP, dBP) data for the purpose of assessing physiological drug effects. Both data sets were obtained from sensitization and post-sensitization days (A1, A2, A3 and A4) and analyzed with Bayesian MLMs using the following model:

response variable ~ session + sensitized + amphetamine + (session|ID)

We dummy-coded parameters session and amphetamine for 1 = sensitized (A4) and 0 = not-sensitized (A1, A2, A3); 1 = amphetamine (received amphetamine) and 0 = not-amphetamine (received placebo). Sessions were represented by categorical predictor variables with 4 levels (1 = A1, 2 = A2, 3 = A3, 4 = A4). Amphetamine administration predictor was included because of interest in acute amphetamine effects after sensitization (A4), when DEQ and physiological measures coincided with the amphetamine challenge for both groups. The purpose of this analysis served as a cross-check that sensitization was working.

Heart rate & blood pressure

Before building a multilevel Bayesian model, we prepared the data so that we calculated the difference between the baseline and each other measurement of the respective parameter within each session separately. Afterwards we checked the minimum and maximum difference between baseline and the respective parameter, and selected the highest of the absolute values. This value represented the peak difference in heart rate during heart rate measurement and the peak difference in cytosolic and diastolic blood pressure during blood pressure measurement. Then, the statistical model was built as described above. Based on previous findings, we expected elevated heart rate and blood pressure in amphetamine-challenged and / or sensitized participants, although the expectation of the effect was relatively low (Boileau et al., 2006; O'Daly, Joyce, Stephan, Murray, & Shergill, 2011).

Drug effect questionnaire

We processed the data for the DEQ in such a way that we calculated the difference between the reported peak effect and the baseline for each DEQ-item and session. After applying the Bayesian model, we examined if there was an effect of sensitization and amphetamine on the subjective experience of drug effect. We expected an increased self-reported drug effect when participants were sensitized and / or amphetamine challenged.

3.2 fMRI DATA ANALYSIS

fMRI data from testing session (A4) and sensitized group were pre-processed and analyzed in MATLAB using SPM12[3] software package.

3.2.1 Pre-processing

We followed the SPM12 manual[4] steps to pre-process the MRI data, including slice-time correction, realignment, unwarping, co-registration and uniform segmentation. Prior to the above steps, the MRI data were converted to NiFTi format. The pre-processing steps were performed separately for each subject. Afterwards, the images were spatially normalized to Montreal Neurological Institute (MNI) space and smoothed with a 3D Gaussian kernel of 4 mm full-width at half-maximum (FWHM) to allow comparison between subjects.

First, we used slice-time correction to correct differences in image acquisition time between sampled slices that resulted from acquiring images in interleaved mode. Our reference slice was acquired in the middle of the sequence (i.e. at TR/2). Since moving the subjects can lead to large motion artefacts in the functional images, resulting in a loss of sensitivity and specificity, we used realignment and unwarping methods. In realignment, the first scan of each participant's session was realigned to the first scan of the first session, by 6 parameters (3 degrees for rotations and 3 mm for translations). Afterwards all images of a session were realigned to the first image of that session. The subjects' movements introduce strong geometrical distortions, where unwarping was used to correct susceptibility-by-movement interactions. Co-registration was then used to link the anatomical information of the functional images to the structural image, with the purpose of achieving better anatomical localization. Segmentation based on tissue probability maps was performed to separate different tissue types (gray matter and white matter). Bias correction was then used to correct for the inherent intensity heterogeneity of MRI, facilitating normalization. Normalization helps to establish a voxel-to-voxel match between the brains of different subjects, which then allows comparison of brain activity between subjects. We normalized and transformed T1-weighted anatomical images into the MNI template of uniform segmentation. The parameters of the normalization output were then applied to all functional images. We used smoothing to increase the signal-to-noise ratio by suppressing noise and the effects of residual differences in gyral and functional architecture, ultimately resulting in normally distributed data, better spatial overlap and increased sensitivity to effects of similar magnitude to the kernel.

[3] Wellcome Centre for Human Neuroimaging, London, UK, https://www.fil.ion.ucl.ac.uk/spm/
[4] `https://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf`

3.2.2 First-level analysis

For the MIST task, a general linear model (GLM) was used to perform a statistical analysis to determine the voxels activated by stimulation. The GLMs of the first-level analysis were performed at the individual level. Since we were interested in the changes in neural activity while performing the stress task using the block design, and in the differences between the control condition (no-stress block) and the experimental condition (stress block), our GLM model was conducted using two regressors of interest: No-Stress (Block) and Stress (Block). The regressors were modelled using the contrast between when participants received feedback (whether positive or negative, dependent on the block) and when they rest. We also included six realignment parameters as regressors of no interest to account for movement-induced variance. Before fitting the model to the data, the regressors were convolved with a canonical hemodynamic response function. We applied a high-pass filter with a cut-off frequency of 128 Hz to eliminate low frequency signal drifts. We corrected the regressors for serial correlations using a first order autoregressive model. Finally, the following contrast images were generated for each subject, ready for group-level analyses:

- Regressor 1: No-Stress / Control Block
- Regressor 2: Stress / Stress Block

3.2.3 Group-level analysis

Our primary interest was to compare neural activity in a stressful condition between the placebo and amphetamine groups. Because the groups were unequally distributed, we analyzed only contrast images of subjects from the experimental group. We therefore first wanted to perform a whole-brain analysis to compare the changes in neural activation between No-Stress and the Stress condition/block within the sensitized amphetamine group to investigate the effect of stress on brain activity. Second, we examined neural activity for each condition separately in the regions of interest (ROIs): hippocampus, caudate nucleus and putamen to examine what drives the effect of No-Stress vs. Stress. Due to the small sample size, we performed non-parametric alternatives to regular t-tests using the Statistical nonParametric Mapping (SnPM) toolbox.

We performed a whole-brain analysis comparing differences between control and stress blocks with paired sample t-test. Multiple comparison correction was used to control the number of false positives to obtain reliable results. Statistical maps were corrected for multiple comparison correction at a family-wise error (FWE) of $p < 0.05$ with a maximum number of permutations run by a series of Monte Carlo simulations of 5,000. None of the clusters survived the threshold cluster correction in this case.

To examine each condition (control and stress) separately, we performed a one sample t-test in the regions of interest. The mask images for the ROI analysis were created using the

WFU_PickAtlas toolbox[5]. Since we were interested in two regions, the hippocampus and the ventral striatum (caudate nucleus and putamen), we created the image masks separately. For the hippocampus, we chose the Hippocampus area, Left + Right, 3D with the dilatation of 1. The Ventral striatum mask was created using Caudate Body, Caudate Tail, Caudate Head and Putamen, Left + Right, 3D with the dilatation of 1. The masks were then inserted as implicit masks. Again, multiple comparison correction was used to control the number of false positives to obtain reliable results. The maximum number of permutations run by a series of Monte Carlo simulations was set at 5,000. Statistical maps were then corrected for the stress block using a familywise error of pFWE = 0.05 and a cluster-defining threshold of 4.2968 (p = 0.001). We did not choose variance smoothing, so the t-statistics here are not pseudo t-statistics. We then extracted each subject's ß-coefficients from the Stress block for the hippocampus and ventral striatum for further within-group analysis. In the Control block, none of the clusters survived the FWE cluster correction.

3.3 PHYSIOLOGICAL, SUBJECTIVE AND fMRI DATA ANALYSIS

Finally, we were interested in the correlation between changes in neural activity during the stress block and physiological and subjective ratings of drug effects. The physiological and subjective data on drug effects were processed as described in the corresponding section above. Because we had only one participant in the placebo group, we examined only the data from the amphetamine-sensitized group. We obtained Beta coefficients from the hippocampus and ventral striatum during the stress block. Within the amphetamine-sensitized group we took the difference between session A4 (sensitized) and session A1 (not-sensitized) for the physiological and subjective data to assess drug effects and looked for a possible correlation between the given difference and neural activity in the hippocampus and ventral striatum during the stress block. We tested for a statistically significant relationship between neural activation during the stress block in the sensitized group, the physiological and behavioral measurements with the Spearman's correlation method.

4 RESULTS

4.1 Heart rate & blood pressure

Results showed no strong effects of sensitization or amphetamine administration on participants heart rates:

[5] https://www.nitrc.org/projects/wfu_pickatlas/

Sensitization: β = 0.66, 95% CI = [-4.15, 5.37], dt = 0.04, 95% CI = [-0.29, 0.36]; Amphetamine: β = 0.44, 95% CI = [-4.45, 5.15], dt = 0.03, 95% CI = [-0.32, 0.33]).

Sensitization and amphetamine administration only slightly increased participants' heart rates, and the positive effect seemed to be weak. The means of the posterior distribution of the regression coefficients for sensitization and amphetamine administration were both positive. Credible intervals varied from strongly positive to strongly negative effects, which reflects a high amount of uncertainty. The percentage of posterior distribution above 0 point was almost equal for sensitization (59% > 0 > 41%) and amphetamine (57% > 0 > 43%) to increase or decrease heart rate. Since the effect sizes approached 0, the observed positive effect of sensitization and amphetamine administration remains inconclusive.

As for heart rate, we found positive effects for sensitization and amphetamine administration on participants blood pressure:

Sensitization: β =1.37, 95% CI = [-2.86, 5.48], δt = 0.15, 95% CI = [-0.39, 0.69]; Amphetamine: β = 1.91, 95% CI = [-2.95, 6.71], δt = 0.22, 95% CI = [-0.40, 0.88].

The percentage of posterior distribution above 0 points for both sensitization (71%) and amphetamine administration (74%) suggests an increase in participants' blood pressure, although the effects were also associated with a large uncertainty.
Our results point to a positive effect of sensitization and amphetamine administration on blood pressure but also do not exclude negative effects. The effects were also associated with small effect sizes and large uncertainties, therefore the effects of sensitization and amphetamine administration on participants' blood pressure remains preliminary.

4.2 Drug effect questionnaire

The effects of sensitization on the subjective experience of the administered drug, as measured by DEQ were weakly positive but uncertain. The analysis showed that sensitization did affect perceived subjective effects of the administered drug after sensitization in terms of a specific elevated sensation/feeling due to amphetamine:

feel: β = 0.46, 95% CI = [-2.20, 3.00], δt = 0.18, 95% CI = [-0.91, 1.47]),
high: β = 0.85, 95% CI = [-2.25, 3.84], δt = 0.29, 95% CI = [-0.73, 1.20]),
like: β = 0.47, 95% CI = [-2.13, 3.04], δt = 0.17, 95% CI = [-1.04, 1.33]),
more: β = 0.18, 95% CI = [-1.96, 2.26], δt = 0.06, 95% CI = [-0.90, 1.18]).

Weakly positive effects of sensitization on the general subjective drug effect experience were reflected in a percentage of the posterior distribution above 0 points, where the feeling of wanting more of a drug being the least exaggerated (feel: 62% > 0; high: 68% > 0; like: 61% > 0; more: 55% > 0). The plausibility of the effects of sensitization on all four dimensions was either strongly negative or strongly positive, although this reflects a degree of inaccuracy. The δt values also reflect highly uncertain effect sizes.

In comparison to sensitization, amphetamine administration showed a stronger effect on the subjective experience of the administered drug and also a clear trend of a positive effect, although the effect was still associated with large uncertainty. The effect of amphetamine administration on the subjective experience of the drug was shown to affect the sense of "feel" (feel: β = 1.15, 95% CI = [-1.15, 3.41], δt = 0.44, 95% CI = [-0.59, 1.47]), being high (high: β = 1.09, 95% CI = [-2.10, 4.22], δt = 0.29, 95% CI = [-0.65, 1.33]), like the drug (like: β = 1.17, 95% CI = [-1.16, 3.41], δt = 0.44, 95% CI = [-0.57, 1.50]) and wanting more drug (more: β = 0.60, 95% CI = [-1.38, 2.47], δt = 0.26, 95% CI = [-0.74, 1.24]). As mentioned previously, the percentage of the posterior distribution above the 0 showed a clear positive trend (feel: 81% > 0, high: 72% > 0, like: 80% > 0, more: 71% > 0), although the credible intervals are wide, which again reflects a rather large amount of uncertainty and the possibility of both, positive and negative effects of amphetamine administration on the subjective experience of the drug.
Our findings indicate that sensitization and amphetamine administration influence the subjective experience of the administered drug, with acute amphetamine appearing to have a stronger positive effect. Although large uncertainties are evident in both conditions, the results should be taken with caution.

4.3 fMRI

We compared whole-brain neural activity between control and stress block within an amphetamine-sensitized group with paired sample t-tests. Analysis showed differences between the stress and control (stress > control) conditions in specific brain regions, including the hippocampus and ventral striatum. After threshold cluster correction with pFWE < *0.0*5, none of the clusters survived, indicating that the difference was not statistically significant.

Figure 1: Images demonstrating the differences in the general model for the whole brain using the contrasts of stress and control conditions (stress > control) before threshold cluster correction. The cross is located on the hippocampus [MNI xyz: -30 -10 -20]. The t-maps are displayed in the sagittal, coronal, and horizontal planes.

Figure 1

General model for the whole brain (stress > control) (Štamulak K. 2021).

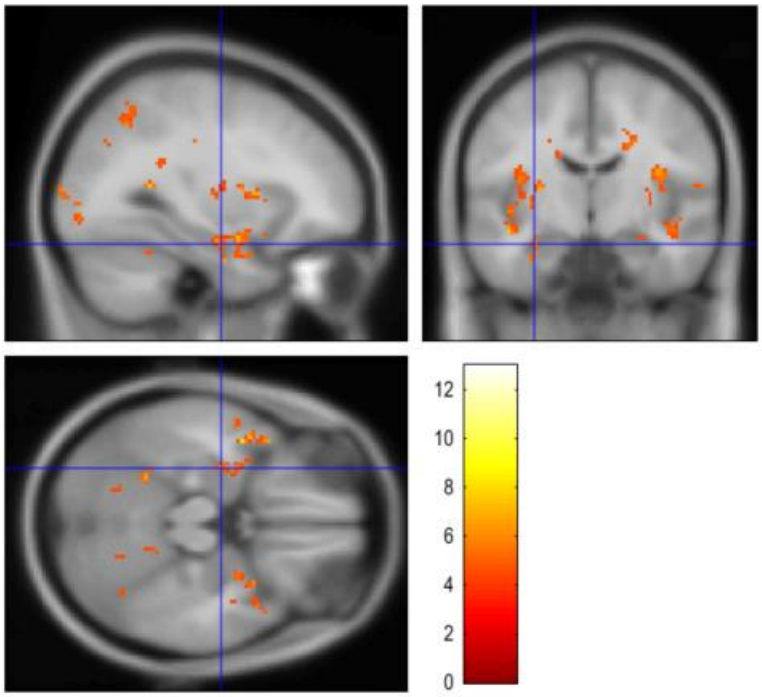
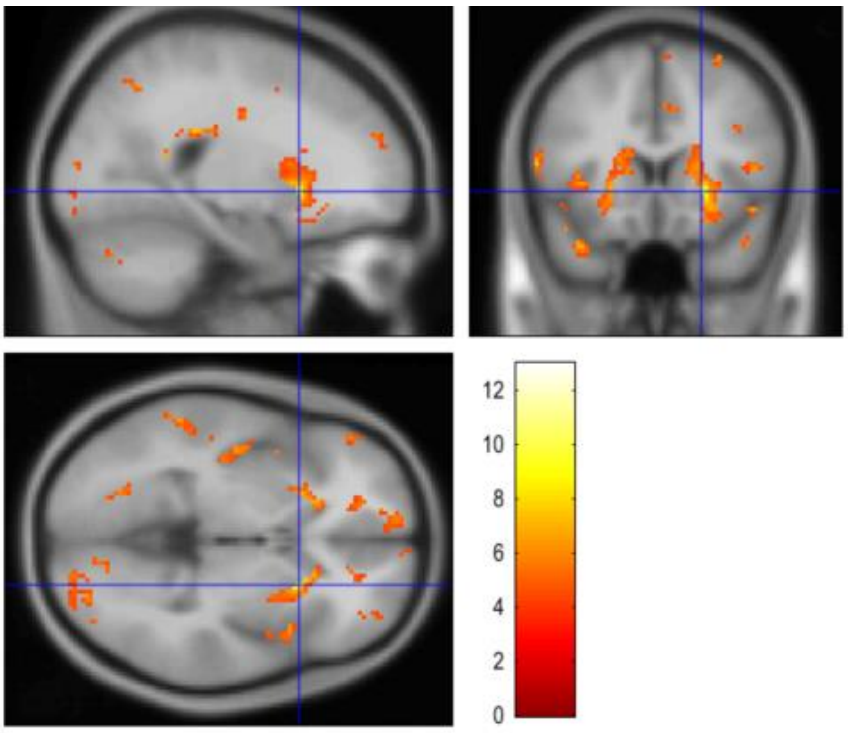


Figure 2: Images demonstrating the differences in the general model using the contrasts of stress and control conditions (stress > control) before threshold cluster correction. Cross is located on the striatum [MNI xyz: 22 16 -02]. The t-maps are displayed in the sagittal, coronal, and horizontal planes.

Figure 2

General model for the whole brain (stress > control) (Štamulak K. 2021).



We could not examine brain activation in the control block for the amphetamine sensitized group, because none of the voxels survived correction for multiple comparison (pFWE > 0.05). In contrast, examination of the stress condition survived the multiple comparison correction (pFWE = *0.0*5, *cluster-defining threshold* at *4.296*8 (*p = 0.001*), therefore we obtained ß-coefficients for both ROIs; hippocampus and ventral striatum, which were then ready for further analysis. Unfortunately, we were unable to perform a comparison for statistically significant differences between control and stress condition, as we could not obtain ß-weights for the control block.

Lastly, we examined the correlation between changes in neural activity during physiological measurements of the stress block and ratings of subjective drug effects. The results of the correlation analysis between ROIs and physiological measurements are shown in Table 1 and the p-values in Table 2. The results of the correlation analysis between ROIs and DEQ are shown in Table 3 and the p-values in Table 4.

Table 1

Spearman's correlation coefficients between ROI beta coefficients and physiological data. Štamulak K. 2021.

	HR	dBP	sBP	striatum	HC
HR	1.000				
dBP	0.724	1.000			
sBP	0.122	-0.323	1.000		
striatum	0.342	0.240	-0.429	1.000	
HC	**0.708**	0.623	-0.024	-0.167	1.000

Table 2

Correlational p-values between ROI beta coefficients and physiological data. Štamulak K. 2021.

	HR	dBP	sBP	striatum	HC
HR	< 0.001				
dBP	0.042	< 0.001			
sBP	0.774	0.435	< 0.001		
striatum	0.408	0.568	0.289	< 0.001	
HC	**0.050**	0.099	0.955	0.693	< 0.001

Table 1 presents the Spearman's correlation coefficients between the ROIs (ventral striatum and hippocampus) and heart rate (HR), diastolic blood pressure (dBP) and systolic blood pressure (sBP), while Table 2 shows the corresponding p-values. As can be seen from the tables, the only correlation existed between the changes in heart rate and the HC ($r = 0.708$, *p*-value = 0.050). As seen from the tables, changes in heart rate are associated with neural activity during the stress condition. Concerning systolic and diastolic blood pressure as a result of amphetamine sensitization, we can conclude that they are not associated with changes in neural activity during the stress condition.

Table 3

Spearman's correlation coefficients between ROI beta coefficients and DEQ data. Štamulak, K. 2021.

	feel	high	like	more	striatum	HC
feel	1.000					
high	0.689	1.000				
like	0.110	-0.280	1.000			
more	0.291	-0.176	0.830	1.000		
striatum	**0.807**	**0.699**	-0.060	-0.048	1.000	
HC	-0.193	-0.157	-0.265	-0.431	-0.167	1.000

Table 4

Correlational p-values between ROI beta coefficients and DEQ data. Štamulak, K. 2021.

	feel	high	like	more	striatum	HC
feel	< 0.001					
high	0.059	< 0.001				
like	0.796	0.501	< 0.001			
more	0.485	0.677	0.011	< 0.001		
striatum	**0.015**	**0.054**	0.887	0.910	< 0.001	
HC	0.647	0.711	0.526	0.286	0.693	< 0.001

Table 3 represents the Spearman's correlation coefficients between the ROIs (ventral striatum and hippocampus) and the features of the DEQ questionnaire (*feel*, *high*, *like* and *more*), while Table 4 presents the corresponding *p*-values. As can be seen from the tables, there was a statistically significant ($p < 0.05$) correlation between changes (A4-A1) in perception of the "feeling" as well as being "high" on the drug and neural activity during the stress condition in the striatum ($p_{feel} = 0.015$; $p_{high} = 0.054$), but not in the hippocampus ($p_{feel} = 0.647$; $p_{high} = 0.711$). Other correlations were not statistically significant. In conclusion, as a result of amphetamine sensitization, changes in drug sensation (feeling the drug and being high on the drug) between sessions A4-A1 appear to be strongly correlated with brain activation in the striatum during the stress condition ($r_{feel} = 0.807$, $p_{high} = 0.015$; $r_{high} = 0.699$, $p_{high} = 0.711$).

5 DISCUSSION

We performed a first MR study of cross-sensitization between amphetamines and stress response in humans. We examined brain activity during stress challenge in the d-Amphetamine sensitized group and sought to find correlations between brain activity and indirect indicators of sensitization in the form of changes in physiological and psychological effects. Our purpose was to understand the neuroanatomical background of dopaminergic sensitization and thus, to better understand the aetiology of schizophrenia.

Our results are consistent with previous studies, in which amphetamine sensitization seems to enhance subjective responsiveness to the administered drug. In contrast, but also consistent with previous studies, physiological sensitization effects (heart rate and blood pressure) seem to be weak (O'Daly, Joyce, Tracy, Azim, Stephan, Murray, Shergill, 2014; O'Daly, Joyce, Stephan, Murray, & Shergill, 2011). It is important to emphasize that there is a great deal of uncertainty associated with our results. Nevertheless, we can assume that amphetamine sensitization was successful to some extent. Furthermore, we assume that the findings on psychological and neural measures reflect some degree of amphetamine sensitization.

Unfortunately, results on changes in whole-brain neural activity as a result of stress remain inconclusive, although many studies reported that stressful arithmetic challenges with negative evaluative feedback resulted in hippocampal deactivation (Dedovic et al. 2009; Pruessner et al. 2008; Castro et al. 2015) and increased neural activation in the ventral striatum (Dedovic, D'Aguiar, Pruessner, 2009). Nevertheless, our data showed activation across the ventral striatum and deactivation in the hippocampus during the stressful arithmetic challenge with negative evaluative feedback. Unfortunately, we cannot conclude that the (de)activation was a consequence of stress induction. We should emphasize that this result is consistent with the idea that hippocampal activation plays an important role in inhibiting the HPA axis and, moreover, in attenuating cortisol release (Ledoux & Daw 2018). Concerning correlation between the HC and the HR, we could not prove that deactivation in the HC was a consequence of stress induction. Changes in HR were too small and the positive effect of sensitization on HR seemed to be weak, therefore we cannot draw any conclusions about correlation between the HC and HR.

At this point, it seems important to mention that the Montreal Imaging Stress Task we used in this study was designed to induce psychological stress in the context of functional imaging. The study reported elevated cortisol levels during the stress task, suggesting that the task was indeed perceived as stressful (Dedovic et al. 2005). Unfortunately, we could not analyze the cortisol levels obtained during the stress task due to the COVID-19 situation, but it definitely suggests an avenue for further investigation. Since we only analyzed data from the amphetamine sensitized group, we cannot draw conclusions about differences in brain

activity between the sensitized and placebo groups. Here, we refer to results of a study by Castro et al. (2015), which showed increased activation in the HC during the stress task in healthy controls compared to schizophrenia patients. This suggests a possible expectation for further examination that amphetamine sensitization, mimicking the neurochemical background of schizophrenia, together with the stress task, could result in larger deactivation of the HC, thereby not providing inhibitory input to the HPA and consequently resulting in higher cortisol release and DA activity, potentially leading to increased risk of psychosis.

In summary, we found (de)activation in the ventral striatum and hippocampus during the stress task in the amphetamine sensitized group, which could be the consequence of perceived stress and amphetamine sensitization. If we could demonstrate this and compare the results with the placebo group, we could confirm whether stress cross-sensitizes with amphetamines, meaning that it runs through the same mechanism. This mechanism involves sensitization of the DA system as a result of either stressful event(s) or drug abuse. Furthermore, sensitization could lead to augmented behavioral and neurochemical patterns, including greater deactivation in the hippocampus, which could potentially lead to disinhibition of the HPA axis (the glucocorticoid-mediated feedback inhibition), resulting in higher cortisol release and DA activity, which could then potentially lead to the development or exacerbation of psychotic symptoms (Dedovic, D'Aguiar, Pruessner, 2009). Further research would be needed to draw conclusions of this nature.
Finally, we found an association between the difference in subjective experience of the drug and the changes in brain activity during the stress task. The changes in subjective experience of the drug may reflect amphetamine sensitization, in which participants reported a higher experience in *feeling* of the drug and being *high* on the drug on the last day of the study compared to the first day of the study, albeit with some degree of uncertainty, as mentioned several times before. Other studies of amphetamine sensitization reported an enhanced amphetamine-like experience and amphetamine-induced euphoria (O'Daly, Joyce, Tracy, Azim, Stephan, Murray, Shergill, 2014), which is in line with our findings.

The correlation between the difference in subjective experience of the drug after sensitization, and the changes in brain activity in the ventral striatum during the stress task suggests a perspective that could be explained as the possibility that amphetamine sensitization is reflected in higher susceptibility to stress due to cross-sensitization, since the study by Booij et al. (2006) reported that repeated exposure to amphetamine increases responses to stress. Therefore, it is reasonable to assume that amphetamine sensitivity correlates with stress response in the participants of our study.

We found greater activation of the ventral striatum, where some studies reported a significant association between stress and ventral striatum (Dedovic, D'Aguiar, Pruessner, 2009). Interestingly, the study by O'Daly et al. (2014) reported a negative correlation

between sensitization-induced happiness and striatal activity. At this point, we should turn to the context of the relationship between stress and happiness to better understand our findings. Schiffrin and Nelson (2010) found a significant inverse relationship between happiness and perceived stress. Although the subjective experience of the drug in our study, namely the *feeling* of the drug and being *high* on the drug, doesn't indicate anything about an emotional charge of the drug (whether positive or negative), we induced some degree of stress that could be augmented by amphetamine sensitization.

Finally, it seems reasonable to say, although we cannot claim, that participants had an enhanced subjective feeling of the drug as a result of sensitization and an increased stress response as a result of amphetamine-stress cross-sensitization, which correlated highly with changes in brain activity in the ventral striatum. Regarding ventral striatum activity, there is evidence of augmented secretion of dopamine in the striatum following stress exposure as a consequence of sensitization of the mesolimbic dopamine system (Nagano-Saito et al., 2013; Pruessner, Champagne, Meaney, & Dagher, 2004).

Let us bring our findings into the context of the etiology of schizophrenia. The DA hypothesis posits that certain DA pathways are overactive in schizophrenia (Lieberman et al. 1997; Seeman 1987), which could be the consequence of repeated drug exposure and/or life stressors. On the other hand, the *endogenous sensitization* hypothesis postulates that a sensitized DA system is intrinsic to the disease (Peleg-Raibstein, Yee, Feldon, Hauser, 2009), which could be a consequence of early life stress (Walker, Mittal, Tessner, 2008; Walker & Diforio, 1997). We used amphetamine to induce sensitization of the striatal DA system, following the regime by Booij et al. (2006). In our study, an elevated subjective experience of the drug is indicative of successful sensitization. The study by Booij et al. (2006) also showed cross-sensitization between amphetamine and stress, where both stress and d-amphetamine activate the HPA axis, resulting in increased cortisol levels. We therefore induced a psychological stressor in amphetamine-sensitized participants, where we expected an elevated stress response. There is evidence for synergistic activation of the HPA axis and the DA circuit during stress, in which glucocorticoid secretion may increase DA activity, particularly in the mesolimbic system (Mizrahi et al. 2012; van-Winkel et al., 2008).

Based on the evidence of some studies, stress induction in our study should lead to activation of the HPA axis, elevated cortisol release, and also elevated striatal DA release, which would be higher in the amphetamine-sensitized participants compared to the placebo group (Mizrahi et al., 2012; Walker & Diforio 1997, Walker et al. 2000). It is now known that schizophrenia is associated with elevated cortisol, dysregulation of the HPA axis and elevated striatal DA release, and it has been observed that corticosteroids and DA agonist drugs to induce psychotic symptoms (Walker & Diforio 1997, Walker et al. 2000). After

stress induction, we found changes in the neural activity of the ventral striatum, which was also demonstrated in some studies reviewed by Dedovic (2009). Ventral striatum activity is positively correlated with enhanced DA release, based on evidence from some studies (Pessiglione et al., 2006; Knutson and Gibbs, 2007 Schott et al., 2008), therefore we can propose a possible outcome of elevated striatal DA release in our study. Unfortunately, we were unable to provide evidence for the latter mechanisms, but we can suggest it as a possible outcome based on the method and procedure of our study, which mimics the etiology of schizophrenia. This therefore remains an open question and may be addressed with future research.

5.1 LIMITATIONS

We encountered many obstacles during the study, but these limitations may serve as a guide when conducting further studies on this particular topic:

First, because our study took place during the beginning of the COVID-19 pandemic, we were unable to test as many participants as we had planned. Therefore, our main limitation is the small sample size, which led to large uncertainties and small effect sizes in the analysis of subjective drug assessment and physiological data.

Second, in addition to the small sample size, our groups were unevenly distributed, which prevented us from performing what is probably the most important part of this work, namely, comparing neural activation during the stress task between amphetamine and placebo groups. We therefore limited ourselves to analyzing only the data from the amphetamine group.

Third, we collected saliva to measure cortisol levels before, during, and after the fMRI scanning and performance of the MIST task, which would serve as an indirect indicator of the stress response and could probably give important insights into sensitization dynamics. Unfortunately, biochemical analysis of saliva has not yet been performed.

6 CONCLUSIONS

Our study represents the first amphetamine-stress cross-sensitization MR study in humans. Amphetamine was used to pharmacologically manipulate dopamine levels or specifically induce dopaminergic sensitization. When sensitized, our participants were exposed to psychological stress during the fMRI session. This procedure was designed to better understand the etiology of schizophrenia, which involves complex mechanisms of HPA

activity, cortisol levels, DA activity and neural brain activation. Sensitization seems to affect both physiological and subjective drug experience, albeit with large uncertainty. Second, the elevated subjective experience of amphetamine after sensitization seems to correlate with elevated neural activity in the striatum during the stress task, possibly indicating sensitivity of the mesolimbic dopamine system.

Unfortunately, we cannot draw conclusions about dopamine levels, but we can further hypothesize that elevated dopamine levels following amphetamine sensitization and stress exposure would correlate with enhanced subjective drug experience and activity in the ventral striatum. Finally, this study points to a promising avenue to investigate the role of dopaminergic sensitivity in the etiology of schizophrenia.

7 DALJŠI POVZETEK V SLOVENSKEM JEZIKU

Psihoaktivne substance, vključujoč amfetamine, kokain, opiate, Δ9-tetrahidrokanabinol in alkohol, predstavljajo raznolike skupine spojin, ki se razlikujejo po svojih nevrokemičnih in vedenjskih učinkih (Robinson and Berridge 1993; Willeit 2016). Znano je, da večkraten vnos tovrstnih substanc vodi do tolerance, tj. zmanjšanega odziva na drogo, vendar pa v določenih primerih pride do povečanja njihovih učinkov. Slednji fenomen imenujemo senzitizacija (Mayer and Quenzer 2005; Robinson and Berridge 1993). V farmakološkem kontekstu je senzitizacija definirana kot okrepljen učinek na ponavljajočo administracijo substance (Mayer and Quenzer 2005; Willeit 2016). Natančneje, senzitizacija označuje neasociativni učni proces, pri katerem ponavljajoča se izpostavljenost dražljaju vodi do postopnega ojačanja vedenjskega in nevrokemičnega odziva (Willeit, 2016). Študije na živalih so na primer pokazale, da ponavljajoča se občasna administracija amfetamina postopno povečuje gibalno aktivnost in stereotipno vedenje (Robinson and Becker 1986; Robinson and Berridge 1993). Poleg tega, so študije na ljudeh pokazale, da ponavljajoča se izpostavljenost nizkim odmerkom amfetamina postopno povečuje budnost in evforijo ter povečuje odziv na stres (Strakowski 2001; Booij et al. 2006).

Vedenjska senzitizacija amfetaminov in podobnih psihostimulansov nastane kot posledica občasne administracije substanc in je opredeljena kot povečanje vedenjskega učinka psihostimulansov ob ponovni uporabi. Jasno je, da osnovni mehanizmi ponavljajočega se vnosa amfetamina vodijo do dolgoročnih sprememb v vedenju, kjer naj bi spremenjena dopaminergična nevrotransmisija igrala ključno vlogo pri krepitvi odvisnosti in vedenjskih stimulativnih učinkov psihostimulansov (Robinson and Berridge 1993; Boileau et al. 2006; Pierce and Kalivas 1997). Znano je, da vedenjska senzitizacija vključuje spremembe v ventralno-tegmentalni regiji. Administracija amfetaminov stimulira dopaminske receptorje v VTA, kar sproži kaskado molekularnih dogodkov in sprememb v nevronski plastičnosti, ki

posledično povečajo sproščanje dopamina (Boileau et al. 2006). Trajne spremembe na dopaminergičnih D1 receptorjih v VTA prispevajo k povečanju glutamata in zmanjševanju prenosa GABA (Venzina 1996; Pierce and Kalivas 1997). Tovrstne spremembe spodbujajo vzdraženje dopaminergičnih nevronov v mezoakumbensu. Študije so pokazale, da spremembe v presinaptičnem in postsinaptičnem prenostu dopamina v NAcc in striatumu prispevajo k izražanju vedenjske preobčutljivosti na psihostimulanse. Te spremembe vključujejo povečan zunajcelični dopamin v NAcc in striatumu, zmanjšano število dopaminergičnih transporterjev, zmanjšanje vezavnih mest dopaminskih transporterjev in povečano občutljivost D1 receptorjev (Pierce and Kalivas 1997).

Spremembe v dopaminergični nevrotransmisiji so povezane tudi z nastankom psihotičnih simptomov pri shizofreniji (Boileau 2006; Peleg-Raibstein et al. 2008). Znani pa so tudi primeri zdravih posameznikov, pri katerih so se ob večkratni uporabi amfetaminov razvili psihotični simptomi, ki spominjajo na simptome paranoidne shizofrenije (Robinson and Berridge 1993). Nekatere nevrološke študije so pokazale večje sproščanje dopamina po akutnem amfetaminskem izzivu pri bolnikih s prvo epizodo shizofrenije v primerjavi z zdravimi posamezniki. Te ugotovitve podpirajo hipotezo o endogeni senzitizaciji pri shizofreniji, kjer je dopaminergična senzitizacija bistvena in odgovorna za nastanek psihotičnih simptomov (Peleg-Raibstein et al. 2008).

Znano je, da pre- in perinatalni stres pomembno prispeva k razvoju in poslabšanju nevropsihiatričnih motenj (Booij et al. 2006). Na primer, prenatalne okužbe, stresorji v zgodnjem življenjskem obdobju in urbano življenje so pogostejši pri ljudeh s shizofrenijo kot pri splošni populacij. Tovrstni dražljaji iz okolja pogosto povzročijo povečano reaktivnost na blag stres. Ta tako imenovana stresna senzitizacija lahko povzroči razvoj shizofrenije s preobčutljivostjo na stres (Yuii, Suzuki & Kurachi, 2007). Stresni odziv poteka preko dveh različnih mehanizmov; prvi poteka preko simpatičnega-adrenomedularnega sistema, drugi pa preko osi hipotalamus-hipofiza-nadledvična žleza. Oba sta tesno povezana z dopaminskih sistemom. Na drugi strani pa osnovni nevrokemični mehanizem senzitizacije na stres vključuje nevrokemijsko preobčutljivost dopaminskega sistema, pri katerem ponavljajoča se izpostavljenost življenjskim stresorjem ali psihostimulansov napreduje v povečan nevrokemični stresni odziv, ki vključuje HPA os in DA. Kljub temu, da natančni mehanizmi še vedno niso povsem raziskani je znano, da je za senzitizacijo ključna povezava med aktivacijo HPA osi in dopaminergične mreže. Dokazi kažejo da lahko izločanje glukokortikoidov poveča aktivnost dopamina, zlasti v mezo-limbičnem sistemu. Študije kažejo, da je za bolnike s shizofrenijo značilna disregulacija HPA osi, ki vključuje povišano izhodiščno raven kortizola, in da se v akutnem obdobju bolezni dopaminergični sistem prekomerno odziva na okoljske dražljaje. Hkrati celo izpostavljenost zmernim stresnim dogodkom lahko povzroči prekomerno sproščanje DA ter pospešitev ali ponovitev bolezni pri ranljivih posameznikih (Mizrahi et al. 2012; Winkel,

Stefanis and Myin-Germeys, 2008). Študije na živalih na primer kažejo, da akutni psihološki in/ali fizični stres vodi do sproščanja kortikalnega DA in s tem zmanjša sproščanje DA v striatumu, kar kaže na povezavo med dopaminergičnim sistemom in HPA osjo (Howes et al. 2016; Pani et al. 2000). Nevroanatomska regulacija stresnega odziva vključuje limbično in prefrontalno območje. Študije na glodavcih kažejo, da hipokampus in predfrontalna skorja zagotavljata inhibitorno funkcijo HPA osi. Pri ljudeh je stresni aritmetični izziv z negativnimi povratnimi informacijami privedel do povečanega sproščanja kortizola in deaktivacijo hipokampusa (Ledoux & Daw 2018; Pruessner et al., 2008). Iz zgoraj navedenih izsledkov študij je jasno, da senzitizacija na psihostimulanse in stres potekata preko istih poti in nevrokemičnih mehanizmov. Dokaz o navzkrižni senzitizaciji med amfetamini in stresom pri ljudeh pa so dokazali Booji in sodelavci (2006) v študiji, ki je pokazala, da tako stres kot amfetamini aktivirata HPA os in z njo povečano sproščanje kortizola. Kljub temu, da so nekateri mehanizmi dopaminergične senzitizacije že znani, pa do danes še ne obstaja nobena raziskava, ki bi v senzitizacijo dokazala tudi na funkcionalnem nevroanatomskem nivoju.

Namen pričujoče magistrske naloge je bil izvesti prvo fMRI študijo navzkrižne senzitizacije med amferamini in stresom. Bolj specifično, zanimal nas je učinek amfetaminske senzitizacije na stresni odziv in možgansko aktivnost. Devet zdravih odraslih moških oseb je bilo razporejenih v testno (n = 8) in kontrolno (n = 1) skupino, kjer so prejeli tri odmerke amfetamina oz. placeba. Po latentnem obdobju 14 dni so v fMRI skenerju reševali MIST test, ki inducira stres. Zadnji dan so vsi udeleženci ne glede na skupino prejeli odmerek amfetamina. Po vsakem odmerku amfetamina oz. placeba smo udeležencem izmerili krvni tlak in srčni utrip, udeleženci pa so nato reševali še DEQ vprašalnik. Odkrili smo šibke pozitivne učinke senzitizacije na amfetamin ter povečano subjektivno doživljanje droge. V nadaljevanju smo odkrili korelacijo med povečano subjektivno zaznavo droge in nevronsko aktivacijo v ventralnem striatumu med izpostavljenostjo stresu. Naši rezultati kažejo na navzrižno senzitizacijo stresa in amfetamina, ki hkrati vključuje specifične možganske regije. Študija ponuja obetavne načine za boljše razumevanje etiologije shizofrenije na podlagi hipoteze o dopaminergični preobčutljivosti.

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IZJAVA O AVTORSTVU MAGISTRSKEGA DELA

Spodaj podpisana Katarina Štamulak, z vpisno številko 89172051, vpisana v študijski program Biopsihologija, 2. stopnja, sem avtorica magistrskega dela z naslovom:

Effects of Amphetamine-induced Sensitization on Stress Response and Brain Activity

S svojim podpisom zagotavljam, da je predloženo magistrsko delo izključno rezultat mojega lastnega dela. Prav tako se zavedam, da je predstavljanje tujih del kot mojih lastnih kaznivo po zakonu.

Soglašam z objavo elektronske verzije magistrskega dela v zbirki »Dela FAMNIT« ter zagotavljam, da je elektronska oblika magistrskega dela identična tiskani.

Katarina Štamulak

APPENDICES

APPENDIX A *Bayesian Model Results*

We only report constant effects that are shared across subjects i.e. population-level. Chains were converged at R = 1.00

Heart rate

Table A.1: Population-level effects of model for heart rate.

Model formula: *HR ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CI	Rˆ
Intercept	25.87	[17.08, 34.69]	1
sessionA4	-0.56	[-5.12, 4.08]	1
sensitised	0.66	[-4.15, 5.37]	1
amph_admin	0.44	[-4.45, 5.15]	1

Blood pressure

Table A.2: Population-level effects of model for diastolic blood pressure.

Model formula: *BP ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CI	Rˆ
Intercept	-5.22	[-11.17, 0.94]	1
sessionA4	0.81	[-3.46, 5.05]	1
sensitised	1.37	[-2.86, 5.48]	1
amph_admin	1.91	[-2.95, 6.71]	1

DEQ-item feel

Table A.3: Population-level effects of model for DEQ-item feel.

Model formula: *feel ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CrI	Rˆ
Intercept	0.71	[-1.61, 3.09]	1
sessionA2	-0.06	[-1.51, 1.38]	1
sessionA3	-0.6	[-1.96, 0.78]	1
sessionA4	-0.97	[-3.49, 1.69]	1
sensitised	0.46	[-2.20, 3.00]	1
amph_admin	1.15	[-1.15, 3.41]	1

DEQ-item high

Table A.4: Population-level effects of model for DEQ-item high.

Model formula: *high ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CrI	Rˆ
Intercept	1.43	[-1.63, 3.07]	1
sessionA2	0.89	[-1.51, 1.43]	1
sessionA3	0.84	[-1.95, 0.80]	1
sessionA4	1.56	[-3.54, 1.61]	1
sensitised	0.85	[-2.25, 3.84]	1
amph_admin	1.09	[-1.16, 3.41]	1

DEQ-item like

Table A.5: Population-level effects of model for DEQ-item like.

Model formula: *like ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CrI	Rˆ
Intercept	1.43	[-1.63, 3.07]	1
sessionA2	0.89	[-1.51, 1.43]	1
sessionA3	0.84	[-1.95, 0.80]	1
sessionA4	1.56	[-3.54, 1.61]	1
sensitised	0.47	[-2.13, 3.04]	1
amph_admin	1.17	[-1.16, 3.41]	1

DEQ-item more

Table A.6: Population-level effects of model for DEQ-item more.

Model formula: *more ~ session + sensitised + amphetamine + (session|ID)*

β	Estimate	95% CrI	Rˆ
Intercept	1.73	[-0.23, 3.77]	1
sessionA2	-1.53	[-2.47, -0.54]	1
sessionA3	-1.64	[-2.58, -0.63]	1
sessionA4	-0.86	[-2.91, 1.20]	1
sensitised	0.18	[-1.96, 2.26]	1
amph_admin	0.58	[-1.38, 2.47]	1