硕士学位论文

作者姓名： Sinem Güneri

中方指导教师: 罗非

丹方指导教师: Jørgen Scheel-Krüger

学位类别: M.Sc.

学科专业: Neuroscience and Neuroimaging

研究所: 中国科学院大学中丹学院

MK-801诱导精神分裂症大鼠模型的诱发痛和自发痛评估

By

Sinem Güneri

A Thesis Submitted to

The University of Chinese Academy of Sciences

In partial fulfillment of the requirement

For the degree of

M.Sc. in Neuroscience and Neuroimaging

Sino-Danish College, UCAS

学位论文中文摘要
Graduate Thesis Abstract (in Chinese)

一些临床和研究表明，与正常人相比，精神分裂症患者对急性疼痛的敏感性降低，同时慢性疼痛的发生率更高。但是，目前关于精神分裂症和疼痛的研究大多基于人类实验。除了 Boyette et. al. (2011) 采用机械痛评估精神分裂症模型大鼠的神经病理痛的疼痛行为外，还没有基于对NMDAR拮抗剂介导的精神分裂症动物模型疼痛评估的研究。

本研究通过 MK-801 慢性给药在大鼠身上建立精神分裂症模型，并采用新颖客体识别任务，旷场测试及糖水偏好测试用于检验精神分裂症模型的建立；同时检验了热痛及机械痛诱发 的疼痛行为及福尔马林引起的自发性痛行为；结果显示，注射过 Mk -801 的大鼠对机械痛和热痛的敏感性降低，对福尔马林引起的持续性疼痛的敏感性增加。此外，我们也采用了CFA 注射诱导大鼠慢性炎症痛模型，并观察到经过 MK -801 给药的大鼠慢性疼痛持续时间更长。基于这些发现，并考虑到疼痛与精神分裂症在皮质区域改变的相似性，我们初步探 讨了二者关系的潜在机制。

关键词 Keywords：精神分裂症, MK-801, 慢性疼痛, 自发痛, 诱发痛

学位论文英文摘要

Graduate Thesis Abstract (in English)

Several clinical and research reports indicated that Schizophrenia patients have decreased pain sensitivity to acute pain as well as high occurrence of chronic pain compared to normal individuals. Unfortunately, the information concerning the relationship between Schizophrenia and pain are mostly based on human studies. As far as known, there is only publication by Boyette et. al. (2011) that evaluated mechanical allodynia in NMDAR antagonism Schizophrenia rat model with neuropathic pain. The present study investigated pain sensitivities in distinct pain testing implementations in Sprague Dawley rats, which have been subchronically MK-801 administrated (0.5 mg/kg, twice a day for 7 days followed by a 7 days washout period) to present the translational appropriateness to mimic Schizophrenia-like behaviors. The validity of this model was presented with behavioral tests - novel object recognition test (NOR), sucrose preference test (SPT) and open field test (OFT) - in MK-801-injected rats prior to pain testing. The results revealed that MK-801-treated rats showed hyposensitivity to thermal-, and mechanical-induced evoked pain; hypoalgesia in phase I, and increased hyperalgesia in phase II of formalin-induced spontaneous pain; as well as increased thermal, and mechanical hyperalgesia in CFA-induced chronic pain model, compared to control rats. Moreover, the persistency of pain was observed with MK-801 treatment in the phase II of formalin test and thermal hyperalgesia in CFA-test. Based on these results, possible underlying mechanisms and cortical areas are mentioned by taking the similarities of pain and Schizophrenia into account.

关键词 Keywords：Schizophrenia, MK-801, evoked and spontaneous pain, chronic pain

ACKNOWLEDGEMENT

I am thankful to all my professors with all my heart in Sino-Danish College whose broadened my horizon with sharing their peerless experience and knowledge. I am especially thankful to my supervisors Prof. Jørgen Scheel-Krüger and Prof. Fei Luo for their supervision, enlightening discussions, inspirations, and support. Also extra thanks to Prof. Jørgen Scheel-Krüger for very detailed revision and language correction of the thesis; and to Prof. Fei Luo for giving advices to conduct the experiments; and revision of data analyses and representations.

It is a pleasure to thank to Xinhe Liu, Jifang Li, Xiaoxiao Lin, Wenxiao Gong, Liajia Gu, Wen Ye and Yuzheng Wang who contributed this thesis for helping me to take care the rats, perform experiments and do statistical analyses.

I also thank to my parents, Sevinç and Ömer Sabri Güneri and my best friend, Sinem Kadıoğlu for their emotional support and simply for their existence. My most sincere gratitute is to my sister, Simge Güneri, for being the inspiration of the thesis topic, and for her contributions to my personal growth.

This thesis is dedicated to whom suffer from mental disorders & their relatives; and knowingly or unknowingly contribute to my education and give me the motivation to go further in my research life.

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

ABBREVATIONS

5-HT: 5-hydroxytrypamine

AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

ANOVA: Analysis of Variance

CeAl: Latero-Capsular Division of Central Nucleus of Amygdala

CFA: Complete Freund's Adjuvant

COMT: Catechol-O-Methyltransferase

DA: Dopamine

DISC: Disrupted in Schizophrenia

DLPFC: Dorsolateral Prefrontal Cortex

DSM I to IV-TR: Diagnostic and Statistical Manual of Mental Disorders, 1st edition to 4th edition, Text revision

EPSP: Excitatory Post-synaptic Potential

FGA: First Generation Antipsychotics

FS: Fast spiking

GABA: Gamma Aminobutyric Acid

GAD: Glutamate Decarboxylase

GAD1: Glutamic Acid Decarboxylase

GluRs: Glutamate Receptors

IASP: International Association for the Study of Pain

ICD-10: 10th revision of the International Statistical Classification of Diseases and Related Health Problems

IL: Interleukin

ITI: inter-trial interval

KCNK2 or (TREK-1): Potassium Two Pore Domain Channel Subfamily K Member 2

KCNK4 or (TRAAK): Potassium Two Pore Domain Channel Subfamily K Member 4

kv3.4 or KCNC4: Potassium voltage-gated channel subfamily D member 4

kv4.3 or KCND3: Potassium voltage-gated channel subfamily D member 3

LTP: Long-term Potentiation

MATRICS: Measurement and Treatment Research to Improve Cognition in Schizophrenia

mGluRs: Metabotropic Glutamate Receptors

NB: Nucleus Basalis

NMDAR: N-Methyl-D-aspartic acid or N-Methyl-D-aspartate receptors

NRG: Neuregulin

OB: Olfactory Bulbectomy

PAG: Periaqueductal Gray

PCP: Phencyclidine

PFC: Prefrontal Cortex

PV: Parvalbumin

PWL/T: Paw Withdrawal Latency or Threshold

SGA: Second Generation of Antipsychotics

TNF: Tumor Necrosis Factor

TRP-V: Transient Receptor Potential- Vanilloid

TRPA1: Transient Receptor Potential Ankyrin 1

TURNS: Treatment Units for Research on Neurocognition and Schizophrenia

UCMS: Unpredictable Chronic Mild Stress

VTA: Ventral segmental area

α7nACh: Alfa-7 Nicotinic Acetylcholine

CHAPTER 1: INTRODUCTION

SCHIZOPHRENIA

Concept and definition

In the beginning of 20th century, Emil Kraepelin - was a well-known German psychiatrist with his modern approaches to mental illnesses - proposed a modern concept for a 'psychotic thought disorder' for the first time by subdividing it into two groups as single entities: *manic depression* – as an episodic psychosis - , and *dementia praecox* – as a chronic state with cognitive disruption. Although, the plural term ''Schizophrenias'' was first coined by a Swiss psychiatrist Eugen Bleuler who was closely interested in Kraepelin's conceptualization of dementia praecox. He was thinking that the chronicity and deterioration in this thought disorder are inevitable, and proposed more convenient consensus meaning ''fragmenting mind'' (Schizo = fragmenting ; phren= mind) [1].

Schizophrenia is a complex neuropsychiatric syndrome that has relatively high prevalence appearing in 0.5% to 1% of people worldwide [1]. To the contrary of awareness of this global burdensome, there has always been a definitional chaos as from Kraepelin and Beuler ab inito the etiology remained puzzling. As a matter of fact, despite all definitions given in the past, DSM I to IV-TR provides reliable, auxiliary and diagnostic identification based on its manifestations rather than its biomarkers. It is significant to state that DSM IV-TR does not provide a single-shot definition due to the symptomatic diversity [2]. By common consent, Schizophrenia affects perception, emotions and cognition triggering the state of failure in comprehension of 'what is real and what is not?'.

Manifestations

The followings are three main symptoms of Schizophrenia [1]:

Positive symptoms:

- ✓ *Hallucinations*
- ✓ *Delusions*
- ✓ *Abnormal thought processing*
- ✓ *Repeated bizarre or disorganized behaviours*

Negative symptoms:

- ✓ *Alogia*
- ✓ *Affective flattening (Blunted effect)*
- ✓ *Anhedonia*
- ✓ *Asociality*
- ✓ *Avolition*
- ✓ *Apathy*

Cognitive symptoms:

Impairment in following:

- ✓ *Semantic and Explicit Memory*
- ✓ *Attention*
- ✓ *General intelligence & Information Processing Speed*
- ✓ *Visual and verbal learning*
- ✓ *Executive functions such as problem solving, planning, abstract thinking and planning*

The matter should be laid emphasis on is also patterns of manifestations that are extremely diverse in Schizophrenia that its diagnosis is not based on united symptoms but rather composition of them. A disease characterized by its manifestations rather than biological deficits could be limited by itself. On the other hand, homogeneity was achieved by a classification system provided by ICD-10. By this way, Schizophrenia was categorized as two classes of subtypes including paranoid, catatonic and hebephrenic Schizophrenias; as well as Type I (positive), Type II (negative) and Type III (mixed) Schizophrenias[2].

Along the main manifestations, it is worthy to mention depression as a symptom. Since negative and positive dimensions of psychosis accompanies depression [3] - is considered as subtype in the ICD-10 - [1], it is not surprising that 40% of Schizophrenia patients experience depression [4].

Unmistakably, psychosis-associated positive symptoms are most frequently and first-seen experiences among Schizophrenia patients. Consequently, they had used to be called as first-rank symptoms as primary influence in diagnosis [1, 5]. However, they are not discriminative in diagnosis because they appear in psychosis as well. By the DSM-IV, negative symptoms and cognitive deficits has involved in the diagnosis of Schizophrenia; and accepted as the core features [6, 7]. Moreover, Strauss et. al. (1993) proposed that cognitive regression is closely

bound with negative and positive symptoms while all are correlated with psychological deficits. It is understood that cognitive deficits are one of the first indications to diagnose Schizophrenia as Euler and Kraepelin had mentioned from the very beginning [1, 8]. Even though, cognitive deficits are not unique to Schizophrenia but 73 to 98% of patients experience it, and eventually affects patient's daily life making these deficits to be more predictive than positive symptoms[9, 10].

PATHOGENESIS OF SCHIZOPHRENIA

Genetics Factors and Epigenetics

Several genome sequencing studies among twins and first-degree relatives of Schizophrenia have performed. According to monozygotic twin studies, the possibility of developing Schizophrenia is 30% - 80%, while first-degree family members of Schizophrenics have risk around %8. These findings indicate that neurodevelopmental factors should be involved as well as genetics in Schizophrenia, whereas even monozygotic twins do not have 100% concordance[11].

Some of the first candidate susceptibility genes discovered in Schizophrenia are monoamine oxidase A (MAOA), catechol-O-methyltransferase (COMT), dopamine transporter (SLC6A3), protein phosphatase 1, regulatory subunit 1B (PPP1R1B), dystrobrevin-binding protein 1 (DTNBP1), and neuregulin 1(NRG1) genes [12] due to the popularity of dopamine hypothesis. Although, when this theory had started to fade away; attention was shifted to neurodevelopment, calcium channel, and glutamatergic signaling pathway genes. Therefore, the correlation between genes; and behavioral, cognitive and cytoarchitectural abnormalities in Schizophrenia have become stronger. New susceptibility genes became on focus by the rising importance on NMDA/GABA hypofunction. Some of them are followings : disrupted in schizophrenia 1 (DISC1) , neuregulin 1 (NRG 1), calcium voltage-gated channel auxiliary subunit beta 2 (CACNA1C), DAA0 (D-amino acid oxidase), DAOA (D-amino acid oxidase activator), dystrobrevin binding protein 1 (DTNBP1) gene, glutamic acid decarboxylase (GAD1), central α7-nicotinic acetylcholine receptor (CHNRA7) and glutamate metabotropic receptor 3 (GRM3) genes [11, 13-15].

Alterations in several genes led abnormalities in dopaminergic, GABAergic and glutamatergic pathways are known. Particularly, alteration of the genes affecting glutamate receptors are crucial in the development and progress of Schizophrenia. Recently, it is hypothesized that

epigenetic changes contribute NMDAR hypofunction in Schizophrenia. Several studies have showed that NMDAR subunit switch is essential during the early neurodevelopment, and transition of the subunits makes NMDARs accessible to environmental and genetic factors resulting epigenetic alterations in the promoter region of NMDARs. Also, NMDAR hypofunction can lead to epigenetic alterations as schematized in figure 1. That is why, the abnormality in genes expressing some molecules affecting NMDARs and neurodevelopment have role in negative symptoms and cognitive deficits directly, and in positive symptoms indirectly by dopaminergic pathway[12, 13, 16].

Neuroanatomy

Post-mortem and neuroimaging studies showed that the following neuropathological abnormalities are likely to be observed in Schizophrenia patients: enlarged lateral and third ventricles, decreased total brain volume, decreased thalamus; amygdala; hippocampus; parahippocampus; substantia nigra; and internal globus pallidus [1]. Moreover, abnormalities based on arrangement of neurons and cytoarchitectural degeneracy were observed in about 10-20% of Schizophrenia patients. Pyramidal neuron disarrangement in hippocampus, particularly CA1-CA3; disarray of neurons and abnormal cytoarchitectures were detected in parahippocampus of Schizophrenia patients. Also, an absolute prominent region in Schizophrenia - is frontal lobe - has reduced density, size and number of neurons[17] usually called as hypofrontality.

As a matter of fact, cognitive deficits are the results of cumulative abnormality not only in prefrontal areas but also in inferior parietal lobule, amygdala, superior temporal gyrus, medial temporal lobe, basal ganglia, thalamus, corpus callosum and cerebellum during neurogenesis and neurodevelopment; which are the processes that proceed from prenatal life to late adulthood [1, 11].

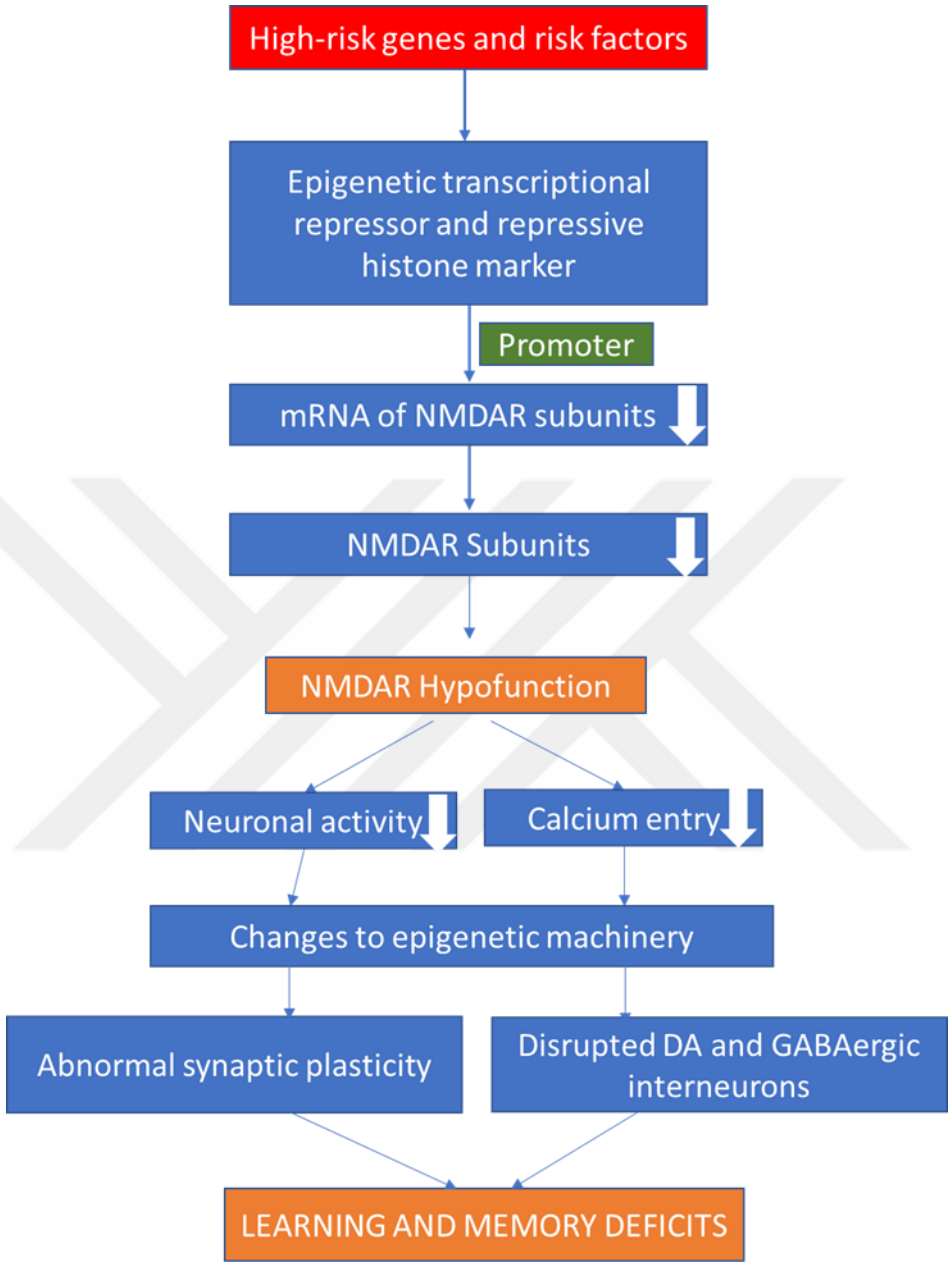

Figure 1: *The relationship between NMDAR hypofunction and epigenetic mechanisms. High risk genes and factors resulting epigenetic changes in the promoter regions of NMDARs. Epigenetic alterations cause decreased NMDAR subunit expression ended up NMDAR hypofunction in pyramidal neurons. The alterations in neuronal activity and calcium influx lead to disruption in DA and GABAergic interneurons due to feedback loop. Therefore, all together trigger cognitive deficits including learning and memory deficits. For instance, NMDAR antagonist, MK-801 leads epigenetic mechanism alterations in medial PFC by increasing the phosphorylation of histone 3 resulting chromatin compensation and decreased gene expression. (This figure is modified from the figure included in the study of Snyder et. al. (2019) [16]*

Neurotransmission

Dopamine and Serotonin

Initially, prominent neurotransmitter considered a cause for Schizophrenia was dopamine (DA) due to following observations: the alleviation of psychotic-positive symptoms by first generation antipsychotics (FGAs or typical antipsychotics) acting via D2 receptor antagonism, and drugs such as LSD (Lysergic acid diethylamide) or amphetamine triggering DA activity leading psychosis and aggravates Schizophrenia-like behaviours [15, 18, 19]. Thereby, it has been hypothesized that hyper-expression of DA in the brain was the fundamental reason of Schizophrenia [11]. The first times, researchers had had little information about dopaminergic projections. Yet, it is discovered that DA pathway can be divided into four systems related to its projections: mesocortical, mesolimbic, tuberoinfular, and nigrostriatal. While dopamine activity is excessive in mesolimbic system (VTA innervating mPFC and nucleus accumbens etc.) related to elevated positive symptoms, it is decreased in mesocortical system (substantia nigra innervating striatum) associated to negative symptoms. Furthermore, FGAs block the nigrostriatal system and hypothalamic DA expression. The blockage of nigrostriatal system which innervates the basal ganglia (caudate nucleus and putamen) produces unwanted extrapyramidal side effects and hypothalamus - is innervated by tuberoinfular system- causes endocrine side effects. [14, 20].

While FGAs alleviate the positive symptoms, this effect has been not achieved for negative and cognitive symptoms. That is why, a revision of hyper-dopaminergic hypothesis was considered followed by seeking new responsible neurotransmitters. Consequently, demanding in treatment of negative symptoms and especially cognitive deficits, and the extrapyramidal side effects of FGAs led to the introduction of second generation of antipsychotics (SGAs or atypical antipsychotics). On the other hand, SGAs provide differential receptor profiles having an impact on serotonin (5-hydroxytrypamine [5-HT]), DA and norepinephrine (α-adrenergic) receptors [11, 15]. In spite of the fact that DA and serotonin are expressed in similar brain areas. However, while serotonin is widespread, dopamine is more localized. That is why, serotonin has other and more multiple modulation of brain functions. For instance, Haloperidol -is used to be a FGA- blocks strong DA type-2 receptors (D2Rs) resulting the alleviation of delusions and hallucinations; however, clozapine and risperidone (SGAs) blocks both serotonin type-2 ($5\text{-}HT_{2)}$ and D2Rs having little but tolerable

level in treating negative and cognitive symptoms [19]. Despite the reveal of the roles of new neurotransmitters, there is no drug licensed for cognitive and negative symptoms [21, 22]

Hypotheses: Dopamine dysfunction and NMDAR/GABA hypofunction

It is known that several neurotransmission systems are involved in pathopsychology of Schizophrenia notwithstanding the recent prominent hypothesis was used to be based on hyper-dopamine activity that is thought to be a result of NMDAR (N-Methyl-D-aspartic acid or N-Methyl-D-aspartate receptors) hypofunction recently. [22]. This finding is supported by animal studies by the NMDAR antagonists that trigger dopamine release. Moreover, the key cortical areas of DA system are target areas for NMDAR antagonists supporting negative feedback hypothesis on NMDAR hypofunction[14].

As shown in figure 1, GABA (γ -aminobutyric acid) fast-spiking (FS) and parvalbumin (PV) containing interneurons project to glutamatergic pyramidal neurons. Mainly, AMPARs (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) are responsible of excitatory postsynaptic potential (EPSPs) in pyramidal cells and NMDARs are just related to synaptic plasticity that specifically responsible of learning. Yet, NMDARs contributing EPSPs exist on PV-containing FS-interneurons that affects neurotransmission on pyramidal cells via a recurrent feedback inhibition mechanism. So, when there is enough release of glutamate on NMDARs on these interneurons - are highly sensitive to glutamate -, the inhibitory output would be relayed to pyramidal cells. The NMDAR antagonists (e.g. MK-801) are able to inhibit the glutamate excitation of the otherwise GABAergic inhibition on pyramidal cells leading disinhibition of the glutamate pathway directed towards the VTA with the consequence of an increase in VTA DA release. Subsequent swelling of pyramidal neurons following to cortical disinhibition are found to be the damage to parvalbumin GABA interneurons as well as an effect that is considered to be the reason of cognitive deficits in Schizophrenia by some researchers. After all, it remains a mystery about why some other agents do not lead Schizophrenia symptoms in humans; although, they do disinhibition as well as NMDAR antagonists. There are two existing hypotheses: one is that these agents may decrease both inhibitory output and input on/of interneurons which cause a cancellation of effect and other is that NMDAR antagonists may selectively decrease EPSPs in interneurons. Thus, there is also one more fact that interneurons are regionally distinctive to NMDAR antagonists. For instance, NMDAR antagonists are more likely to alter the inhibitory feedback in PFC, entorhinal cortex and thalamus than hypothalamus [14, 22-24] .

In Schizophrenia, GABAergic hypofunction and decreased level of GABA synthesizing enzyme glutamate decarboxylase (GAD) are demonstrated in post-mortem cortical tissues. GABA function is mainly limited to PV-containing basket and chandelier interneurons, but other type of GABAergic interneurons has role additionally. For instance, somatostatin neurons innervate the dendrites of GABAergic interneurons to filter out uninterested behavioural distractors affecting attention that can interfere with memory functioning. Indeed, this ability is disrupted in multi-cortical areas projecting to PFC (prefrontal cortex) of patients with Schizophrenia [25]. As PV is Ca2+ buffering protein, reduced GAD67 observed in PV-containing interneurons depends on the blockage of Ca2+ entry through the NMDA channels [26, 27]. Eventually, the resulted higher level of free Ca2+ leads delayed GABA release causing decrease in inhibitory output. Therefore, NMDA channels are the sensors for activity of pyramidal cells because of the fact that hyper-activity of pyramidal cells is occurred as a consequence of the low activity of NMDARs that triggers less expression of GABA and PV in interneurons. [28, 29]. The feedback loop between FS interneurons and pyramidal cells generates gamma frequency oscillations (30-100 Hz) that maintains the communication between cortical areas [25, 30]. The reduced gamma power alters the behavioural state led negative and cognitive symptoms of Schizophrenia. Thus, NMDAR antagonism triggering decrease in gamma oscillations demonstrated in DLPFC (dorsolateral prefrontal cortex) and hippocampus , especially CA1, with reduced GAD67 expression[14]. Chung et. al. (2016) hypothesized that impaired memory and decreased gamma oscillations in DLPFC of Schizophrenics may be resulted from pyramidal neuron inhibition by PV interneurons. [30].

Several studies signified a special role of hippocampus and ventral tegmental area (VTA) in NMDAR/GABA hypofunction. As illustrated in Figure 2, there is a positive feedback loop between VTA and hippocampus via hippocampal nucleus accumbens - ventral pallidum – VTA neuronal network. Disinhibition on FS-interneurons elevates dopamine levels as a result of increased VTA activity that led investigations through the relationship between memory functioning, and the loop of hippocampus-VTA system. It is well-known that hippocampus is accepted in memory control and also in detection of novel information altogether with the prefrontal cortex. Detecting a new information results increased activity in VTA which sends dopaminergic projections to hippocampus and prefrontal cortex for initiations of cognitive and /or motor actions. Therefore, if there is a failure in VTA-hippocampus/prefrontal loops (Figure 2) , it eventuates hyper-DA activity which affects cognitive systems such as working memory, with the projections to DLPFC. [27]. It has been demonstrated that PFC neurons are

responsible of working memory as well via its distributed communication network with cortical areas. Moreover, these PFC pyramidal neurons are highly innervated by PV containing interneurons. These interneurons are associated with reversal learning and memory which can be disrupted by NMDAR antagonism [29]. It refers to the fact that working memory is related to gamma oscillations in PFC which its frequency increases in proportion to load in normal individuals. Nevertheless, lower gamma oscillations is observed in both psychosis and Schizophrenia manifesting that its decrease is not a result of chronicity or treatment approaches in Schizophrenia[25].

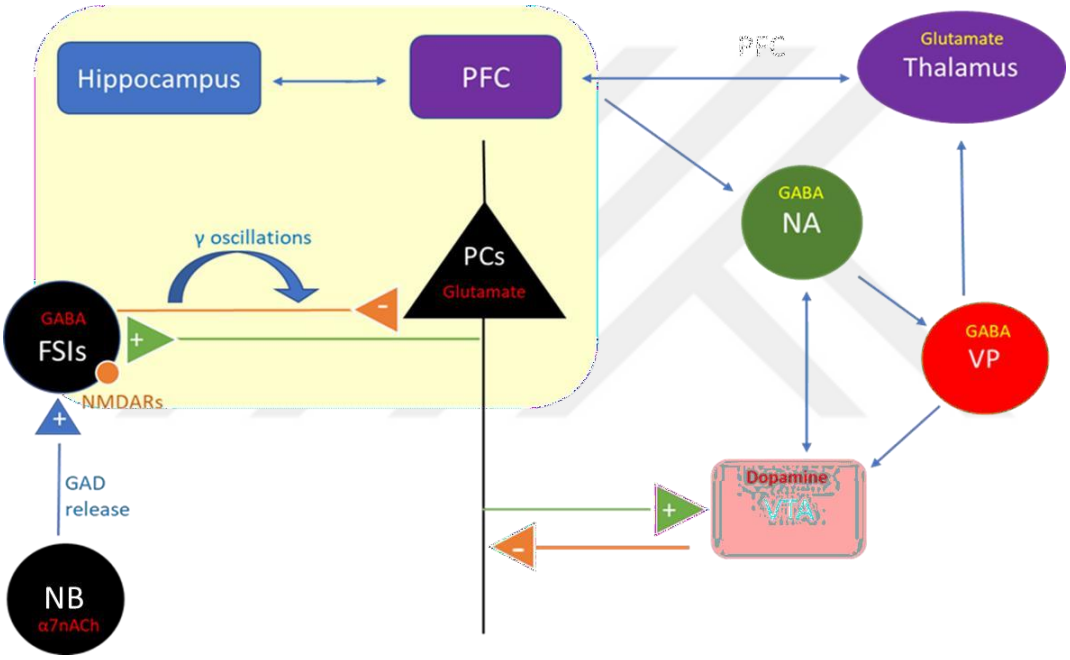

Figure 2*: Homeostatic feedback loops and concerning cortical areas are illustrated. (i) Negative feedback between fast-spiking interneurons and pyramidal cells; (ii) and positive feedback between Hippocampus-VTA via Hippocampal NA – VP – VTA neuronal network. Triangles represent whether it is an excitation or inhibition according to (+) and (-) marks, respectively. On the other hand, both green and orange triangles on the edge of lines represent inhibitory mechanism (through green = inhibitory input ; through orange = inhibitory output) Abbreviations: Fast-spiking interneurons (FSIs); Pyramidal cells (PCs); Ventral segmental area (VTA); Nucleus basalis (NS); alfa-7-nicotinic acetylcholine (α7nACh); Prefrontal Cortex (PFC); Nucleus Accumbens (NA); Ventral pallidum (VP)* (*This* picture was modified from Jørgen Scheel-Krüger's presentation)

Sub-chronic and chronic administration of NMDARs antagonists in rodents demonstrated these implications [28, 29]. Moreover, acute treatment of an NMDAR antagonist leads immediate inhibition in negative feedback temporarily, while constant treatment is able to

decrease the efficacy of inhibition by reducing cortical GAD67 and parvalbumin mRNA[26]. Although, these decreases with constant treatment reflect a neurodevelopmental effect since they were not observed in post-pubertal period [31].

PFC is highly communicative with other important higher order cortical areas (the temporal, parietal, insular, entorhinal cortices; and hippocampus) and to subcortical structures (the mediodorsal thalamic nucleus, nucleus accumbens (NA), and ventral pallidum (VP), VTA and various brain stem regions including the serotonergic raphe nuclei). Moghaddam et. al. (1990) mentioned for the first time: dopamine innervation of mPFC have more impact on SGAs or FGAs than nucleus accumbens and striatum have. Nonetheless, dopamine dysfunction in PFC has fundamental role in negative and cognitive symptoms of Schizophrenia [20, 23]. Another study by Moghaddam et. al. (2012) provided that intra-PFC injection of NMDAR antagonists may increase memory deficit associated to Schizophrenia supporting NMDAR hypofunction hypothesis of Schizophrenia [32]. Moreover, McLean and her colleagues (2017) have showed that sub-chronic administration of NMDAR antagonists may increase extracellular DA in PFC, while treatment with SGAs (i.e. sertindole and risperidone) improves memory deficit in NMDAR antagonist-induced Schizophrenia rats [23]. Moreover, some cognitive impairments were ameliorated by D1-like receptor agonists. For instance, haloperidol acts on D2-like receptors enhancing more DA release in striatum and nucleus accumbens than mPFC, while clozapine cause more DA release in mPFC than in the cortico-limbic system[20]. Since the enhanced effect of SGAs (especially clozapine) is known, facts which as above-mentioned support evidences on the importance of PFC role in cognition. Thereby, DA activity of PFC are not elevated due to NMDAR antagonism whereas control rats exhibit increased DA in same area [23, 32]. It shouldn't be forgotten the role of hippocampus as well as PFC that regulate DA activity with glutamatergic projections to the midbrain (see figure 1) [27]. Thus, it is worthy to state that the extensive and reciprocal interactions between glutamate and dopamine make difficult to understand which is primary; however, it is obvious that both of them have impact on the abnormalities in the other system[33]

Furthermore, enhanced binding of α7 nicotinic receptors has been found to alleviate cognitive deficits and turn back gamma oscillations into normal frequency, whereas these receptors located on FS interneurons and modulate GABA release. Consistent with that, nicotine can reverse disinhibitory output caused by NMDA antagonists[23].

ANIMAL MODEL OF SCHIZOPHRENIA BASED ON NMDAR ANTAGONISM

It is widely known that there is no golden standard Schizophrenia animal model method due to being far from understanding the etiology of Schizophrenia. As a matter of fact, a single model cannot represent the entire Schizophrenia spectrum, and it is not possible to elicit all symptoms within a single animal model, certainly due to the less developed prefrontal cortex in lower animal species as rodents. Furthermore, as Schizophrenia patients do not have all symptoms but rather different set of symptoms representing various endotypes, an animal model eliciting just some key abnormalities would be relevant. Certainly, modelling the positive symptoms are not very possible because animals cannot; and if the possibility exist, they are not able to self-report hallucinations and delusions by anything else than via their motor acts. In this point, the faith of animal modeling is totally dependable on motor actions reflexing negative, and cognitive symptoms[26, 34, 35] Clementz et. al. has demonstrated district subtypes on Schizophrenics based on the cortical biomarkers found to be not correlated to the clinical diagnosis [36]. As the greatest purpose is to advance treatment of Schizophrenia and measure antipsychotic effects multidimensionally, it has been proposed that patients with district subtypes should have diversified treatments for maximum efficacy. That is why, representing these subtypes in animal modeling of Schizophrenia gained key role that is proposed to be models of certain subtypes of schizophrenia[1, 26, 37].

The effective treatment to cognitive deficits of Schizophrenia is an unmet necessity. To develop cognitive-enhancing drugs, MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) and TURNS (Treatment Units for Research on Neurocognition and Schizophrenia) programs have been initiated. Setting an animal model mimicking cognitive impairments as well as negative symptoms are real challenges in a unique 'human disorder'. Since cognitive enhancement is not likely to be shown in 'normal' animals; pharmacological, genetic and neurodevelopmental approaches are adapted depending on this purpose. For instance, while social isolation after weaning is to focus on novel therapies, NMDAR antagonism remain another focus on searching for mechanisms to improve cognition[22, 28].

NMDA Receptor Antagonists

In 1950s, glutamate receptor (GluR) antagonists were used as anaesthetics and even analgesics [38]. Consequently, their psychotic effects – found to be very similar to

Schizophrenia - on mentally healthy individuals led researcher's attention shift to these compounds for the development of preclinical models. Among GluRs, NMDAR antagonists became foremost due to their ability to exacerbate negative and positive symptoms as well as cognitive impairments of Schizophrenia. This effect could be seen even after a single dose of those anesthetics [14, 15, 29, 39].

Most prominent and well-known NMDAR antagonists are Ketamine, Phencyclidine (PCP) and MK-801 (Dizocilpine). Some others are Diphenidine, 2-Methoxydiphenidine, Dexoxadrol, Cyclazone, (-)- Pentazocine etc. [34, 38]. By favor of microelectrophoresis, both glutamate receptor agonist and some antagonists were administered into neurons to underlying the selectivity - or potency – by comparing the physicochemical properties of compounds. The potency comparisons provided researchers to discover the potential doses that inducing psychotomimetic effects, for example, by 0.05 mg/kg MK-801 [34, 40]; 0.1 mg/kg PCP; 2 mg/kg Ketamine in rats [38].

NMDARs are coupled to voltage-dependent ion channels, which are activated by the coordination of two independent sides: glycine or D-serine binding sites, and a glutamate recognition side that modulates local pH and redox state (Figure 3A). NMDAR activation generates Ca^{2+} permeability through the channels, and Ca^{2+} influx is inhibited by Mg2+ ions or uncompetitive NMDAR antagonists that bind distinct side from recognition side (represented as C in figure 3A). This binding is deep within the channel (Figure 3B) and dependable on the amino and carboxylic moieties of glutamate.

Electrophysiological studies demonstrated that Ketamine is voltage dependent more than MK-801, while voltage-dependency of PCP is intermediate compared them. Also, cognitive and negative symptoms of Schizophrenia induced by different non-competitive NMDA antagonists may show differentiation due to potency differences in the subtypes of NMDARs. These subtypes are pairs of GluN1 and GluN2 (two GluN2A, GluN2B, GluN2C or GluN2D subunits). While glutamate binds to GluN2 subunit, D-serine or glycine binds to GluN1 subunits to induce conformational change that ended with channel opening [41]. Recently, it has shown that ketamine and PCP are more potent on GluN1/GluN2B-D than GluN1/GluN2A, whereas MK-801 is equipotent to all subtypes. Interestingly, GluN2A and GluN2B have role in synaptic activity of higher cortical areas [38]. These subunits play important roles in mimicking Schizophrenia with cognitive deficits by altering glycine or D-serine binding side of NR1 subunit or reducing NR1 subunit. Moreover, decreased concentration of glycine and

D-serine; increased level of endogenous antagonists; reduced channel expression and the subunits are markers of NMDA hypofunction [24].

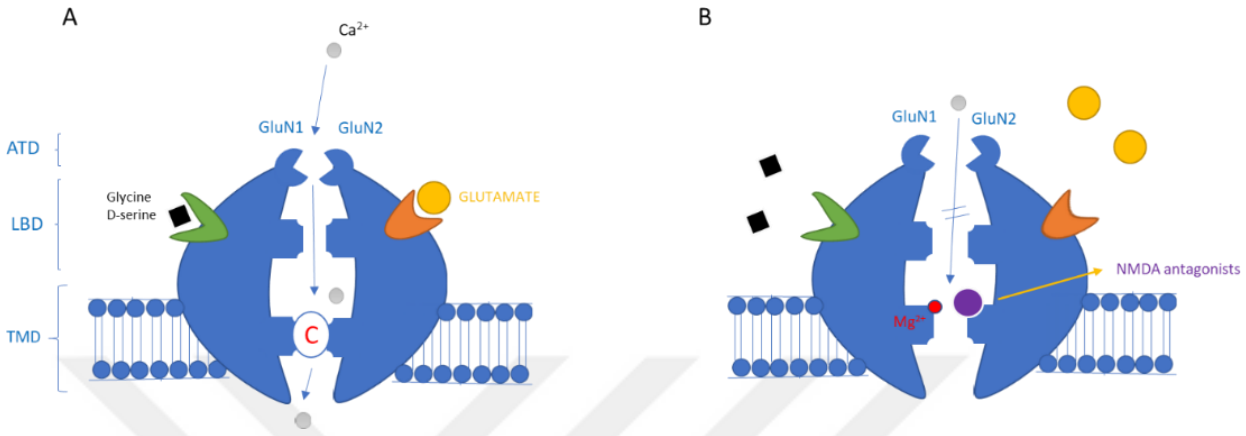

Figure 3: *Na+/K+ Channels coupled with NMDARs (A) Activation of ion channel by glutamate and glycine or D-serine resulting calcium flow through channel. C represents the binding side for NMDAR antagonists detailed in B. Moreover, amino terminal (ATD); ligand-binding (LTD); transmembrane (TMD) domain; GluN1 and GluN2 are represented. Between GluN1 and GluN2, there is negative allosteric binding side. (B) Inhibition of channel due to magnesium binding or non-competitive NMDAR antagonists through C that inhibits calcium flow through channel. (*This figure has been re-drawn by combining two figures from the article of Mercier et. al.;(2015)*)*

The featured question is that '' if these anesthetics can imitate the positive symptoms, even better than dopamine agonists (e.g. amphetamine), then why not only glutamate antagonists be targeted to develop treatments as that they additionally trigger negative symptoms and cognitive deficits?'' Thus, NMDAR agonists (e.g. mGluRs) have been considering for treatments [15, 21]. The clinical trials conducted on Schizophrenic patients having concurrent SGAs have been performed to test glycine site agonists. For instance, Sarcosine - is a glycine transport inhibitor - achieved to decrease positive and negative symptoms while alleviating cognition [42]. Moreover, the first and successful medication to treat Schizophrenia is NMDAR, mGlu2/3, agonist that is not based on dopamine antagonism[33].

PCP and MK-801

In the study of Cadinu et. al. (2017), one of the biotypes of Schizophrenia described by Clementz et. al. was mimicked with sub-chronic treatment of PCP (2 mg/kg). The authors adopted the following regime: 7 days injection for twice a day and afterwards 7 days washout

[23]. This model was successful to produce poorest cognition, negative symptoms and pathology of Schizophrenia; while these manifestations were alleviated by SGAs but not by FGAs. In this model, decreased expression of PV protein in PFC and hippocampus is observed (also with MK-801 [15]) that can be attenuated by a novel treatment is still under development. They also evaluated the cognitive domains affected in Schizophrenia including visual memory deficits that showed by Novel object recognition test (NOR) [15], whereby PCP-injected rats couldn't discriminate between familiar and novel object. MK-801 and PCP share behavioural effects and binding features. It is a fact that MK-801 is by far the most potent compound among NMDAR antagonists to displace PCP[38]. King et.al (2004) reported that NOR deficits can be observed by 0,05 mg/kg acute administration of MK-801 with 2h inter-trial interval (ITI) [43]. Furthermore, Ashby et. al. (2009) proposed similar Schizophrenia model with 0,05 mg/kg MK-801 in Sprague Dawley rats and testing motor activity [40]. Therefore, MK-801 has been shown to produce increased motor activity plus learning and memory impairments that are tested variety of tests in several species including rodents, zebrafish and non-human primates [44]. Impaired recognition following to NMDARs antagonist administration can be restored by SGAs (i.e. clozapine; 1-5 mg/kg), D-serine (800 mg/kg) and a glycine transporter inhibitor (NFPS; 0.3–1.0 mg/kg). Although, FGAs (haloperidol; 0.03–0.1 mg/kg) are failed to restore cognitive impairments (i.e. MK-801-induced impairment) [15, 42].

As well as cognitive deficits, acute administration of NMDAR antagonist (i.e. PCP) in rodents are relevant to generate abnormal pre-pulse inhibition (PPI) of startle response, sensorimotor deficits and increased locomotion. Regarding to particular cognitive deficits, it is worthy to state that sub-chronic regime impairs performance on tasks such as recognition memory and attentional set-shifting tasks, which is based on hippocampal and frontal functioning [29]. Attenuated level of PV in GABAergic interneurons is associated to disrupted reversal learning directly, as a consequence of hyper-dopaminergic state in PFC which is a reflection of GABAergic hypofunction in frontal and hippocampal areas. The reversal learning tests in rodents and primates suggested that these cognitive disturbances are observed by PCP administration, it is considered that it fulfills both DA and glutamate related neurotransmission abnormalities. [28, 29].

Acute versus Sub-chronic/Chronic administration

It is worthy to mention that chronic or sub-chronic administration of MK-801 provides more valuable replication of neuropathogenesis and manifestation of Schizophrenia, which is basically a chronic illness, than acute treatment. Even though, single dose of treatment trigger Schizophrenia-like behaviours, it should be interpreted as psychosis or transient neurochemical alterations [27]. It has been observed that the sub-chronic studies, with the same regime as Cadinu et. al. applied, leads robustness in cognitive; social behaviour; and neuropathological impairments associated to Schizophrenia. Reversal of the effect are inevitable for acute exposure of MK-801 and sub-chronic or chronic treatment is necessary to maintain the effects [15] supported by imaging of disrupted neurodevelopment of GABA interneurons in rat PFC after exposure to continuous MK-801 in 35-63 days old rats[44].

PAIN

According to the IASP (International Association for the Study of Pain), pain is described as ‘‘unpleasant emotional and sensory experience related to potential or actual tissue damage’’ [45]. The mechanism of pain perception -is still elusive- and can be examined by dividing it in two types: evoked pain and spontaneous pain. Evoked pain is self-evident nociceptive pain that appears from nociceptor responses to real or potential damage as IASP suggested. Come to that the definition is spontaneous pain is much complicated than its consensus (fluctuating firing) and it has no definition in IASP. Bennett et. al. (2012) defines it as ‘internal and self-contained with respect to neurons in the somatosensory system’ [46].

The process of mediating chemical, thermal or mechanical stimuli by sensory neurons is called Nociception. Nociceptors are peripheral sensory neurons detecting noxious stimuli, selectively injurious stimuli and then carrying it to CNS (central nervous system). There are 3 types of nociceptors depending on the features of response to stimuli: C-fibers that is associated with unmyelinated axons conducting electrical, mechanical and thermal stimuli by transduce into action potentials; Aδ-fibers that are associated with myelinated axons which conduct info very fast; and sleeping nociceptors that are activated by extreme stimulation which is related to increased spontaneous activity. Sleeping nociceptors are only sensitive to noxious thermal stimuli but in extreme cases of injury, they response to mechanical stimuli as well. Aδ-fibers are divided into two types: high threshold mechanoreceptors and polymodal receptors. While mechanoreceptors respond thermal and mechanical stimuli with

high and low thresholds, respectively; polymodal receptors respond the both stimuli with vice versa. Mechanoreceptors sensitize the pain in the development of tissue damage by dropping threshold. Polymodal receptors are responsible of generating first acute pain against noxious thermal stimuli; however, mechanoreceptors are required for first mechanical pain to mechanical stimuli[47-50].

It is interesting that while momentary/acute pain is helping organisms to protect the body from damage, chronic/persistent pain is so deleterious that people even prefer to die instead of suffering for long. So, acute pain is a present from evolution to defense from nociceptive stimuli as a consequence of normal nervous system mechanism. On the other hand, chronic pain appears in tissue/nerve damage persisting long due to pathological disruption within nervous system. In case of acute pain, a response to the stimuli from peripheral nerve fibers is amplified as a sensory input and is relayed with normal output to descending areas to restore the nociceptive pain. Persistent/chronic pain is related to the alteration of peripheral nerve fibers following to insistent and impairing stimuli that can be originated from injury or disease. This persistency is originated from increased spontaneous firing and subsequent altered neurotransmission systems leading to abnormal output through CNS by descending projections. On the other hand, normal output is under control by inhibitory and excitatory synaptic transmissions (i.e. NMDARs, glycine receptors, GABA-ARs). The spontaneous firing changes glutamatergic neurotransmission by inducing prolonged and increased amplitude of EPSCs, and increased tyrosine phosphorylation of NMDAR subunits which means upregulation of NMDAR activity[41, 48].

Inflammatory pain arises from the responding to tissue injury and subsequently to inflammation in which there is an obligatory shift from protecting body from noxious stimuli to address a problem concerning the consequences of damage. In this stage, sensory nervous system converts the responsiveness and becomes prolonged over-sensitivity to both noxious and innocuous stimuli during the process of healing. Throughout inflammation, hyperalgesia (an increased sensitivity to pain); or allodynia (a pain due to innocuous stimuli) is developed[51]. Primary hyperalgesia usually occurs following to skin incision leading to muscle, joint or visceral etc. damage due to increased excitation of CNS neurons, while secondary hyperalgesia occurs at neighbouring areas of injury due to increased excitation of nociceptors. Therefore, primary and secondary hyperalgesia are related to peripheral and central sensitization, respectively [46, 52]. The inflammatory pain disappears when the initial tissue damage is repaired. Although, if tetrodotoxin-resistant sodium channel Nav1.8

expressing nociceptors are ablated, it cause neuropathic pain[51] which is a consequence of loss of nervous system function due to nerve damage.

Peripheral sensitization is defined as alterations in the chemical environment of the nerve fiber due to inflammatory mediators. On the other hand, central sensitization is altered neuronal responses/ synaptic plasticity to subsequent inputs after peripheral sensitization [48, 51]. Several neurotransmitters are involved in these procedures such as AMPARs, NMDARs, GABA-Ars and Glycine receptors etc. In the develop mechanism of central sensitization, dorsal horn neurons depolarized due to over activation of C-fibers that eventuate lost magnesium blockage from NMDARs. Nonetheless, other receptors permit calcium influx activating calcium dependent kinases. The kinases phosphorylate NMDARs inducing glutamate binding and decreased GABA levels. That is why, NMDAR antagonists are good analgesics that alleviate central sensitization[47].

Cortical Representation of Pain

Brain imaging techniques provided multiple cortical areas activated during pain stimulation. To the response on noxious and innocuous pain, the most prominent and well-known cortical areas are S1 and S2 somatosensory cortices, thalamus, anterior cingulate cortex (ACC) and insular cortex (IC). Moreover, the other district cortical areas preferentially participated the dimensions of pain perception. Although, the strong interaction between affective-motivational and sensory-discriminative aspects is obvious [53].

Meta-analyses revealed that PFC, mainly known for its government of executive functions, has a great role in pain processing. Intensity and spatial dimension of pain are discriminated by vertical projections from insular cortex to PFC; and by dorsal projections from posterior parietal cortex to DLPFC, respectively; while both projections activate anterior cingulate cortex. Anterior insula innervates PFC and posterior insula is connected to S2 somatosensory cortex indicate the significance of insular cortex in pain. In allodynia and neuropathic pain, insular cortex and S2 somatosensory cortex are the contributors to pain sensitization. It is known that medio dorsolateral thalamus - PFC network is the origin of chronic pain that is caused by abnormal thalamic inputs potentially owing to reduced GABA. That is because the periaqueductal gray (PAG) that is a center of primary pain modulation receiving projections mainly from mPFC and innervating to Thalamus and other controlling cortical areas for pain relief. Nociceptive stimuli cause increased gamma oscillations in insular cortex and PFC and

herewith increased activity of GABAergic neurons. This process is called as nociception as aforementioned which is reversed by mPFC via glutamatergic activity [54]. Long-lasting disinhibition of nociceptive neurons in mPFC is prevented by acute NMDAR antagonist (i.e. MK-801) or mGluR administration in animal studies. Moreover, it has been observed that acute administration of NMDAR antagonists decreases mechanical allodynia while improving cognition and motor control in a mice neuropathic pain model. Although, acute injection of AMPA in PFC only concludes with analgesic effect. It is interesting that animals with neuropathic pain exhibit increased mGluR5 in prelimbic cortex that its decrease concludes depressive behaviours and hypersensitivity to mechanical stimulation. Moreover, VTA suppresses nociceptive responses in PFC including anterior cingulate and prelimbic cortices by its dopaminergic projections, particularly with binding to D2-like receptors [53-58].

SCHIZOPHRENIA AND PAIN

A theory: Insensitivity to Acute Pain in Schizophrenia

Several clinical and research reports demonstrated that Schizophrenia patients have decreased pain sensitivity with the prevalence of 52% to 91% up to age, gender, ethnicity etc. [45, 59]. The very first, in 1919, Kraepelin mentioned this phenomenon in his book, 'Dementia praecox and paraphrenia', such following [60]:

> ''*… it appears also that patients become less sensitive to bodily discomfort; they endure uncomfortable positions, pricks of a needle, injuries... bum themselves with their cigar, hurt themselves, tear out the hair from their genitals*…'' (p.34) and ''*… it was shown that the absence of pain reaction could be ascertained only in 36 percent* …'' (referring pupillary reaction to pain; p.78)

It can be assumed that insensitivity to pain had been known for long time; however; it was neglected for a while. That is also owing to the fact that some Schizophrenia patients had never reported that they suffer pain maybe because of verbal communication difficulties [61, 62], and what is worse, self-observation of decreased pain sensitivity in mentally disordered patients is likely to be not confidential. Solely, the prevalence of pain complaints was observed much less for Schizophrenia than other mental disorders and several clinicians including surgeons and psychiatrics, have reported such cases that called attention to this issue [63]. In 1982, an MD, Fishbain from Psychiatry Emergency Service has reported some cases, including a case as the following[64] :

'' … her mental status examination was compatible with the diagnosis of acute schizophrenic episode. The physical examination, including vital signs and the checking of all reflexes and motor/strength tests, was within normal limits. No visible signs of injury were noted, and no pain response was elicited…Two days after admission, the patient suddenly began to complain of right ankle pain… '' (note that referring to antipsychotics here); ''… *Radiographs of the right ankle showed an uncomplicated fracture of the lateral malleolus…"*(p.58/631, Annals of Emergency Medicine)

In 2008, Virit et. al. published an article to mention the severity of pain insensitivity of Schizophrenia by a single case about a patient diagnosed with Schizophrenia over 20 years. The patient had put his hand on burning flames of gas to get warm and then he realised his arm was on fire, but he expresses that he had not experienced pain during this incident. He was admitted to emergency with his relatives and his arm had to be amputated, since his, tendons, muscles and even nerve fibers and bone tissues were injured due to burn. As well as this case, several cases have been reported that some Schizophrenia patients defining their supposed-to-be severe pains as slight pains were actually life threating conditions (i.e. cancer, bone fractures etc.)[64, 65] That is interesting that even several decades ago, there is such observations, even ended with the patients' death. For instance, Marchand et. al. (1959) has mentioned that absence of pain to acute perforated peptic ulcer and acute appendicitis presented 21% and 37% of Schizophrenics respectively. So, most of these patients came to see physicians when surgical intervention late in that phase of disease. With estimation, 95% of healthy individuals with pain present their complaints[63] which is a life-saving mechanism. Marchand et. al. also emphasized this insensitivity in another perspective and proposed that Schizophrenics have ''asymbolia" for pain [45, 62]. It was re-proposed by other authors as 'lack of motivation-affective aspect of pain correlated to limbic system and frontal lobe' [61], followed by the idea of that cognitive impairments may hinder the ability of recognise and express pain in Schizophrenia patients[66]

Studies about pain perception in Schizophrenia used nociceptive stimulation to measure the thresholds. All patients participated such studies had to be diagnosed by the criterions according to DSM and ICD. Although, while some patients were having antipsychotics, some others were drug-free that cause a heterogeneity within these studies. Meta-analysis by Potvin et. al. (2008) indicated that hypoalgesia involves both medicated and drug-free patients [67]. Since current antipsychotics have multi-action properties such as being efficient to treat anxiety disorders, some type of depression and even improve sleep

parameters, they are also analgesics, which is not surprising because the act of these drugs are mainly on dopamine and serotonin [19, 68]. Associated with neuropathology of Schizophrenia, experiencing pain insensitivity is not that much shocking as for that the alteration in neurotransmitter pathways in prominent cortical areas (i.e. PFC) of Schizophrenia [61]. As Dworkin et. al. (1994) mentioned more than a decade ago, NMDAR administration can lead hypoalgesia due to its analgesic property. They hypothesized that NMDAR hypofunction triggers pain insensitivity in Schizophrenia[63].

A theory: High prevalence of chronic pain – Is it a consequence of increased sensitiveness to chronic pain?

Interestingly, despite insensitivity to pain, the prevalence of physical comorbidities ended with pain in Schizophrenia that is higher than normal individuals as Kraepelin also stated in his book [60]:

> ''Headaches are frequent … Sensitiveness to pain seems not infrequently diminished…'' (p.77)

The consistency of pain should be more than 3 months to be categorized as chronic pain in clinic, while at least 6 months experience of pain is required in researches [56]. The occurrence of chronic pain among healthy population is approximately 20-40%. Among psychiatric disorders, chronic pain was used to investigated including in depression, post-traumatic stress disorder etc. On the other hand, the studies on chronic pain Schizophrenia are limited in number [69]. Due to some form of chronic pains reported by patients have potential to be appeared due to hypochondriacal ideas, until last few decades, chronic pain complaints were accepted as exaggeration or agitation etc. consistent with the manifestations of Schizophrenia [62, 63, 70]. In the study of Almeida et. al. (2013), the prevalence of chronic pain is reported as 36.6% among Schizophrenia patients; although, they also mentioned other studies with diversified prevalence and indicated that this is because of the varied definition of chronic pain of different authors. The common sense associated to chronic pain is its increased occurrence in Schizophrenia [69]. That is quite interesting that the chronic experiencing areas are generally orofacial (39.4%) [61]; so much so that some researchers, including Kraepelin and Beuler, have thought migraine-like headaches or orofacial pains may be an early indication of Schizophrenia [45, 59].

Pain evaluation in Schizophrenia animal models

Despite the observed relationship between pain and Schizophrenia, there is only one study conducted belongs to Boyette et. al. (2011) that evaluated mechanical pain thresholds in NMDAR antagonism-based Schizophrenia rat model as far as known [71]. The interpretations on pain are mostly based on human studies and surveys [69], while very less number of them are based on chronic pain. Evaluation of pain on animals are necessary and even mandatory, if this puzzled relation is desired to end as one of the manifestations of Schizophrenia.

As above stated, Boyette et. al. showed decreased mechanical pain response in chronically administrated PCP-induced Schizophrenia rats experiencing chronic pain due to L5 nerve ligation[71]. Notwithstanding as far is known, there is no spontaneous pain behaviour evaluation so far and consequently, evoked versus spontaneous pain behaviours in Schizophrenia animal model have never compared.

Schizophrenia and Depression

According to meta-analysis conducted by Engels et. al. (2014), Schizophrenia patients with indication of pain insensitivity was higher than depression regardless of mentioning the type of pain. Similarly, chronic pain appears in Schizophrenia is lower than depression and some other psychiatric disorders (i.e. hysteria, drug addiction, mania) [61] In addition to consideration of Schizophrenia and depression as two different entities, depression were reported 20% to 60% of Schizophrenia cases as for that the course and endotype of Schizophrenia. Note that depression can be presented in all stages of Schizophrenia [72], while 80% of patients were observed to have depression one or more time point in the early stage of Schizophrenia. According to Upthegrove et. al. (2016), depression is underestimated in Schizophrenia, even though it has been defined as sub-manifestations by DSM-V, not only owing to the safety concerns (i.e. depression-driven suicides) or decreased quality of life, but also because of high-demand in cross-sectional understanding [4]. According to Calgary Depression Scale for Schizophrenia (CDSS), some of the negative symptoms of Schizophrenia may appear as a consequence of depression [26] such as anhedonia, which is diminished pleasure seeking[4]. Conversely, depression patients are also at high risk of experiencing psychosis-like behaviours and schizophrenia, while it has tendency to appear prior to psychotic symptoms in Schizophrenia[4, 72]

The comorbidly of depression in Schizophrenia were related to some genes [72], such as DISC1 and DISC2 genes playing great roles in the etiology of Schizophrenia that were originally found translocated in a Scottish family who have 1 Schizophrenia, 1 bipolar and 10 depression experiencing members [37]. In addition to these genes, NMDAR subunit gene (GRIN1) and GPM6A gene (Glycoprotein that modulates hippocampal stress) were also indicated. Also, Fatemi et. al. mentioned that the reduction of GAD67 was observed both in depression and Schizophrenia[73]. These findings showed that some NMDAR correlated abnormalities are shared in the neuropathophysiology of depression and Schizophrenia.

From aforementioned approach, it is possible to observe depression in NMDAR-induced Schizophrenia rat model. Therefore, results concerning the nociceptive thresholds and agent-induced inflammatory pain responses in Schizophrenia should be similar with depression and pain comparison indicated by Shi et. al. (2010), Su et. al. (2010) and Wang et. al. (2016) [74-76]

AIMS OF THE STUDY

AIM 1 – Set a rat model of Schizophrenia:

The first aim was setting a Schizophrenia rat model with sub-chronic MK-801 administration. To show whether this rat model was set or not; novel object recognition (NOR) test, open field test (OPT) and sucrose preference test (SPT) have performed to indicate the behavioural alterations including lack of novelty interest, anxiety and anhedonia in rats, respectively. While NOR test has performed before all pain experiments, open field test and sucrose preference test have only performed for the rats used only in acute nociceptive and formalin-induced pain experiments as seen in figure 4. The purpose of such preference was based on the observation of NOR paradigm's sufficiency alone to provide the information of whether Schizophrenia rat model is set or not.

AIM 2 – Evaluation of pain responses:

The main purpose of pain evaluation is to provide consistency with human studies that were resulted with insensitivity to physical pain in Schizophrenia. First, thermal- and mechanical-induced evoked pain thresholds were measured to show the difference of acute pain tolerance between control and MK-801-induced Schizophrenic rats. Second, to make an appropriate interpretation, nociceptive and ongoing pain experiences were measured

holistically by triggering formalin-induced spontaneous pain. At least but not least, CFA-induced inflammatory pain model was set and afterwards, nociceptive behaviours under chronic pain were assessed. As a whole, evoked versus spontaneous and acute versus chronic pain behaviours were investigated.

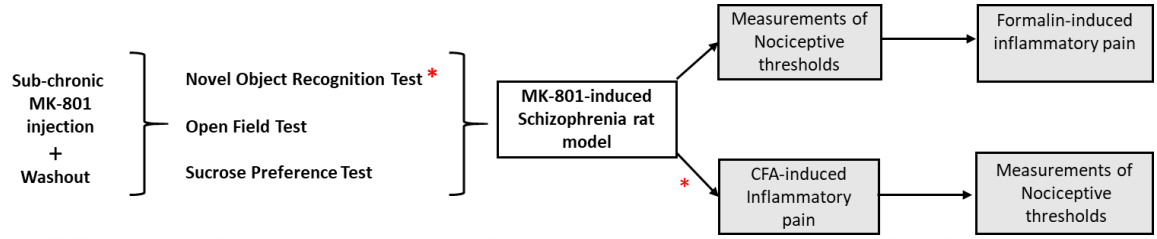

Figure 4: *Experimental design summarizing the route followed through aims. * indicates that novel object recognition test was the only behavioural test applied on the rats in which nociceptive CFA-induced chronic pain model was adopted.*

CHAPTER 2:MATERIALS AND METHODS

Animals

Six weeks-old Sprague-Dawley rats (male; 200–250 g; n=39) were purchased from the Academy of Military Medical Science, Beijing, China. The gender, age and breed of the rats were adopted and/or inspired from the study of Cadinu et. al. [21] All rats were socially housed as group of 4 to 5 per cage in a controlled environment with a standard 12/12 h light/dark cycle, with ambient temperature set at 22 ± 2°C. Food and water were ad libitum, and sawdust were used for animal bedding. Animals were habituated for at least one week and were softly handled everyday before the onset of the experiment, and once a week after the experiments due to necessary cage changes. All experimental procedures were reviewed and approved by the International Review Board of the Institute of Psychology, Chinese Academy of Sciences.

Drugs

(+)-MK-801 hydrogen maleate [(5R, 10S) - (+)-5-methyl-10, 11-dihydro-5I-dibenzo [α, β] purchased from Selleckchem, China. Formaldehyde (formalin) and CFA (Complete Freund's Adjuvant) were purchased from Sigma Aldrich, St. Louis, MO.

MK-801 Injection

Sub-chronic N-methyl-D-aspartic acid receptor (NMDA-R) antagonist treatment is preferred to set a Schizophrenia model in rats because of its ability to produce behavioural and cognitive changes in animals. Among NMDA-R antagonists, a non-competitive MK-801, is favored owing to the study of Ashby et. al. (2010) in connection with appropriate progress of its Schizophrenia model with cognitive deficit indication. Consequently, the dosage and the regime of MK-801, the animal breed, age and gender enforced same as the study to catch similar in Schizophrenia model. 1 week acclimated 6 weeks old Sprague Dawley rats (n=52) were injected intraperitoneally with either 0,5 mg/kg MK-801 (n=32) dissolved in 0,9% saline, or 1 ml/kg saline (n=20). The implemented regime was twice a day in every 10-12 hours for 7 days followed by 7 days washout period [2].

Behavioural Tests

To indicate the translational link of Schizophrenia with MK-801-injected rats, 3 main and adequate tests are conducted as all translations of behavioural alterations could not exhibited due to limited time in this study[15, 77].

Novel Object Recognition Test

To indicate whether Schizophrenia model was set in rats or not, novel object recognition (NOR) Test was performed notwithstanding it is recommended by Cadinu et.al. (2018) due to the ability of testing novelty preference and its self-sufficiency- it does not require any external stimulant in so far as it is only based on the animal's natural curiosity to novel [21, 53]. Regarding to behavioural alterations, in order to proceed the rats into the pain testing, it is expected that saline-injected rats prefer novel object more than familiar object, and MK-801-injected rats prefer the familiar object more than the novel object. To maintain the certain number of rats for appropriate statistical analysis, new rats with expected behaviours concerning Schizophrenia model were added to the groups.

Objects and Apparatus

2 identical pairs of objects were purchased from Tencolor, a woodworking company in China. The color, shape and portion of the objects were selected wisely according to the study of Antunes et.al [53] In the company, the objects were colored with healthy and opaque dyes: one pair was brown and other pair was grey. The shape of the objects has chosen to be about

grey pair was cylinder-shaped and brown pair was rectangular-shaped. The portion of rectangular objects were 15 x15 x25 cm3 placed into the chamber on its square surface. For cylinder objects, the sphere surface contacted the floor of chamber was 15 diameters with 25 cm height. After the record with a single rat, a new rat was placed into chamber after its floor and/or objects were cleaned with 70% ethanol and were wiped with tissue to get rid of non-evaporated alcohol and feces. Both pairs were used for test in return in order to be sure the preferences were not because of the properties of objects but their novelty in the environment.

The apparatus was a round chamber located approximately 1.5m below the mounted camera. To avoid reflection, suspended environment was provided by the favor of a very thick curtain and a chamber had chosen with all surfaces in black. The portion of the chamber was in a such way that its height and its diameter were 60 cm [53].

Software and Analysis

The videos were recorded within Water-Maze Software 3 and were subsequently transferred to AnyMaze 6. In protocol area of Any Maze, video sources were added, and apparatus were created according to individual video sources by drawing a map on chamber and defining the diameter of chamber with ruler. To well define the position of objects in the chamber, square and concentric grids with diameters of 50 cm and 2 cm respectively were added. As a matter of fact, grid settings were done to define zones efficiently which was necessary for the introduction of definition of exploration to the software. Two different zones were set to let the software to identify two separated objects as familiar and novel. Since the grid diameters were known, the diameter of the zone was considered as 'object diameter+4cm' or 'radius+2cm' and these zones were highlighted. Moreover, only the head directions were considered as the definition of exploration described as snout existence within this zone including sniffing or touching with snout. Consequently, sitting next to the object or touching with tail etc. would not been identified as exploration. [53] Then, testing duration and number of trials were determined as 5-10 minutes and 5, respectively. In experiment area, the treatments and ID of animals were noted and afterwards testing were performed.

Phases and Implementation

NOR Test is comprised of habituation, familiarization and test phases. Additionally, 24 hours retention time was applied [53, 54]. It is crucial to state that NOR test had been applied all the

rats had been used in experiments. In the habituation phase, all rats were placed individually into the chamber one by one with no objects for 5 minutes and the chamber were cleaned before each rat's trial. After that all rats were taken back to their cages for 24 h. Then by retention time, to conduct the familiarization phase, first the identical rectangular objects were placed into the chamber 8-10 cm far from its wall while both objects were vis a vis. So, rats were left in the chamber individually alone for 10 minutes leading them to explore these objects in familiarization phase followed by 24h retention time. Later, one of whichever types of object had been introduced was switched with one of the other type of objects to present rats with one familiar and one novel object. After testing, rats were taken back to their cages until investigation of pain behaviours. [53, 55, 56]

Calculations

Time spent to explore:

Familiar object is denoted as F and Novel object is denoted as N

Recognition or Preference Index (PI) = (N/ (N+F)) *100

PI as a value in percentage:

50 - 100% refers to more curiosity for N than F

0 - 50% refers to more curiosity for F than N

Open Field Test (OFT)

The general purpose of Open field test is to assess anxiety. It was performed only on first cohort of animals (n=17; 9 MK-801-injected, and 8 saline-injected) which were going to be used to assess nociceptive and formalin-induced pain responses. The same apparatus used for novel object recognition (a circular apparatus with 60 cm height and 30 cm radius) was used as an open field. Rats were tested for 5 minutes 24 hours prior to NOR. Time spent in center and peripheral datum were analyzed using Any Maze. In protocol section, video sources were selected and consequently the apparatus for each rat were created. Afterwards, two different zones were created as center and outer areas by selecting a radius grid with 20cm radius. It was technically 25-50% of chamber as stated by Gould et. al. (2009) [78] Same as NOR, the entrance of animals' head into the zones was considered. 5 trials were conducted, and their average were taken for calculations.

Sucrose Preference Test (SPT)

Sucrose Preference test is to assess anhedonia, inability to feel pleasure, were performed because of the similar reason of OFT in the same cohort of rats with OFT. Anhedonia is a common symptom of depression, and Schizophrenia. As depression has 40% of prevalence in Schizophrenic patients [4], and the relationship between depression and pain were investigated in previous studies [50], SPT was performed because of our general purpose of evaluation of the relationship between Schizophrenia and pain.

In SPT, sucrose (purchased from Sigma Aldrich) solution was prepared being about to containing 1% sucrose. This test was only applied to the animals used in second part of the study to emphasize MK-801-injected rats anhedonia. Each rat was taken from their home cage and put into individual cages for acclimation. During this period, animals had access to food and two water bottles for 24 h until the deprivation of them for another 24 hours. [59] 2 bottles, one with sucrose solution (1%) and the other with tap water, were prepared and the volume of liquid inside of them were noted as at the beginning both bottles contained same volume. Thereafter, these bottles were presented to animals for 6 hours followed by the removal of the bottles from the cages and hence the calculation of new volumes. [59] The preference percentage for sucrose solution was calculated according to the following formula:

%sucrose preference = (sucrose solution consumption / (sucrose solution consumption + water consumption) x 100. [51]

Measurement of Pain Behaviours

Beforehand both MK-801 or saline, and CFA injections on rats, thermal and mechanical-induced thresholds had been measured as baseline with Hargreaves Test and VonFrey test, respectively. Accordingly, they were repeated to observe the variation following the washout period. Since, formalin injection has a potential impact on Schizophrenia model, baseline for formalin-induced inflammatory pain test had not been performed.

Hargreaves' Test

Hargreaves' test, also called Plantar test, were used to observe rats' peripheral response, lifting the paw, to thermal stimuli. Hargreaves apparatus and devices were purchased from Stoelting (Wood Dale, IL) including followings:

- *Controller that enables the manipulation of some parameters including heat intensity and maximum display time of the heat (cut-off time). 25 seconds for baseline and 45 seconds for testing and non-chronic pain baseline were set by the experimenter.*
- *Counting device that enables to illustrate the time until a rodent showing response to heat by lifting its targeted paw.*
- *Plexi-glass panel that holds maximum 8 rats at the same time and supplies an optimal environment for thermal conductance.*
- *Supporting columns that keeps the panel high above*
- *Transparent enclosures that are half cylinder-shaped compartments allowing to individually house up to 500 g weighted rats. It also eliminates unnecessary movement during testing.*
- *Heat source that provides radiant heat as a stimulus.*

Animals were taken from the holding room to testing room gently. The plexi-glass and enclosures were cleaned with 70% ethanol to make sure that there was no odor left from past experiment. Afterwards, rats were placed into the enclosures on the glass platform and were acclimated for 10-15 minutes to make sure that they won't change their position due to curiosity to new environment during the testing. Then the source of radiant heat was situated below the glass platform and the heat intensity was set according to desire which was chosen as 48 units for our tests in the controller. Thus, testing was conducted by guiding and fixing heat source to the paws in turn for each rat and the time of tolerating heat was recorded manually by the favor of counting device. The tests were repeated 5 times and the last 3 trials were payed regard to the average of response. Moreover, 5 minutes interval was applied among the rats' testing. Thereafter, the rats were taken back their cages and the procedure were iterated for other rats subsequent to cleaning the platform and enclosures with 70% ethanol to remove odor, urine and feces.

VonFrey Test

VonFrey test, was used to examine the peripheral response of rats to VonFrey hair by lifting their paws. Sensory probes, VonFrey hairs (filaments), were purchased from Stoelting (Wood Dale, IL) and other materials used were followings:

- *Panel with metal rods that enables the experimenter to have access to paws.*
- *Supporting columns that keeps the panel high above*

- *Transparent enclosures that are half cylinder-shaped compartments allowing to individually house up to 500 g weighted rats. It also eliminates unnecessary movement during testing.*
- *A tray that is below the panel at the middle of supporting columns. The faces and urine falling from among metal rods ends on the tray.*

Animals transferred from the holding room to testing room were placed into previously cleaned enclosures on the panel with metal rods. Afterwards, rats were acclimated for 10-15 minutes to make sure that they won't change their position frequently due to curiosity to new environment during the testing. From the second and third metatarsals of the plantar hind paws, different amount of forces were applied with VonFrey hairs. According the withdrawal of paws, the following hair application were determined either through increased force or decreased force. A withdrawal of paw after a non-withdrawal paw trial or vice versa were considered as first-time reaction since the behaviour was changed. Including this trial, paw responses were measured 5 times and withdrawal thresholds for each rat were calculated by CellG software. A range of force delivered by filaments were 3.61-5.18 log unit or 0.41–15 g.

Formalin Test

The purpose of the test is to constitute acute tissue injury-induced pain in rats. Following were the materials and reagents used:

- *Formaldehyde (formalin) that was purchased from Sigma Aldrich, St. Louis, MO.*
- *Chamber that is a 30 x 30 x 30 cm box which put on the glass platform above a video-camera. Its roof includes holes with 5 mm diameters was black and interior walls were white opaque glass.*
- *0.9% (w/v) saline*
- *1-ml syringes with 27-G, ½ -in needle*
- *Ethanol (70%, v/v) and tissue paper*
- *A pair of thick gloves: In order to do the injection, the technique adopted was using a thick glove where the experimenter pushes rat's upper body into it and then it enables to clutch the animals' right hind paw to do the injection without any other individual's aid.*

Prior to formalin injections, rats were habituated in the test chamber individually for 10-15 minutes. 500 µl formalin (37.5%) dissolved in 20 ml of 9% saline (2.5%), while 100µl was

injected subcutaneously into distal tips of the two basal tori of the right hind paw [60] [61]. Thereafter, injected rat was taken into the chamber to monitor its behaviours for 70 minutes. The observation was stopped on time and the rat was taken back to its cage. Before, doing another injection and observation, the glass platform and the chamber were cleaned with 70% ethanol and tissue paper. This procedure was repeated for other rats as well. Different phases are evaluated within each features: Phase I (evoked pain); 0-15 min, and Phase II (Spontaneous pain); 15-70min [79]

Complete Freund's Adjuvant Test

The purpose of the test was set chronic inflammatory pain in both saline and MK-801 injected animals. The following were the materials and reagents used:

- *CFA (Complete Freund's Adjuvant)* that was purchased from Sigma Aldrich. Until the injection day, it was stored in -20 °C. (1 mg/ml of Mycobacterium tuberculosis, heat killed and dried)
- *1-ml syringes with 27-G, ½ -in needle*
- *Ethanol (70%, v/v) and tissue paper*
- *A pair of thick gloves* that is required to handle the animals. Contrast to formalin injection, it was used to hold rats' and their ipsilateral paws by an experimenter while other experimenter was doing the injection.

Animals were taken from holding room to testing room and the rats were taken out of the cages one by one at every turn. First of all, the plantar surface on the footpad was cleaned 70% ethanol. Then one of the experimenters had wore a pair of thick gloves held the rat from axillary fossa with one hand and clutched its ipsilateral to be hind paw with other hand while its tail was taken between ring finger and pinkie finger to limit animal's movement. While this experimenter was holding the rat still in optimal position, the other experimenter injected 100µl CFA subcutaneously into distal tips of the two basal tori of the right hind paw by moving the injector smoothly 3-4 mm through inside. [62] Since CFA is viscose, it takes time to inject all inside the injector. Therefore, the withdrawal of the injectors was executed gently and slowly, which also decreased the possibility of leakage of the drug. Each rat replaced calmly into their home cages which had been layered thickly with sawdust to make them as much as comfortable. Then thermal and mechanical hyperalgesia were tested on the days 14th, 21st and 28th after CFA-injection.

Statistical analysis

For statistical analyses and graph illustrations, GraphPad prism 5.0 and Statistica 8 were used. Student's t-test were performed when only two groups were compared, and multi-factor analysis of variance (ANOVA) was preferred for conditions with two or three parameters. Bonferroni analysis was adopted as post-hoc test. The datum was illustrated as means ± SD. The statistical significance was set at $P < 0.05$

CHAPTER 3: RESULTS

BEHAVIOURAL RESULTS:

Novel Object recognition – Lack of novelty interest in MK-801 rats

Regarding to PI (Preference index) of novel and familiar object in figure 5A and 5B, MK-801 rats are lack of novelty interest because they exhibit more than 50% interest to familiar object, and less than 50% interest to novel object ($p<0.0001$); while control rats are considered to have novelty preference because they show less than 50% interest towards familiar object, and more than 50% interest to novel object ($p<0.0001$). The individual preferences to novel object are represented in Table 1 to present above mentioned interests of rats individually. The standard error of means in PI of novel object are 69.45 ± 3.462 and 29.46 ± 2.869 for control and MK-801 rats, respectively.

PREFERENCE PERCENTAGE (%) OF NOVEL OBJECT

1ST COHORT (n=17)	***C***	*59.09*	*56.74*	*54.62*	*53.10*	*67.16*	*75.22*	*53.49*	*63.71*			
	MK	*11.49*	*11.39*	*43.94*	*39.46*	*40.45*	*43.38*	*32.68*	*33.21*	*29.89*		
2nd COHORT (n=22)	***C***	*63.71*	*100*	*68.98*	*53.64*	*54.10*	*65.89*	*85.04*	*92.20*	*86.54*	*75.22*	91.08
	MK	*46.40*	*45.45*	*37.66*	*28.05*	*33.72*	*23.00*	*15.81*	*11.10*	*35.31*	*7.02*	19.71

Table 1: *The preference percentage (%) to novel object both for control and MK-801 rats. 1st cohort of rats used for acute nociceptive and formalin-induced pain testing, and 2nd cohort of rats used for CFA-induced chronic pain model.*

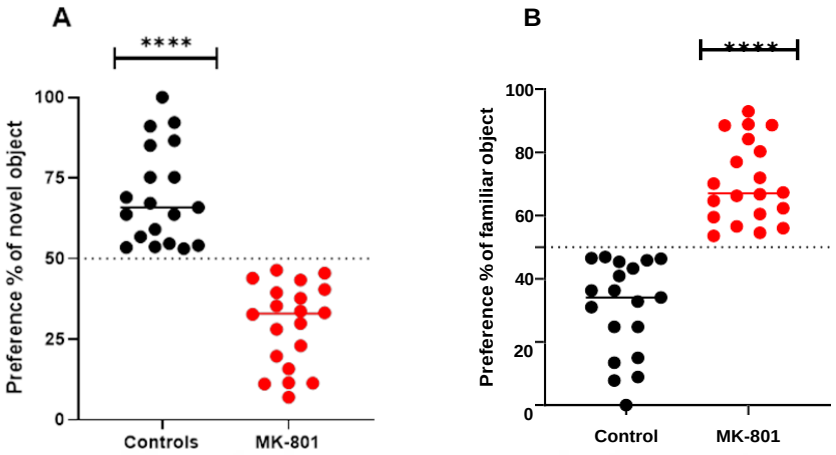

Figure 5: *Novel Object Recognition Test Results. (A) Novel Object Preference (%). (B) Familiar Object Preference (%). Student's t-test: p<0.0001 for both indicated with ****

The MK-801 rats did not display lack of novelty interest and the control rats did not present novelty preference are excluded from the cohorts and are not included into the statistical representations of PI of novel object recognition test. To have enough number of rats to run the statistical tests, more rats are added to the groups.

OFT and SPT – Anxiety and Anhedonia in MK-801-injected rats

As aforementioned, open field test (OFT) and sucrose preference test (SPT) are performed on 1st cohort of rats (n=17) which are used in the first set of experiments consisting of acute nociceptive and formalin-induced pain assessments. These behavioural tests are performed before novel object recognition test (NOR) and therefore, MK-801 rats excluded from NOR (n=4) due to their preference of novel object was greater than familiar object are used as well. Although, the datum belongs to the control rats excluded from NOR (n=2) due to their lack of novelty interest did not added into these tests' statistical analysis because of the fact that comparison among these rats and MK-801 rats would have not been appropriate since these supposed-to-be control rats did not present 'normal' internal curiosity towards novel object.

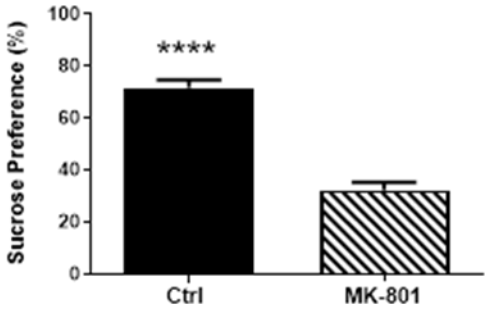

Figure 6: *The percentage of sucrose preference percentage. **** as p<0.0001*

Therefore, these tests did not perform for the rats used in setting CFA-induced chronic inflammatory pain model since NOR, itself, is sufficient to represent the Schizophrenia model. In figure 6, the mean percentages of sucrose preference are represented: 71.61±3.129 and 32.31±2.947 for control and MK-801 rats, respectively ($p<0.0001$). As seen in table 2, same 9 MK-rats show anhedonia and anxiety among 10 MK-801 rats; while only 1 rat (in red) is extremely hedonic and it does not experience anxiety Regarding to the open field test results, MK-801 rats spent more time in the peripheral areas of the chamber significantly compared to center with $p<0.001$ indicating their anxiety as demonstrated in the figure 7. [78, 80]. The mean of times spent in center and peripheral are 14.06 ± 1.149 and 45.94 ± 1.148 for MK-rats, respectively. Although, control rats spent more time within center than peripheral area with $p<0.05$ showing that they do not experience anxiety. The mean of times spent in center and peripheral are 34.11 ± 2.116 and 25.96 ± 2.135.

SUCROSE PREFERENCE PERCENTAGE (%)		**TIME SPENT IN CENTER (SEC)**		**TIME SPENT IN PERIPHERAL (SEC)**	
Control	**Control**	**MK-801**	**Control**	**MK-801**	**Control**
82.73	17.35*	12.88	17.00	47.10	17.00
68.79	36.93*	16.50	24.8	43.50	24.8
63.01	29.88*	11.18	27.56	48.80	27.56
74.03	25.92*	20.56	22.34	39.44	22.34
81.32	40.25*	10.32	30.28	49.68	30.28
76.87	31.39*	10.14	31.38	49.86	31.38
57.19	24.64	13.78	19.82	46.22	19.82
68.93	50.79	28.00	34.46	14.00	34.46
	39.57	16.82		43.18	
	44.88	14.32		45.68	

Table 2: *The individual sucrose preference index (%) and time of spend (seconds) of rats. * represent the MK-801 rats showed lack of novelty interest in the NOR test while the rest are excluded-to-be rats due to their exhibition of novelty interest in NOR.*

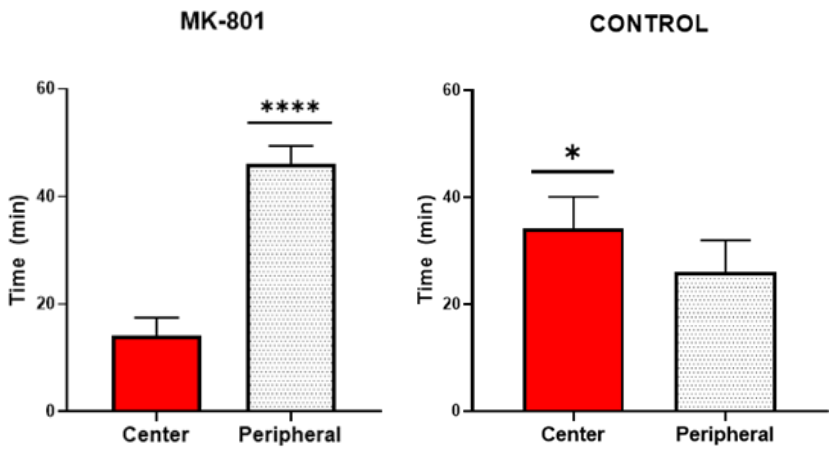

Figure 7: *The anxiety states of both MK-801 and control rats are illustrated by Open field test results concerning the time spent in center and peripheral (outer) parts of the chamber.*

FIRST SET OF EXPERIMENTS

Nociceptive pain thresholds in MK-induced and control rats.

Mechanical (figure 8A)-induced paw withdrawal thresholds (PWTs) and thermal (figure 8B) -induced paw withdrawal latencies (PWLs) are evaluated by 2-way ANOVA with Bonferroni post-doc tests. In figure 8, Control baseline and control treatment measurements indicate that there is no response differentiation with saline treatment to mechanical and thermal stimuli, while they do not display any worthy to mention difference between the paws ($p<0.05$). In figure 7A, the relationship among MK-801 baseline group (PWTs: ~11g) and all control groups are not meaningful, as expected. On the other hand, MK-801-treated rats exhibit significant increase both in left and right paws ((Left paw: 18.65±1.606; Right paw: 18.25±2.268) with $p<0.01$ and $p<0.001$; respectively compared to both control treatment and MK-801 baseline rats (figure 8B). In figure 7B, thermal-induced PWLs are not variable within control baseline and treatment groups; moreover, the variance among paws between them are not meaningful ($p<0.05$) as well. Notwithstanding, the PWLs of MK-801 treated group shows significant increase compared to all control group and MK-801 baseline (~13 sec), consistent with mechanical pain results. The p value for this significance is $p<0.001$ for both paws (Left paw: 19.55± 0.8428; Right paw: 19.56± 0.701). Interestingly, the increase percentage to both acute nociceptive pains is approximately 30% with the treatment of MK-801.

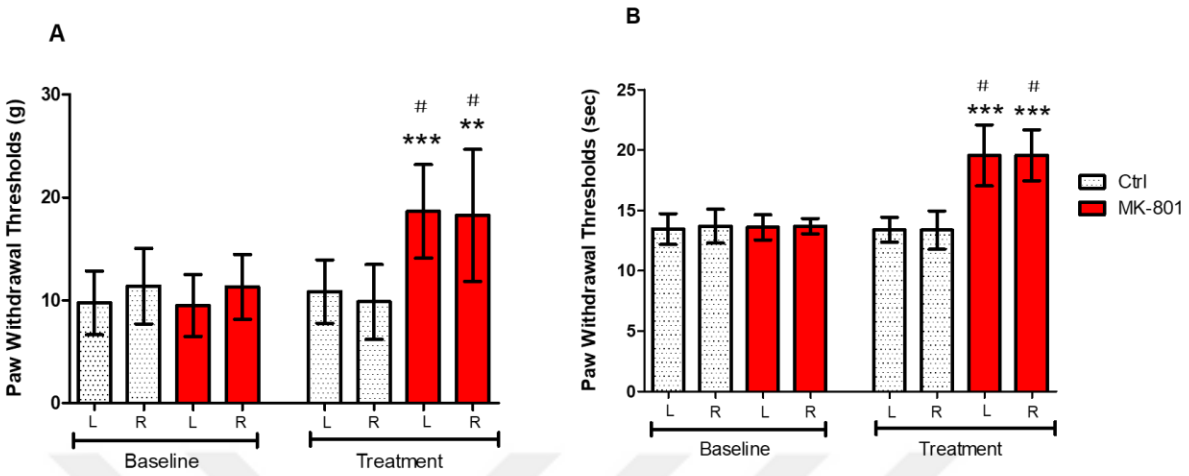

Figure 8: *Mechanical (A)- and Thermal (B) induced pain thresholds are illustrated. # is used to indicate that the significance for increased thresholds in MK-801-induced rats with Schizophrenia is both compared with control group. Abbreviations: Left paw as L and right paw as R. ***: p<0.001; **: p<0.02*

Formalin-induced Spontaneous Pain

In formalin-induced pain responses, there is sensitivity differentiation between MK-801 and control groups both within phases of formalin as illustrated in figure 8 and 9. The drastic decrease in sensitivity is observed from 5th to 10th minute in figure 8 with p~0.017. After a steady increase in time period of 15-25 sec which is within the Phase II indicating the development of inflammation-induced hyperalgesia. While the hyperalgesia of MK-801 rats continues to increase, the control group's hyperalgesia gets ready to decrease. After 25 minutes, the hyperalgesia of MK-801 rats decrease slightly through 70th minute. Even though, there is no significance from 25th to 60th minute of post-injection ($p<0.05$) in the comparison of single time points; the significant decrease within whole phase II time points are indicated in figure 9. appears at 60th minute that control group experience a drastic decrease ($p>0.05$). When control group is losing their sensitivity over the time and eventually reach '0' sec of licking at 60th minute, the spontaneous pain sensitivity of MK-801 group slightly decreases through 70th minute that is observable by the high bevel angle of the line.

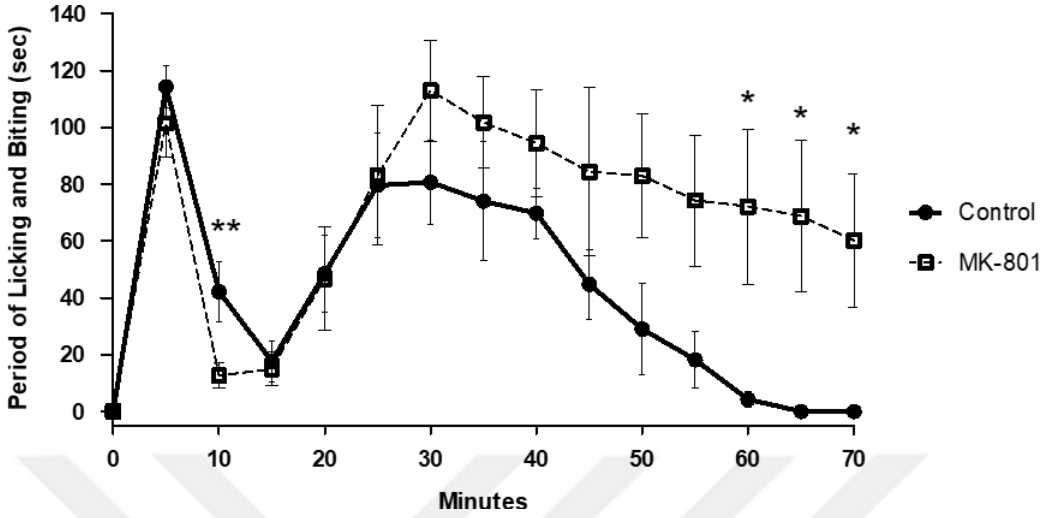

Figure 9: *Formalin-induced spontaneous pain responses are illustrated. There is a significant difference between treatment groups at 10th minute (p=0.017) due to sudden drop of the licking period of Schizophrenic rats. Another significance appeared at 60th minute to 70th minute with p<0.05 indicating the persistency of spontaneous activity. **: p<0.02 and *: p<0.05*

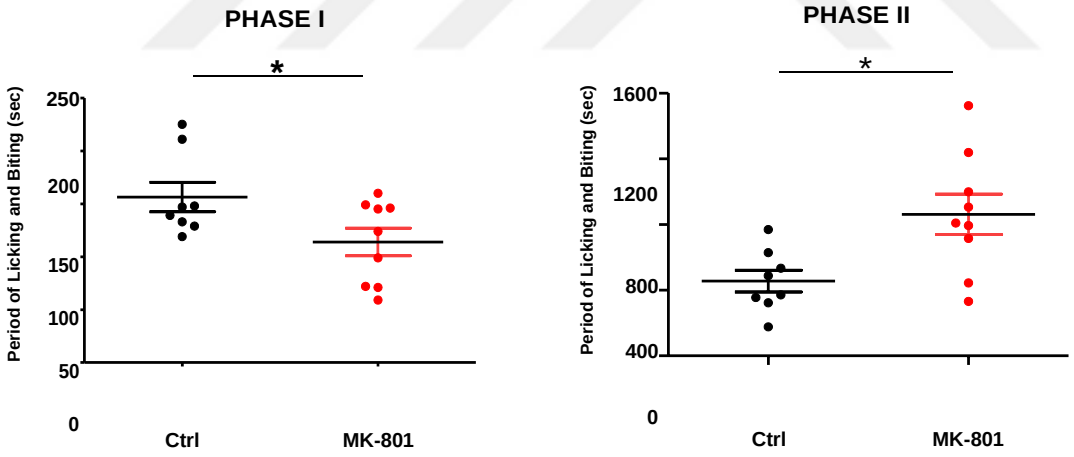

Figure 10: *Phase I (0-14 sec) and Phase II (15-70sec) of formalin induced spontaneous pain responses. *: p<0.05*

SECOND SET OF EXPERIMENTS

Analyses concerning thermal and mechanical hyperalgesia in the chronic pain model of CFA-injection are conducted via 3-way ANOVA, with Bonferroni post-doc test.

THERMAL HYPERALGESIA									MECHANICAL HYPERALGESIA								
WITHIN GROUPS									**WITHIN GROUPS**								
	CONTROL				**MK-801**					**CONTROL**				**MK-801**			
Contra vs Ipsi	*ns*	******	******	******	*ns*	******	******	******	***Contra vs Ipsi***	*ns*	*****	***	*ns*	*ns*	*****	******	******
t	*B*	*14*	*21*	*28*	*B*	*14*	*21*	*28*	***t***	*B*	*14*	*21*	*28*	*B*	*14*	*21*	*28*
WITHIN PAWS									**WITHIN PAWS**								
	IPSILATERAL				**CONTRALATERAL**					**IPSILATERAL**				**CONTRALATERAL**			
Ctrl vs MK	******	****	*ns*	*****	******	******	******	******	***Ctrl vs MK***	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
t	*B*	*14*	*21*	*28*	*B*	*14*	*21*	*28*	***t***	*B*	*14*	*21*	*28*	*B*	*14*	*21*	*28*

Table 3: *The interactions within groups in relation of contralateral and ipsilateral paws, and within paws in relation of control and MK-801 groups are illustrated. Abbreviations: B as baseline, t as pre and post-CFA injection time points, 14; 21 and 28 as the days of measurement after CFA-injection, ns as non-significant. Significance: ****, p<0.0001; ***, p<0.001; **, p<0.02; *, p<0.05; ns, p>0.05*

The PWLs to thermal stimuli

In Table 3 and figure 11, comparison between contralateral and ipsilateral paws in both control and MK-801 groups showed significance at baseline, 14th day, 21st day and 28th days measurements ($p<0.0001$ for all). Moreover, the comparison of contralateral PWLs among the control and MK-801 groups revealed that at every time point measured the significant difference with $p<0.0001$. Also, ipsilateral PWLs showed significance between control and MK-801 groups, except 21st day measurement, as illustrated in figure 11; while the p-values were differentiated for all as following: baseline with $p<0.0001$, consistent with contralateral PWLs; 14th day with $p<0.02$; and 28th day with $p<0.001$. When the meaningful changes for the ipsilateral PWLs of control and MK-801 rats were observed in all post-CFA measurements with $p<0.0001$ as seen in figure 11 and table 3. To understand the changes over the time; measurements at 14th, 21st and 28th time points are compared directly with baseline measurement separately represented in figure 13A. Significances did not change in any post-CFA measurements for contralateral PWLs both for control and MK-801,while the percentage change of ipsilateral PWLs were significant with $p<0.0001$ for all post-CFA measurement time points in figure 13A. All together, comparison among PWLs of both groups' rats signified that

MK-801 rats experience CFA-induced chronic inflammatory pain more persistent than control rats. While control rats restore the pain from 21st day measurement to through last measurement; thermal hyperalgesia of MK-801 group continues to decrease through last measurement.

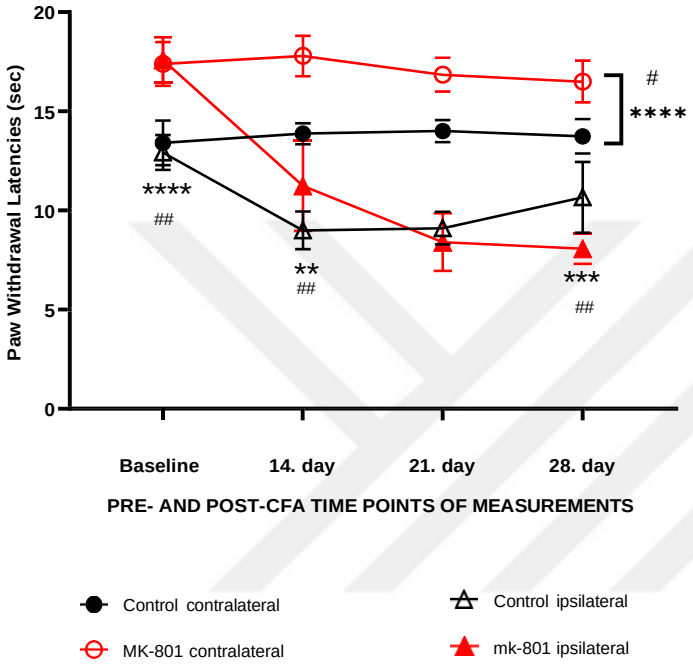

Figure 11: *Thermal hyperalgesia in CFA-induced chronic inflammatory pain model. # indicates that single interactions in contralateral paws at every time points are significant between control and MK-801 rats with p<0.0001. Moreover, it represents the significance between ipsilateral and contralateral paws both for control and MK-801 groups for all measurement times except baseline. ## signifies the significant difference of ipsilateral PWLs between control and mk-801 rats. Significance: ****, p<0.0001; ***, p<0.001; **, p<0.02; *, p<0.05*

The PWTs to mechanical stimuli

As illustrated in Table 3 and figure 12, MK-801 rats showed significant difference between ipsilateral and contralateral paws at 14th, 21st and 28th days of measurements with p<0.0001; although, control group displayed differentiated significance only at 14th and 21th post-CFA measurements with p<0.001 and p<0.05, respectively. In contrast to thermal hyperalgesia, either contralateral or ipsilateral PWTs of control and MK-801 rats do not show significant difference at any pre- and post-CFA measurement times; that is why, the percentage changes in PWTs from baseline to all post-CFA measurements separately are illustrated in the figure 13B. From baseline to 14th day measurement, the ipsilateral PWT significance between control and MK-801 rat is with p<0.0001; while 21st day measurement differs significantly from baseline with p<0.02. Although, no significance was found from baseline to 28th measurement with p>0.05, indicating the increased hyperalgesia of MK-801 rats were restored. Concerning to the persistency to CFA-induced chronic pain, the figure 12B shows the individual PWTs at pre and post-CFA time points indicating that both control and MK-801

rats are likely to restore the hyperalgesia; although, since MK-801 rats have increased hyperalgesia compared to the control rats, the time of pain restoration may take more time.

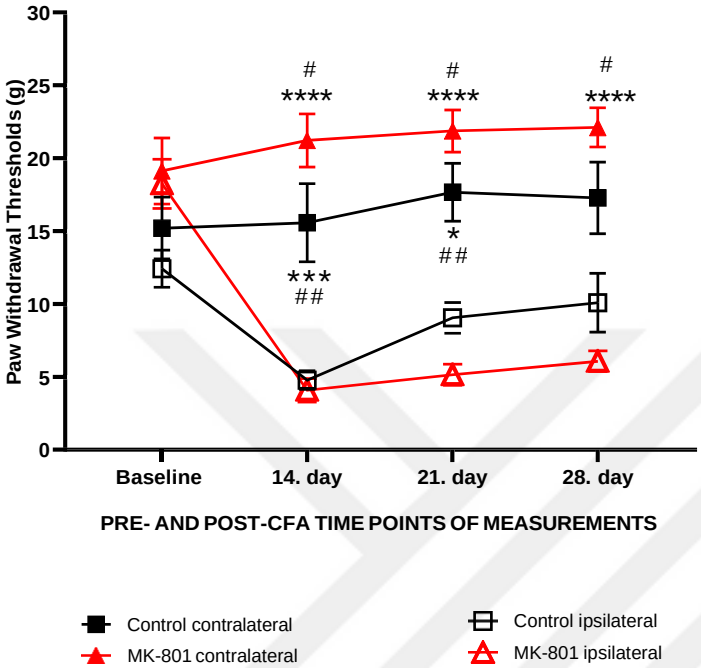

Figure 12: *Mechanical Hyperalgesia in CFA-induced chronic inflammatory pain model. # indicates the difference between contralateral and ipsilateral paws of MK-801 rats with p<0.0001. ## signifies the difference between contralateral and ipsilateral paws of control rats with p<0.001 and p<0.05 for 14th and 21st day of post-CFA measurements, respectively. ****: p<0.0001; ***: p<0.001 and *: p<0.05*

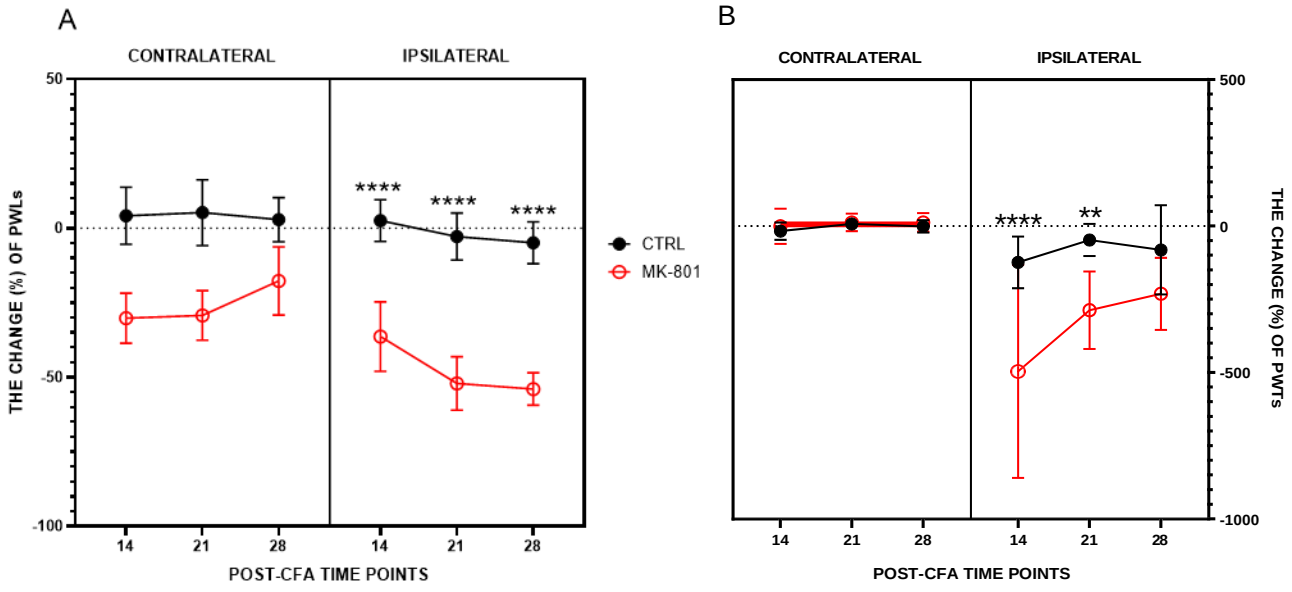

Figure 13: *The percentage change of PWLs for both groups from baseline measurement to 14th,21st, and 28th days measurement of post-CFA injection one by one are represented for both paws. # represents the comparison between control and MK-801 group. Significance; ****: p<0.0001; and **: p<0.02*

CHAPTER 4: DISCUSSION

The mechanisms of pain, and cognitive deficits associated to Schizophrenia are highly correlated to NMDAR/GABA hypofunction in similar cortical areas, particularly PFC, VTA and hippocampus [22, 55]. To explicating the differentiated pain thresholds and the prevalence of distinct pains in patients with Schizophrenia, setting a NMDAR antagonism induced Schizophrenia rodent model is considered as advisable. The present study is the first research to compare evoked and spontaneous pain responses in MK-801-induced Schizophrenia rat model with cognitive impairments.

OUTLOOKS TO THE FINDINGS

Schizophrenia Rat Model Associated to Abnormal Neurotransmission

The Schizophrenia rat model induced by sub-chronic treatment of MK-801 with the aforementioned regime and dosage is considered to be set according to the results of behavioural tests. In novel object recognition (NOR) test, MK-801-injected rats exhibit more curiosity towards familiar object than novel object indicating the lack of novelty interest[40]. Moreover, open field and sucrose preference tests are showed that MK-801 injection leads other behavioural alterations correlated with anxiety and Anhedonia respectively [81, 82]. The purpose of using NOR in all pain testing, and using other tests only for the rats used in first set of experiments (acute nociceptive and formalin-induced pain testing), was that the rats (n=9) experience anxiety and anhedonia included the rats (n=3) did not present lack of novelty interest in NOR test. Therefore, it has been considered after testing the first cohort of animals which were used for first set of experiments, only the rats exhibited the expected behaviours (as MK-801 rats show lack of novelty interest, and control rats show novelty interest) in NOR alone was able to present the presence of MK-801-induced Schizophrenia. That is the reason why only NOR used in second set of experiments.

Concerning to NOR testing, animals' mobility is an impact on the preference to novel and familiar objects. That is why, the locomotion of rats within NOR testing phase is analyzed; although, the significance is not found which is probably due to the fact the testing phase kept in 5 minutes to prevent the effect of hyper-activity by animals.

Acute nociceptive pain thresholds

The pre-treatment measurement indicates that MK-801 and control rats have similar PWTs and PWLs to mechanical and thermal stimuli, respectively. Moreover, the comparison of pre- and post-treatment measurements of control rats show no difference. On the other hand, MK-801-injected rats exhibit increased PWLs and PWTs to thermal and mechanical stimuli, compared to its pre-treatment measurements as well as pre- and post- measurements of control group. In general, MK-801-injected rats show approximately 30% increase in insensitivity to both stimuli. Although, the insensitivity of right paw of MK-801-injected rats to mechanical pain is lesser compared to left paw. (right, $p<0.02$ and left, $p<0.001$) The separate PWTs averaging of paws might cause such difference and generally right paw of PWTs might have higher variance leading lower p value than left paw's due to also few rats in number. On the other hand, the force applied while poking the paws by experimenter does not seem to affect it significantly because of the design of VonFrey hairs in such way that they do not apply more force when pushed more but rather they bend because, the force is just proportional to the filament's diameter and inversely to length. Although, cannot applying the force into same spot and subsequently movement of sensory surfaces or logarithmic scale of measurement etc. may contribute such difference due to non-automated system[83]. Generally, the significant insensitivity in MK-801-injected rats may correlate with the disrupted activation of the nociceptors and the ion channels.

Responses to Formalin-induced Spontaneous Pain

Holistically, MK-801 rats display significant insensitivity in the Phase I (0-15) with the force of needle through tissue which is a mechanical-induced nociceptive stimuli [84, 85] and the increased hyperalgesia in average is observed in MK-801 rats compared to control rats in the Phase II (15-70), as represented in figure 9. When time points are interpreted separately, the hyperalgesia of control and MK-801 rats does not differ significantly within 15-25 minutes. Afterwards, control group displays steady level of hyperalgesia for a while followed by a decrease through '0' at 60^{th} minute. Although, hyperalgesia of MK-801 rats does not decrease drastically but instead slightly supported by the time point: 60^{th} minute of post-injection that control rats reach '0', licking period of MK-801 rats is around 80 seconds ($p<0.05$). Basically, the development of spontaneous pain is similar for these groups; however, the restoration to hyperalgesia is persistent for MK-801 group.

Differentiated sensitivities in CFA-induced Chronic pain model

Thermal Hyperalgesia

In contralateral PWLs, control and MK-801 rats differ significantly at pre- and all post- CFA measurement due to the increased antinociception as above mentioned. It was an expected result since the biochemical changes are limited to injected paw [86]. For ipsilateral PWLs, control and MK-801 rats presented differentiated responses in every measurement, except baseline which is only a sign of the effects of sub-chronic MK-801 administration. At 14^{th} day, the hyperalgesia experienced by MK-801 rats are higher than control group's ($p<0.02$) followed by non differentiation between groups at 21^{st} day; although, MK-801 rats show increased hyperalgesia compared to control group at 28^{th} day of post-CFA measurement. Apparently, while hyperalgesia of control group starts to restore after a while, hyperalgesia of MK-801 rats continue to increase over the time indicated in figure 11 and table 3, supported by the changes (%) from baseline to post-CFA measurements separately as ipsilateral PWLs show significance between control and MK-801 rats with $p<0.0001$ for all time periods. Consequently, MK-801 rats experience increased thermal hyperalgesia in chronic inflammatory pain model with increased persistency as expected so far from the human studies that indicated the increased prevalence of chronic pain in Schizophrenia patients with around 36% occurrence [69].

Mechanical Hyperalgesia

In contrast to the findings in the increased antinociception, control and MK-801 groups did not show difference in terms of contralateral PWTs. Although, taking the average of separate PWTs of rats might leading high variance is above mentioned as well as other possible reasons [83]. Moreover, ipsilateral PWTs of control and MK-801 rats also do not exhibit significant difference at post-CFA measurements, which may be a consequence of drastic decrease of hyperalgesia of MK-801 rats. By other words, difference between contralateral and ipsilateral PWT of MK-801 rats are significant with $p<0.0001$ for all post-CFA measurement while this difference for control rats is just significance for 14^{th} and 21^{st} days of post-CFA measurements with $p<0.05$; and there is no significance in the last measurement, as represented in Table 3 and figure 12. The changes from baseline to post-CFA measurements shows that the significant is appeared at 14^{th} day, decreased at 21^{st} day and disappeared at 28^{th} day measurement indicating that mechanical hyperalgesia of MK-801 rats also restore the pain within this period of measurements in contrast to thermal hyperalgesia

which was still increasing. Due to the increased hyperalgesia experienced by MK-801 rats compared to control rats, the restoration of pain takes more time and presents as 'more persistent' compared to control rats. Another possibility is few rats used in number prevent to represent the significances since the high variance can affect the p-value dramatically.

The different profiles of thermal and mechanical hyperalgesia in CFA-induced chronic pain model might be owing to the occupation of distinct nociceptive fibers and ion-channels as well as how these have been affected due to exposure to sub-chronic MK-801 administration which are discussed below.

NOCICEPTORS AND THE ION-CHANNELS

The nociceptive activity related to peripheral information is relayed to higher cortical areas by nociceptors which projects laminae of dorsal horn of Spinal Cord. The primary afferent projections through lamina I and V of dorsal horn are responsive to noxious stimuli via Aδ (or Aβ)-fibers and C fibers. The response to acute thermal-induced pain is related to the activity of first Aδ polymodal fibers and then C fibers, while the activation of the transient receptor potential- vanilloid- (TRPV) ion channel family located on these fibers is contributor for pain transduction. Particularly, TRPV1 is very outstanding for heat sensation; although, other channels also contributes it such as TRPV2, 3, 4 etc. [50] For instance, TRPV3, that its activation on keratinocytes transmits thermal input to Aδ fibers via ATP indicated on hot plate test [87]. Although, the nociceptive responses to mechanical stimuli are very diverging due to the variety of stimuli affecting different channels on the nociceptors. The high threshold mechanoreceptors and C-fibers are known with their response to the acute mechanical-induced pain. The role of Na^{2+} and K^{2+} channels in mechanical pain response is still under investigation, but the contribution of activated potassium channels KCNK4(TRAAK) and KCNK2(TREK-1) are demonstrated in VonFrey tests [48, 50]. These two potassium channels also contribute to heat sensation fairly. In addition, the role of TRPV4, TRPV1, TRPA1 (ankyrin 1), kv3.4 (KCNC4) and kv4.3(KCND3) are reported by their contributions to acute mechanical nociception. However, the main contributions of these receptors within this study are quite elusive. Even though, these receptors respond mechanical stimuli, either the deprivation of these individual channels may not change the response, or they haven't been tested on VonFrey test[48].

In formalin test, the emerging pain is correlated to mechanical force of needle through the footpad, and when inflammation takes over the tissue, the spontaneous pain appears that point. In contrast, CFA test is based on testing the nociceptive responses on the rats suffering from chronic inflammatory pain. For both settings, when tissue damage by inflammation is emerged; it leads to formation of 'inflammatory soup' within the damaged area by the recruitment of some factors including neurotransmitters, i.e. serotonin and glutamate; cytokines, i.e. interleukin-1β (IL-1β); and other substances i.e. ATP, substance P, NGF etc. This recruitment is activated by nociceptors as well as non-neuronal cells such as keratinocytes. Nociceptors express some receptors more to recognize the proinflammatory agents to enhance the excitation in order to increase the sensitivity. So, the binding of mediators within inflammatory soup to these receptors on the nociceptors (i.e. TRPV, acid sensitive ion channels (ASIC), Two-P Potassium Channels etc.) cause the inflammatory pain to be modulated. For instance, NGF (Neuregulin) acts on C-fibers to produce hypersensitivity to both mechanical and thermal stimuli. In the transduction of nociceptive information within the nociceptors, TRP family of channels play great role in both mechanical and thermal pain; although, their contribution in sensitization is still unclear. TRPV1 and 3 channels are indicated to be the main contributor in thermal hyperalgesia on hot plate test; while TRAK-1 and TRAAK interaction has been shown to lead mechanical hyperalgesia in VonFrey test. Additionally, role of TRPV4 is fairly clear that it produces both mechanical and thermal hyperalgesia, whereas it is not responsive to acute mechanical stimuli. Plus, both TRPV3 and TRPV4 are observed to be activated by the mediators within inflammatory soup slightly increase PWLs of thermal hyperalgesia, but not demonstrated on hotplate test. As well as above mentioned channels, sodium channels on sensory neurons have role in pain induction [48, 50]. Particularly, $Na_v1.7$ is essential for to increase the hypersensitivity caused by formalin and CFA and for acute antinociception. [88]Holistically, the differentiated responses from thermal and mechanical hyperalgesia within CFA-induced pain model might be explain by the recruitment of different channels emerging in the case of chronic pain.

In conclusion, all these ion channels on nociceptors can be affected by the factors affecting NMDA channels such as membrane lipids and signal transduction lipids such as arachidonic acid[89], since these channels are co-exist with NMDA channels[90]. As the communication within cortical areas are supplied by projecting fibers and the faith of receptors on fibers are extremely important. In a hypothesis associated to the etiology of Schizophrenia, the abnormalities in membrane lipids and some essential fatty acids play role in the disruptions

of channel activations. For instance; arachidonic acid increases the magnesium block and potentiate NMDAR currents into glutamatergic pyramidal neurons by acting directly on the receptor[89]. Therefore, Schizophrenia model with MK-801 might triggered changes in some lipids related to membrane may affect the activation of NMDA and other receptors during the development of Schizophrenia

What is the correlation of these channels with NMDAR hypofunction theory of Schizophrenia? The role of increased glutamatergic neurotransmission and cortical disinhibition are implicated in central sensitization. As acute pain is relayed to dorsal horn neurons as EPSCs by the activation of AMPARs, but if the tissue damage is occurred with tissue or nerve damage, the neurotransmitters on the nociceptors depolarize postsynaptic neurons to activate NMDA channels followed by increased connectivity within dorsal horn neurons and nociceptors. Aβ-fibers mostly related to light touch; and the C and Aδ fibers -high threshold mechanoreceptors responding tactile stimuli generate primary hyperalgesia as a consequence of the over-activation of glutamatergic neurotransmission dorsal horn of spinal cord. By the time, this chronic pain condition may lead the death of GABAergic interneurons in laminae I to III of dorsal horn because of peripheral nerve damage[48, 91, 92]. If there has been already loss of inhibitory GABAergic interneurons and subsequent increased central glutamate release due to the sub-chronic MK treatment, that would provide a dramatic substrate for an increase in hyperalgesia as observed for MK-801 rats. The recent study by Huang et. al. (2019) demonstrated that TRPA1 and TRPV1 - playing role in chronic inflammatory pain induced by CFA-injection - have been indicated to potentiate glutamatergic input to lamina I of dorsal horn neurons[93]. That is why, the response of the some members of TRP and KNCK (Potassium Two Pore Domain Channel Subfamily K) might be impaired to thermal and/or mechanical stimuli in both acute and chronic pain [94]

Insensitivity: A consequence of the alterations in the cortical areas

Basically, nociceptive stimuli generate significant activation in primary and secondary somatosensory cortices, cingulum, insular cortex, prefrontal cortices (PFCs) including dorsolateral and medial PFCs, ventrolateral orbital cortex, amygdala and other cortical areas[55]. As increased gamma power in several cortical areas, particularly mPFC, is considered, acute mechanical stimuli decreasing gamma power for the antinociception in mPFC possibly to protect the tissue from further damage has been indicated by Fu et al. [95]. The increased activity of dorsolateral and medial prefrontal cortex accompanies the pain

sensitization by its glutamatergic projections to periaqueductal gray (PAG); and it projects to serotoninergic nucleus raphe magnus which contribute pain relief by blocking the response coming from nociceptors to dorsal horn of spinal cord. Since increased glutamatergic activity, particularly with NMDARs, in the mPFC plays a role in antinociception but in contrast increased GABAergic activity in the mPFC leads to increase in pain[55]. It is worthy to mention the role of cingulum since GABAergic neuron density in anterior cingulate cortex is decreased in Schizophrenia patients indicated by post-mortem studies[96]. It is known that increased activity of glutamatergic projections from medial dorsal thalamus to anterior cingulate cortex innervating PAG elevates the nociception [97]; while activity of dopaminergic projections from Ventral tegmental area (VTA) inhibits it [98]. In the study of Hamani et. al. (2011), the disrupted subcallosal cingulum (SCG) projections to PFC, nucleus accumbens, amygdala, hippocampus, PAG and other cortical areas due to depression is observed to come with consequences such as interference of pain processing [99]. All together, the possible explanation of decreased sensitivity to acute nociceptive pain within this study is that MK-801 rats already have long-lasting disinhibition of neurons, particularly in mPFC that is highly communicative with other cortical areas, than control rats and acute pain may reduce 'already' decreased GABAergic activity resulting more insensitivity to acute nociceptive pain in MK-801 rats [98, 100].

Transition from antinociception to hyperalgesia with formalin-induced pain model

Aforementioned discussion regarding to increased antinociception in MK-801 rats are also related to the first pain induced by formalin within Phase I (0-15) [84, 85]. Although, when untreated pain becomes insistent and impairing and starts damaging tissue with subsequent inflammation; the spontaneous pain is observed. Therefore, the sensory system starts to exaggerate the response to both innocuous and noxious stimuli in order to keep the tissue from further damage while it is repairing the present damage [57]. The route followed in the transition from antinociception to hyperalgesia seems to same for both MK-801 and control rats (as observed within 15-25 min); but then the hyperalgesia of control rats starts to be restored, while hyperalgesia of MK-801 rats are more persistent probably due to loss of GABAergic prefrontal interneuronal neurotransmission. In normal conditions, insistent pain leads over activation of nociceptive neurons that ends up with the lost of magnesium blockage from NMDARs and consequently abnormal glutamate release [39]. That is why, acute administration of NMDAR antagonists is good approach to treat chronic pain. In spite

of the fact that NMDAR antagonism-based Schizophrenia model has already triggered biochemical changes in the cortical areas, and it changed the synaptic plasticity permanently in the present study. Therefore, there is no possibility of pain to be modulated and decreased just because of the current brain status of NMDAR hypofunction. That is why, increased hyperalgesia may be observed instead of pain relief [86]. Therefore, MK-801 rats having persistent increased hyperalgesia show that healing process to tissue damage is slowed down correlated to cytokines and other inflammatory mediators within inflammatory soup.

A Hypothesis on cortical areas of MK-801 rats with increased hyperalgesia in chronic pain model.

Insular and somatosensory cortices are activated continuously during pain stimulation and respond to non-nociceptive pain during chronic pain. Insular cortex, primary and secondary somatosensory cortices, medial and dorsolateral PFCs, anterior cingulate cortex (ACC), medio dorsolateral thalamus and other cortical areas are indicated with their role in chronic pain [55, 101]. A LTP is a form of synaptic plasticity related to learning and memory, chronic pain can be considered as a persistent sensory memory. Induction of LTP on glutamatergic synapses in the ACC is indicated to possible by the activation of NMDARs specifically by the binding of GluN2A and GluN2B preferring NMDAR antagonists[101]. Note that MK-801 is equipotent to all subtypes of NMDAR subunits [38]. The systemic or direct administration of NMDAR antagonists, specifically selective to GluN2B, to ACC inhibits hyperalgesia related to peripheral inflammation. Interestingly, GluN2B containing NMDAR increase in insular cortex and PFC is also observed after peripheral nerve injury indicating why there would be a enhanced neurotransmission and subsequently chronicity[101].

In normal condition, the abnormal neurotransmission due to continuous sensoric stimulation in the pathway of brainstem, and spinal cord: medio dorsolateral thalamus > insular cortex and parietal cortical regions; primary (S1) & secondary (S2) somatosensory cortices > mPFC, DLPFC and anterior cingulate cortex (ACC) > nucleus accumbens >ventral pallidum > medio dorsolateral thalamus – ACC/DLPFC and oscillations among these cortical areas are responsible of the chronicity of pain. Also, projections to mPFC from VTA and locus coeruleus as well as projections to nucleus accumbens from VTA contributes the facilitation of chronicity within the hippocampal nucleus accumbens –ventral pallidum – thalamic cortical loops. [55]. Then a question arises: what makes pain modulation decreased in PAG relaying abnormal output to dorsal horn of spinal cord eventuating central sensitization? The answer

is neuronal damage within peripheral or central nervous system induce central sensitization, which is an activity-dependent synaptic plasticity with involvement of various neurotransmitters, changed properties of ion-gated channels and increased activity of kinases. Therefore, increased synaptic strength facilitate the activation of nociceptive neurons to induce their responsiveness while decrease their thresholds[51] Furthermore, neuroimaging studies showed that loss of functional connectivity between PAG and other cortical areas, leading the less modulation of pain, supporting why the control group experience hyper-sensitivity [55]. Although, what making responses to spontaneous pain aggravated with MK-801 administration remains to be elusive.

Moreover, the increased functional connectivity between amygdala and PFC in chronic pain is observed. The disinhibition of neurons within latero-capsular division of central nucleus (CeAl) of amygdala occurs because of the mPFC disinhibition innervating the paracapsular intercalated interneurons of amygdala, which are dopamine hyperpolarized to cause disinhibition in CeAl [102]. The amygdala is responsible of fear and fear conditioning affective-motivational aspects of cingulate cortex driven pain avoidance and motor reaction, since pain is highly correlated to emotion-driven avoidance and escape behaviours. Pedersen et. al. (2007) proposed that GABAergic activity of the amygdala CeA affects sensory-discriminative aspect in neuropathic pain model as well as affective-motivational dimension of pain. The authors think that the sensory dimension of the pain is affected because of the neuroplastic changes in CeA is triggered [55, 103]. All together, the increased hyperalgesia with chronic pain model in MK-801 rats are facilitated by aggravated abnormal communication within cortical areas which have already decreased or increased functional connectivity may be a key to understand the leading abnormal transduction of pain information.

Dopamine and Noradrenaline

With regard to dopaminergic activity, different DA receptors contribute Schizophrenia with/without pain. While D1-like receptor (D1Rs) occupation is related to post-synaptic excitation, D2-like receptor (D2Rs) binding has inhibitory property. The densities of DA receptors vary but both are high in striatum and lower in hippocampus and amygdala. Moreover, while D1-like receptors are denser than D2-like receptors in PFC and anterior cingulate cortex, vise versa for thalamus. It has been indicated that sensitivity to acute thermal nociception and formalin-induced tonic pain are increased by one of the FGAs,

Haloperidol which acts on D2-like receptors [104]; while apomorphine – a D2 agonist - decreases nociceptive pain. Since haloperidol increases acute nociception, the increased antinociception in MK-801 rats are not surprising at all. Moreover, raclopride – a SGA which acts as a D2R antagonist - is indicated to prevent the analgesic effect of morphine and substance P in tonic pain inhibition by the mesolimbic system including VTA and nucleus accumbens[105]. That literally means SGAs do not let the pain relief in case of tonic pain experience. Moreover, increased sensitivity thresholds are observed with the administration of D2Rs antagonist, eticlopride, that is also able to decrease antinociception led by D2R selective agonists. Therefore, is that mean Schizophrenia treatment makes chronic pain more persistent due to D2R antagonism? Consistent with, the D2-like receptor agonists augment the dose-dependent attenuation of acute pain and allodynia to mechanical stimuli induced by nerve injury could not achieved with D1-like receptor agonists in anterior insula and anterior cingulate cortex [104].

PFC is able to mediate the DA release in nucleus accumbens by functional connectivity of mesocortical and mesolimbic projections[106]. Since high affinity to D2-like receptors with MK-801 administration ends up with Schizophrenia-like behaviours -such as stereotypy and hyperlocomotion-; increased D2-like receptors in PFC and nucleus accumbens may contribute to the increased antinociception in MK-801 rats in the present study[107]. Although, alterations in DA signalling with chronic pain is quite confusing and depending on the type of pain. While some patients with chronic pain displayed decreased dopamine binding with noxious stimuli as normal individuals without chronic pain, in other patients displayed no change or increased binding with noxious stimuli indicating the abnormal DA system. Interestingly, DA binding increased positive correlated in case of self-reportings [104]. Low affinities of D2-like receptors as seen after high levels of dopamine release; however, reduced D1/D2 ratio are also found in the patients with different chronic pain syndromes such as burning mouth and atypical facial pain syndromes[105]. Therefore, it is hard to interpret the changes within DA system in MK-801 rats with chronic pain.

The genetic researches suggest that the variation of the different allele occurrence in COMT gene affects dopamine signalling [104]as well as the activity of noradrenergic activity within the PFC from locus coeruleus in midbrain[108]. Norepinephrine has also a role in the development of Schizophrenia and depression; even though the certain actions of α1-adrenoceptors in their etiology is elusive. α-adrenoceptors contribute the persistency of allodynia and hyperalgesia by their increased activity leading aggravation of spontaneous

pain [55]. Systemic administration of MK-801 (0.3 mg/kg) lead to increased DA, serotonin (5-HT) and noradrenalin transmission in nucleus accumbens by cortical disinhibition of PFC projections to VTA, raphe nucleus and locus coeruleus. It may be suggested that α-adrenoceptors contribute the increase in hyperalgesia in MK-801 rats, probably by inducing DA release [109].

Cytokines

The transition from sensory to emotional state of pain is based on the rearrangement of circuity within limbic system, therefore the role of hippocampus can not be underestimated. As well-known abnormal hippocampal activity in animals with chronic pain showed impaired working memory and reduced LTP due to increased D2R bindings. It has been observed that the expression of cytokines is observed to be either increase or decrease in case of neuropathic pain. While tumor necrosis factor (TNF), IL-1b and IL-4 are decreased in hippocampus after few hours of peripheral nerve injury, IL-6 and IL-1 are increased. Absence of IL-1 leads reduced LTP, while absence of IL-6 is responsible of the LTP decrease maintenance. Interestingly, both ILs have impact on learning indicating that the cognitive impairments may be increased more due to chronic pain in Schizophrenia model, not only because of GABAergic disinhibition in several cortical areas but also cytokines. The impact of cytokines is consistent with the 'cytokine model of Schizophrenia' proposed by Girgis et. al. (2014). The authors proposed this model to explain the role of prenatal infections in Schizophrenia and the model suggests that cytokines are involved to responding the normal and non-infectious elements of neurodevelopment. IL-6 and TNF-α have showed influence in cytotoxicity, neurotransmission including glutamatergic and dopaminergic systems and cognition. These cytokines are secreted by glial cells that are responsible of the production of metabolites activating NMDARs. This can be a direct link between immune system activation and NMDAR hypofunction [110]. IL-10 and IL-1β expressions are increased in VLO (ventrolateral orbital cortex) of neuropathic rats. VLO plays a role in nociception by receiving projections from PFC and sending projections to the PAG. Both increased IL-1β expressions and glutamate release in PFC of neuropathic rats exhibits memory impairment as well as depression [51, 55]. Therefore, the aggravation of hypersensitivity in Schizophrenic rats to nociceptive pain during the experience of chronic pain can be explained by the role of cytokines both in Schizophrenia and pain.

Depression and Schizophrenia: What are the common features in terms of pain?

Luo and colleagues have performed researches about the pain sensitivities in UCMS (unpredictable chronic mild stress) or olfactory bulbectomy (OB) rat models of depression. These authors indicated that OB and UCMS rats exhibit significantly higher PWLs to acute thermal stimuli as well as decreased thermal hyperalgesia to inflammatory-induced spontaneous pain compared to control rats. Therefore, OB and UCMS rats were observed to decreased pain sensitivity to both evoked and spontaneous pain; while the chronic pain of OB and UCMS rats is not persistent compared to the control groups as in the MK-801-induced Schizophrenia model of this study. These authors also suggested that the chronic inflammatory pain exhibiting animals are more likely to exhibit depressive-like behaviours indicated by anhedonia in sucrose preference test [74-76]. Both depression-like rats and MK-801 rats exhibit insensitivity to acute nociceptive pain; although, they sharply differentiated in the responses to formalin and CFA-induced tonic pain.

CONCLUSION

Evaluation of thermal and mechanical stimuli-evoked and formalin-induced spontaneous pain in the rats - received sub-chronic MK-801 administration that induced the symptoms translational to Schizophrenia – has showed that MK-801-injected rats experienced increased acute antinociception and increased tonic pain sensitivity. Experienced insensitivity to acute pain was in concordance with the clinical reportings and human researches [45, 59-63, 65, 67]; although, there was no reportings/interpretation to spontaneous pain behaviours in humans, except the fact that the presence of spontaneous pain was used to be accepted/assumed as a form of hallucination [62, 70, 111]. Moreover, increased nociceptive hyperalgesia in chronic inflammatory pain is found with increased persistency with Schizophrenia rat model. Even though, there is no reporting - as far as known - concerning to the sensitivity to chronic pain in Schizophrenia patients, the high prevalence of chronic pain in Schizophrenia is signified by a few publications [60, 69].

BIBLIOGAPHY

1. Weinberger, D.R. and P. Harrison, *Schizophrenia* 3ed. 2011. 737.
2. Tandon, R., et al., *Definition and description of schizophrenia in the DSM-5.* Schizophrenia Research 2013. **150**(1): p. 3-10.
3. Stefanis, N., et al., *Evidence that three dimensions of psychosis have a distribution in the general population.* Psychological medicine, 2002. **32**(2): p. 347-358.
4. Upthegrove, R., S. Marwaha, and M. Birchwood, *Depression and schizophrenia: cause, consequence, or trans-diagnostic issue?* Schizophrenia Bulletin, 2017. **43**(2): p. 240-244.
5. Rössler, W., et al., *Psychotic experiences in the general population: A twenty-year prospective community study.* Schizophrenia Research 2007. **92**(1-3): p. 1-14.
6. Marder, S.R. and S. Galderisi, *The current conceptualization of negative symptoms in schizophrenia.* World Psychiatry, 2017. **16**(1): p. 14–24.
7. Bowie, C.R. and P.D. Harvey, *Cognitive deficits and functional outcome in schizophrenia.* Neuropsychiatric Disease and Treatment 2006. **2**(4): p. 531–536.
8. Strauss, M.E., *Relations of Symptoms to Cognitive Deficits in Schizophrenia.* Schizophrenia Bulletin, 1993. **19**(2): p. 18.
9. Gold, J.M., *Cognitive deficits as treatment targets in schizophrenia.* Schizophrenia Research 2004. **72**: p. 21-28.
10. Tripathi, A., *Cognitive Deficits in Schizophrenia: Understanding the Biological Correlates and Remediation Strategies.* Clinical Psychopharmacology and Neuroscience, 2018. **16**(1): p. 7-17.
11. Nihart, M.A., *The Neurobiology of Schizophrenia.* Journal of the American Psychiatry Nurses Assosiation, 1996. **2**: p. 7.
12. Howes, O.D., et al., *The Role of Genes, Stress, and Dopamine in the Development of Schizophrenia.* Biological Psychiatry, 2017. **81**(1): p. 9-20.
13. Van de Leemput, J., et al., *Genetics of Schizophrenia: Historical Insights and Prevailing Evidence*, in *Advances in Genetics*. 2016, Elsevier. p. 99-141.
14. Lisman, J.E., et al., *Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. .* Trends in neurosciences, 2008. **31**(5): p. 234-242.
15. Neill, J.C., et al., *Animal models of cognitive dysfunction and negative symptoms of schizophrenia: Focus on NMDA receptor antagonism.* Pharmacology & Therapeutics, 2010. **128**(3): p. 419-432.
16. Snyder, M.A. and W.-J. Gao, *NMDA receptor hypofunction for schizophrenia revisited: Perspectives from epigenetic mechanisms.* Schizophrenia Research, 2019: p. 11.
17. Iritani, S., *Neuropathology of schizophrenia: A mini review.* Neuropathology of Drug Addictions and Substance Misuse, 2007. **27**: p. 604–608.
18. Abi-Dargham, A., et al., *Baseline and Amphetamine-Stimulated Dopamine Activity Are Related in Drug-Naïve Schizophrenic Subjects. .* Biological Psychiatry, 2009. **65**(12): p. 1091–1093.

19. John, L. and M.J. H, *Antipsychotic medication in schizophrenia: a review.* British Medical Bulletin, 2015. **114**: p. 11.
20. Moghaddam, B. and B.S. Bunney, *Acute effects of typical and atypical antipsychotic drugs on the release of dopamine from prefrontal cortex, nucleus accumbens, and striatum of the rat: an in vivo microdialysis study.* Journal of neurochemistry, 1990. **54**(5): p. 1755-1760.
21. Talpos, J.C., *Symptomatic thinking: the current state of Phase III and IV clinical trials for cognition in schizophrenia.* Drug Discovery Today, 2017.
22. Cadinu, D., et al., *NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update.* Neuropharmacology, 2018. **142**: p. 41-62.
23. McLean, S.L., et al., *Dopamine dysregulation in the prefrontal cortex relates to cognitive deficits in the sub-chronic PCP-model for schizophrenia: A preliminary investigation.* Journal of Psychopharmacology, 2017. **31**(6): p. 660-666.
24. Thomas, E.H., et al., *The Influence of the Glutamatergic System on Cognition in Schizophrenia: A Systematic Review.* Neuroscience and Biobehavioral Reviews, 2017. **77**: p. 369-387.
25. Dienel, S.J. and D.A. Lewis, *Alterations in cortical interneurons and cognitive function in schizophrenia.* Neurobiology of disease, 2018.
26. Feifel, D. and P.D. Shilling, *Modeling Schizophrenia in Animals*, in *Animal Models for the Study of Human Disease.* 2017, Elsevier. p. 587-617.
27. Neill, J.C., et al., *Acute and chronic effects of NMDA receptor antagonists in rodents, relevance to negative symptoms of schizophrenia: A translational link to humans.* European Neuropsychopharmacology, 2014. **24**(5): p. 822–835.
28. Neill, G.P.R.a.J.C., *Modelling the cognitive and neuropathological features of schizophrenia with phencyclidine.* Journal of Psychopharmacology, 2016. **30**(11): p. 1141 –1144.
29. Abdul-Monim, Z., J.C. Neill, and G. Reynolds, *Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat.* Journal of Psychopharmacology, 2007. **21**(2): p. 198–205.
30. Chung, D.W., K.N. Fish, and D.A. Lewis, *Pathological basis for deficient excitatory drive to cortical parvalbumin interneurons in schizophrenia.* American Journal of Psychiatry, 2016. **173**(11): p. 1131-1139.
31. Belforte, J.E., et al., *Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes.* Nature neuroscience, 2010. **13**(1): p. 76.
32. Moghaddam, B. and J.H. Krystal, *Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans.* Schizophrenia bulletin, 2012. **38**(5): p. 942-949.
33. Howes, O., *Glutamate and dopamine in schizophrenia: an update for the 21st century.* J Psychopharmacol, 2015. **29**(2): p. 97–115.
34. Liu, W., et al., *Psychotomimetic effects of different doses of MK-801 and the underlying mechanisms in a selective memory.* Behavioural Brain Research, 2017.

35. Powell, C.M. and T. Miyakawa, *Schizophrenia-Relevant Behavioral Testing in Rodent Models: A Uniquely Human Disorder?* BIOL PSYCHIATRY, 2006. **59**(12): p. 1198–1207.
36. Clementz, B.A., *Identification of Distinct Psychosis Biotypes Using Brain-Based Biomarkers.* Am J Psychiatry., 2016. **173**(4): p. 373–384.
37. Kellendonk, C., E.H. Simpson, and E.R. Kandel, *Modeling cognitive endophenotypes of schizophrenia in mice.* Trends in Neurosciences, 2009. **32**(6): p. 347-358.
38. Lodge, D. and M. Mercier, *Ketamine and phencyclidine: the good, the bad and the unexpected.* British Journal of Pharmacology, 2015. **172**(17): p. 4254–4276.
39. D.Luby, E., *Study of a New Schizophrenomimetic Drug- Sernyl.* Archives of Neurobiology and Psychiatry, 1959. **81**: p. 7.
40. Ashby, D.M., et al., *Subchronic MK-801 treatment and post-weaning social isolation in rats: differential effects on locomotor activity and hippocampal long-term potentiation.* Behavioural Brain Research, 2010. **212**(1): p. 64-70.
41. Salter, M.W. and G.M. Pitcher, *Dysregulated Src upregulation of NMDA receptor activity: a common link in chronic pain and schizophrenia.* The FEBS journal, 2012. **279**(1): p. 2–11
42. Karasawa, J.-i., K. Hashimoto, and S. Chaki, *d-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats.* Behavioural Brain Research, 2008. **186**(1): p. 78–83.
43. King, M.V., *5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation—an effect sensitive to NMDA receptor antagonism.* Neuropharmacology, 2004. **47**: p. 195–204.
44. Kovacic, P. and R. Somanathan, *Clinical physiology and mechanism of dizocilpine (MK-801).* Oxidative Medicine and Cellular Longevity, 2010. **3**(1): p. 13-22.
45. Singh, M.K., L.L. Giles, and H.A. Nasrallah, *Pain Insensitivity in Schizophrenia: Trait or State Marker?* Journal of Psychiatric Practice, 2006. **12**(2): p. 90–102.
46. J, B.G., *What Is Spontaneous Pain and Who Has It?* The Journal of Pain, 2012. **13**(10): p. 921-929.
47. Schug, S.A., H.C. Daly, and K.J. Stannard, *Pathophysiology of Pain*, in *Mechanisms of Vascular Disease: A Reference Book for Vascular Specialists [Internet]*. 2011, University of Adelaide Press.
48. Basbaum, A.I., et al., *Cellular and molecular mechanisms of pain.* Cell, 2009. **139**(2): p. 267-284.
49. Woolf, C.J. and Q. Ma, *Nociceptors—noxious stimulus detectors.* Neuron, 2007. **55**(3): p. 353-364.
50. Dubin, A.E. and A. Patapoutian, *Nociceptors: the sensors of the pain pathway.* The Journal of clinical investigation, 2010. **120**(11): p. 3760-3772.
51. Costigan, M., J. Scholz, and C.J. Woolf, *Neuropathic pain: a maladaptive response of the nervous system to damage.* Annual review of neuroscience, 2009. **32**: p. 1-32.
52. Council, N.R., *Mechanisms of Pain*. Recognition and Alleviation of Pain in Laboratory Animals. 2009: National Academies Press
53. Hofbauer, R.K., et al., *Cortical representation of the sensory dimension of pain.* Journal of neurophysiology, 2001. **86**(1): p. 402-411.

54. Ji, G., et al., *Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation.* Journal of Neuroscience, 2010. **30**(15): p. 5451-5464.
55. Ong, W.-Y., C.S. Stohler, and D.R. Herr, *Role of the prefrontal cortex in pain processing.* Molecular neurobiology, 2019. **56**(2): p. 1137-1166.
56. Treede, R.-D., et al., *A classification of chronic pain for ICD-11.* Pain, 2015. **156**(6): p. 1003.
57. Apkarian, A.V., et al., *Prefrontal cortical hyperactivity in patients with sympathetically mediated chronic pain.* Neuroscience letters, 2001. **311**(3): p. 193-197.
58. Onozawa, K., et al., *Amygdala-prefrontal pathways and the dopamine system affect nociceptive responses in the prefrontal cortex.* BMC neuroscience, 2011. **12**(1): p. 115.
59. Bonnot, O., *Are Patients With Schizophrenia Insensitive to Pain? A Reconsideration of the Question.* Clin J Pain, 2009. **25**: p. 244–252.
60. Kraepelin, E., *Dementia praecox and paraphrenia.* Eng.: Livingstone, 1919.
61. Engels, G., *Clinical Pain in Schizophrenia: A Systematic Review.* American Pain Society, 2014.
62. Watson, G., P. Chandarana, and H. Merskey, *Relationships between pain and schizophrenia.* The British Journal of Psychiatry, 1981. **138**(1): p. 33-36.
63. Dworkln, R.H., *Pain Insensitivity in Schizophrenia: A Neglected Phenomenon and Some Implications.* Schizophrenia Bulletin, 1994. **20**(2): p. 235-248.
64. Fishbain, D.A., *Pain Insensitivity in Psychosis.* Annals of Emergency Medicine, 1982.
65. Virit, O., H.A. Savas, and A. Altindag, *Lack of pain in schizophrenia: a patient whose arm was burned and amputated.* General Hospital Psychiatry, 2008. **30**(4): p. 384–385.
66. Stubbs, B., et al., *Decreased pain sensitivity among people with schizophrenia: a meta-analysis of experimental pain induction studies.* Pain, 2015. **156**(11): p. 2121–2131.
67. Potvin, S. and S. Marchand, *Hypoalgesia in schizophrenia is independent of antipsychotic drugs: A systematic quantitative review of experimental studies.* Pain 2008. **138**(1): p. 70–78.
68. Calandre, E.P. and F. Rico-Villademoros, *The role of antipsychotics in the management of fibromyalgia.* CNS drugs, 2012. **26**(2): p. 135-153.
69. de Almeida, J.G., et al., *Chronic pain and quality of life in schizophrenic patients.* Official Journal of the Brazilian Psychiatric Association, 2013. **35**(1): p. 13-20.
70. Merskey, H., *Psychological aspects of pain.* Postgraduate Medical Journal, 1968. **44**(510): p. 297.
71. Boyette-Davis, J. and P.N. Fuchs, *Chronic administration of phencyclidine produces decreased sensitivity to mechanical stimulation in the absence of altered affective behavior: Implications for pain processing in schizophrenia.* Neuroscience Letters 2011. **498**(2): p. 153– 157.
72. Samsom, J.N. and A.H. Wong, *Schizophrenia and depression co-morbidity: what we have learned from animal models.* Frontiers in Psychiatry, 2015. **6**: p. 13.
73. Fatemi, S.H. and T.D. Folsom, *The Neurodevelopmental Hypothesis of Schizophrenia, Revisited.* Schizophrenia Bulletin, 2009. **35**(3): p. 528–548.
74. Shi, M., J.-Y. Wang, and F. Luo, *Depression Shows Divergent Effects on Evoked and Spontaneous Pain Behaviors in Rats.* J Pain, 2010. **11**(3): p. 219-229.

75. Wang, N., et al., *Increasing Pain Sensation Eliminates the Inhibitory Effect of Depression on Evoked Pain in Rats.* Front. Behav. Neurosci. , 2016. **10**: p. 183.
76. Su, Y.-L., et al., *The effect of depression on the thermal nociceptive thresholds in rats with spontaneous pain.* Neurosci Bull, 2010. **26**(6): p. 429-436.
77. Gilmour, G., et al., *Diverse and often opposite behavioural effects of NMDA receptor antagonists in rats: implications for "NMDA antagonist modelling" of schizophrenia.* Psychopharmacology, 2009. **205**(2): p. 203-216.
78. Gould, T.D., D.T. Dao, and C.E. Kovacsics, *The open field test*, in *Mood and anxiety related phenotypes in mice*. 2009, Springer. p. 1-20.
79. Mohammad-Zadeh, M., *Modulation of Different Phases of Formalin Test by Force Swim Stress.* Basic and Clinical Neuroscience, 2014. **5**(4): p. 5.
80. Seillier, A. and A. Giuffrida, *Evaluation of NMDA receptor models of schizophrenia: divergences in the behavioral effects of sub-chronic PCP and MK-801.* Behavioural brain research, 2009. **204**(2): p. 410-415.
81. Vardigan, J.D., et al., *MK-801 produces a deficit in sucrose preference that is reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive allosteric modulator CDPPB: relevance to negative symptoms associated with schizophrenia?* Pharmacology Biochemistry and Behavior, 2010. **95**(2): p. 223-229.
82. Pelizza, L. and A. Ferrari, *Anhedonia in schizophrenia and major depression: state or trait?* Annals of General Psychiatry, 2009. **8**(1): p. 22.
83. Lambert, G.A., G. Mallos, and A.S. Zagami, *Von Frey's hairs--a review of their technology and use--a novel automated von Frey device for improved testing for hyperalgesia.* J Neurosci Methods, 2009. **177**(2): p. 420-6.
84. Il-Ok Lee, Y.-S.J., *Effects of Different Concentrations of Formalin on Paw Edema and Pain Behaviors in Rats.* J Korean Med Sci 2002. **17**: p. 81-5.
85. Abbott, F.V., K.B. Franklin, and R.F. Westbrook, *The formalin test: scoring properties of the first and second phases of the pain response in rats.* Pain, 1995. **60**(1): p. 91-102.
86. Ren, K. and R. Dubner, *Inflammatory models of pain and hyperalgesia.* ILAR journal, 1999. **40**(3): p. 111-118.
87. Mandadi, S., et al., *TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP.* Pflügers Archiv-European Journal of Physiology, 2009. **458**(6): p. 1093-1102.
88. Nassar, M.A., et al., *Nociceptor-specific gene deletion reveals a major role for Nav1. 7 (PN1) in acute and inflammatory pain.* Proceedings of the National Academy of Sciences, 2004. **101**(34): p. 12706-12711.
89. Kloda, A., B. Martinac, and D.H. Adams, *Polymodal Regulation of NMDA Receptor-Channels.* Channels, 2007. **1**(5): p. 334-343.
90. Chahl, L.A., *TRP's: links to schizophrenia?* Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2007. **1772**(8): p. 968-977.
91. Moore, K.A., et al., *Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord.* Journal of Neuroscience, 2002. **22**(15): p. 6724-6731.

92. Polgár, E., et al., *Loss of neurons from laminas I-III of the spinal dorsal horn is not required for development of tactile allodynia in the spared nerve injury model of neuropathic pain.* Journal of Neuroscience, 2005. **25**(28): p. 6658-6666.
93. Huang, Y., et al., *Endogenous TRPA 1 and TRPV 1 activity potentiates glutamatergic input to spinal lamina I neurons in inflammatory pain.* Journal of neurochemistry, 2019.
94. Fenton, W.S., J. Hibbeln, and M. Knable, *Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia.* Biological psychiatry, 2000. **47**(1): p. 8-21.
95. Fu, B., et al., *Acute and chronic pain affects local field potential of the medial prefrontal cortex in different band neural oscillations.* Molecular pain, 2018. **14**: p. 1744806918785686.
96. Jadi, M.P., M.M. Behrens, and T.J. Sejnowski, *Abnormal gamma oscillations in NMDAR hypofunction models of schizophrenia.* Biological Psychiatry, 2015. **79**(9): p.716-726.
97. Harte, S.E., C.A. Spuz, and G.S. Borszcz, *Functional interaction between medial thalamus and rostral anterior cingulate cortex in the suppression of pain affect.* Neuroscience, 2011. **172**: p. 460-473.
98. López-Avila, A., et al., *Dopamine and NMDA systems modulate long-term nociception in the rat anterior cingulate cortex.* Pain, 2004. **111**(1-2): p. 136-143.
99. Hamani, C., et al., *The subcallosal cingulate gyrus in the context of major depression.* Biological psychiatry, 2011. **69**(4): p. 301-308.
100. Wood, P.B., *Role of central dopamine in pain and analgesia.* Expert review of neurotherapeutics, 2008. **8**(5): p. 781-797.
101. Bliss, T.V., et al., *Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain.* Nature Reviews Neuroscience, 2016. **17**(8): p. 485.
102. Marowsky, A., et al., *A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function.* Neuron, 2005. **48**(6): p. 1025-1037.
103. Pedersen, L.H., J. Scheel-Krüger, and G. Blackburn-Munro, *Amygdala GABA-A receptor involvement in mediating sensory-discriminative and affective-motivational pain responses in a rat model of peripheral nerve injury.* Pain, 2007. **127**(1-2): p. 17-26.
104. Jarcho, J.M., et al., *Pain, affective symptoms, and cognitive deficits in patients with cerebral dopamine dysfunction.* PAIN®, 2012. **153**(4): p. 744-754.
105. Mitsi, V. and V. Zachariou, *Modulation of pain, nociception, and analgesia by the brain reward center.* Neuroscience, 2016. **338**: p. 81-92.
106. Del Arco, A., et al., *Stimulation of D2 receptors in the prefrontal cortex reduces PCP-induced hyperactivity, acetylcholine release and dopamine metabolism in the nucleus accumbens.* Journal of neural transmission, 2007. **114**(2): p. 185-193.
107. Bubeníková, V., et al., *The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating.* Pharmacology Biochemistry and Behavior, 2005. **80**(4): p. 591-596.
108. Taylor, B.K. and K.N. Westlund, *The noradrenergic locus coeruleus as a chronic pain generator.* Journal of neuroscience research, 2017. **95**(6): p. 1336-1346.

109. Yan, Q.-S., et al., *Dizocilpine (MK-801) increases not only dopamine but also serotonin and norepinephrine transmissions in the nucleus accumbens as measured by microdialysis in freely moving rats.* Brain research, 1997. **765**(1): p. 149-158.
110. Girgis, R.R., S.S. Kumar, and A.S. Brown, *The cytokine model of schizophrenia: emerging therapeutic strategies.* Biological psychiatry, 2014. **75**(4): p. 292-299.
111. Engel, G.L., *"Psychogenic" pain and the pain-prone patient.* The American journal of medicine, 1959. **26**(6): p. 899-918