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VASOPRESSIN-DEFICIENT BRATTLEBORO RAT AS A SCHIZOPHRENIA MODEL: VALIDATION AND POSSIBLE CONTRIBUTING MECHANISMS TO THE DEVELOPMENT OF SCHIZOPHRENIA-LIKE BEHAVIOR

Ph.D. thesis

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1 Abbreviations

5-HT	serotonin
AcH3K9	acetylated lysine 9 of histone 3
ACTH	adrenocorticotropic hormone
AVP	arginine vasopressin, antidiuretic hormone
BDNF	brain-derived neurotrophic factor
Bdr	blind-drunk mouse
BLA	basolateral amygdala
BNST	bed nucleus of stria terminalis
CA1/2/3	field cornu ammonis 1, 2 or 3 of hippocampus
CeA	central amygdala
COMT	catecholamine O-methyltransferase
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DDAVP	1-desamino-8-D-arginine vasopressin
di/di	homozygous diabetes insipidus, missing functional vasopressin
DISC1	disrupted-in-schizophrenia
DP	dorsal peduncular cortex
DSM	Diagnostic and Statistical Manual of Mental Disorders
EEG	electroencephalography
EPM	elevated plus maze
GAD1	glutamate decarboxylase 1
GLS	glutaminase

HAB	high anxiety-related behavior based upon EPM
HC	hippocampus
HPA	hypothalamic pituitary adrenocortical axis
IL	infralimbic cortex
KO	knock-out
LAB	low anxiety-related behavior based upon EPM
LH	lateral hypothalamus
LS	lateral septum
LSD	dorsolateral septum
LSI	lateral septum, intermediate part
LSV	ventrolateral septum
MAM	maternal methylazoxymethanol
MeA	medial amygdala
MIA	maternal immune activation
MK-801	dizocilpine, NMDA receptor antagonist
MS-USV	maternal separation-induced ultrasonic vocalization
MWM	Morris water maze
nAChR	nicotinic acetylcholine receptor
NMDA	N-methyl-D-aspartate, ionotropic glutamate receptor
NOR	novel object recognition
NRG1	neuregulin-1
OXT	oxytocin
PCP	phencyclidine, NMDA receptor antagonist

PFC	prefrontal cortex
poly (I:C)	polyinosinic-polycytidylic acid
PPI	prepulse inhibition
PrL	prelimbic cortex
PVN	paraventricular nucleus of hypothalamus
RI	resident-intruder test
RRL	righting reflex latency
SCN	suprachiasmatic nucleus
SCZ	schizophrenia
SD	social discrimination
SIADH	syndrome of inappropriate antidiuretic hormone release
SNAP-25	synaptosomal-associated protein (25 kDa)
SON	supraoptic nucleus
USV	ultrasonic vocalization
V1a, -1b, -2R	vasopressin receptor type 1a, -1b, 2
vGluT1/2	vesicular glutamate transporter 1/2
vHC	ventral hippocampus

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2 Introduction

Schizophrenia (SCZ) is a chronic mental disorder, which has not only a negative influence on the patient's life, but also on its entire family. Moreover, SCZ has serious economic disadvantages. According to a systematic review - depending on the country - the total societal cost of one patient with SCZ varied from 5,818 to 94,587 $US in 2015 (Jin et al., 2017). Interestingly, according to the most detailed studies there is no sex difference in its prevalence (Saha et al., 2005; Perala et al., 2007).

Take into consideration the high prevalence of SCZ worldwide (0.4 - 0.87 %, depending on geographical, environmental factors and the type of prevalence estimate used (Saha et al., 2005; Perala et al., 2007; Kahn et al., 2015)) and the failures in its treatment further studies are needed on the field. Animal models provide great opportunity to study the mechanism and to identify new targets for treatments. We aimed to fully characterize a vasopressin (AVP)-deficient rat model.

2.1 Schizophrenia

2.1.1 Symptoms

SCZ symptoms can be grouped into three main categories: *positive* (behaviors not seen in healthy people: hallucinations, delusions, disorganized speech and behavior), *negative* (disrupted normal behaviors like diminished emotional expression, avolition) and *cognitive* ones (disturbances in working and spatial memory) (Diagnostic and Statistical Manual of Mental Disorders: DSM-V) (Insel, 2010). Signs of SCZ may also include non-specific symptoms such as social withdrawal, irritability or sleep disturbances. According to DSM-V, at least two out of the five major symptoms (delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, or negative symptoms) have to last more than a month to diagnose SCZ and one of them has to be delusions, hallucinations or disorganized speech.

2.1.2 Main brain areas implicated in schizophrenia

There is no single brain area which is responsible for the diverse symptoms, rather this disorder is due to the deficit of networks (Alamian et al., 2017; Dauvermann et al., 2017).

Moreover, positive, negative and cognitive symptoms can be linked to overlapping, but not necessarily identical brain areas.

SCZ is characterized by pronounced *cortical gray matter loss* (Dietsche et al., 2017). Indeed, auditory-verbal hallucinations are associated with cortical thickness on the area of *temporal gyrus* responsible for auditory perception (Kose et al., 2018). A further cortical area linked to hallucination is the *prefrontal cortex* (PFC), where dopamine is released in excessive manner (Laruelle et al., 1996). The decrease in cognitive ability is also accompanied by hypo-activation of the PFC (Birrell et al., 2000; Kumar et al., 2017). Both dopamine D1 receptor function, and N-methyl-D-aspartate (NMDA) mediated glutamate transmission is impaired in PFC (Laruelle, 2014). On the other hand, D2 receptor in the basal ganglia including striatum may be responsible for the extrapyramidal side effect of antipsychotics rather than for the antipsychotic effect (Joyce et al., 1997). Cortical atrophy can be also characterized by increased *ventricle*-to-brain ratio (Van Assche et al., 2017).

Hippocampus (HC) is also implicated in SCZ with smaller CA1 volumes and CA1 hyperactivation (Nakahara et al., 2018). Anterior HC is important during psychological stress (Herman et al., 1992), and was found to be smaller in hyponatremic SCZ patients (in contrast to posterior HC or amygdala) (Goldman et al., 2007). On the other hand, lesions of the ventral HC (vHC) during development is proved to be a useful animal model of SCZ extensively studied as a model of positive symptoms (see 1.3.1.) (Moser, 2014).

2.1.3 Risk factors

The main causes of SCZ are still unclear, but there are several theories for understanding this complex disease. Engel's Biopsychosocial Model (Engel, 1978) (**Figure 1.**) describes that SCZ develops (1) on the basis of genetic predisposition, which is supported by the fact that schizophrenic patients' offspring are more likely to develop symptoms than others with healthy parents (Hameed et al., 2016). (2) An early negative environment contributes to the development of SCZ as well (Bearden et al., 2018). Environmental changes can regulate gene expression through epigenetic processes (for detailed review see: Zelena, 2012a). In the individuals sensitized by genetic and

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epigenetic variants, the (3) environmental challenges, stressors can trigger the appearance of schizophrenic symptoms.

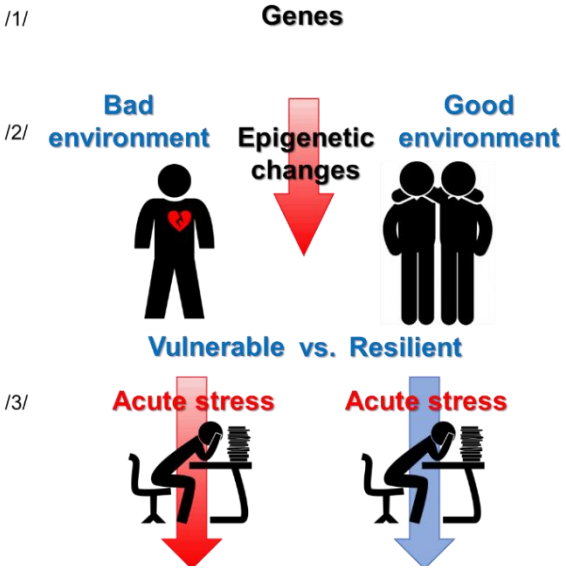

Figure 1. Engelôs (source: p own figure)

2.1.3.1 Genetics

Twin studies demonstrated that SCZ is highly heritable with around 80% heritability (Gejman et al., 2010; Jones et al., 2011). However, no single gene was found to be responsible for the phenotype of the disease (Demeter et al., 2016).

The closely related glutamatergic and dopaminergic hypotheses of SCZ are based upon the role of these neurotransmitter systems in the appearance of SCZ symptoms (Khlghatyan et al., 2018). There is an interplay between these systems as dopamine signaling may impact neuronal activity by modulating the glutamate neurotransmission (Laruelle, 2014). Indeed, in schizophrenic patients, changes were detected in gene expressions related to glutamate and dopamine. For example in SCZ patients there is an underexpression of catecholamine O-methyltransferase (COMT, metabolic enzyme of dopamine –among other catecholamines), NMDA, vesicular glutamate transporter 1 (vGluT1) and glutamate decarboxylase 1 (GAD1, regulating GABA synthesis) genes and there is an overexpression of vGluT2 and glutaminase (GLS) genes (for review see: Zelena, 2012a). Neuregulin-1 (NRG1) is a leading SCZ susceptibility gene (Harrison et al., 2006), and it is also connected to the glutamatergic system. Indeed, polymorphism of a single nucleotide of the NRG1 gene leading to enhanced NRG1 signaling contributes to glutamatergic receptor hypofunction (Hahn et al., 2006). One of the most studied gene in connection with SCZ is the dysbindin (DTNBP1) (Harrison et al., 2005), which has a well-known role in the regulation of D2 and D3 receptor signaling (Schmieg et al., 2016).

2.1.3.2 Environment ïepigenetics

If there is a genetic predisposition, negative environmental challenges - through epigenetic changes - may increase the risk of SCZ during the pre-, peri- and postnatal period. The environmental challenge can be, for example, perinatal hypoxia, urban environment, use of cannabis or a trauma (van Os et al., 2010). However, there are still controversial studies in connection with cannabis use; some of them found genetic predisposition to the use of cannabis and the development of SCZ (for review see (Ortiz-Medina et al., 2018)). Epigenetic process is any mechanism that is not based on a change in the nucleotide sequence of DNA but regulates gene expression.

Three major mechanism exist for epigenetic reprogramming. (1) The DNA methylation occurs on cytosine nucleic acids that are followed by guanine in the DNA sequence (i.e., CpG sites) and is rather targets specific single genes, mostly resulting in reduced expression. (2) In contrast, changes in chromatin structure may occur via covalent modification of histone and may increase (via histone acetylation) or decrease (via histone methylation) the access of large portions of DNA to the natural subcellular machinery that governs transcription and translation (Wolffe et al., 1999). (3) Besides, there are microRNAs with various functions (Sato et al., 2011; Török, 2021).

Several studies have shown that epigenetic changes may also be involved in the development of SCZ (Roth et al., 2009; Svrakic et al., 2013). In this regard, hypomethylation of COMT and vGluT2 genes, and hypermethylation of NMDA, vGluT1, GAD1 and GLS genes can be observed in SCZ patients (for review see: Zelena, 2012a). Moreover, Aoyoma et al. (Aoyama et al., 2014) using a pharmacological model of SCZ (piperidine hydrochloride (PCP) in mouse) found decreased level of acetylated lysine 9 of histone 3 (AcH3K9) in the PFC, and a previous observation found a decrease of AcH3K9 in human SCZ patients (Gavin et al., 2008).

2.1.3.3 Triggers

Psychosis can be interpreted as a lack of adaptability to environmental factors as described by the stress vulnerability model. If the individual is vulnerable – evolved by genetic and epigenetic factors – appearance of SCZ symptoms can be triggered also by low stress (Zubin et al., 1977). Urban environment (Kahn et al., 2015) and cannabis use

(Henquet et al., 2008) can both contribute to the susceptibility through epigenetic changes and trigger the appearance of the symptoms.

2.2 Importance of modeling disorders

Available therapies cannot improve cognitive deficits and have small effect on negative symptoms of SCZ (Sanchez et al., 2017), therefore new drugs should be developed. According to the current state of science, animal models are essential for the preclinical investigation of a new drug to explore the underlying mechanisms as well as to be able to develop new treatment options. The use of valid animal models for a given human condition allows the preclinical efficacy of candidate drugs to be assessed. By definition, an animal model is considered valid if it is similar to the human condition in etiology, pathophysiology, symptoms, and response to therapy (Van Dam et al., 2006). Ideally, all the listed conditions would be met in the models, but unfortunately, usually only a few aspects of a disease can be modeled in animals. This statement is exponentially true of mental illnesses: we will never be able to find human-like complexity in another species. On the other hand, a good animal model can provide answers to questions during preclinical phase that no other method is currently able to do, and with their help we can get one step closer to understanding the given disease.

2.2.1 Validation of an animal model

Validity of an animal model is indispensable for valuable translational relevance. Face-, construct-, and predictive validity can be distinguished. *Face validity* means that the animal model mimic core behavioral symptoms shown in the given human disorder. It can be tested by different behavioral measurements or monitoring methods (for SCZ see: **Table 1.**). According to *construct validity*, in animal models, neurochemical and structural defects have to be similar to human patients. *Predictive validity* requires therapeutic effectiveness similar to that in humans (Willner, 1986). An animal model that meets the validation criteria as much as possible can lead to more reliable preclinical research that benefits both economy and society (Varga et al., 2010).

Table 1. Main symptoms of schizophrenia and the corresponding behavioral tests and monitoring methods used in animal studies (Török et al., 2019); Abbreviations: EEG: electroencephalography, MWM: Morris water maze, PPI: prepulse inhibition, USV: ultrasonic vocalization.

Categories	Human symptoms	Tests in animals
Positive symptoms	hallucinations, delusions, disorganized speech	open field: NMDA receptor antagonist-induced hyperlocomotion
	disorganized behavior (attention deficit)	latent inhibition, PPI, attentional set shifting
Negative symptoms	diminished emotions	USV, forced swim, tail suspension, sucrose preference
	social withdrawal	three chamber test, social avoidance, social interaction, resident-intruder test
	sleep disturbances	EEG
Cognitive deficit	working memory	Y-maze, object recognition
	spatial memory	T-maze, MWM, Barnes maze

2.3 Animal models of schizophrenia

Three main subtypes of preclinical models of SCZ can be distinguished. In neurodevelopmental models, risk factors of a given disease occur in prenatal, early postnatal or early adolescent age of the animal. Therefore, young adulthood is usually the characteristic time for the onset of the symptoms. In the case of pharmacological animal models symptoms of the disease that we would like to model appear after an invasive procedure, by introducing an active ingredient into the body. Genetic models, in turn, carry one or more gene mutations specific to the disease to be modeled, thus, the symptoms of the given disorder can be observed. A summary about animal models of SCZ can be found in **Table 2**.

Table 2. Animal models of schizophrenia and possible contribution of vasopressin. Abbreviations: AVP: arginine vasopressin; Bdr: blind-drunk mouse; DISC1: disrupted-in-SCZ; KO: knock-out; MAM: maternal methylazoxymethanol; MIA: maternal immun activation; MK-801: disocilpine, NMDA receptor antagonist; PCP: phencyclidine; PPI: prepulse inhibition; PVN: paraventricular nucleus of hypothalamus; PWSI: postweaning social isolation; SCZ: schizophrenia; SON: supraoptic nucleus; V1aR/V1bR: vasopressin receptor types; vHC: ventral hippocampus.

Category	Model	Involvement of vasopressin	Reference
Neurodevelopmental models	MIA	hypothalamic V1a mRNA elevation in susceptible	(Morais et al., 2018)
	MAM	amnionic AVP elevation	(Oosterbaan et al., 1985)
	prenatal stress	decreased AVP positive cell number in PVN	(de Souza et al., 2013)
	vHC lesion	enhanced stress-induced AVP secretion; AVP injection alleviated forgetting	(Mitchell et al., 2004; Goursaud et al., 2006)
	PWSI	AVP decreased in PVN, increased in SON; site-specific alterations in V1aR expression	(Ruscio et al., 2007; Pan et al., 2009; Tanaka et al., 2010; Hawken et al., 2013; Hiura et al., 2018)
Pharmacological models	PCP	reduced V1aR binding; high dose: decreased plasma AVP	(Zerbe et al., 1983; Tanaka et al., 2003)
	MK-801	AVP analogue (NC-1900) decreased NMDA receptor antagonist-induced hyperlocomotion and social deficit	(Wisniewski et al., 1996; Artemowicz et al., 1998; Matsuoka et al., 2005; Sato et al., 2005)
	Ketamine	SCZ-like behavior through AVP-producing SON cells	(Nissen et al., 1994)
Genetic models	DISC1	V1a mRNA decreased in hypothalamus	(O'Tuathaigh et al., 2017)
	Bdr	disrupted AVP rhythms	(Oliver et al., 2012)
	V1aR KO	SCZ-like behavior (impaired social interaction)	(Bielsky et al., 2004; Egashira et al., 2004; Egashira et al., 2007; Egashira et al., 2009; Li et al., 2009; Masuki et al., 2013)
	V1bR KO	impairments in PPI, restored by atypical antipsychotics	(Egashira et al., 2005; Egashira et al., 2009)
	Brattleboro rat	point mutation in AVP; SCZ-like symptoms	(Boer et al., 1982; Greer et al., 1982; Williams et al., 1983, 1985; Brown et al., 1989; Engelmann et al., 1994a; Feifel et al., 2001; Feifel et al., 2004; Shilling et al., 2006; Feifel et al., 2007; Mlynarik et al., 2007; Feifel et al., 2009; Schank, 2009; Cilia et al., 2010; Fodor et al., 2014; Varga et al., 2014; Varga et al., 2015; Csikota et al., 2016; Demeter et al., 2016; Fodor et al., 2016a; Fodor et al., 2016b; Zelena, 2018)

2.3.1 Neurodevelopmental models

According to some old hypotheses, SCZ is thought to be a neurodevelopmental disorder (Collin et al., 2018). Based upon this assumption intrauterine exposure to various

insults including *infections* is used. Indeed, maternal immune activation (*MIA*) is a widely used model of SCZ (Bergdolt et al., 2018; Minakova et al., 2018). Maternal polyinosinic-polycytidylic acid (poly (I:C)) or lipopolysaccharide or strains of influenza injections may induce harmful epigenetic changes, especially in genetically predisposed animals (Estes et al., 2016).

Prenatal application of the mitotoxic agent, methylazoxymethanol (*MAM*) (Jones et al., 2011), as well as maternal malnutrition are also widespread models (Moser, 2014).

Prenatal stress, like maternal electric footshock or corticosterone injections may also induce SCZ-like symptoms (Hill, 2016).

Another intervention employed during the early postnatal period are brain lesions. Most commonly the vHC is removed in the early stage of development (*vHC lesion*), causing SCZ-like symptoms (Tseng et al., 2009). Unfortunately, this model has approximately 15% mortality and 30% unsuccess rate (Jones et al., 2011). Moreover, a 1997's study showed that antipsychotic administration do not improve the negative symptoms of animals, however, reduced their NMDA receptor antagonist-induced hyperlocomotion, the only behavior interpreted to model hallucination (Sams-Dodd et al., 1997).

Further models are based upon postnatal distress and diminished social contact by postweaning *social isolation* rearing (Jones et al., 2011; Moser, 2014). Indeed, social deprivation of rat/mice pups from the age of weaning by placing them alone in separate cages alters brain development and causes SCZ-like behavioral deficits (e.g. disrupted PPI) in adulthood (Weiss et al., 2001).

2.3.2 Pharmacological models

Administration of amphetamine or NMDA receptor antagonists (phencyclidine or piperidine hydrochloride: PCP, MK-801, ketamine) can cause SCZ-like symptoms (Murray, 2002; Pedersen et al., 2014; Chaki et al., 2015). These drugs are psychotomimetics and may induce hallucinations in humans, therefore, are good candidates to model positive symptoms in animals (Meltzer et al., 2013). Besides, they may also induce a number of other SCZ-like symptoms (anhedonia, reduced prepulse inhibition /PPI, which is a neural mechanism regulated by forebrain structures that inhibits the processing of external sensory, cognitive, and motor information; with PPI

test, sensorimotor gating deficit can be detected/, disrupted memory function etc.). However, possible interactions between drugs have to be tested and SCZ-inducing agents may be a limiting factor in the development of new treatments in these models (Jones et al., 2011).

2.3.3 Genetic models

Although there is not a known gene responsible for SCZ per se, knocking out (KO) many candidate genes may lead to developmental disturbances and these "two hits" (genes + early environment) together may lead to enhanced vulnerability (Leung et al., 2016). Moreover, in these models no acute interventions are necessary to induce SCZ-like symptoms (in contrast see pharmacological models) and do not have to deal with unsuccessful operations or infectious agents. Thus, new drugs can be examined more easily and reliably in these models.

Disturbances of the dopaminergic system is a core feature of SCZ. Therefore, mutation of this system seemed to be a reliable model. Indeed, knocking out *COMT*, *dopamine transporter* or nuclear receptor related 1 protein (*Nurr1*, a transcription factor implicated in the development of midbrain dopaminergic neurons) all led to the development of some SCZ-like symptoms (Hill, 2016).

Glutamatergic system is also in the center of interest. Especially, *GRIK4* KO, containing a mutation in the kainate receptor 4 subunit, is prone to memory disturbances (Lowry et al., 2013). The α7-nicotinic acetylcholine receptor KO mice (*Ű-7nAChR* KO) also have deficits in their NMDA receptors, which may contribute to their SCZ-like behavioral alterations.

In humans, several high risk genes were identified and used later to model the symptoms in mice (Hill, 2016). *DISC1* (disrupted-in-SCZ) disruption may lead to enhanced susceptibility to SCZ (among other psychiatric illnesses). DISC1 KO mice have cognitive disturbances especially in PFC-based spatial working memory (delayed non-matched place task) (Koike et al., 2006). *NRG1* belongs to the epidermal growth factor family, and has an essential physiological role confirmed by the fact that – similarly to its receptor, the *ErbB-4* - its complete deletion is lethal (Leung et al., 2016). However, heterozygous NRG1 KO mice exhibit several SCZ-like symptoms including hyperlocomotion and memory disturbances (Stefansson et al., 2002).

Reelin is a matrix glycoprotein and its level is reduced in postmortem SCZ brains (Guidotti et al., 2000). However, reelin KO mice show only mild SCZ-like disturbances (Tremolizzo et al., 2002).

Synaptosomal-associated protein of 25 kDa (*SNAP-25*) is an important component of the synaptic machinery (Antonucci et al., 2016) known to regulate exocytosis (Noor et al., 2017). Synaptic dysfunction is a key pathomechanism of SCZ (Egbujo et al., 2016; Osimo et al., 2018): mutation of SNAP-25 (in blind-drunk (Bdr) mouse model) may lead to SCZ-like circadian and cognitive dysfunctions (Ohira et al., 2013; Tam et al., 2015).

Conditional forebrain KOs of the brain-derived neurotrophic factor (*BDNF*) show even typical sex-dependent effects with male-specific hyperactivity and female-specific depressive phenotypes (Monteggia et al., 2007).

A new model is based upon the connection between SCZ and cannabis use. With the help of this model it was found that mice overexpressing cannabinoid receptor interacting protein 1 in their vHC region (with the help of a lentivirus) show several signs of SCZ-like behavior (Perez et al., 2018).

A promising genetic model of SCZ might be the **vasopressin (AVP) deficient Brattleboro rat** (di/di, homozygous for diabetes insipidus, **Figure 2.**). Indeed, AVP was associated to SCZ disorder at many points (Török et al., 2019). Furthermore, Brattleboro rat mimics core behavioral symptoms of SCZ (Braff et al., 1990; Engelmann et al., 1994a; Schank, 2009; Varga et al., 2014; Demeter et al., 2016; Fodor et al., 2016b; Schatz et al., 2018), shows molecular changes (Shilling et al., 2006; Gavin et al., 2008; Demeter et al., 2016) and has response to antipsychotic treatment similar to that in humans (Feifel et al., 2004; Feifel et al., 2007; Feifel et al., 2009; Cilia et al., 2010; Feifel et al., 2011).

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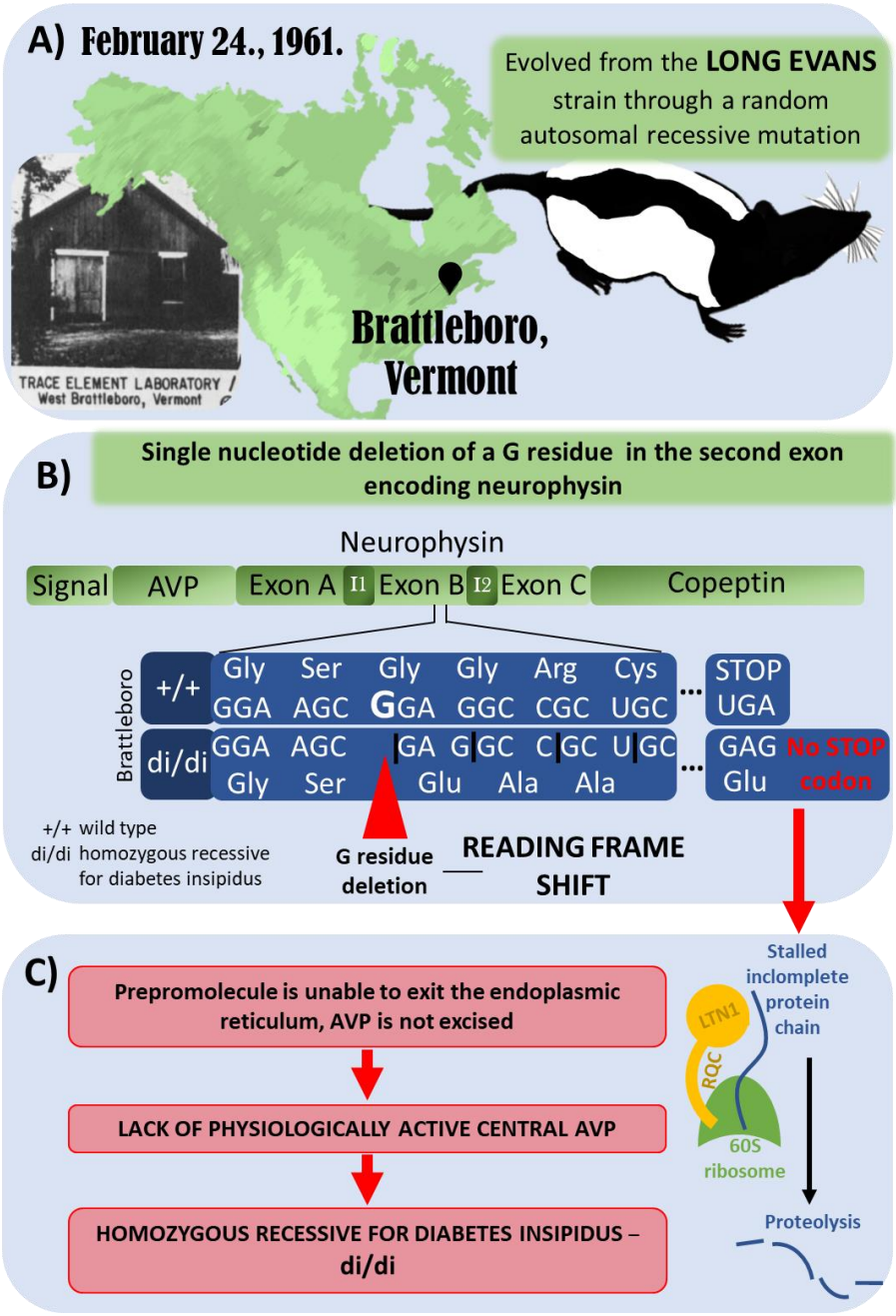

Figure 2. Development of the Brattleboro rat strain. A) Brattleboro rat strain was discovered in 1961 in Brattleboro, Vermont. It evolved from the Long-Evans rat strain through a random autosomal recessive mutation. **B)** The genetic mutation of Brattleboro strain is a single nucleotide deletion of a G residue in the second exon of neurophysin gene. For that reason, a reading frame shift develops, which results a different C-terminus for the precursor hormone. **C)** Due to the lack of stop codon, the mRNA cannot leave ribosomes after translation, and the incomplete protein chain also possibly stick in the ribosome. As a consequence, the ubiquitin-ligase (LTN1) part of the ribosome-associated quality control complex (RQC) will induce proteolysis. All in all, vasopressin (AVP) is not excised, which means there is no physiologically active central AVP. It has to be emphasized, that AVP is produced in a different way in the peripheral organs (e.g. colon, liver, kidney, testis), therefore, Brattleboro rats are not complete AVP-KO animals. However, their

blood lacks AVP (which is produced in magnocellular, neurosecretory hypothalamic cells), that is why central diabetes insipidus appears with polydypsia and polyuria. (Sawyer et al., 1964; Valtin, 1982; Schmale et al., 1984b; Schmale et al., 1984a; Defenouillere et al., 2016).

2.3.4 Complex models

For the preclinical examination of SCZ, it is most advantageous if -according to Engel's biopsychosocial model- all three risk factors are present in one animal model. Thus, genetic predisposition is associated with epigenetic changes and negative environmental challenges.

In this regard, postnatal vHC lesion can be combined with social isolation rearing, however, vHC and PCP combination resembles more the three hit theory (Jones et al., 2011).

A genetic model, if exposed to an early environmental challenge and therefore causing epigenetic changes in its nervous system, could be the most appropriate animal model for SCZ. This is why we widely investigated the AVP-deficient Brattleboro rat.

2.4 Vasopressin and schizophrenia

2.4.1 Vasopressin: main functions, receptor subtypes

AVP is a nonapeptide (it consists of nine amino acids); as a hormone it is released into the general circulation from the posterior pituitary and form the peripheral vasopressinergic system, also called hypothalamic–neurohypophysial system (Csikota et al., 2016). This is the main regulator of water resorption in the kidney, playing an important role in salt-water homeostasis. However, AVP may be released within the brain forming the central vasopressinergic system and regulating the activity of other neurons. In this regard many other, parvocellular neuron population is important. This latter system is implicated more in circadian regulation, anxiety, depression, learning and memory, as well as social behavior.

AVP may target three different receptor-types: V1a (vascular) and V1b (hypophyseal) utilize the Gq-phospholipase C pathway, while V2 (kidney) is operating through Gs-adenylate cyclase pathway. In mice and rats oxytocin (OXT) and AVP receptors are not as selective for their ligands as in humans, there is a crosstalk between these systems (Sala et al., 2011). Beside classical agonists and antagonists, pharmacological chaperons can

modify AVP effect (Mouillac et al., 2014). These cell permeable chaperons may help to restore the plasma membrane location and function of a possible mutated receptor.

V2 receptor (V2R) is the predominant form in the kidney regulating salt-water homeostasis, while V1bR can be found primarily on the anterior lobe of the pituitary being implicated in stress-axis regulation. V1aR is the dominant form in the central nervous system being associated with various behavioral and cognitive effects (Young et al., 1999a). In most species this receptor subtype can be found in amygdala, bed nucleus of stria terminalis (BNST), lateral septum (LS), hypothalamus and brainstem. In Rhesus monkey both the mRNA (in situ hybridization) and ligand binding (autoradiography) was detected on several cortical sites including PFC, cingulate, pyriform-, and entorhinal cortex, as well as on presubiculum and mamillary bodies (Young et al., 1999a). Further studies confirmed the wide distribution of V1a receptor (V1aR) also in rat cortical areas (Yamazaki et al., 1997).

However, it is hard to interpret results of animal studies related to AVP levels, because there are quite huge differences between species in AVP synthesis and receptor distribution in the brain. For example, in most of the rodents AVP positive neurons were found in BNST and medial amygdala (MeA), but not in Syrian hamsters (Caldwell et al., 2016). In this later species there is no AVP synthesis in parvocellular neurons, but magnocellular neurons –beside the posterior pituitary- project also centrally (Ferris et al., 1992). In vertebrates, species-specific OXT- and AVP-like receptor effect had been also observed on modulation of the dominant forms of sociosensory processing. For example, rodents' olfaction is the dominant mode of sociosensory processing, while in primates vision and audition are the dominant ones (Johnson et al., 2017). That could be one reason why the results are sometimes contradictory between different studies.

2.4.2 Vasopressin expressing brain areas implicated in schizophrenia

Vast majority of AVP is synthesized in two magnocellular cell groups, in the supraoptic nucleus (SON) and in the paraventricular nucleus of hypothalamus (PVN) (Iovino et al., 2016) (**Figure 3.**). The magnocellular neurons of these nuclei send their axons to the posterior pituitary, where the neuropeptide is stored and released to the general circulation and act as a hormone (its contribution to SCZ see 1.4.1.). However,

the same neurons might secrete AVP from their soma and dendrites to the extracellular fluid (Ludwig et al., 2005) called ‘intranuclear release’ or ‘intra-hypothalamic release’ (Douglas et al., 1994; Ludwig et al., 1998). This intranuclearly released neuropeptide might reach adjacent areas (e.g. LS, MeA) through volume transmission in physiologically relevant concentrations and regulate neuronal activity and thereby behavior (Engelmann et al., 2004; Landgraf et al., 2004; Albers, 2015; Ludwig et al., 2015).

Parvocellular neurons might also synthesize AVP. In the medial part of PVN AVP

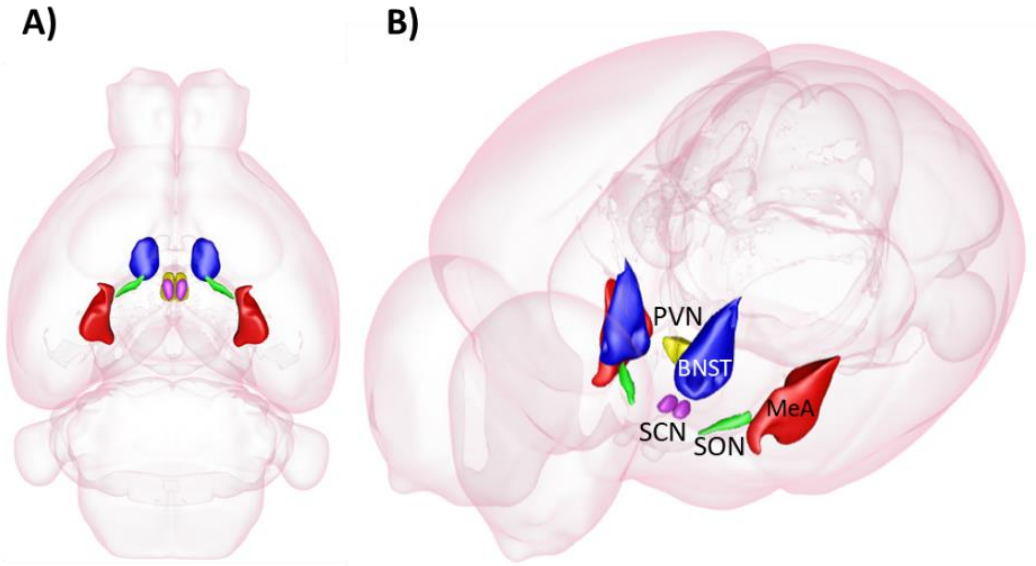

Figure 3. Vasopressin-producing brain regions in rodent. A) bottom view; **B)** side view. MeA (red): medial amygdala, BNST (blue): bed nucleus of stria terminalis, PVN (yellow): paraventricular nucleus, SON (green): nucleus supraopticus, SCN (purple): nucleus suprachiasmaticus; Source: (Török et al., 2019); https://scalablebrainatlas.incf.org/composer/?template=ABA_v3

might be co-localized with corticotropin-releasing hormone (CRH) and they reach the median eminence and the long portal vessels together and can interact on the anterior pituitary to regulate the adrenocorticotropic hormone (ACTH) secretion, the hypophyseal component of stress regulation (Raadsheer et al., 1993). However, this AVP is implicated more in the development of depression, not in development of SCZ (Zelena, 2012b).

One of the first discovered neurotransmitter of suprachiasmatic nucleus (SCN) was also the AVP, confirmed later in many species, including humans (Kalsbeek et al., 2010). Neuronal activity of AVPergic cells of the SCN shows daily variation and may control neuroendocrine (stress and gonadal axis) and other (e.g. sleep-wake) rhythmic changes

(Li et al., 2009). Although disturbances of this endogenous clock is more typical to depression, but was also implicated in SCZ (Trbovic, 2010). Its importance is supported by jet-leg induced exacerbation of psychosis and seasonal changes in the prevalence of psychotic episodes.

Another brain area synthesizing AVP is the amygdala, especially the medial part (MeA) (Rood et al., 2011). This area is important in regulating social behavior. Human studies have suggested that AVP modulates medial PFC-amygdala circuitry during emotion processing (Zink et al., 2010). Vasopressinergic neurons can be found in other areas of the social behavior neural network including BNST and LS. There is a negative correlation between AVP mRNA levels in BNST and social play in male juvenile rats (Paul et al., 2014). The LS seems to have the most dominant role in regulating social recognition and social memory through AVP (Liu et al., 2001; Gabor et al., 2012). Administration of AVP antagonist into LS impaired social memory, but did not affect object or spatial memory (Bielsky et al., 2005). Thus, AVP receptors in LS may have a specific effect on memory processes. Individual recognition is regulated by AVP projections from MeA and BNST to LS (Bluthe et al., 1990).

In the temporal cortex, which has a critical role in the development of auditory hallucinations in SCZ, Frederiksen et al. found lower AVP levels in patients compared with controls. Surprisingly, differences in the hypothalamus were not found (Frederiksen et al., 1991).

2.4.3 Possible effects of vasopressin on schizophrenia symptoms

2.4.3.1 Positive symptoms

Animal studies have suggested that chronic administration of an AVP analogue, NC-1900 decreased NMDA receptor antagonist-induced hyperlocomotion, the only behavior which is accepted as a model of hallucinations (Matsuoka et al., 2005). Although there is no study which suggests that AVP level may contribute to the development of positive symptoms of SCZ in humans, Rubin et al. have found that in untreated SCZ women, serum AVP is associated with more prominent positive symptoms (Rubin et al., 2013).

2.4.3.2 Negative symptoms

Many studies suggested that AVP may contribute to the appearance of negative symptoms, such as anxiety, mood disruption, social withdrawal and sleep disturbances.

Indeed, JNJ-17308616, a V1aR antagonist, reduced maternal separation-induced USV in 11-day-old rats (Bleickardt et al., 2009). Moreover, this maternal separation-induced USV correlated with the hypothalamic AVP levels in rats and mice selectively bred for low (LAB) and high (HAB) anxiety-related behavior based upon their behavior on elevated plus maze (EPM) (Lukas et al., 2015). This USV was consider as a sign of anxiety. In this regard, V1aR KO mice exhibited reduced anxiety (Bielsky et al., 2004; Egashira et al., 2007) and V1aR antagonist was found to be anxiolytic (Bleickardt et al., 2009). Moreover, in the previously mentioned PPI test the V1aR gene has an important role in response to auditory stimuli (Levin et al., 2009).

Elevated levels of AVP (in the plasma as well as in PVN and SON; both at mRNA and protein levels) are associated with higher appearance of clinical depressive symptoms (Zelena, 2012b; Kormos et al., 2013; Csikota et al., 2016). Therefore, AVP antagonist were develop with the hope to treat depression (Holmes et al., 2003; Griebel et al., 2005). Despite many positive preclinical data, clinical efficacy was far beyond the expectations (Griebel et al., 2012). Nevertheless, these drugs were developed against V1bR, however – as mentioned before – V1aR might more implicated in the development of SCZ-like symptoms.

Indeed, AVP receptors have been found on all of the brain areas of social behavior neuronal network as well as on mesocorticolimbic dopamine system (Caldwell et al., 2016). These networks control social behavior in mammals according to the hypothesis of Newman (Newman, 1999). Indeed, olfactory bulb, an important player of social recognition contains AVP as a main regulator (Tobin et al., 2010; Wacker et al., 2010). In hamster, AVP injection into the medial preoptic-anterior hypothalamus, LS, BNST or periaqueductal gray area increased, while V1aR antagonist decreased territorial marking (Hennessey et al., 1992), supporting the role of this receptor subtype in social behavior. Interestingly, gonadal hormones (especially testosterone) may influence AVP effect on flank marking (Albers et al., 1995). In prairie voles, the best studied social animal model, septal AVP fiber density showed alteration according to the paternal behavior and V1aR

antagonist reduced the appearance of paternal responsiveness (Wang et al., 1994). Moreover, central infusion of AVP increased partner preference which was antagonized by a V1 antagonist (Winslow et al., 1993b). All in all, AVP is implicated in the regulation of social behaviors and may underlie the species differences of *monogamous* prairie *vole* (Microtus ochrogaster) and the *polygamous* montane *vole* (Microtus montanus) and different life strategy (Wang et al., 1998). In respect to a special form of social behavior, aggression is also regulated by AVP (Haller, 2013). Moreover, AVP has a very important role also in human social behavior. It regulates aggression, pair-bonding as well as stress-responsiveness (Heinrichs et al., 2009).

Regarding sleep regulation, the AVP-content of the SCN, the central circadian clock is remarkable. The correlation between the lack of AVP in the SCN and a deterioration of sleep-wake rhythms makes it likely that AVP contributes to sleep disturbances detectable also in SCZ patients (Kalsbeek et al., 2010). Indeed, in a mouse model of SCZ (blind-drunk: Bdr, see earlier) disturbed AVP rhythms in the SCN contributed to the phase advanced and fragmented circadian rhythms, resembling disturbed sleep pattern in SCZ (Oliver et al., 2012). Moreover, V1aR KO mice show reduced circadian rhythmicity of locomotor activity (Li et al., 2009). In healthy humans AVP administration (intranasal, sometimes intravenous) was consistently found to increase the mismatch negativity, an important event related potential linked also to SCZ (see 1.4.1.) (Born et al., 1998).

2.4.3.3 Cognitive symptoms

The contribution of AVP to memory formation was suggested already in 1966 by de Wied and Bohus, who found that posterior pituitary extract lead to prolonged memory retention in a passive avoidance paradigm (de Wied et al., 1966). Later studies confirmed that AVP facilitates memory processes (de Wied, 1976; Kovacs et al., 1979), however, one author found intraventricular injection of AVP to be ineffective in a passive avoidance task (Sahgal et al., 1982). Antagonizing the V1aR results in memory impairment both in mice (Bielsky et al., 2004) and in rats (Nephew et al., 2008). As memory disturbance is a key abnormality of Alzheimer's disorder, it is not surprising, that a significant AVP decrease has been found in the cerebrospinal fluid (CSF) (Mazurek et al., 1986b) and in many brain regions of Alzheimer's patients (Mazurek et al., 1986a;

Goudsmit et al., 1992). A study in rats found centrally administered AVP being protective against amyloid-beta protein-induced memory decline in the MWM (Pan et al., 2013), further supporting the positive effect of centrally released AVP on memory formation.

The memory enhancing effect of systemically administered AVP may be secondary due to elevation in blood pressure (Koob et al., 1989). However, a direct, central effect may be additive to this, most probably through the LS (Engelmann et al., 1994a). Dense AVPergic innervation of the HC may also underlie the behavioral role of AVP (Cilz et al., 2019).

In respect to SCZ Aydin et al. did not find any AVP level differences between SCZ and healthy controls in connection with cognitive tasks, but we have to add that these patients were on antipsychotics (Aydin et al., 2018). Indeed, another research group have found that antipsychotics can normalize plasma AVP levels in patients with SCZ, which could explain the previous results (Raskind et al., 1987). In contrast, Rubin et al. examining untreated patients have found that AVP is also important in cognitive functioning, however, only in women (Rubin et al., 2013). We have to mention that there are contradictory results in CSF samples: there was no difference in CSF AVP concentrations between SCZ with and without neuroleptics and healthy controls (Beckmann et al., 1985).

2.4.4 Treatment with vasopressin and analogues

As early as 1937 SCZ patients were treated with an AVP analogue, pitressin (intramuscular) (Forizs, 1952a, b; Cross et al., 1982). Symptoms were improved in 40% of patients resulting in a more social and interested attitude and patients could leave the clinic. In another study lysine-8-vasopressin was used for 3 weeks and decreased the thinking disorder, but agitative and aggressive side-effects were observed (Korsgaard et al., 1981). However, in a similar study this disorientation occurred only in few patients with beneficial effects on emotional withdrawal (Vranckx et al., 1979). Later Iager et al. demonstrated that chronic treatment with desmopressin (1-desamino-8-D-arginine vasopressin: DDAVP) (3-month, double-blind, placebo-controlled trial on 10 patients), another AVP analogue, could improve the Negative Symptom Rating Scale total score and cognitive functions (Iager et al., 1986). Later on a 20 days DDAVP treatment (on 10

patients again) was also effective on negative symptoms and partly on memory disturbances as well (Brambilla et al., 1989). This effect was found to be central as several peripheral parameters including electrolytes and blood pressure was unchanged during the therapy (Brambilla et al., 1986). Intranasal administration of AVP (400 ng, 4 times per day) improved the positive and negative syndrome scale and memory in first-episode SCZ patients (Geng et al., 2017). Beside these, synthetic AVP has improved the condition of paranoid SCZ patients (Bakharev et al., 1984).

The complexity and time course of learning and memory processes and the influence of other factors (anxiety, attention etc.) make it unlikely that a single peptide, like AVP will obviously influence all aspect of this process (Jolles, 1983). Therefore, it is somewhat surprising that AVP administration to healthy humans increased one or other aspect of memory processes in many studies with only few exceptions (25 vs 7 article, for details see Table 1 in (Born et al., 1998)).

Animal studies have suggested that a single subcutaneous or intraventricular administration of AVP increased memory performance in rats (de Wied, 1976). Furthermore, in a pharmacological SCZ rat model chronic administration of an AVP analogue improved social behavior and decreased NMDA receptor antagonist-induced hyperlocomotion, the only behavior which is accepted as a model of hallucinations (Matsuoka et al., 2005). Interestingly, a 1999 study described the importance of AVP in social behavior: mice that are transgenic (knock-in) for the highly social, monogamous prairie vole V1aR gene showed increased affiliative behavior after icv. AVP administration (Young et al., 1999b). For summary see **Figure 4**.

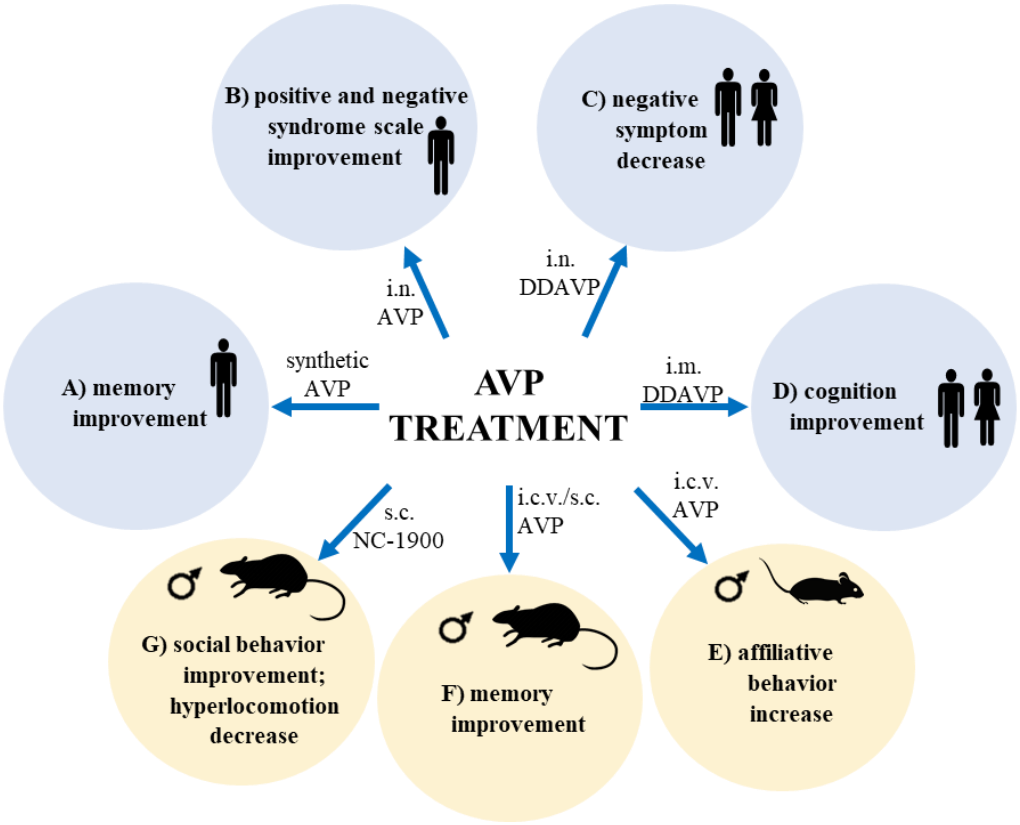

Figure 4. Effects of treatments with vasopressin or vasopressin analogues. Abbreviations: AVP: arginine-vasopressin; DDAVP: 1-desamino-8-D-arginine vasopressin; NC-1900: AVP analogue; V2R: vasopressin receptor type 2; i.c.v.: intracerebroventricular; i.m.: intramuscular; i.n.: intranasal; s.c.: subcutaneous (administration); References: **A)** (Bakharev et al., 1984) **B)** (Geng et al., 2017) **C)** (Iager et al., 1986; Hosseini et al., 2014) **D)** (Brambilla et al., 1986) **E)** (Young et al., 1999b) **F)** (de Wied, 1976) **G)** (Matsuoka et al., 2005)

2.5 Animal models of schizophrenia and vasopressin

Several studies confirmed the contribution of this nonapeptide to SCZ-like behavior of rodents. However, the outcome may highly depend on gender and early environmental manipulations (Perkeybile et al., 2015), and can be different (sometimes even opposite) depending on which brain area the intervention takes place (Ruscio et al., 2007). A summary about animal models of SCZ and its association with AVP can be found in **Table 2**.

2.5.1 Neurodevelopmental models

In case of poly (I:C)- induced *MIA* model of SCZ the susceptible mouse strain showed elevation of V1aR mRNA levels in their hypothalamus (Morais et al., 2018).

Interestingly, maternal *MAM* treatment induced amnionic AVP elevation, but it is thought to be connected only with growth retardation, not with development of psychotic symptoms (Oosterbaan et al., 1985).

On the other hand, prenatal stress (restraint during late pregnancy) with long term SCZ-like consequences (e.g., social deficit) is accompanied by a decrease in the number of AVP positive cells of the PVN (but not of the SON) (de Souza et al., 2013). Moreover, after maternal social stress during late pregnancy, the social deficit of female offspring went parallel with BNST and LS AVP mRNA decrease without changes in males (Grundwald et al., 2016).

vHC is known to regulate the stress axis, including the AVP secretion of the PVN (Herman et al., 1992). Indeed, *lesion of vHC* resulted in enhanced AVP and stress hormone secretion to auditory stressor (Mitchell et al., 2004), as well as blunted negative hypothalamic pituitary adrenocortical (HPA) axis feedback (Goursaud et al., 2006); similar phenomenon was observed in hyponatremic SCZ patients (Goldman, 2009). Moreover, HC got AVPergic innervation from the MeA. This might be the anatomical background why AVP injection into the vHC alleviated forgetting, and immunoneutralization of endogenous AVP on the same site resulted in impairment of retrieval and relearning (Metzger et al., 1993). However, other authors found that dorsal HC is more implicated, as its lesion, as well as local AVP immunoneutralization prevented the memory enhancing effect of intracerebroventricular AVP injection, an effect utilizes V1 receptors (Alescio-Lautier et al., 1998).

Social isolation rearing induces profound changes in the AVPergic system of prairie voles, but not that much in the sister nonapeptide OXT (Ruscio et al., 2007). Specifically, AVP content of the PVN was decreased, while in SON, the main source of AVP secretion, was increased in socially isolated animals compared to control. Moreover, their V1aR expression showed also site-specific alterations (Hiura et al., 2018). Enhanced anxiety of socially isolated prairie voles was accompanied by enhanced AVP expression in their PVN (Pan et al., 2009). Interestingly, this is in contrast to their lower AVP content (see earlier) and with rats, where social isolation-induced anxiety was contrasted by reduced AVP immunoreactivity in PVN. However, this later observation was true for the parvocellular subdivision, while the mRNA increase was detected mainly above the magnocellular cells, known to be involved in peripheral AVP release rather than HPA

axis regulation (Tanaka et al., 2010). A further link between social isolation rearing and AVP is the postweaning social isolation-induced polydipsia in adult, food restricted rats (Hawken et al., 2013).

2.5.2 Pharmacological models

There is a general knowledge, that glutamate – among others– through its NMDA receptor (the main target of pharmacological SCZ models) can influence the synthesis and release of AVP (Morsette et al., 1998).

PCP (2mg/kg/day for 14 days intraperitoneally) reduced V1aR binding in several brain regions together with impaired social interaction (Tanaka et al., 2003). Higher doses of PCP (10mg/kg subcutaneously) may also induce diuresis through a fall in AVP plasma levels (Zerbe et al., 1983). However, this later interaction might be indirect, as AVP decrease might triggered directly by a rise in blood pressure.

The AVP analogue NC-1900 ameliorated the *MK-801*-induced hyperlocomotion and social deficit (Matsuoka et al., 2005). Interestingly, this interaction was bidirectional, as MK-801 attenuated the NC-1900-induced prolongation of step-through latency in passive avoidance task (Sato et al., 2005). Similar results were found with AVP and MK-801, the later attenuating the positive effect of AVP on consolidation of conditioned avoidance responses and on retrieval of memory in passive avoidance situation (Wisniewski et al., 1996; Artemowicz et al., 1998). Nevertheless, these results confirm the role of AVP in the development of SCZ-like symptoms after MK-801 administration.

Ketamine, the widely used anesthetic is known to induce SCZ-like behavior as well (Becker et al., 2003; Haaf et al., 2018) and AVP-producing SON cells may contribute to this effect (Nissen et al., 1994).

2.5.3 Genetic models

Disruption of *DISC1* in male (but not in female) mice – beside all the symptoms of SCZ-like behavior- lead to reduction in hypothalamic V1a mRNA levels (O'Tuathaigh et al., 2017). Contradictory, co-deletion of NRG1 with DISC1 eliminated the observed changes. Interestingly, NRG1 is co-localized with AVP in SON, median eminence and pituitary stalk (Zhao et al., 2015). In a preclinical study with NRG-1 and DISC1 mutant mice, disruption of DISC1 reduced hypothalamic mRNA expression of V1a, but just in

males. Surprisingly, there were no correlations between disruption of NRG1 and AVP levels (O'Tuathaigh et al., 2017).

The SNAP-25 deficient Bdr mice has disturbed AVP rhythms as mentioned before (Oliver et al., 2012).

Although there is no data about AVP levels in other KO animals, clear interaction exists between the implicated genes and AVP. For example, α7-nAChR receptor (Hatton et al., 2002), as well as BDNF (Ohbuchi et al., 2009) are important modulators of neuronal activity in SON, known to secrete AVP. Additionally, a bidirectional interaction between reelin and AVP was also supposed (Carter, 2007).

V1aR KO mice did not show any sign of hyperlocomotion (Egashira et al., 2009). In contrast, the probability to move during a 60 min observation period is even lower than that of the wild type counterparts supported also by central V1aR antagonist injections (Masuki et al., 2013). The circadian pattern of the locomotor activity is also reduced (Li et al., 2009). However, these mice reveal impaired anxiety (EPM, marble burying, startle) together with impaired social interaction (Egashira et al., 2007) and social recognition (Bielsky et al., 2004), PPI and spatial learning deficit (not in the MWM, but in the 8-arm radial maze test (Egashira et al., 2004)) (Egashira et al., 2009). The anxiolytic phenotype suggest a direct role of V1aR on social interaction not confounded by anxiety (Egashira et al., 2009). Altogether, this mouse strain can be a good model of SCZ.

V1bR KO mice have normal locomotion, anxiety, and learning abilities (Egashira et al., 2009). In respect to some SCZ-like symptoms social and PPI deficit can be detected. The PPI disruption was even restored by atypical antipsychotics (Egashira et al., 2005), therefore, this model could be also important in the preclinical studies of SCZ.

2.5.3.1 Vasopressin-deficient Brattleboro rats

Similarly to cortical and hippocampal atrophy in SCZ patients, there is also a brain atrophy in di/di **Brattleboro rats** (Boer et al., 1982; Greer et al., 1982).

As a possible positive symptom, Williams et al. described hyperlocomotion in AVP-deficient Brattleboro rats (Williams et al., 1983, 1985), however, only females were studied in this experiment, and they used Long Evans rats as controls, which may be genetically distant from di/di rats. Schank found this hyperlocomotion already in 10-day-old pups both in comparison to normal or heterozygous rats (Schank, 2009). However,

none of the studies were investigated the possible molecular mechanisms, therefore there is still no data if glutamatergic system is responsible for this phenomenon in AVP-deficient Brattleboro rats. Thus, no parallel can be drawn between NMDA receptor antagonist-induced hyperlocomotion in pharmacological animal models and increased locomotion of vasopressin-deficient Brattleboro rats; further studies are needed to explore this interesting phenomenon.

Furthermore, in our previous studies we did not find any difference between AVP-deficient and heterozygous animals in open field or rotarod tests (Mlynarik et al., 2007) as well as in comparison with +/+ animals during a 24h telemetric observation (Demeter et al., 2016). We have to add, however, that ketamine-induced hyperactivity was lower in our AVP-deficient rats, too (unpublished data).

As a sign of sensorimotor gating deficit PPI disturbances were repeatedly described in this strain. First suggestion came from Feifel et al. who could reverse PPI deficit of Brattleboro rats with acute and subchronic haloperidol (Feifel et al., 2001), clozapine (Feifel et al., 2004) and risperidone (Feifel et al., 2007) treatment and found even dopamine receptor disturbances in the brain of Brattleboro rats as a possible contributing mechanism (Shilling et al., 2006). Later, researchers of the Glaxo Smith company repeated these results (Cilia et al., 2010) and confirmed this strain as a good model of SCZ. However, we have to mention, that PPI models just a small area of SCZ symptomatology and can be altered in other disorders as well e.g., obsessive compulsive disorder and Gilles de la Tourette's syndrome (Kohl et al., 2013).

As a negative symptom, maternal separation-induced USV is diminished in Brattleboro rats (Zelena, 2018). This might be interpreted as a sign of reduced anxiety (Varga et al., 2015). Indeed, this strain shows signs of reduced anxiety and depression (Williams et al., 1985; Mlynarik et al., 2007; Csikota et al., 2016; Fodor et al., 2016b). Feifel et al. also described social withdrawal and memory dysfunction as negative symptoms in Brattleboro rats (Feifel et al., 2009). The social discrimination problems were repeatedly described (Engelmann et al., 1994a; Demeter et al., 2016) not only in males, but also in females (Fodor et al., 2016a) and was reversed not only by antipsychotic treatment (Feifel et al., 2009), but also by AVP administration into the LS (Engelmann et al., 1994a). The social deficit was already detectable in adolescent rats (Schatz et al., 2018) and even in younger animals, the 10-day-old pups (Schank, 2009).

The effect of AVP on aggressiveness is brain area specific. Therefore, it is not surprising, that the complete lack of AVP in Brattleboro rats did not affect aggressiveness in reproductively experienced males, however, it decreased the share of violent attacks in reproductively inexperienced males without affecting total attack counts suggesting a specific role of AVP in violent aggression (Fodor et al., 2014).

Another negative symptom should be the lower sleep rhythm amplitudes of Brattleboro rats (Brown et al., 1989).

In relation to cognitive symptoms, there is a global memory impairment in Brattleboro rats (e.g., active avoidance in shuttle box, novel object recognition (NOR)) (Varga et al., 2014), which is not present in females, only in male rats (Fodor et al., 2016b).

These changes resembling clinical symptoms in SCZ patients are likely due to the lack of AVP in di/di rats leading to developmental disturbances as well as a chronic stress-like state due to the constant thirst. All in all, this model may be the most suitable to explore the broad role of this neuropeptide in SCZ-like disturbances covering "two-hit" of the Engel's biopsychosocial model (see **Figure 1**.).

DOI:10.14753/SE.2022.2647

3 Objective

Our main aim was to confirm the role of centrally released AVP in the development of SCZ-like behavior using AVP-deficient (di/di) Brattleboro rat.

First (**Exp. 1**) to ensure predictive validity of the model we investigated if antipsychotic treatment (acute, subacute or chronic) normalize SCZ-like behavioral alterations (memory impairment, social avoidance, PPI-deficit) in adult animals (Torok et al., 2021a).

Rat pups, due to their more permeable blood-brain-barrier (BBB) and low body weight seem to be a good model for novel antipsychotic testing. In relation to SCZ maternal separation-induced ultrasonic vocalization (MS-USV) can be considered as a symptom of reduced communication. We tested if the reduced MS-USV of di/di pups can be improved by antipsychotic treatment (**Exp.2**) (paper is under evaluation in Neuropsychopharmacology). Sedative (by righting reflex and negative geotaxis) and stress hormone elevating (ACTH, corticosterone) side effects were excluded. Next (**Exp.3**) the possible contributing AVP receptor type was investigated in MS-USV (Torok et al., 2021b).

To study possible brain mechanisms, we investigated (**Exp.4**) the contribution of magnocellularly released AVP in social behavior using AVP rescue in SON by adeno-associated viral vector (paper is under evaluation in Frontiers in Endocrinology). As another possible mechanism (**Exp.5**) epigenetic changes (histone AcH3K9 modification) were also examined in the PFC, nucleus accumbens, LS and HC of the Brattleboro rat brain (Demeter et al., 2016).

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4 Methods

4.1 Subjects

Brattleboro rats were maintained at the Institute of Experimental Medicine in a colony started from breeder rats from Harlan, Indianapolis, IN, USA.

We compared 56-70 days old male AVP-deficient di/di with homozygous (+/+) control rats (**Exp. 1, 4 and 5**). For details of the breeding see (Zelena et al., 2009c). Rats were housed 2 animals/cage under standard laboratory conditions (temperature: 23±1°C; relative humidity: 50-70 %) with rat chow and tap water available ad libitum. In Experiment 4, animals were housed isolated for safer healing of surgical wound.

In **Exp. 2 and 3** we compared 7-8-day-old Brattleboro pups from the same litters with homozygous AVP deficient (di/di) fathers and heterozygous (di/+) mother rats; in this way di/+ and di/di rats were born (Bohus et al., 1998; Zelena et al., 2003a). The litter size was not controlled and in this case the gender was not taken into consideration. The genotype of the pups was assessed after the experiment upon the AVP content of the pituitary by radioimmunoassay. In **Exp. 3** Wistar rats were also used: they were purchased from Charles River (Budapest, Hungary) and were mated in the local animal facility. Pups were housed with the dam in their home cage under standard laboratory conditions (temperature: 23±1°C; relative humidity: 50-70 %). The day/night schedule was 12/12h, with lights on at 07:00h.

All experiments were carried out between 10:00 and 14:00 in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and were reviewed and approved by the Animal Welfare Committee of the Institute of Experimental Medicine, Budapest, Hungary.

4.2 Experiment 1.: Do antipsychotic drugs can improve behavioral deficits in Brattleboro rat?

4.2.1 Manipulations

Rats were injected subcutaneously with an antipsychotic drug (for summary of the properties see **Table 3.**) or vehicle every day for 15 days. On the 1st (D1, acute), 8th (D8, subacute), and 15th (D15, chronic) experimental days rats were injected 30 minutes before the behavioral tests. Different doses were used for *aripiprazole* (5 mg/kg; Gedeon Richter Plc., Budapest, Hungary), *clozapine* (1 mg/kg; Sigma Chemical Co., St. Louis, MO),

haloperidol (0.1 mg/kg; Sigma Chemical Co., St. Louis, MO), *olanzapine* (0.3 mg/kg; Gedeon Richter Plc., Budapest, Hungary), and *risperidone* (0.25mg/kg; Sigma Chemical Co., St. Louis, MO) (n~6-8 in every group). Aripiprazole was dissolved in 5% acetic acid-saline solution while other drugs in 50 µl of 1N HCl per ml of saline solution and titrated with 3N NaOH to pH 6-7. Control animals got vehicle (HCl or acetic acid solution).

4.2.2 Experimental design

Social discrimination was tested comparing control, haloperidol, clozapine, olanzapine, and risperidone treated animals, while aripiprazole treatment was done separately using appropriate controls. The five different treatment was conducted in five "shifted" series testing approx. 20 animals/day containing rats from each treatment groups while the injections of the series were overlapping. As there was no significant difference between the controls (HCl or acetic acid containing saline) during subsequent testing HCl containing vehicle was used only. Then - in line with other drugs - we studied the effect of aripiprazole instead of clozapine because this later antipsychotic drug has a similar receptor binding profile to olanzapine and has been extensively tested in previous preclinical studies. (Feifel et al., 2007; Cilia et al., 2010).

Social avoidance and PPI tests were conducted on the same day right after each other in control, aripiprazole, haloperidol, olanzapine and risperidone treated animals. To avoid influential effect of one test over the other, half of the animals underwent first social avoidance followed by PPI, while the other half was tested in PPI first.

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Table 3. Antipsychotic drug receptor binding profiles according to (Siafis et al., 2018) and applied drug concentrations compared with concentrations used in clinical practice. Abbreviations: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, 5-HT_7: serotonin receptor subtypes; α_1, $\alpha_{2A\text{-}2C}$: adrenergic receptors; $D1_{\text{-}4}$: dopamine receptor suptypes; $H_{1\text{-}3}$: histamine receptor subtypes; M_1, M_3: muscarinic receptor subtypes.

Antipsychotics	Antagonism or inverse agonism	Partial agonism	Concentration used in our experiment (/day)	Concentration used in rats according to the literature	Concentration used in clinical practice (/day)
Aripiprazole	D_4, H_1, 5-HT_{2B}, α_1, α_{2A}, α_{2B}, α_{2C}	D_2, D_3, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, 5-HT_7	5 mg/kg	0.1-5 mg/kg (Ganella et al., 2017; Ebrahimzadeh et al., 2019; Hereta et al., 2020)	5-30 mg (Topolov et al., 2016; Prommer, 2017)
Clozapine	D_1, D_2, D_3, D_4, H_1, H_2, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, 5-HT_7, M_3, α_1, α_{2A}, α_{2B}, α_{2C}	5-HT_{1A}, 5-HT_{1B}, M_1	1 mg/kg	0.1-15 mg/kg (Feifel et al., 2009; Cilia et al., 2010)	100-600 mg (Pardis et al., 2019)
Haloperidol	D_1, D_2, D_3, D_4, H_2, 5-HT_{1B}, 5-HT_{2A}, 5-HT_7, α_1, α_{2A}, α_{2B}, α_{2C}		0.1 mg/kg	0.1-1 mg/kg (Korpi et al., 1984; Gnegy et al., 1994; Halici et al., 2009; Ikemura et al., 2012)	3-30 mg (Lustig et al., 2005; Buchanan, 2007)
Olanzapine	D_1, D_2, D_3, D_4, H_1, H_2, H_3, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, 5-HT_7, M_3, α_1, α_{2A}, α_{2B}, α_{2C}	5-HT_{1B}, M_1	0.3 mg/kg	0.3-7.5 mg/kg (Zangrando et al., 2013; Vickers et al., 2015; Stanisavljevic et al., 2017)	2.5-25 mg (Cuesta et al., 2001; Keefe et al., 2007)
Risperidone	D_1, D_2, D_3, D_4, H_1, H_2, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_7, α_1, α_{2A}, α_{2B}, α_{2C}		0.25 mg/kg	0.06-1 mg/kg (Cilia et al., 2010; Celikyurt et al., 2011)	0.5-11mg (Cuesta et al., 2001; Keefe et al., 2007)

4.2.2.1 Social discrimination test

Social discrimination was performed in a new environment (plexi cage with bedding, 41.3 × 26 × 29.8 cm, GeoMaxi, Ferplast, Italy) (Varga et al., 2014). Subjects were habituated to the experimental cage for 1 h (**Figure 5.**). 25-30 days old male Wistar rats were used as stimulus animals. The test started with a 4 min sampling phase, when a juvenile (Stim1) was introduced to the test-cage. After 4 min Stim1 was removed and kept individually in a fresh cage with food and water ad libitum. Thirty minutes later - during the 4 min choice phase - test subjects were faced with the already familiar Wistar pup, Stim1, and an unknown, similar one (Stim2). Tests were videotaped and investigatory behavior was analyzed by an event analyzing software (H77) by an experimenter blind to treatment conditions. To allow the observer to distinguish between the two juveniles, one of them was marked with green lines (Edding 30 permanent marker, odorless, green, Edding AG, Germany) at least 30 min before testing. To exclude preference or aversion toward the marked animals we randomized the marking between Stim1 and Stim2. Investigatory behavior defined as direct action towards the juvenile rat including anogenital sniffing, hunting, licking, close pursuing and pawing. A significantly longer investigation duration of Stim2 versus Stim1 during choice phase is interpreted as a manifestation of an intact recognition memory (Engelmann et al., 1995). The discrimination index (DI) was calculated as follows:

DI = (time percentage Stim2 - time precentage Stim1)/(time percentage Stim 1 + time percentage Stim 2).

The result of the index may change between -1 and 1, where 0 = no discrimination. Normally, the animals spend more time with the new stimulus (novelty effect), thus, the index ≤0 is a sign of memory deficit.

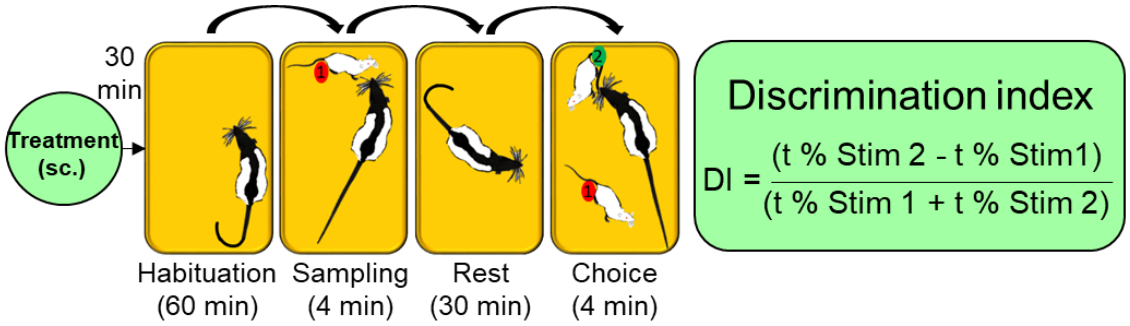

Figure 5. Experimental design of social discrimination test. Thirty minutes after antipsychotic treatment animals were habituated to the new environment for 60 minutes. During 4 min sampling phase, a juvenile was put into the cage where the experimental animal habituated. After 30 min rest, in the 4 min choice

phase the already known and an unknown juvenile was put into the cage. Discrimination index was calculated with the formula above.

4.2.2.2 Social avoidance test

Social avoidance test was introduced by our group as a measure of social anxiety (Leveleki et al., 2006) (**Figure 6.**). The test cage contained two subcompartments connected by a sliding door. The cages were open on the upper part; their walls were 40 cm high. The experimental animal was placed into the smaller cage (surface: 15 cm by 50 cm) for 3 min habituation. The larger cage (surface: 40 cm by 40 cm) was divided to two equal compartments by a transparent, perforated plastic wall. The distant compartment contained an unfamiliar, adult +/+ male rat. After the habituation period, the sliding door was removed, and the experimental animal was allowed to explore the small as well as large cages for 5 min. Plastic cages were cleaned with water after each test. The test apparatus did not permit physical contact between the experimental and stimulus animals. Subjects had a clear view of the stimulus rat when the door was removed, but not before. Behavior was videorecorded from above and analyzed by an experimental blind to treatment conditions. Three variables were recorded: the duration of visits made to the compartment containing the opponent, time spent with social interaction, and the latency of social interaction.

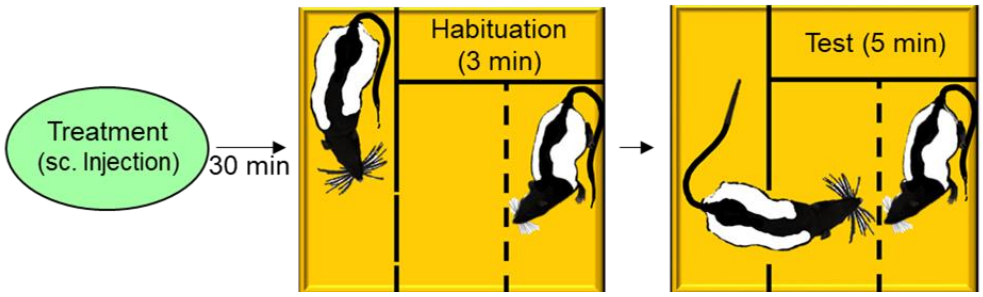

Figure 6. Experimental design of social avoidance test. Thirty minutes after treatment experimental animal was placed into a smaller cage (surface: 15x50 cm) for 3 min habituation. A larger cage (surface: 40x40 cm) was divided to two equal compartments by a transparent, perforated plastic wall. The distant compartment contained an unfamiliar, adult +/+ male rat. After the habituation period the sliding door was removed, and the experimental animal was allowed to explore the small as well as large cages for 5 min.

4.2.2.3 Prepulse inhibition (PPI) test

We performed the test as described previously (Fodor et al., 2016a) (**Figure 7.**). After a weight calibration, subjects were placed in a test cage inside a sound attenuated chamber (Acoustic Startle setup, Coulbourn Instruments, LLC). Following 5 min habituation, subjects were presented a 40 ms long, 120 dB acoustic stimuli ("noise", referred as pulse) for five times in every 20 s to standardize startle. Five trial types were then presented during testing: pulse alone, pulse preceded 80 ms by a 20 ms prepulse of varying intensity (73, 77 or 81 dB "tone"), and a trial with no prepulse but 0 dB pulse. Each trial-type was presented five times in a randomized order. The program automatically recorded the startle responses. Response to the 0 dB pulse was the weight of the subject and was subtracted from subsequent startle response data. Mean of the startle response to the 120 dB pulse without prepulse was calculated for every subjects and served as acoustic startle response. This response was considered 100%, from which PPI was calculated by the following formula: PPI = 100 – (startle after prepulse / startle without prepulse * 100). Mean PPI values were given for different prepulse intensities. In the present study the different prepulse intensity had no significant effect and did not modify the effect of genotype or treatment either, therefore we use the average of the three prepulse intensity for each day and treatment.

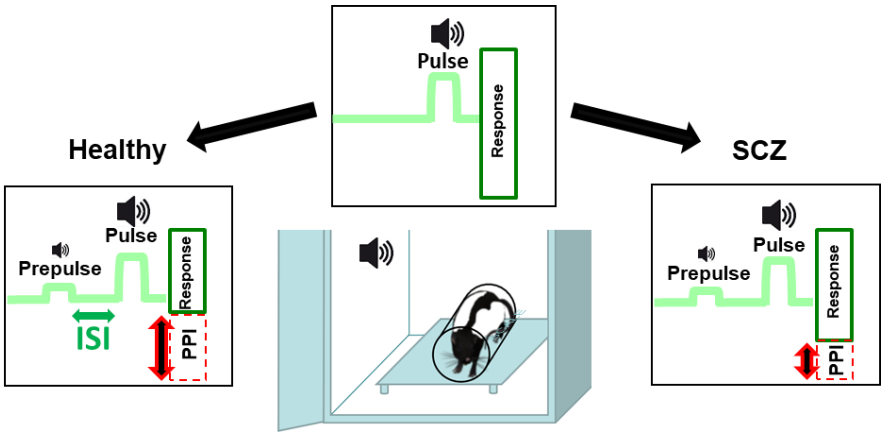

Figure 7. The prepulse inhibition (PPI) test. Subjects were placed into a test cage inside a sound attenuated chamber and startle response was measured with a scale. When animal get a 120 dB acoustic stimuli ("noise", referred as pulse), startle response can be detected. If a prepulse precedes pulse, this startle response decreases. The amount of decrease is the prepulse inhibition: prepulse inhibits the response to pulse. If schizophrenia (SCZ)-like behavior occurs, PPI is lower than in case of 'healthy' subjects.

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4.3 Experiment 2.: Ultrasonic vocalization measurement of Brattleboro rat pups during antipsychotic treatment

4.3.1 Manipulations

Pups were injected subcutaneously with 1µl/g antipsychotic drug or vehicle 30 minutes before the MS-USV test. Different doses were used for *haloperidol* (0.1 mg/kg, 1 mg/kg; Sigma Chemical Co., St. Louis, MO), *clozapine* (1 mg/kg, 10 mg/kg; Sigma Chemical Co., St. Louis, MO), *olanzapine* (0.3 mg/kg, 3 mg/kg; Gedeon Richter Plc., Budapest, Hungary), *risperidone* (0.25mg/kg, 1mg/kg, 0.05 mg/kg, 0.1 mg/kg; Sigma Chemical Co., St. Louis, MO) and *aripiprazole* (0.5 mg/kg, 5 mg/kg, 50 mg/kg; Gedeon Richter Plc., Budapest, Hungary). Haloperidol, clozapine, olanzapine and risperidone were dissolved in 50 µl of 1N HCl per mL of saline solution, while aripiprazole was dissolved in 5% acetic acid-saline solution and titrated with 3N NaOH to pH 6-7. Control animals got vehicle (HCl or acetic acid).

4.3.2 Experimental design

4.3.2.1 Maternal separation-induced ultrasonic vocalization

On the day of the study 7-8-day-old rat pups and their dam were moved from the animal housing room to another room and left undisturbed for at least 1 h before initiating the behavioral test. In order to minimize maternal effects, pups from the same litter were randomly assigned to treatments. Pups from at least three different mothers were used for each experiment. Pups received an injection of the drug or vehicle (1µl/g) one after the other in every 12 min, they were marked and returned to the dam and littermates. Thirty minutes after the administration one pup was brought to a soundproof room and placed in a 2l glass beaker without bedding and heating. USV was measured for 10 min. Individual calls during this period were detected using an ultrasound-sensitive frequency division detector (CDB205, CIEL Electronique, Nice, France) fixed on a holder 25 cm above the bottom of the glass beaker coupled to a computer. The distance between the USV detector and the beaker was 10 cm. Vocalizations were recorded using a free Audacity 2.0.5. software and stored on a personal computer. In previous studies, the large

portions of ultrasonic vocalizations emitted by 8-day-old rats were found from 30 kHz to 50 kHz (Allin et al., 1971; Blumberg et al., 2000). Thus, signals were filtered and the power spectrum was analyzed ranging from 30 kHz to 50 kHz. Data were automatically counted using a Rat Call Counter software (developed by S. Zsebők). The threshold value was set at a signal amplitude of 0.4 V to exclude background noise. Total number and total duration of calls per session were measured. In addition, USV frequency was calculated as the total number /10 min (**Figure 8.**).

4.3.2.2 Righting reflex

Immediately after MS-USV, *righting reflex latency (RRL) test* was used for investigating sedative effect of the drugs (Zelena et al., 2009a). Offspring were placed into an unusual supine posture on a smooth, flat surface and the latency to return the normal upright position with all four feet on the table was measured manually. Each animal was allowed a maximum of 30 s to return to prone position (**Figure 8.**).

4.3.2.3 Negative geotaxis

Negative geotaxis test investigated impaired motor coordination. After RRL measurement, offspring were placed on a 45° inclined foam-rubber board with their nose pointing downward and its latency to turn 180° to an upright position was recorded in seconds. Each animal was allowed a maximum of 60 s on the apparatus to complete the task. If the rat fell off the plane, the rat was considered to have failed the task and a maximum score of 60 s was assigned (**Figure 8.**).

Both tests were repeated three times at the termination of the measurement of USV and the mean of the three measurements was taken as a typical parameter.

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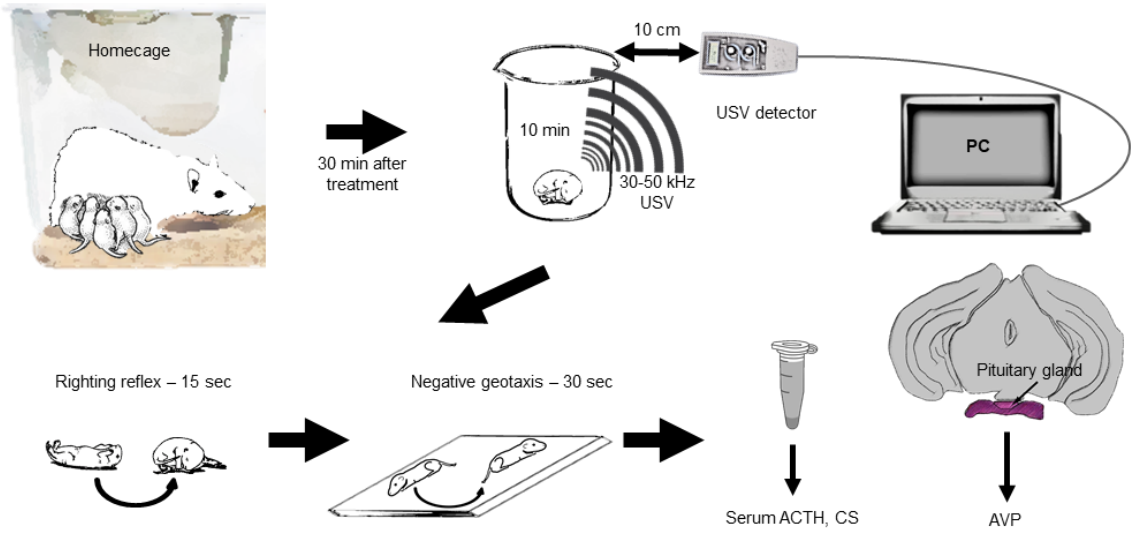

Figure 8. Experimental workflow. Abbreviations: USV: ultrasonic vocalization; AVP: arginine vasopressin; ACTH: adrenocorticotropic hormone; CS: corticosterone (own figure)

4.3.3 Hormone measurements

Pups were immediately decapitated after behavioral measurements and blood was collected on ice-cold Eppendorf tubes and centrifuged at 3 000 rpm for 30 min at 4 ˚C. Serum was stored at -20 ˚C till the hormone assay. From serum samples ACTH and corticosterone concentrations were measured by specific radioimmunoassay (RIA) without previous extraction. Both antibodies were developed in our Institute as described elsewhere (Zelena et al., 1999; Zelena et al., 2003b). The intra-assay coefficients of variations were 4.7% and 7.5%, respectively for the two hormones.

The hypophysis of the pups was also collected to determine the AVP content thereby the genotype. Pituitary samples were stored in 100µl of 0.1 N HCl at -20 ˚C. The preparation of the samples were as following: they were placed in a boiling water bath for 5 min, and then homogenized by ultrasound, centrifuged, and AVP content was measured from the 100-fold diluted supernatant using specific RIA as described earlier (Zelena et al., 2009a). The rabbit antibodies were donated by Dr. M. Vecsernyés (Szent-Györgyi Medical University, Szeged, Hungary). The limit of detection was 1 pg AVP/assay tube. The intraassay coefficients of variation was 10.7%.

All the samples from a particular experiment were assayed in the same RIA to avoid interassay differences.

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4.4 Experiment 3. Vasopressin receptor antagonists on maternal separation-induced ultrasonic vocalization

4.4.1 Manipulations

Brattleboro rat pups born from heterozygous (di/+) mothers and homozygous diabetes insipidus (di/di) fathers were injected ip. with physiological saline 30 min before MS-USV.

Wistar rat pups were injected with V1aR antagonist (SSR49059), V1bR antagonist (SSR149415) or V2R antagonist (SSR121463B) (a generous gift from the Sanofi-Synthélabo company), which were suspended in 0.4 % Tween 80, then injected ip. 30 min before USV in three different concentrations: 3, 10 or 30 mg/kg. In a further experimental series 10 mg/kg V1aR antagonist was mixed with 10 mg/kg V1bR antagonist. Control treatment was the solvent in 1µl/g volume washed with 15µl saline.

4.4.2 Experimental design

4.4.2.1 Maternal separation-induced ultrasonic vocalization

See 4.3.2.1 and **Figure 8**.

4.4.2.2 Righting reflex

See 4.3.2.2.

4.4.2.3 Negative geotaxis

See 4.3.2.3.

4.4.3 Hormone measurements

See 4.3.3. Additionally, only Brattleboro, but not Wistar rat pup hypophysis was collected for determination of the genotype (di/+ or di/di disposition based upon AVP content).

4.5 Experiment 4.: Magnocellular AVP in social behavior

To rescue magnocellular AVP synthesis, AVP-containing adeno-associated virus (AVP-AAV) vector was injected into the SON of AVP-deficient Brattleboro rats (di/di). We compared +/+, di/di and AVP-AAV treated male di/di rats.

4.5.1 Manipulations

Anaesthesia was performed by an intraperitoneal (i.p.) injection of a mixture of ketamine (50 mg/kg, SelBruHa Allatgyogyaszati Ltd., Budapest, Hungary), xylazine (20 mg/kg, Spofa, Prague, Czech Republic) and promethazinium chloratum (0.2 ml/kg, EGIS, Budapest, Hungary) dissolved in physiological saline (Zelena et al., 2009b).

Anaesthetised rats were fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). To rescue local AVP synthesis in the Brattleboro rat, AVP-AAV (Type 2, CMV-GH-SP-AVP-NP-GP-SVPA /CMV: human cytomegalovirus promoter; GH: human growth hormone first intron enhancer; SP: signal peptide; AVP: arginine–vasopressin; NP: neurophysin; GP: glycoprotein; SVPA: SV40 poly(A)/; 100 nl/injection site; for more information about the vector see (Ideno et al., 2003)) was bilaterally injected into the SON of di/di rats (stereotaxic coordinates from Bregma: AP: -0.4 mm, ML: +/-1.8 mm, DV: +9.7 mm) (later referred as di/di-AVP) (Csikota et al., 2016). Controls (both +/+ and di/di animals) were injected with saline on the same day and in the same volume as no proper control virus was available. In contrast to Ideno et al. (Ideno et al., 2003) in the present experiment we did not use the beta-galactosidase containing control vector, as previous studies (Reichel et al., 2016), as well as our preliminary examinations, showed that the galactosidase nucleotide sequence might have a significant impact on behavior. We cannot entirely exclude the possibility that the virus incorporation *per se* induced some unwanted effects in treated animals. However, at present all available data are against this possibility, since AAVs are unable to reproduce, they are non-pathogenic and do not incur an immune response (Zelena et al., 2017).

Only those di/di-AVP animals were included in subsequent experiments, which showed a significant drop in water consumption (10 out of 18 for Exp. 1 and 7 out of 10 for Exp. 2), as in this strain water consumption clearly defines the correct hits (Bienemann et al., 2003; Ideno et al., 2003). Although the cytomegalovirus (CMV) promoter used in the applied AAV construct is not tissue-specific, the presence of prohormone convertase

in the cell is essential to produce mature AVP (for details see (Varga et al., 2015)). Therefore, in our hands transgene-derived AVP was specifically produced in the neurons of the SON of di/di-AVP rats, and its surrounding tissue could not produce mature AVP peptide despite a possible leakage of the vector solution (Geddes et al., 1997; Ideno et al., 2003). We considered the drop of water consumption as a proof for successful functional rescue of AVP synthesis in the SON and we therefore started to test the animals two weeks after treatment. The immunohistochemical data confirmed the rescue (in the SON of +/+ rats 23% of the cells showed AVP positivity, while in di/di-AVP animals 17.5%).

4.5.2 Experimental design

4.5.2.1 Social behavioral experiments

Our studies revealed that the MeA, a nucleus known, among others, for its involvement in the control of social behavior in rodents (Noack et al., 2015), responded to our rescue of AVP synthesis in the SON (see below). We therefore studied the impact of AVP-AAV SON treatment on social behavior. Eighteen days after AVP-AAV injection the animals were tested for their social investigatory behavior and three days later for aggression.

4.5.2.1.1 Social investigation

More than two weeks after AVP-AAV injection, the rats were transferred to a new cage with fresh bedding 1 h before starting the test. The test consisted of a 4-min exposure to a previously not encountered conspecific juvenile (Wistar rat, ~25 days old). The duration of investigatory behavior of the adult towards the juvenile was measured online by a trained observer blind to the animal's group, using an events recorder (EVENTLOG 1.0 written by Robert Hendersen 1986). Investigatory behavior was defined as the direct

action of the adult towards the juvenile rat including anogenital sniffing, hunting, licking, pawing and close pursuit (**Figure 9.**).

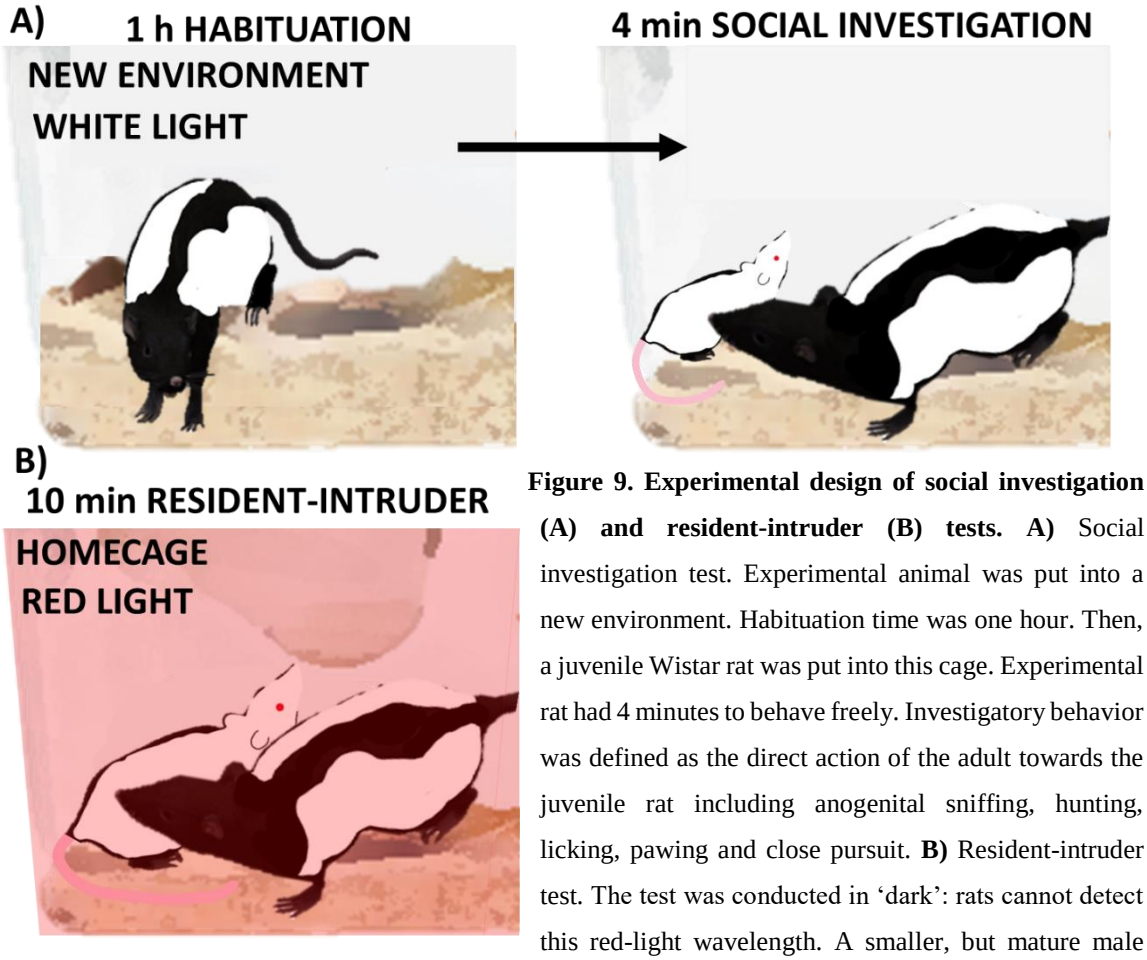

Figure 9. Experimental design of social investigation (A) and resident-intruder (B) tests. A) Social investigation test. Experimental animal was put into a new environment. Habituation time was one hour. Then, a juvenile Wistar rat was put into this cage. Experimental rat had 4 minutes to behave freely. Investigatory behavior was defined as the direct action of the adult towards the juvenile rat including anogenital sniffing, hunting, licking, pawing and close pursuit. **B)** Resident-intruder test. The test was conducted in 'dark': rats cannot detect this red-light wavelength. A smaller, but mature male Wistar rat was put into the homecage of the experimental animal for 20 minutes. Aggressive behavior, namely the bite frequency was measured in this test.

4.5.2.1.2 Resident-intruder test (RI)

Subjects were kept in GeoMaxi cages for three days. Rats were then exposed in these (home) cages to smaller, unfamiliar Wistar opponents for 20 min. Their behavior during the exposure was videorecorded and scored later by an experimenter blind to treatment conditions. Behavioral analysis focused on the consummatory phase of aggressive behavior i.e. on biting attacks (Fodor et al., 2014). The results of the quantitative measures (i.e. attack counts and latency) were present, as we failed to detect differences in qualitative measures (i.e. attack type and context) **(Figure 9.)**.

4.5.3 Immunohistochemistry

Animals were transcardially perfused with 100 ml of chilled phosphate-buffered saline (PBS; pH 7.4) followed by 300 ml ice-cold 4% paraformaldehyde (PFA, Molar Chemicals Ltd. Hungary) solution. After overnight post-fixation brains were transferred into a cryoprotective 30% sucrose solution in PBS for 2 nights at 4°C and then stored at –20°C until sectioning. Sequential 30-µm-thick coronal sections of the hypothalamus containing the SON and PVN were cut with a sliding microtome and divided into 6 parallel slice series.

Brain slices containing the SON and PVN were selected and stained for AVP. The AVP antibody was generated by Tamás Görcs (Nagy et al., 1991; Kocsis et al., 1998). Secondary antibodies were purchased from Jackson ImmunoResearch. Cell nuclei in the slices were visualized by Hoechst 33258 (1:20 000; Sigma-Aldrich). Slides were covered with Mowiol (Sigma-Aldrich).

Images were taken by Nikon C2 confocal laser scanning microscope in the Nikon Microscopy Centre at the Institute of Experimental Medicine in Budapest.

4.6 Experiment 5.: Epigenetic changes in Brattleboro rat brain

Aoyoma et al. (Aoyama et al., 2014) found decreased level of acetylated lysine 9 of histone 3 (AcH3K9) in the PFC in a pharmacological model of SCZ (piperidine hydrochloride (PCP) in mouse). Furthermore, a previous human study found a decrease of AcH3K9 in SCZ cell cultures (Gavin et al., 2008). Therefore, we aimed to investigate this epigenetic change in di/di rats.

4.6.1 Immunohistochemistry

Rats were deeply anesthetized and transcardially perfused with 100 ml saline followed by 300 ml ice cold 4% (w/v) paraformaldehyde (PFA; Molar Chemicals Ltd., Hungary) in phosphate buffered saline (PBS). Brains were removed from the skull, postfixed for 1 day in PFA at 4◦C and cryoprotected in 30% glucose (w/v in PBS) containing 0.1% (w/v) sodium-azide (Sigma–Aldrich, Inc., Hungary). 30 µm frozen sections were cut in the frontal plane on a sliding microtome. The following regions were studied: nucleus accumbens core (AcbC) and shell (AcbS) part, prefrontal cortex (PFC) prelimbic (PrL), infralimbic (IL) and dorsal peduncular (DP) part, dorsal (LSD), ventral (LSV), and

intermediate lateral septum (LSI) and hippocampus cornu ammonis (CA) 1, CA2, and CA3 subregions.

Floating sections were incubated in PBS containing 0.5% Triton X-100 and 0.5% H2O2for 30 min. Non-specific antigens were blocked by 2% bovine serum albumin (BSA; Sigma–Aldrich) in PBS for 30 min at room temperature. Sections were incubated for 72 h at 4◦C with anti-histone H3 (Acetyl-Lys9) antibody (host: rabbit, 1:5000; SAB4500347, Sigma–Aldrich, Inc., Hungary), diluted in blocking solution. After PBS washing sections were incubated in biotinylated secondary antibody for 1 h (anti-rabbit IgG, 1:500, Vector Laboratories). Next, sections were incubated in avidin–biotin complex (1:1000, ABC Vectastain Elite kit, Vector Laboratories) diluted in 0.05 M Tris buffered saline (TBS, pH7.6) for 1 h at room temperature. AcH3K9 immuno-positive cells were visualized by nickel enhanced 3,3'-diaminobenzidine (DAB). Sections were incubated for equal time in Tris-buffered solution containing 0.2 mg/ml DAB, 0.1% nickel-ammonium-sulphate and 0.003% H2O2. Enzymatic reaction was stopped by TBS washing.

Sections were mounted on glass slides in chrome-gelatin solution [0.5% (w/v) gelatin (Sigma–Aldrich) and 1 mM Chromium (III) potassium sulfate dodecahydrate (Sigma–Aldrich)], dehydrated by mixtures of xylol isomers and covered by DPX mounting medium (Sigma–Aldrich, Inc., Hungary). Microscopic images were digitized by OLYMPUS CCD camera, and stained particles were counted by means of the ScionImage software.

4.7 Statistical analyses

Data were analyzed by two-way ANOVA for factor genotype and treatment using the Statistica 12.0 program of StatSoft, Inc., Tulsa, OK, USA. Post hoc comparison of the data from different experimental groups was performed by the Newman– Keuls test or Fisher Least Significant Difference method and the results were presented on the figures. For DI (discrimination index) a single sample t-test against 0 (no discrimination) was conducted for each treatment group separately. Correlations were calculated by the Pearson method. Multiple regression analysis was conducted to determine the

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contribution of behavioral and hormonal changes to MS-USV. Data were present as mean ± S.E.M. The level of statistical significance was take as $p < 0.05$ in all statistical analyses.

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5 Results

5.1 Experiment 1.: Do antipsychotic drugs can improve behavioral deficits in Brattleboro rat?

5.1.1 Social discrimination test

Influence on social interest (**Figure 10.,** left part, **Table 4. A** for statistical details): During the 4 min sampling period, di/di rats spent the same or even more time with investigating a juvenile (Stim1) as control, +/+ rats (genotype effect). Hence, all rats had something to remember.

We have to admit, however, that antipsychotic treatment diminished the time spent with investigation (treatment effect). This was significant for all (acute, subacute and chronic) aripiprazole and haloperidol treatment, but also after repeated clozapine, olanzapine and risperidone treatment. Thus, after repeated treatment we observed sensitization in contrast to the expected habituation. Nevertheless, this - presumably sedative - effect equally affected +/+ and di/di animals (no genotype x treatment interaction). Therefore, any memory difference between genotypes cannot be attributed to this phenomenon. Moreover, even the lowest sampling time was above 20% (roughly 1 min), which should provide enough time for sampling (Engelmann et al., 2011).

Detection of SCZ-like symptoms (**Figure 10.,** right part, **Table 4. B** for statistical details): During the whole observation period (i.e. acute, subacute and chronic treatment) vehicle treated di/di rats had poor short term memory as the value of DI was not significantly differed from zero. In contrast the +/+ control group had intact social memory throughout. Aripiprazole and olanzapine were able to normalize the social memory deficit of di/di rats without influencing the intact memory of the +/+ animals. This effect was independent of the treatment duration. Haloperidol treatment was not effective at all, while the effect of clozapine and risperidone treatment were controversial, significantly increasing the DI in di/di, but reducing it in +/+ rats at most timepoints.

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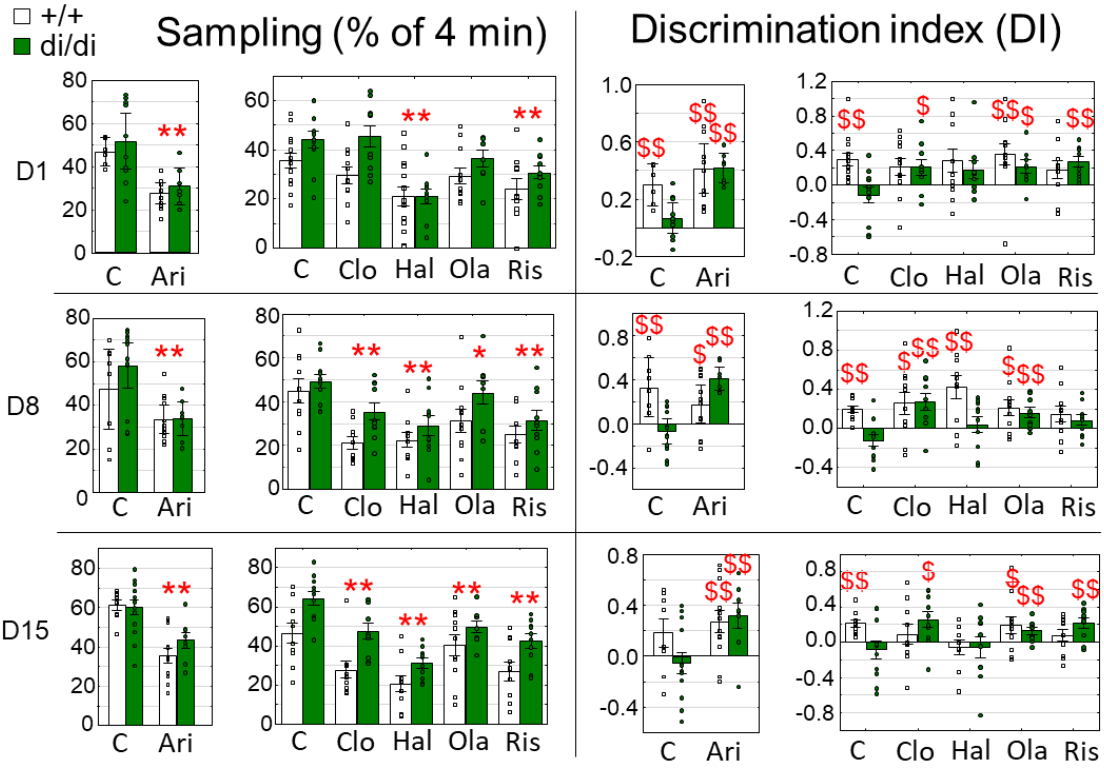

Figure 10. Social discrimination. Left panel: time spent investigating the stimulus juvenile during the 4 min sampling period, analyzed by repeated measure ANOVA; right panel: discrimination index, analyzed by single sample t-test against 0. D1: 30 min after a single treatment, D8: 30 min after the 8th treatment, D15: 30 min after the 15th treatment; n=7-11/group; *p<0.05, **p<0.01 main effect of treatment, compared to control; $p<0.05, $$p<0.01 significant difference from 0. Abbreviations: C: control, Ari: aripiprazole, Clo: clozapine, Hal: haloperidol, Ola: olanzapine, Ris: risperidone

Table 4. p. F or t values of the social discrimination test; **A)**: sampling%. **B)**: discrimination index; Abbreviations: Clo: clozapine, Hal: haloperidol, Ola: olanzapine, Ris: risperidone, Ari: aripiprazole

A)

Repeated Measures ANOVA	Sampling %			
	Clo, Hal, Ola, Ris		Ari	
	F	p	F	p
Genotype	29.920	0.000	0.646	0.427
Treatment	15.975	0.000	35.504	0.000
Genotype*Treatment	0.714	0.584	0.320	0.575
Time	18.661	0.000	13.732	0.000
Time*Genotype	2.894	0.058	0.083	0.921
Time*Treatment	2.752	0.007	0.892	0.415
Time*Genotype*Treatment	0.686	0.703	1.024	0.365

B)

Single sample t test	DI D1		DI D8		DI D15	
	Difference from 0					
	t	p	t	p	t	p
+/+ C	4.104	0.002	5.600	0.000	5.489	0.000
di/di C	-1.224	0.249	-2.012	0.072	-0.812	0.436
+/+ Clo	2.104	0.062	2.279	0.049	0.824	0.431
di/di Clo	2.345	0.044	3.071	0.013	2.812	0.020
+/+ Hal	2.089	0.061	3.598	0.006	-0.664	0.525
di/di Hal	1.825	0.101	0.450	0.663	-0.467	0.652
+/+ Ola	2.849	0.017	2.548	0.029	2.105	0.062
di/di Ola	2.711	0.030	2.999	0.017	3.316	0.011
+/+ Ris	1.747	0.115	1.759	0.112	1.143	0.282
di/di Ris	4.703	0.001	1.587	0.147	3.831	0.004
+/+ C (Ari)	5.273	0.003	4.325	0.008	3.741	0.013
di/di C (Ari)	0.971	0.352	-0.982	0.347	-1.093	0.298
+/+ Ari	5.295	0.000	2.683	0.023	2.790	0.019
di/di Ari	10.465	0.000	9.025	0.000	3.269	0.014

5.1.2 Social avoidance test

Compartment preference (**Table 5**.): None of the animals preferred the unknown, large compartment containing a sexually mature, male stimulus animal (less than 50% time spent here, see **Table 5.A**). During repeated testing (1 week apart) the interest increased (time effect). The treatment (except aripiprazole) significantly reduced the time spent in the "social" compartment, suggesting detrimental side effect. Nevertheless, there was no difference between genotypes and the genotype did not modify the effect of the treatment or time.

Detection of SCZ-like symptoms (**Figure 11.** and **Table 6. A**): On D1 there was a significant difference between vehicle treated di/di and control group in the duration of direct interaction with the stimulus animal. This difference disappeared during subsequent tests. After the 8th vehicle injection the di/di rats started the social interaction significantly later than respective controls. Similar difference was not visible after the 1st or 15th injections.

All treatment reduced the time spent with the stimulus animals. Logically, this interaction started later, except for the aripiprazole treated groups, which was similar to

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control in this respect. Genotype had no effect and did not modify the effects of treatments either.

Table 5. A): Time spent in the large compartment during social avoidance test; **B**): F and p values of repeated measure ANOVA for values in A.

A)

Treatment	Genotype	1st inj.	8th inj.	15th inj.
Control	+/+	33.243±8.846	49.550±8.248	45.063±4.378
	di/di	39.633±12.074	33.913±8.469	34.713±9.774
Aripiprazole	+/+	26.433±10.898	36.843±7.623	44.575±6.420
	di/di	28.443±8.331	26.367±7.283	41.688±7.249
Haloperidol	+/+	0.983±0.983	14.938±6.739	12.013±7.669
	di/di	0.000±0.000	6.375±4.110	14.750±7.113
Olanzapine	+/+	9.0167±5.456	16.114±9.918	29.013±12.660
	di/di	2.650±2.650	10.950±5.644	18.000±8.754
Risperidone	+/+	8.4167±7.058	0.000±0.000	8.100±5.373
	di/di	1.386±1.353	11.671±10.505	7.550±6.916

B)

Repeated Measures ANOVA	Time spent in large compartment %	
	F	p
Genotype	0.860	0.358
Treatment	12.942	0.000
Genotype*Treatment	0.725	0.579
Time	6.165	0.003
Time*Genotype	0.162	0.851
Time*Treatment	1.221	0.295
Time*Genotype*Treatment	1.267	0.270

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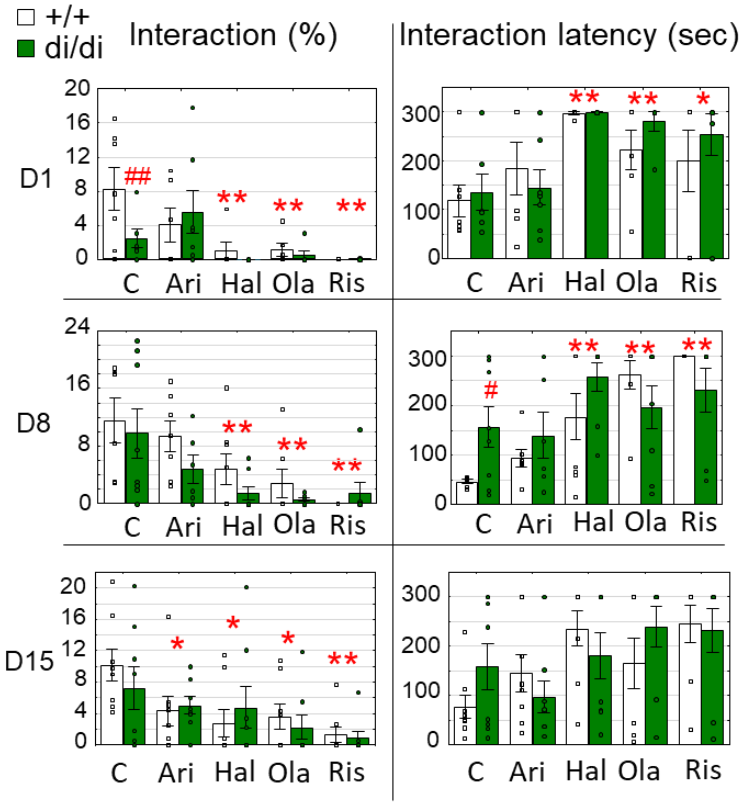

Figure 11. Social avoidance. Left panel: time spent on direct interaction with an adult stimulus animal during 5 min; right panel: latency of the interaction; both analyzed by repeated measure ANOVA. D1: 30 min after a single treatment, D8: 30 min after the 8th treatment, D15: 30 min after the 15th treatment; n=6-8/group; *p<0.05, **p<0.01 main effect of treatment, compared to control; #p<0.05, ##p<0.01 significant difference from +/+, vehicle treated animal. Abbreviations: C: control, Ari: aripiprazole, Clo: clozapine, Hal: haloperidol, Ola: olanzapine, Ris: risperidone

5.1.3 Prepulse inhibition test

Detection of SCZ-like symptoms (**Figure 12.** and **Table 6. B**)*:* AVP-deficient vehicle-treated rats showed decreased PPI on each day of examination compared to +/+ C group.

Effects of antipsychotic drugs: In case of all treatments (except the 15th aripiprazole) the difference between the genotypes disappeared at all studied timepoints. However, the treated di/di rats became significantly different from vehicle treated one after repeated treatment only (except for olanzapine, where single treatment was also effective).

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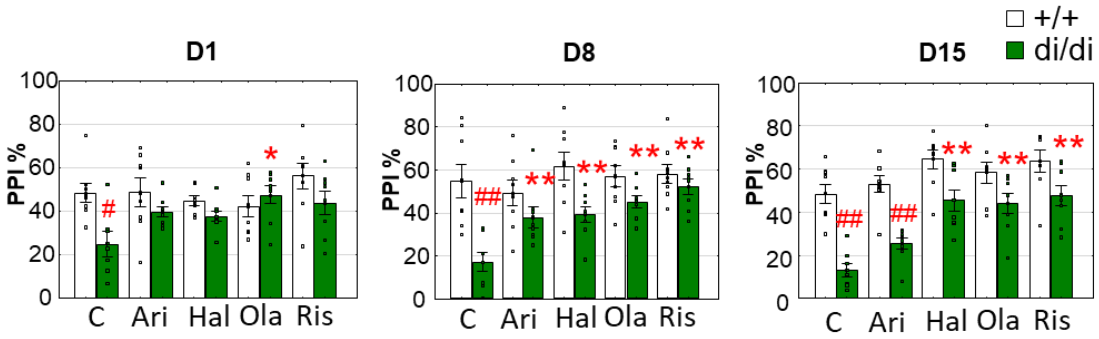

Figure 12. Prepulse inhibition (PPI). PPI data averaged for the three prepulse (73,77,81dB); analyzed by repeated measure ANOVA. D1: 30 min after a single treatment, D8: 30 min after the 8th treatment, D15: 30 min after the 15th treatment; n=7-8/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. Abbreviations: C: control, Ari: aripiprazole, Clo: clozapine, Hal: haloperidol, Ola: olanzapine, Ris: risperidone

Table 6. A): F and p values of the social avoidance test; **B)**: F and p values of the PPI test

A)

Repeated Measures ANOVA	Interaction%		Interaction latency	
	F	p	F	p
Genotype	1.951	0.169	0.472	0.496
Treatment	12.563	0.000	10.814	0.000
Genotype*Treatment	1.039	0.397	0.942	0.448
Time	5.713	0.005	0.945	0.393
Time*Genotype	1.924	0.152	0.057	0.945
Time*Treatment	0.971	0.464	0.778	0.623
Time*Genotype*Treatment	1.100	0.370	1.761	0.095

B)

PPI Repeated Measures ANOVA	PPI (average of 3 prepulses)	
	F	p
Genotype	60.392	0.000
Treatment	9.939	0.000
Genotype*Treatment	4.147	0.005
Time	2.118	0.124
Time*Genotype	6.754	0.002
Time*Treatment	2.060	0.044
Time*Genotype*Treatment	1.147	0.336

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5.2 Experiment 2.: Ultrasonic vocalization measurement of Brattleboro rat pups during antipsychotic treatment

The weight of di/di pups were significantly lower than that of their di/+ littermates ($p < 0.01$) in all experiments (a typical data: di/+: 18.8±0.3g; di/di: 16.7±0.3g). The genotype had a significant effect on the number and duration of emitted calls in every case ($p \leq 0.01$; for detailed statistics see **Table 7.**) with lower levels in di/di pups. The ACTH levels were always significantly lower in di/di rats than in di/+ animals, while corticosterone levels were always higher ($p < 0.01$).

5.2.1 Haloperidol

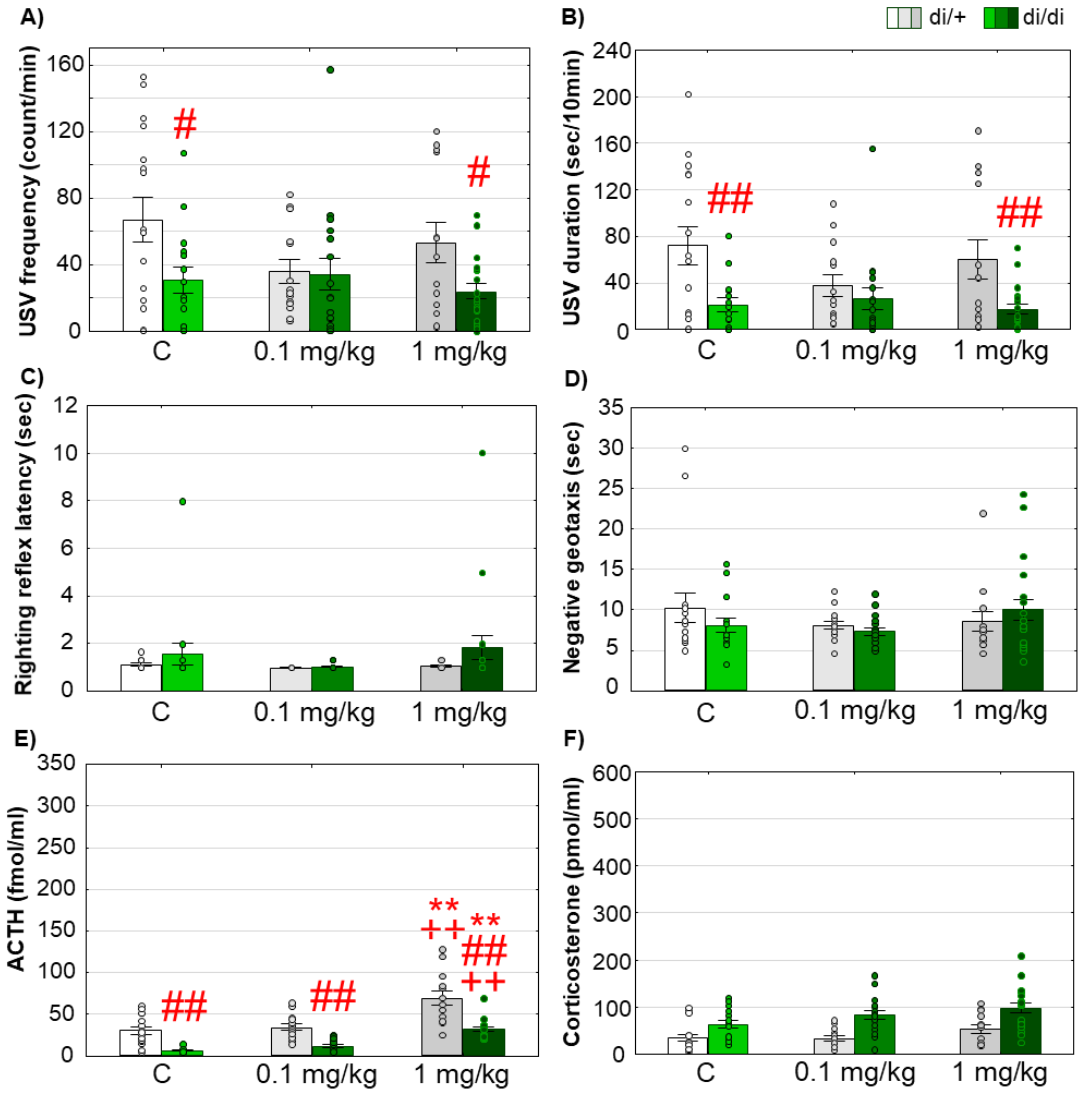

Figure 13. Haloperidol treatment A) The number of calls (count/min) 30 min after treatment; **B)** The duration of calls (sec/10 min) 30 min after treatment; **C)** Righting reflex latency (sec) right after MS-USV

measurement; **D)** Negative geotaxis (sec) right after MS-USV measurement; **E)** ACTH serum concentration (fmol/ml) after MS-USV, righting and negative geotaxis; **F)** Corticosterone serum concentration (pmol/ml) after MS-USV, righting and negative geotaxis. n=13-19/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. ++p<0.05 significant difference from the other treated group.

The 0.1 mg/kg dose of haloperidol reduced MS-USV of the di/+ genotypes to the level of di/di pups (which was unchanged) (**Figure 13. A, B**). After injection of the higher dose (1mg/kg), animals vocalized approximately the same as without any treatments. In the behavior tests there were no any significant changes (**Figure 13 C, D**). Haloperidol dose dependently increased the ACTH and corticosterone levels without interaction with the genotype effect (**Figure 13. E, F**).

Table 7. Statistical data of Exp. 2. Abbreviations: ACTH: adrenocorticotropic hormone; CS: corticosterone; MS-USV: maternal separation-induced ultrasonic vocalization; RRL: righting reflex latency.

	Weight	RRL	Negative geotaxis	MS-USV number.	MS-USV duration	ACTH	CS
Haloperidol (0.1 and 1 mg/kg)							
F(1,88) genotype	82.6 p<0.01	2,7 p=0.1	0.3 p=0.6	8.3 p<0.01	15.4 p<0.01	68.9 p<0.01	30.2 p<0.01
F(2,88) treatment	0.34 p=0.7	1,0 p=0.37	1.1 p=0.34	1.1 p=0.33	0.9 p=0.4	35.3 p<0.01	4.6 p=0.01
F(2,88) interaction	1.5 p=0.23	0,7 p=0.49	1.2 p=0.32	1.86 p=0.16	1.8 p=0.16	1.88 p=0.15	0.7 p=0.47
Clozapine (1 and 10 mg/kg)							
F(1,69) genotype	9.9 p<0.01	2.7 p=0.1	0.8 p=0.36	8.7 p<0.01	8.1 p<0.01	40.72 p<0.01	13.8 p<0.01
F(2,69) treatment	0.28 p=0.75	37.15 p<0.01	16.9 p<0.01	15.5 p<0.01	10.7 p<0.01	6.9 p<0.01	8.8 p<0.01
F(2,69) interaction	1.65 p=0.2	0.15 p=0.85	0.16 p=0.84	2.7 p=0.073	2.4 p=0.09	5.0 p<0.01	3.0 p=0.05
Olanzapine (0.3 and 3 mg/kg)							
F(1,86) genotype	96.1 p<0.01	10.0 p<0.01	0.004 p=0.95	12.96 p<0.01	12.57 p<0.01	88.6 p<0.01	22.8 p<0.01
F(2,86) treatment	2.65 p=0.07	147.5 p<0.01	166.2 p<0.01	34.2 p<0.01	30.6 p<0.01	41.6 p<0.01	12.4 p<0.01
F(2,86) interaction	0.06 p=0.9	2.2 p=0.12	2.3 p=0.11	3.94 p<0.05	4.1 p<0.05	20.3 p<0.01	0.6 p=0.55
Risperidone (0.25 and 1 mg/kg)							
F(1,91) genotype	30.5 p<0.01	6.41 p=0.01	0.29 p=0.59	884.5 p<0.01	7.37 p<0.01	10.54 p<0.01	331.9 p<0.01
F(2,91) treatment	2.3 p=0.1	11.74 p<0.01	16.7 p<0.01	0.94 p=0.39	15.2 p<0.01	8.9 p<0.01	149.6 p<0.01

F(2,91) interaction	0.6 p=0.55	1.83 p=0.17	3.25 p<0.05	0.81 p=0.44	3.4 p<0.05	2.24 p=1.11	58.2 p<0.01
Risperidone (0.05 and 0.1 mg/kg)							
F(1,90) genotype	46.8 p<0.01	15.7 p<0.01	0.77 p=0.38	12.13 p<0.01	19.4 p<0.01	61.9 p<0.01	71.4 p<0.01
F(2,90) treatment	0.46 p=0.62	1.45 p=0.24	1.45 p=0.24	1.14 p=0.32	2.71 p=0.07	12.2 p<0.01	30.9 p<0.01
F(2,90) interaction	1 p=0.37	0.46 p=0.64	0.99 p=0.37	2.27 p=0.1	1.05 p=0.35	5.71 p<0.01	3.95 p<0.01
Aripiprazole (0.5 and 5 mg/kg)							
F(1,134) genotype	68.8 p<0.01	19.3 p<0.01	1.6 p=0.2	11.2 p<0.01	18.9 p<0.01	60.2 p<0.01	97.2 p<0.01
F(2,134) treatment	0.21 p=0.81	4.84 p<0.01	0.29 p=0.75	5.76 p<0.01	4.47 p=0.01	18.9 p<0.01	3.8 p<0.05
F(2,134) interaction	2.93 p=0.056	8.7 p<0.01	4.85 p<0.01	0.46 p=0.63	0.76 p=0.46	2.02 p=0.14	1.72 p=0.18
Aripiprazole (50 mg/kg)							
F(1,56) genotype	7.2 p<0.01	9.8 p<0.01	9.35 p<0.01	5.28 p<0.05	5.09 p<0.05	148.6 p<0.01	28.7 p<0.01
F(1,56) treatment	0.7 p=0.42	2.24 p=0.14	6.2 p=0.01	0.002 p=0.96	0.07 p=0.79	72.6 p<0.01	5.45 p<0.05
F(1,56) interaction	0.09 p=0.76	2.7 p=0.1	5.1 p<0.05	1.66 p=0.2	1.16 p=0.028	32.2 p<0.01	0.09 p=0.76

5.2.2 Clozapine and olanzapine

The lower doses (1mg/kg clozapine and 0.3mg/kg olanzapine) elevated the emitted MS-USVs to the di/+ levels (**Figure 14. A, B; Figure 15. A, B**). Thus, they were effective for the AVP-deficient rats. On the contrary, higher doses (10mg/kg clozapine, 3mg/kg olanzapine) reduced the vocalization of di/+ pups to the di/di level. Nevertheless, the higher, but not the lower doses were sedative during the behavioral tests (**Figure 14. C, D; Figure 15. C, D**) and significantly enhanced the ACTH (**Figure 14.E, Figure 15.E**) and corticosterone (**Figure 14.F, Figure 15.F**) levels. There were no ACTH elevations in di/di animals.

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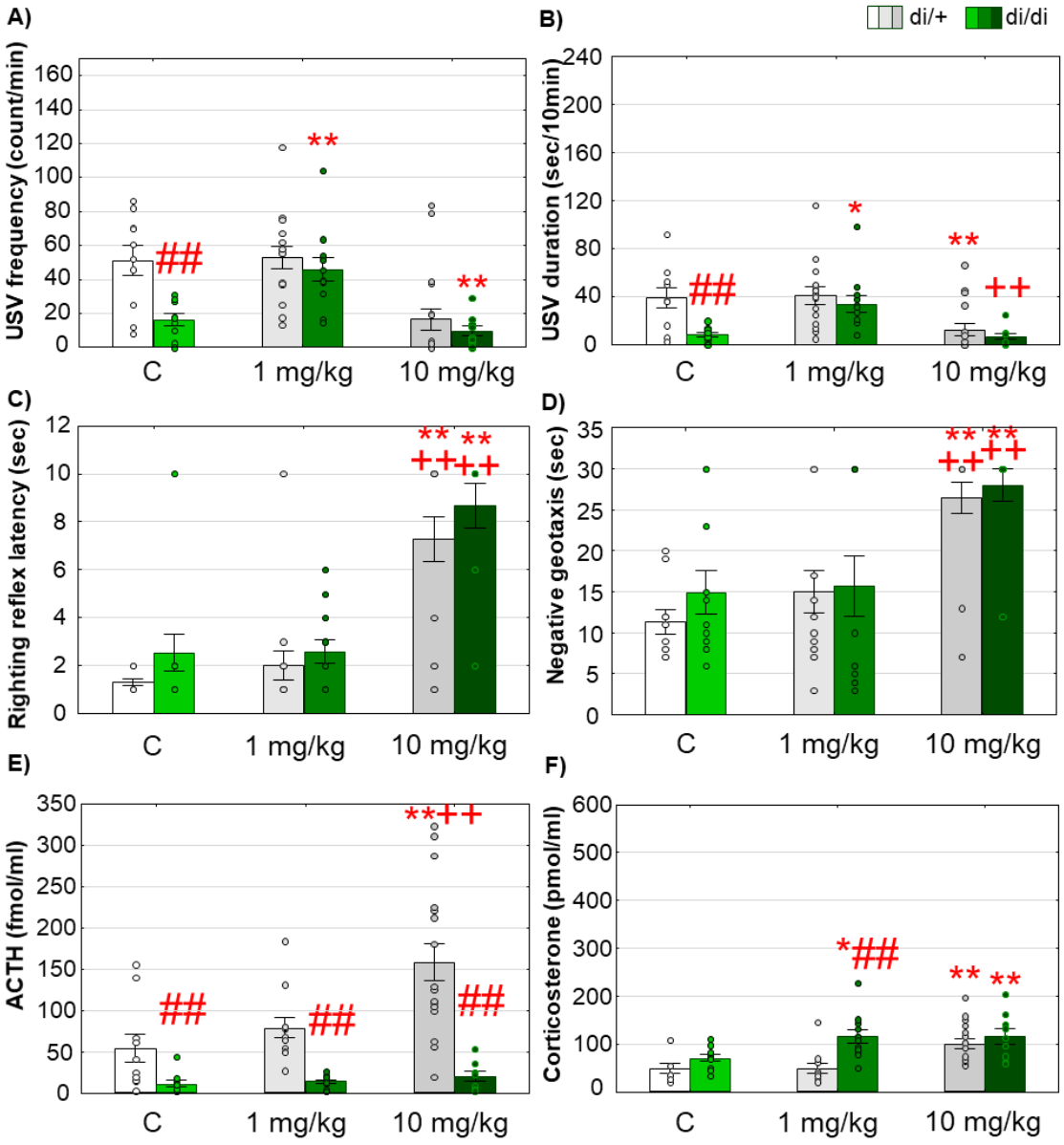

Figure 14. Clozapine treatment. **A)** number of calls (count/min) 30 min after treatment; **B)** MS-USV duration (sec/10 min) 30 min after treatment; **C)** Righting reflex latency (sec) right after MSUSV measurement; **D)** Negative geotaxis (sec) right after MS USV measurement; **E)** ACTH serum concentration (fmol/ml) after MS-USV measurement; **F)** Corticosterone serum concentration (pmol/ml) after MS-USV measurement. n=9-18/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. ++p<0.05 significant difference from the other treated group.

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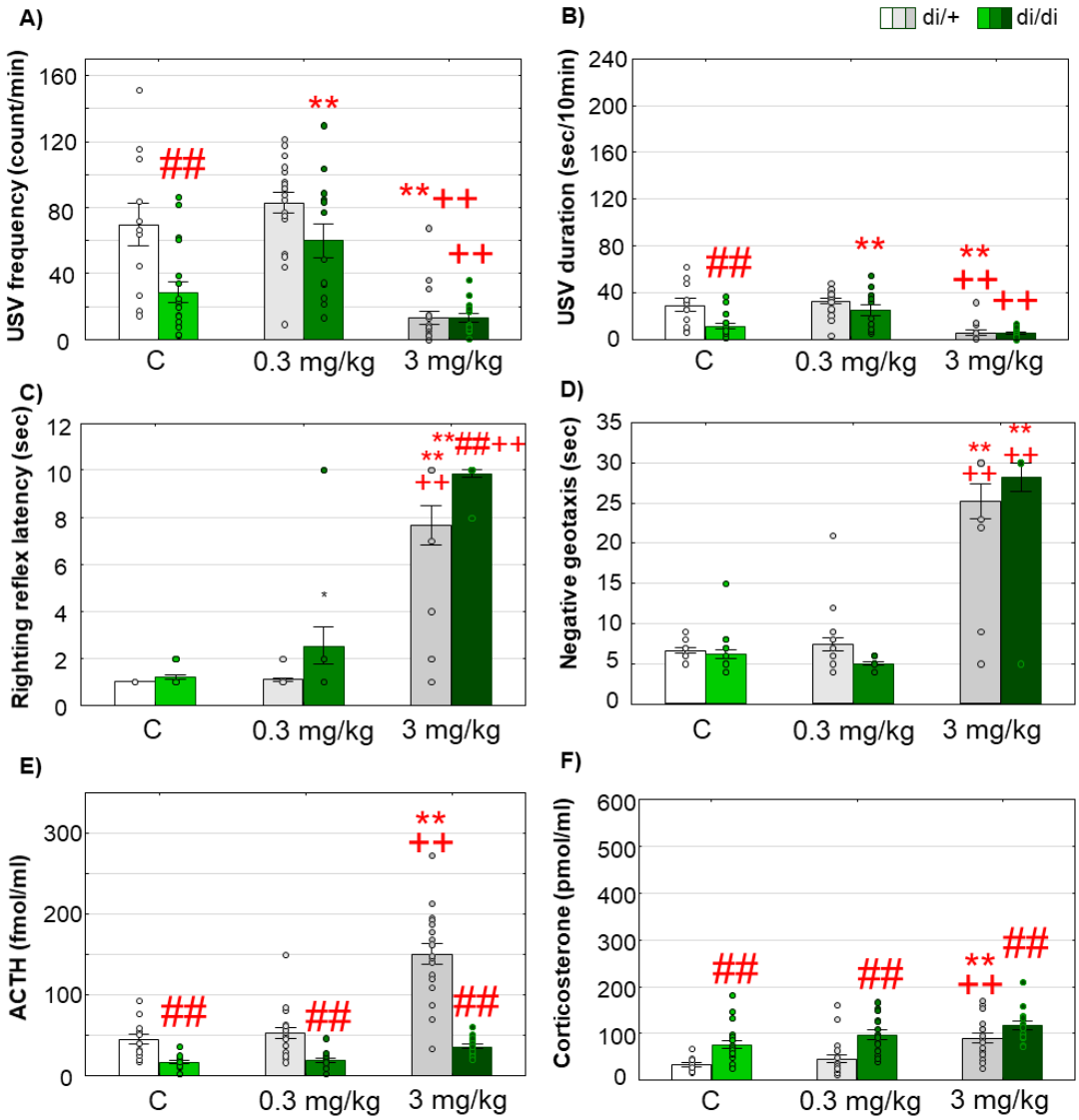

Figure 15. Olanzapine treatment. A) Number of calls (count/min) 30 min after treatment; **B)** MS-USV duration (sec/10 min) 30 min after treatment; **C)** Righting reflex latency (sec) right after MS-USV measurement; **D)** Negative geotaxis (sec) right after MS-USV measurement; **E)** ACTH serum concentration (fmol/ml) after MS-USV measurement; **F)** Corticosterone serum concentration (pmol/ml) after MS-USV measurement. n=12-20/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. ++p<0.05 significant difference from the other treated group.

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5.2.3 Risperidone

0.25mg/kg and 1mg/kg risperidone dose dependently reduced the emitted USV in both genotypes (**Table 8.**). At the same time, the higher dose was sedative on behavioral tests, while even the lower dose increased the ACTH and corticosterone levels. As we supposed that the HPA axis stimulating effect counteracted the direct USV effect, we repeated the experiment with lower doses. Indeed, 0.05 mg/kg significantly elevated the number and duration of emitted calls in di/di animals (**Figure 16. A, B**). Although none of the tested lower doses (0.05mg/kg, 0.1mg/kg) was sedative during behavioral testings (**Figure 16. C, D**), but even the lowest dose elevated the stress hormone (ACTH and corticosterone) levels (**Figure 16. E, F**). AVP-deficient pups showed lower ACTH elevation than their di/+ counterparts with all tested risperidone doses.

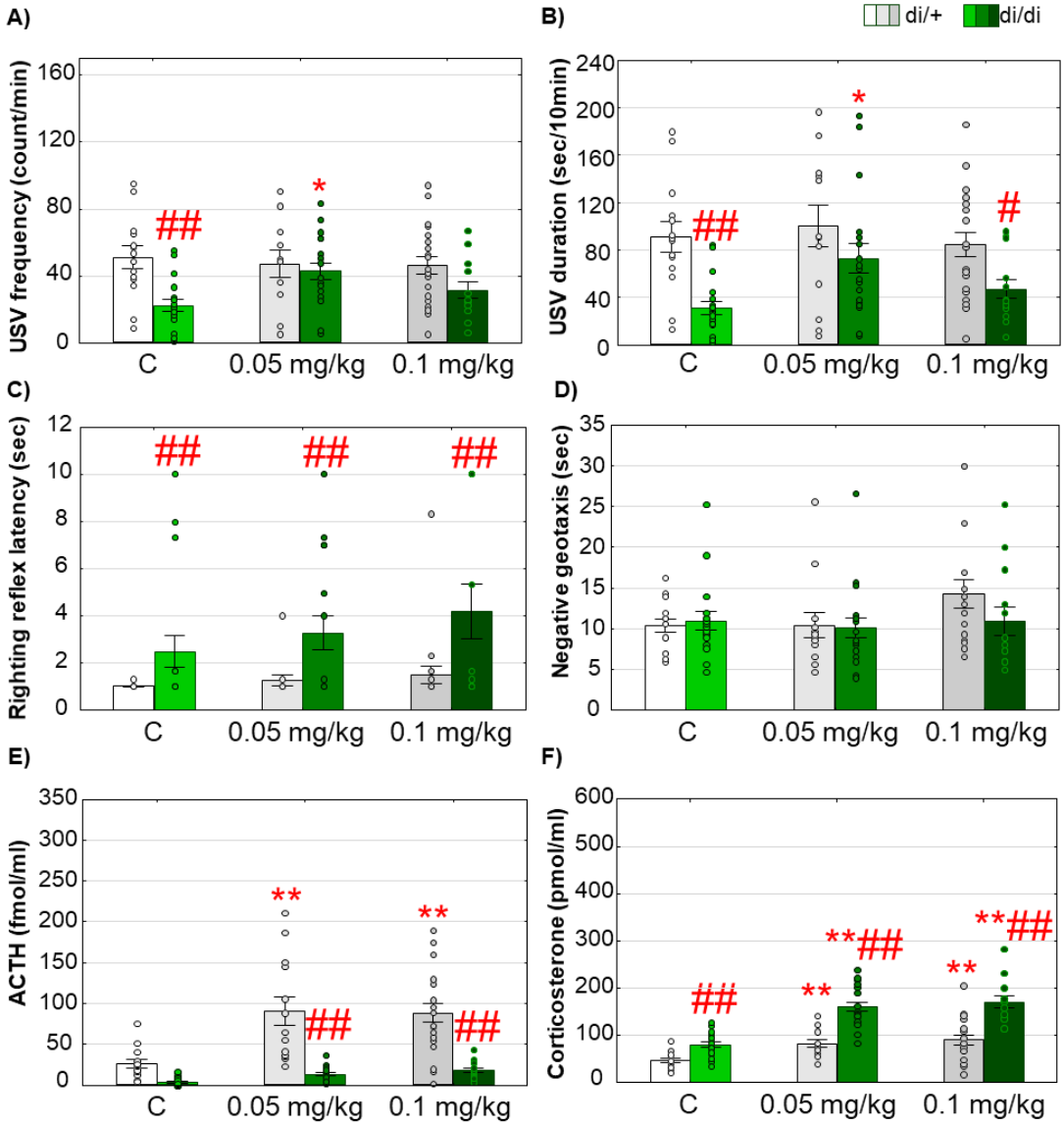

Figure 16. Risperidone treatment. A) USV frequency (count/min) 30 min after treatment; **B)** USV duration (sec/10 min) 30 min after treatment; **C)** Righting reflex latency (sec) right after MS USV measurement; **D)** Negative geotaxis (sec) right after MS USV measurement; **E)** ACTH serum concentration (fmol/ml) after MS USV measurement; **F)** Corticosterone serum concentration (pmol/ml) after MS USV measurement. n=13-20/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. ++p<0.05 significant difference from the other treated group.

Table 8. Results of 0.25 and 1 mg/mg Risperidone treatments

Genotype	Treatment		USV frequency	USV duration	Righting reflex	Negative geotaxis	ACTH (fmol/ml)	Corticosterone (pmol/ml)
di/+	Control	Average	76.8	82.1	1.0	6.5	24.7	31.4
		SEM	12.3	17.0	0.0	0.7	2.4	5.1
di/di	Control	Average	35.4	30.3	1.5	8.5	6.7	71.4
		SEM	8.8	9.0	0.4	1.7	0.8	8.6
di/+	0.25 mg/kg	Average	34.3	44.2	1.4	7.6	134.1	121.7
		SEM	10.8	17.8	0.2	1.2	9.8	21.0
di/di	0.25 mg/kg	Average	28.1	26.0	1.9	9.5	34.3	217.9
		SEM	5.6	6.5	0.4	1.4	3.0	23.7
di/+	1 mg/kg	Average	16.0	19.7	2.5	20.7	176.8	120.0
		SEM	6.5	9.0	0.6	2.6	10.7	10.9
di/di	1 mg/kg	Average	10.7	8.1	4.7	14.2	41.3	261.0
		SEM	2.6	2.3	0.8	2.4	3.1	25.9

5.2.4 Aripiprazole

Aripiprazole in 0.5mg/kg and 5mg/kg doses significantly elevated the number and duration of emitted calls in di/di pups (**Figure 17. A, B**). Neither dose was sedative on behavioral tests (**Figure 17. C, D**), while there was an elevation of righting latency in some di/di animals, while the negative geotaxis latency was reduced in some other AVP-deficient rats. 5mg/kg significantly elevated the serum ACTH levels in all animals with lower levels in di/di pups (**Figure 17. E**). Even a very high dose (50mg/kg) had similar effect, namely "normalized" the USV (**Figure 17. A, B**) without sedative side effects, but with a significant elevation in ACTH concentrations.

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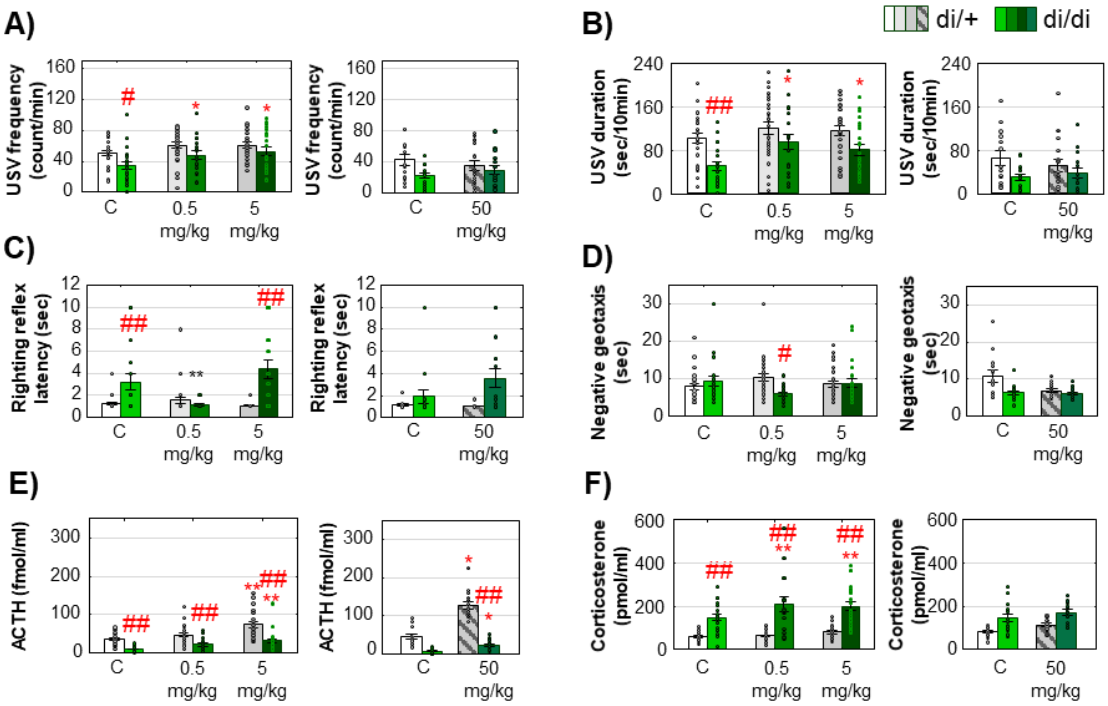

Figure 17. Aripiprazole treatment. A) USV frequency (count/min) 30 min after treatment; **B)** USV duration (sec/10 min) 30 min after treatment; **C)** Righting reflex latency (sec) right after MS USV measurement; **D)** Negative geotaxis (sec) right after MS USV measurement; **E)** ACTH serum concentration (fmol/ml) after MS USV measurement; **F)** Corticosterone serum concentration (pmol/ml) after MS USV measurement. for 0.5 and 5 mg/kg treatment: n=18-28/group; 50 mg/kg treatment: n=14-16/group; *p<0.05, **p<0.01 significant difference from di/di vehicle treated rats; #p<0.05, ##p<0.01 significant difference from respective +/+ animal. ++p<0.05 significant difference from the other treated group.

5.3 Experiment 3. Vasopressin receptor antagonists on maternal separation-induced ultrasonic vocalization

5.3.1 Genetic AVP-deficiency

AVP-deficient Brattleboro rats (di/di) showed decreased MS-USV based upon their number ($F(1,17)=7.600$; $p=0.014$; **Figure 18. A**) and duration ($F(1,17)=7.600$; $p=0.014$; **Figure 18. B**) of emitted calls compared to their heterozygous littermates. Thus, we successfully repeated what we also observed in Experiment 2. In connection their ACTH levels were significantly lower ($F(1,17)=15.380$; $p= 0.001$; **Figure 18. C**), without significant alterations in corticosterone levels ($F(1,17)=1.915$; $p=0.184$; **Figure 18. D**).

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Righting reflex (F(1,17)=1.850; p=0.193) and negative geotaxis (F(1,17)=0.042; p=0.840) values were comparable in the two genotypes (data not shown).

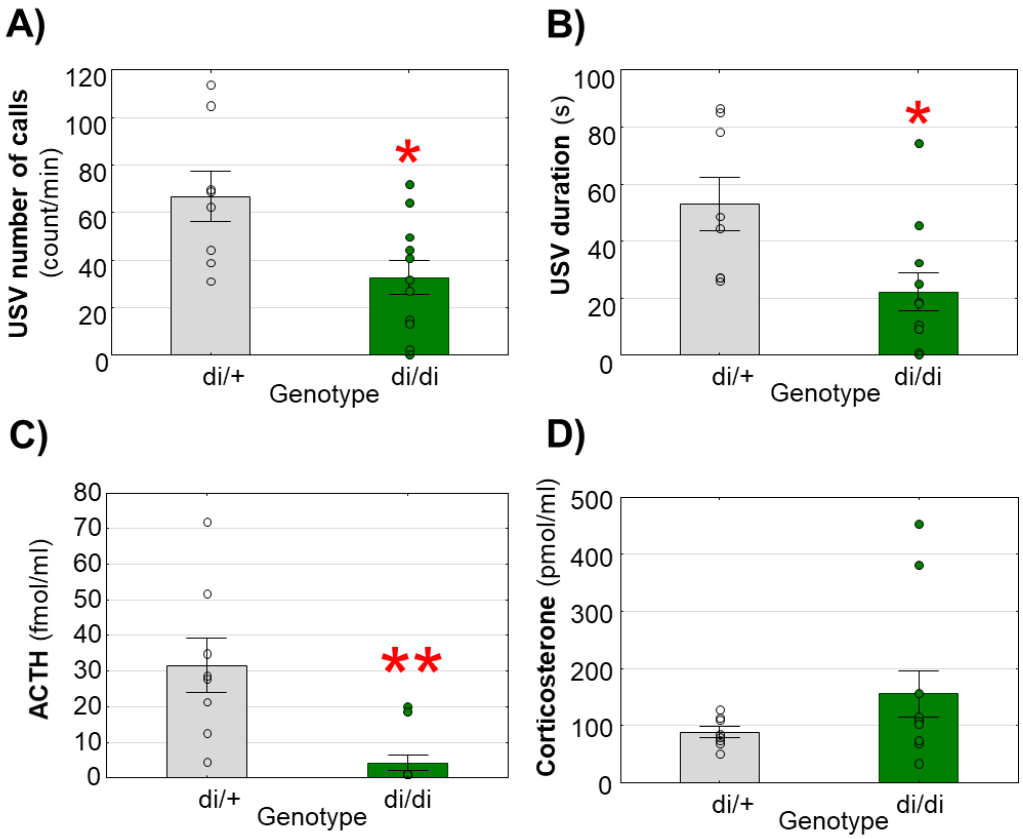

Figure 18. MS-USV and stress-hormone level results of Brattleboro rats. The 7-8-day-old pups emitted reduced ultrasound vocalization to maternal separation measured for 10 min both in **A)** number (count/min) and **B)** duration (sec) of emitted calls 30 min after a single intraperitoneal saline injection. Moreover, they showed reduced **C)** adrenocorticotropin (ACTH, fmol/ml) elevation at the end of separation without significant changes in **D)** corticosterone (pmol/ml) levels. di/+: heterozygous, di/di: homozygous diabetes insipidus, missing functional vasopressin; n=8-11; *p<0.05, **p<0.01 vs di/+ control

5.3.2 Pharmacological AVP-deficiency

5.3.2.1 V1aR antagonist

V1aR antagonist treatment decreased MS-USV in 30 mg/kg concentration only (number of calls: F(3,42)=1.788; p=0.164; Fisher post-hoc Control vs 30 mg/kg: p=0.026; **Figure 19. A**; duration of calls: F(3,42)=1.551; p=0.215; Fisher post-hoc Control vs 30 mg/kg: p=0.040; **Figure 19. B**). ACTH levels showed no alterations (F(3,42)=1.219; p=0.315; **Figure 19. C**), while the corticosterone levels were significantly higher in the group with 30 mg/kg antagonist treatment (F(3,42)=1.857; p=0.152; Fisher post-hoc Control vs 30 mg/kg: p=0.030; **Figure 19. D**).

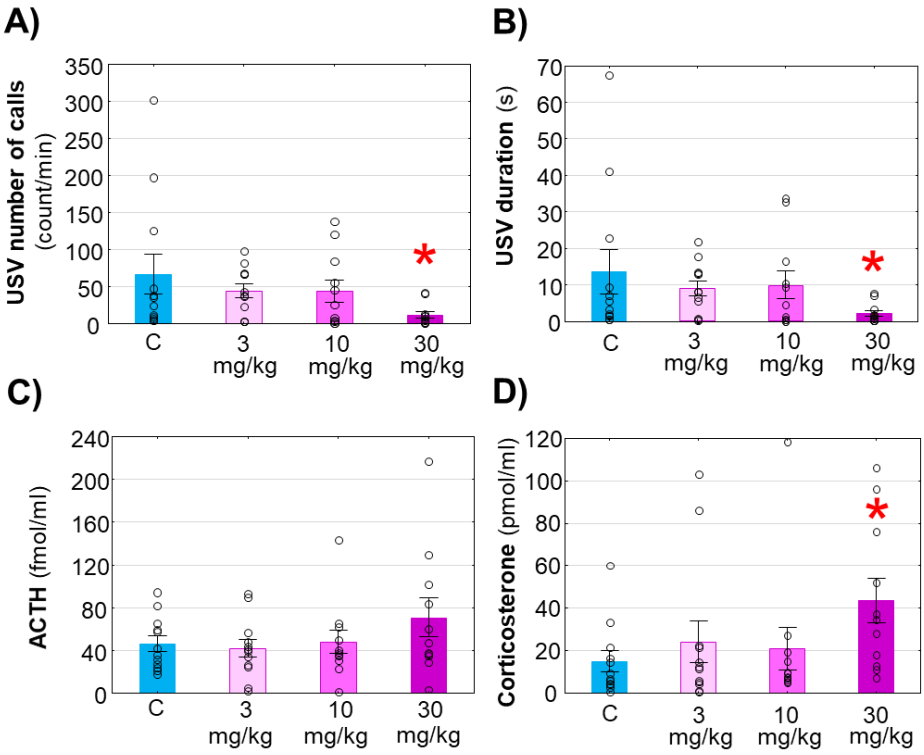

Figure 19. Treatment with SSR49059, a V1a receptor antagonist. 7-8-day-old Wistar rat pups were treated intraperitoneally with 3-10-30mg/kg V1aR antagonist 30 min before a 10 min maternal separation. 30mg/kg significantly reduced the emitted ultrasound vocalization both in **A)** number and **B)** duration without changes in **C)** adrenocorticotropin (ACTH, fmol/ml), but an elevation in **D)** corticosterone (pmol/ml) levels. n=12-14; *p<0.05 vs control

There was no difference between the groups in the latency of righting as well as negative geotaxis (**Table 9**.)

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Table 9. Righting reflex and negative geotaxis values

Antagonist treatment	Time (sec)	Concentration (mg/kg)			
		0	3	10	30
V1aR	Righting	2.333 ± 0.343	2.361 ± 0.230	2.758 ± 0.397	2.212 ± 0.187
	Neg.geo.	19.861 ± 2.687	20.750 ± 2.984	18.212 ± 2.735	16.485 ± 2.929
V1bR	Righting	1.303 ± 0.156	2.279 ± 0.669	1.947 ± 0.303	2.103 ± 0.266
	Neg.geo.	9.194 ± 0.963	8.487 ± 0.880	9.528 ± 1.215	10.100 ± 1.673
V2R	Righting	2.133 ± 0.218	2.267 ± 0.325	2.033 ± 0.195	2.250 ± 0.300
	Neg.geo.	11.967 ± 1.961	13.970 ± 2.707	15.267 ± 3.010	18.667 ± 2.567
V1aR+V1bR	Righting	1.741±0.282		2.750±0.552	
	Neg.geo.	8.296±1.301		7.125±0.900	

5.3.2.2 V1bR antagonist

V1bR antagonist treatment decreased the number (F(3,43)=5.719; p=0.002; **Figure 20. A**) and duration (F(3,43)=4.470; p=0.008; **Figure 20. B**) of emitted calls accompanied by reduced ACTH levels (F(3,19)=3.008; p=0.056; **Figure 20. C**) without changes in corticosterone (F(3,19)=1.230; p=0.326; **Figure 20. D**). There was no difference between the groups in the latency of righting and negative geotaxis (**Table 9**.)

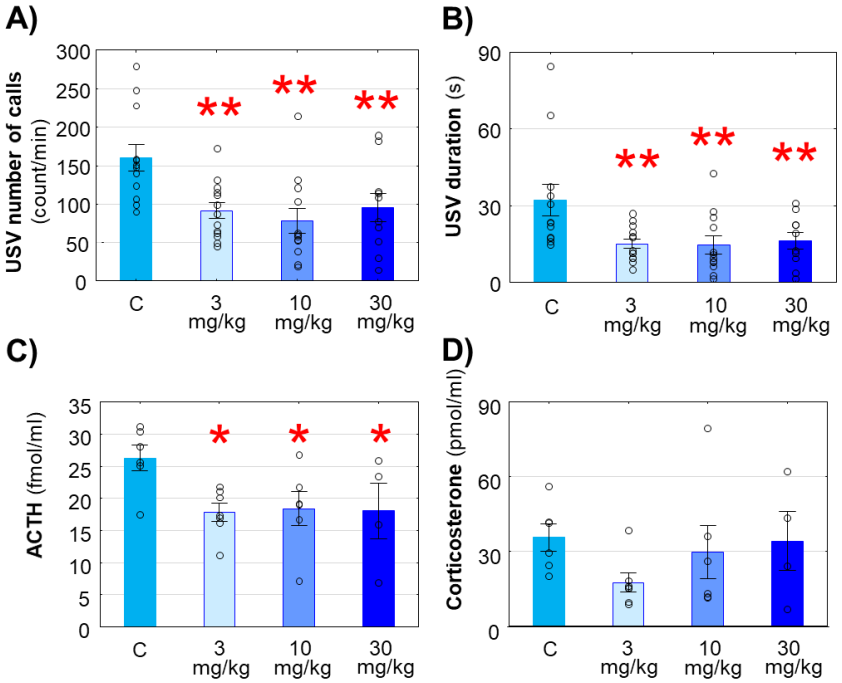

Figure 20. Treatment with SSR149415, a V1b receptor antagonist. 7-8-day-old Wistar rat pups were treated intraperitoneally with 3-10-30mg/kg V1bR antagonist 30 min before a 10 min maternal separation. All doses significantly reduced the emitted ultrasound vocalization both in **A)** number and in **B)** duration

and reduced **C)** adrenocorticotropin (ACTH, fmol/ml) levels without affecting the **D)** corticosterone (pmol/ml) values. n=10-13; *p<0.05, **p<0.01 vs control

5.3.2.3 V2R antagonist

3 mg/kg V2R antagonist enhanced MS-USV (number: F(3,35)=4.891; p=0.006; Fisher post-hoc Control vs 3 mg/kg: p=0.041; **Figure 21. A**; duration: F(3,35)=4.935; p=0.006; Fisher post-hoc Control vs 3 mg/kg: p=0.057; **Figure 21. B**), while the higher doses had no effect on MS-USV. Both stress hormone levels were higher 45 min after a single 30 mg/kg V2R antagonist treatment compared to control injection group (ACTH: F(3,35)=13.321; p=0.000; Fisher post-hoc Control vs 30 mg/kg: p=0.000; **Figure 21. C**; corticosterone: F(3,35)=8.363; p=0.000; Fisher post-hoc Control vs 30 mg/kg: p=0.000; **Figure 21. D**).

There was no difference between the groups in the latency of righting as well as negative geotaxis (**Table 9.**)

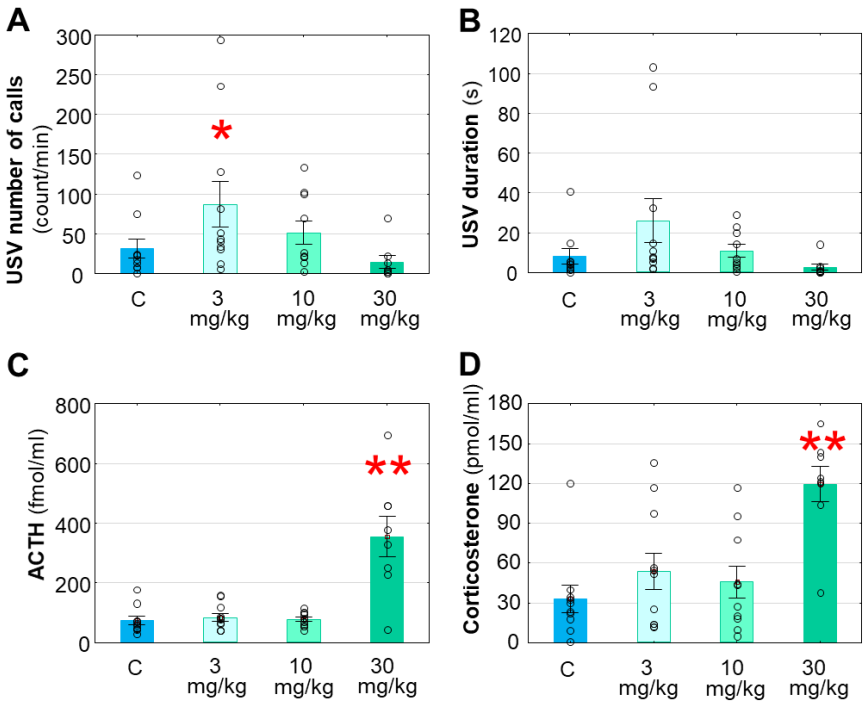

Figure 21. Treatment with SSR121463B, a V2 receptor antagonist. 7-8-day-old Wistar rat pups were treated intraperitoneally with 3-10-30mg/kg V2R antagonist 30 min before a 10 min maternal separation. 3mg/kg significantly enhanced the MS-USV in **A)** number with a tendency in **B)** duration and 30mg/kg

elevated **C)** adrenocorticotropin (ACTH, fmol/ml) and **D)** corticosterone (pmol/ml) levels. n=9-11; *p<0.05, **p<0.01 vs control

5.3.2.4 V1aR+V1bR antagonists

The combination of V1a and V1bR antagonist effectively reduced MS-USV (number of calls: F(1,15)=10.440; p=0.006; **Figure 22. A**; duration: F(1,15)=15.616; p=0.001; **Figure 22. B**), without any effect on stress-hormones (ACTH: F(1,15)=0.008; p=0.931; **Figure 22. C**; corticosterone: F(1,15)=0.001; p=0.982; **Figure 22. D**). The same dose of V1aR antagonist induced 34.3% and 26.8% non-significant reduction in MS-USV number of calls and duration, respectively, while in case of V1b 51.5% and 54.3% significant reduction was visible. The combination induced 57.1% reduction in MS-USV number of calls and 68.53% reduction in duration.

There was no difference between the groups in the latency of righting as well as negative geotaxis (**Table 9.**)

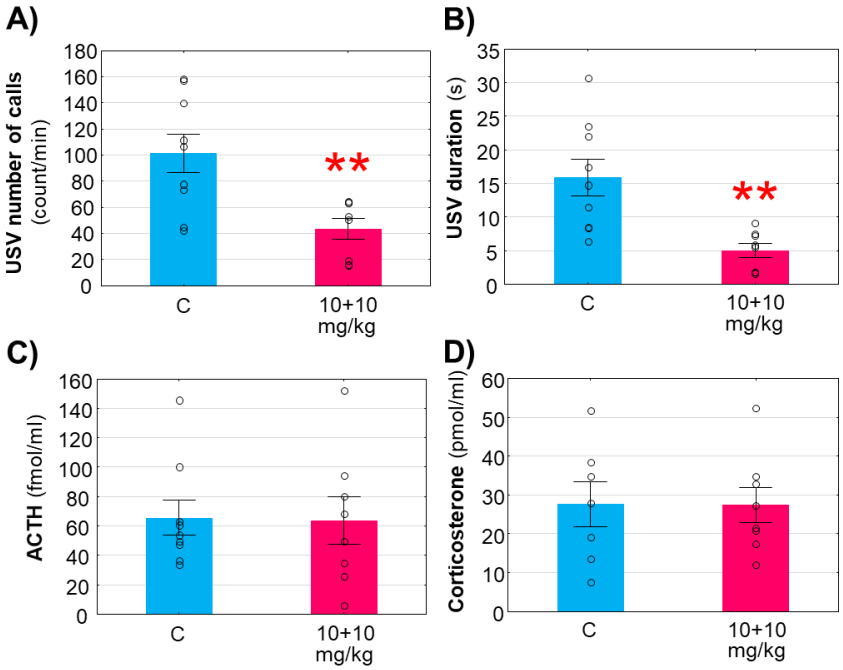

Figure 22. Treatment with SSR49059 (V1a) + SSR149415 (V1b) receptor antagonists. 7-8-day-old Wistar rat pups were treated intraperitoneally with a mixture of 10-10mg/kg V1a+V1b receptor antagonist 30 min before a 10 min maternal separation. The combination significantly reduced the emitted ultrasound vocalization both in **A)** number and **B)** duration of calls without affecting **C)** adrenocorticotropin (ACTH, fmol/ml) and **D)** corticosterone (pmol/ml) levels. n=8-9; **p<0.01 vs control

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5.3.3 Correlations

As it could have been expected the number and duration of emitted calls after maternal separation positively correlated with each other in all experimental series. Interestingly, the same was true for ACTH and corticosterone correlation except in case of Brattleboro animals, where there was no correlation at all. In Brattleboro rats the AVP content of their hypophysis showed a significant positive correlation with the MS-USV number of calls (r=0.556; p=0.017; **Figure 23. A**) and duration (r=0.541; p=0.020). Moreover, in their case the serum ACTH level also showed positive correlation with their MS-USV number of calls (r=0.491; p=0.038; **Figure 23. B**). Interestingly, similar ACTH and MS-USV number of calls correlation was detected after V1bR antagonist treatment (r=0.424; p=0.044; **Figure 23. C**).

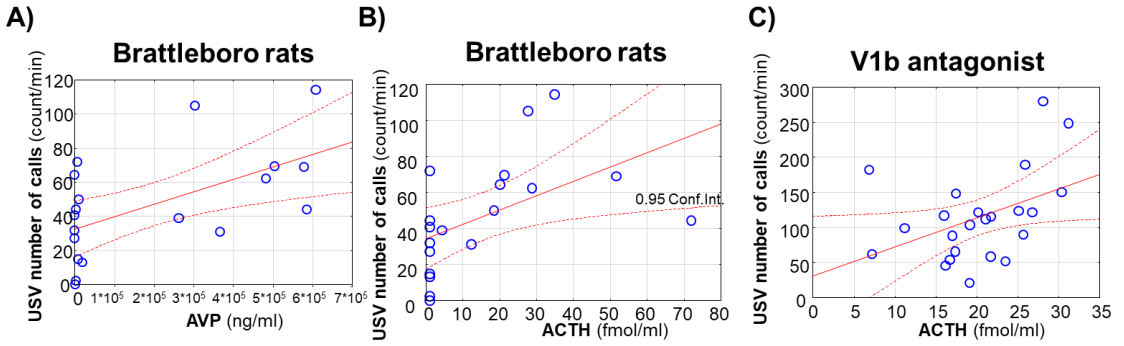

Figure 23. The most important correlations. In Brattleboro pups **A)** the hypophysis vasopressin (AVP) content positively correlated with the emitted number of ultrasound. Similar positive correlation was observable between the serum ACTH levels and the ultrasound number both in **B)** Brattleboro pups and after **C)** V1b receptor antagonist treatment.

5.4 Experiment 4.: Magnocellular AVP in social behavior

In accordance with previous results (Geddes et al., 1997; Ideno et al., 2003) AVP-AAV injection into the SON restored the local AVP synthesis both physiologically (**Figure 24. B**) and functionally (**Figure 24. A**).

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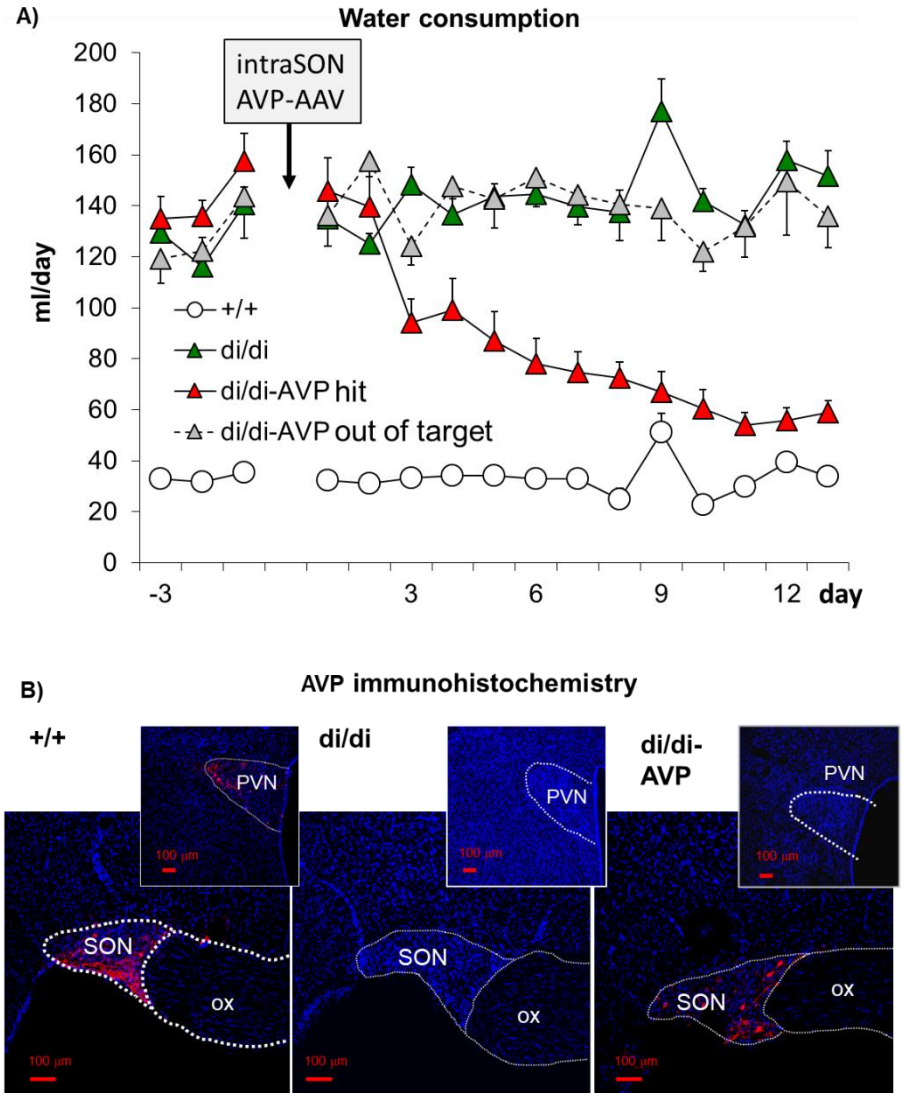

Figure 24. Confirmation of virus-driven rescue of AVP synthesis in the SON of male Brattleboro rats. **A)** In line with previous results, we confirmed that the high water consumption (mean ± SEM) of di/di rats was significantly reduced in a subset of AVP-AAV treated animals. The AVP-AAV animals showing no drop in their water consumption were considered as "no hits" and were excluded from later analysis. The criterion for selection of the "hits" was a positive reduction in water consumption calculated according to the following formula: 100-(average water consumption after operation)/(average water consumption before operation)*100. n=7-8/group; di/di-AVP out of target: n=8; **B)** Microphotographs showing immunohistochemistry for AVP in the brain of representative +/+, di/di and di/di-AVP animals. In the latter AVP synthesis was restored by AVP-AAV treatment in the SON, but not in the PVN. Dotted lines are used to delineate the respective nucleus from the surrounding tissue. Abbreviations: AVP, vasopressin; AVP-AAV, adeno-associated viral vector containing vasopressin sequence; di/di, vasopressin-deficient Brattleboro rat; di/di-AVP, di/di animals with vasopressin synthesis rescue in the SON; OX, optical chiasm; PVN, paraventricular nucleus of the hypothalamus; SON, supraoptic nucleus.

5.4.1 AVP-AAV rescue in SON and social behavior

During social investigation test di/di-AVP rats spent more time investigating the conspecific juvenile than both +/+ and di/di animals (**Figure 26. A**; $F(2,16) = 3.938$, $p = 0.041$).

In the RI test the quantitative measure of aggression (number of bites) was reduced in di/di-AVP rats compared to +/+ ($p = 0.030$), also showing a tendency to be lower in comparison with the di/di group ($p = 0.075$) (**Figure 26. B**; $F(2,18) = 5.923$, $p = 0.051$).

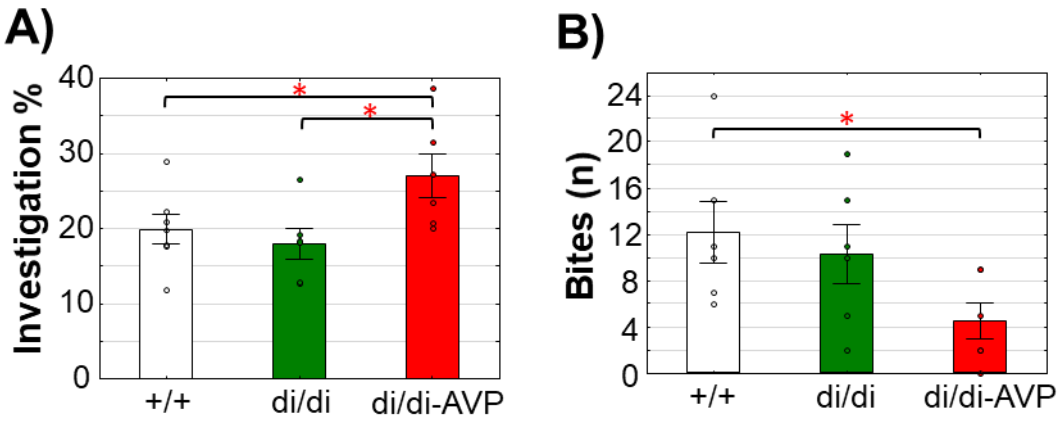

Figure 26. Behavioral parameters measured in Brattleboro rats after AVP synthesis rescue. A) di/di-AVP animals investigated a previously not encountered conspecific juvenile significantly longer than both +/+ ($p < 0.05$) and di/di rats ($p < 0.05$) **B)** In resident-intruder paradigm di/di-AVP animals showed a lower attack frequency than +/+ ($p < 0.05$), with a tendency to be lower also than that of di/di animals ($p = 0.07$; n=6/group). Abbreviations: AVP, vasopressin; di/di, vasopressin-deficient Brattleboro rat; di/di-AVP, di/di animals with vasopressin synthesis rescue in the supraoptic nucleus. * $p < 0.05$

5.5 Experiment 5.: Examination of epigenetic changes in Brattleboro rat brain by immunohistochemistry

The following brain regions were investigated (**Figure 27. E, F, G**): nucleus accumbens core (AcbC) and shell (AcbS) regions, prelimbic cortex (PrL), infralimbic cortex (IL) and dorsal peduncular cortex (DP), dorsal (LSD), intermediate (LSI) and ventral (LSV) part of the LS, as well as CA1, CA2 and CA3 fields of the dorsal HC.

In di/di animals AcH3K9 immunostaining showed significantly less labeled cells in DP of the PFC than in +/+ rats ($F_{genotype}(1,16)= 5.24$; $p < 0.05$) (**Figure 27. A**). In the other investigated PFC areas (PrL and IL) no differences were detected between the genotypes. AcH3K9 immunohistochemistry did not show significant differences between

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di/di and +/+ animals neither in the whole Acb nor in the separately investigated core and shell (**Figure 27. B**).

In LS only in LSV compartment had significantly less AcH3K9 immunopositive cells in di/di than in +/+ rats ($F_{genotype}(1,16)= 5.37$; $p < 0.05$) (**Figure 27. C**).

In CA1 there was significantly more AcH3K9 labeled cell in di/di rats than +/+ ($F_{genotype}(1,15)= 5.81$; $p < 0.05$), while in CA2 and CA3 there was no genotype effect (**Figure 27. D**).

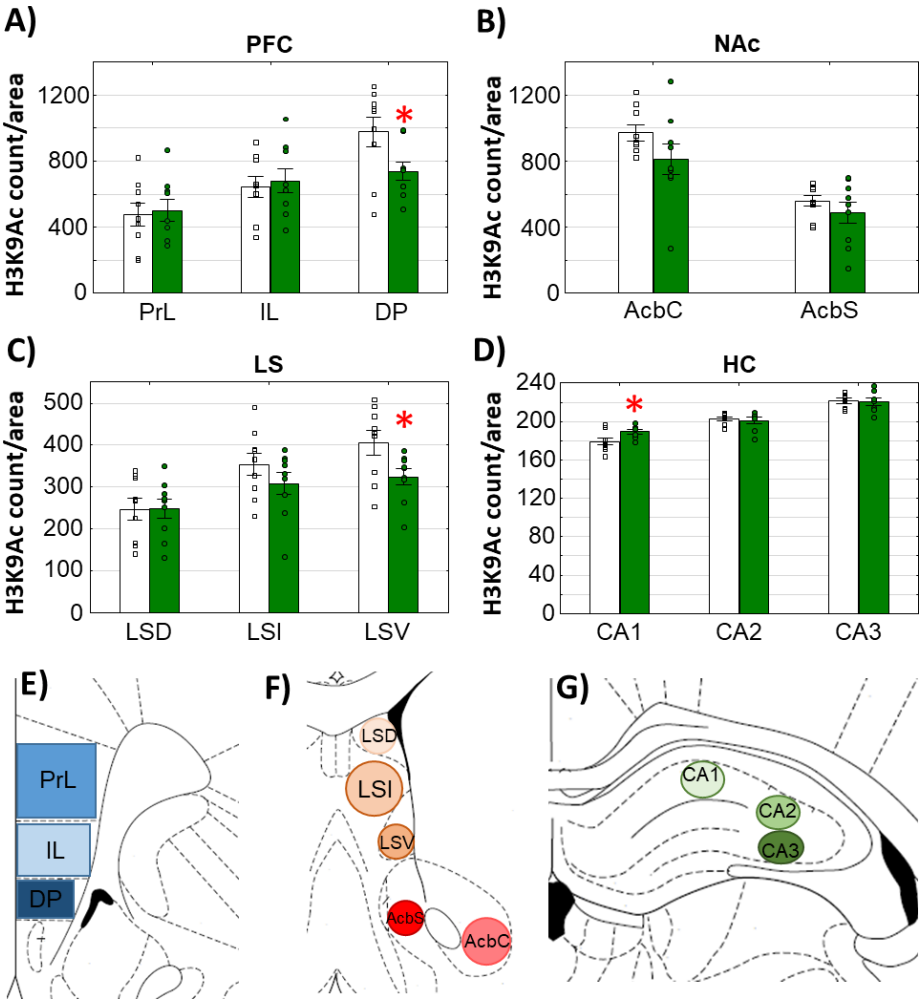
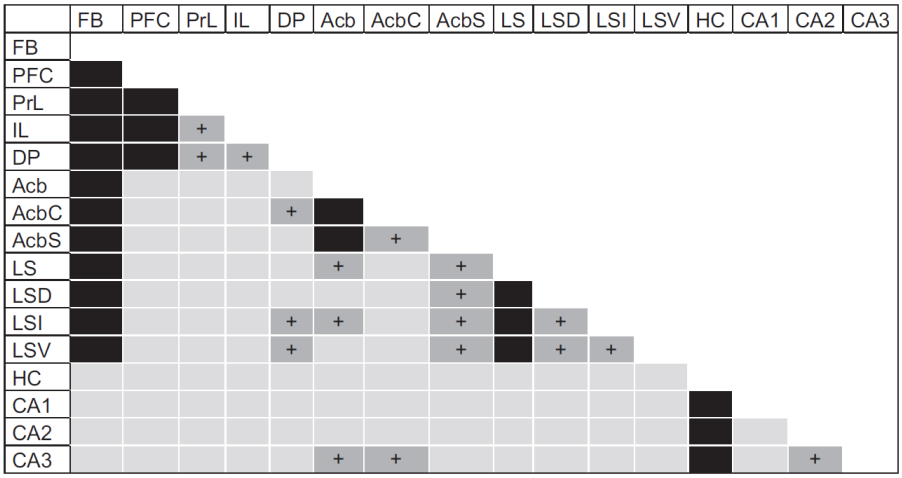


Figure 27. Acetylated lysine 9 of histone 3 (AcH3K9) immunohistochemistry in vasopressin-deficient (di/di) Brattleboro rat brain. A) Among the prefrontal cortex (PFC) regions in the dorsal peduncular part (DP) di/di rats revealed lower levels, while prelimbic (PrL) and infralimbic (IL) region showed equal number of AcH3K9 immunopositive cell number in both genotypes. **B)** Nucleus accumbens (Acb) shell (AcbS) and core (AcbC) did not show significant difference between the genotypes. **C)** In the lateral septum only the ventral (LSV) but not the dorsal (LSD) or intermediate (LSI) parts showed alterations. **D)** In the

CA1 region of the HC the AcH3K9 level was increased. n=8-9/group; **E) F) G)** Position of the investigated nuclei in the rat brain. For better overview only unilateral nuclei was shown. *p < 0.05 genotype difference.

We correlated the values of the investigated brain areas with each other (see **Table 10**). There was a significant positive correlation between DP area and AcbC ($r = 0.47$, $p < 0.05$), LSI ($r = 0.48$, $p < 0.05$) and LSV ($r = 0.55$, $p < 0.05$). The core region of the accumbens significantly correlated with CA3 ($r = 0.61$, $p < 0.05$), while the shell region positively correlated with LSD ($r = 0.50$, $p < 0.05$), LSI ($r = 0.70$, $p < 0.01$) and LSV ($r = 0.50$, $p < 0.05$).

Table 10. Matrix of the correlation among the AcH3K9 immunohistochemically stained brain areas. Light grey cells indicate the non-significant, while dark grey cells the significant correlations. "+" indicates that the correlation is positive. Black filled cells sign the meaningless comparison. FB means frontal part of the brain (PFC + Acb + LS). PFC means the summarized data of PrL + IL + DP. Acb is the summary of AcbC and AcbS, LS is LSD + LSI + LSV, while HC is the summary of CA1 + CA2 + CA3. Abbreviations: Acb, nucleus accumbens; AcbC, Acb core; AcbS, Acb shell; PFC, prefrontal cortex; PrL, prelimbic cortex; IL, infralimbic cortex; DP, dorsal peduncular cortex; LS, lateral septum; LSD, LS dorsal part; LSI, LS intermediate part; LSV, LS ventral part; HC, hippocampus; CA1-3, cornu ammonis of hippocampus.



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6 Discussion

The role of centrally released AVP in the development of SCZ-like behavior was confirmed in AVP-deficient (di/di) Brattleboro rat.

In **Experiment 1** AVP-deficient Brattleboro rats showed memory deficit, social withdrawal and sensorimotor gating deficit and all used antipsychotics successfully normalized the SCZ-like behavior of di/di rats. However, most were effective only after prolonged treatment (**Table 11.**). Regarding social interest (sampling during SD and social avoidance) all drugs reduced it to some extent both in control, +/+ and in di/di animals, suggesting a possible side effect.

Table 11. Schematic summary about the main effects of antipsychotic drugs on different behaviors in vasopressin-deficient Bratleboro rat. a : improvement; ×: impairment; empty: no effect

Antipsychotics	Behavior	Acute	Subacute	Chronic
		Treatment effect		
Aripiprazole	Social memory	a	a	a
	Social anxiety			
	Sensorimotor gating deficit		a	
Clozapine	Social memory	a	a	a
Haloperidol	Social memory	×		×
	Social anxiety	×	×	
	Sensorimotor gating deficit		a	a
Olanzapine	Social memory	a	a	a
	Social anxiety	×	×	
	Sensorimotor gating deficit	a	a	a
Risperidone	Social memory	a	×	a
	Social anxiety	×	×	
	Sensorimotor gating deficit		a	a

With **Experiment 2**, we could confirm our hypothesis that the reduced USV of AVP-deficient Brattleboro rats is indeed a sign of disturbed communication, which can be restored by low, non-sedative doses of atypical antipsychotics. This research is the first which examine the effect of antipsychotics on MS-USV on such a wide scale, only haloperidol-related studies can be found in the literature.

Our results in **Experiment 3** confirmed that genetic AVP-deficiency has an anxiolytic effect already during the early postnatal age, which was not influenced by the mild stress of an ip. saline injection. The positive correlation between the pituitary AVP content and MS-USV further confirmed the participation of this neuropeptide in the separation-

induced vocalization. Pharmacological AVP-effect-deficiency in Wistar rat pups decreased MS-USV and partly altered stress hormone levels.

The hypothesis that intra-SON AVP has an important role in social behavior was confirmed with **Experiment 4**.

Furthermore, we successfully demonstrated that the lifelong frameshift mutation in the gene coding AVP resulted in epigenetic alteration in the frontal part of the brain (DP, LSV) and in the HC (CA1), which might contribute to the appearance of SCZ-like behavior in Brattleboro rats (**Experiment 5.**).

6.1 Predictive validity of vasopressin-deficient Brattleboro rat

6.1.1 Adult Brattleboro rat

In our study acute olanzapine treatment was the most effective on **sensorimotor gating** (i.e. PPI) **deficit**. Similarly, PPI deficit induced by phencyclidine administration was normalized by acute clozapine, but not haloperidol or risperidone treatment (Li et al., 2011). Although many preclinical studies reported an acute effect of a single treatment with antipsychotics (Feifel et al., 2004; Gogos et al., 2008; Feifel et al., 2009), patients with SCZ are treated chronically. Therefore, we investigated the time course of repeated antipsychotic treatment. Indeed, subacute and chronic treatments were more effective regarding all studied antipsychotics (Li et al., 2011). Other animal studies found similar effects of olanzapine on PPI: this atypical drug increased PPI in SCZ models (Zangrando et al., 2013). Furthermore, studies with human patients showed the same effect (Wynn et al., 2007; Martinez-Gras et al., 2009). In contrast to our results with haloperidol treatment, in a 2002 clinical trial SCZ patients exhibited significantly less PPI after treatment with typical (among others haloperidol) than after atypical antipsychotics (clozapine, olanzapine or risperidone) or control subjects (Leumann et al., 2002). However, a number of typical and atypical antipsychotics have been tested in this trial and the results obtained were pooled together, just like in another study (Kumari et al., 2000). Although these drugs have similar receptor-binding profile, there may be large differences in their effects on positive and negative symptoms even within the groups. Therefore, a general conclusion for typical vs. atypical drugs from these data can be misleading (Andrade, 2016). Strain differences in sensitivity cannot be closed out either (Gogos et al., 2008). Indeed, in Balb/c mice a single haloperidol and aripiprazole treatment was more effective

than clozapine, olanzapine or risperidone for restoration of a serotonin 5-HT_{1A} receptor agonist-induced PPI deficit, and may the overall antipsychotic effectiveness was even less pronounced in 129Sv and C57Bl/6 mice. Another difference might be the applied doses, however, in the previously mentioned experiment on mice acute doses were in the same range as in our present study (Gogos et al., 2008).

All used drugs decreased the social interest to some degree, aripiprazole being the less implicated in this regard during the **social avoidance test**. We used rather low doses to avoid sedative side effects (e.g. clozapine 1mg/kg instead of generally used 10mg/kg (Li et al., 2011)) and, indeed, there was no difference in the general activity (e.g. exploration) of the animals. Moreover, the degree of habituation after repeated treatments was far below our expectations.

Human studies described that negative symptoms, like social avoidance respond poorly to medication (Fusar-Poli et al., 2015) e.g. in a clinical trial clozapine and risperidone did not affect social competence (Bellack et al., 2004). Furthermore, a longitudinal study has shown that olanzapine does not improve social functioning (Cuesta et al., 2001). Dorsal raphe dopaminergic neurons may play a crucial role in the regulation of sociability (Matthews et al., 2016). Photoinhibition of this brain area with optogenetics decreased sociability in mice after acute social isolation, while photostimulation improved it. Another study linked dopamine D1 receptor to social behavior: lower levels of dopamine and D1 receptor led to social interaction deficit which could be rescued with dopamine or a D1 receptor agonist (Liu et al., 2017). Clozapine, haloperidol, olanzapine and risperidone also have dopamine D1 receptor antagonist effect which could explain their negative effects on social behavior. On the other hand, aripiprazole has a partial agonist effect on dopamine receptors. Thus, consistently with human literature (Correll et al., 2019), its negative social effect is subtle.

Regarding **social memory** haloperidol treatment could not improve the deficit observable in di/di rats, rather yet worsened the memory of +/+ animals. Similarly, chronic haloperidol treatment in patients may induce working memory deficit (Lustig et al., 2005). The literature confirms that typical antipsychotics, as potent dopamine D2 antagonists, tend to affect only positive symptoms, and are not really suitable for alleviating negative and cognitive symptoms (Lustig et al., 2005; Sanchez et al., 2017). In contrast, clozapine had similar effects in our di/di rats like in a previously described

study: it restored social memory deficit after acute administration (Feifel et al., 2009). However, in this study only the highest, 10 mg/kg dose was effective, but not 1mg/kg used also in our study. The alteration in sampling could not influence the outcome as in case of 1 mg/kg both in our hands and in the above-mentioned experiment clozapine did not reduce significantly the time spent investigating the juvenile. Beyond the difference in appropriate control animals (Long Evans vs +/+) we also interpreted the data differently concentrating on intact memory (DI significantly differed from 0) rather than group comparison, which can explain some discrepancies. In clinical practice, several trials (Stip et al., 2003; Wang et al., 2013a; Topolov et al., 2016) as well as a meta-analysis (Woodward et al., 2005) showed that clozapine, as well as olanzapine, risperidone and aripiprazole could improve memory and learning abilities. The positive effect of olanzapine was especially interesting on social cognition tested by the Social Cue Recognition Test (Roberts et al., 2010). However, in a 2012 study social cognition enhancement have not been associated with first or second generation antipsychotics like haloperidol, clozapine or olanzapine in Facial Expression Recognition Test, the Voice Emotion Recognition Test, the Short Recognition Memory Test for Faces, and the Reading the Mind in the Eyes Test (Kucharska-Pietura et al., 2012).

Actually, this reduced social interest was well modelled in our animals as well (see **Figure 10.** left, **Figure 11.**) and might have also influenced social memory. For example, in case of risperidone the time spent investigating the juvenile during the sampling phase was the lowest during D8 compared to D1 and D15, and at this timepoint the treatment was ineffective on social memory. The ineffectiveness of the subacute versus acute and chronic risperidone treatment could be also explained by pharmacodynamical changes. We might assume that one-week treatment increased the number of - among others - dopamine D2 receptors (Moran-Gates et al., 2007), which lead to reduced receptor occupancy with ineffectiveness of risperidone (Mizuno et al., 2012). Further treatment shifted the receptor-ligand balance toward greater occupancy leading to reappearance of the positive effect on memory.

6.1.2 Brattleboro rat pups

Our results suggest that lower doses of antipsychotic treatment improve communication deficit in di/di rats.

Results in literature showed that in 10-day old rat pups pretreatment with haloperidol reversed USV reduced by cocaine, a potent dopamine reuptake inhibitor (Kehoe et al., 1992; Dastur et al., 1999). In our study haloperidol had no effect on USV, however, a completely different model was used. Cocaine affects dopamine system that regulates reinforcement and reward (Kehoe et al., 1992), which basically means ventral tegmental area dopaminergic neurons. In AVP-deficient rats, however, it is hypothesized that the most marked effect of AVP deficiency on the dopamine system is in the amygdala: AVP is a potent enhancer of dopamine utilization in that brain region (van Heuven-Nolsen et al., 1984). Another study suggests that dopamine D1 and D2 receptors may differentially modulate USV emission (Dastur et al., 1999): selective D2 receptor antagonist, sulpiride decreased USV rate of rat pups, but D2/D3 receptor antagonist, raclopride and the selective D1 receptor antagonist (SCH 23390) had no effect on it. Our results are similar to those found with raclopride and SCH 23390. This may be explained by the fact that haloperidol is an antagonist of all three dopamine receptors mentioned (Siafis et al., 2018). They also found that high doses of three dopamine receptor agonists (D1, D2/D3, D3) reduced USV (Dastur et al., 1999), which also suggests that the dopamine system plays an important role in the regulation of USV.

Lower doses of clozapine, olanzapine, risperidone and aripiprazole restored MS-USV in our study. Antipsychotic drugs (in these doses) do not have anxiogenic effects, furthermore there are data for their anxiolytic effects (Manzaneque et al., 2002; Biojone et al., 2011). Although it seems controversial that a less anxious/depressive state is accompanied by a more schizophrenic phenotype, there are theories suggesting that depression and SCZ are different from normal into two opposite direction, and may be distributed across a dimensional spectrum (Buckley et al., 2009). Thus, - based upon behavioral and hormonal data - we can rule out the possibility that the treatment-induced elevation of the USV was because of the elevated anxiety level of the treated pups, rather this may be related to the improvement of the communication deficit.

The abovementioned atypical antipsychotics have some properties in common that cannot be said for haloperidol: they act on serotonin receptors to a much greater extent.

There are experiments which support the connection that MS-USV is under control of the serotoninergic system (Olivier et al., 1998; Iijima et al., 2005). The effects of antipsychotics on serotonin receptors are extremely complex (Siafis et al., 2018), and their effects on MS-USV have not yet been studied. It can be hypothesized that our results can be explained by the effect of the partial agonism of the 5-HT_{1A} receptors, or by the effect on the 5-HT_7 receptor. It has been found that 5-HT_{1A} receptor agonist decrease MS-USV in rat pups separated from their mother (Groenink et al., 2008). Beside this, the potent 5-HT_7 receptor antagonist amisulpride improved ketamine-induced social withdrawal in rats (Holuj et al., 2015). Therefore 5-HT_7 receptors might have a role in social communication. However, further studies are needed to elucidate the exact mechanism of action.

As for the effects on stress hormone levels, the stressor-induced (i.e. maternal separation-induced) ACTH levels were lower and corticosterone levels were higher in di/di rats. The functional activity of HPA system is reduced in homozygous Brattleboro rats: the concentration of ACTH in the plasma and in the adenohypophysis is markedly reduced with reductions in adrenal weight (Buckingham et al., 1980). The role of AVP is important in the ACTH secretion regulation during most acute stimuli, but the contribution of AVP to stress-induced HPA activity is context dependent (Makara et al., 2012).

In our previous study we confirmed the hypothesis that during the perinatal period, AVP may be the main regulator of the HPA axis (Zelena et al., 2009a): the stressors-induced ACTH rise was always smaller in di/di pups. Furthermore, the resting corticosterone levels of di/di pups were higher. This phenomenon might have relevance also in humans: stress-related increases in cortisol levels exacerbate the abnormality in dopamine neurotransmission that underlies vulnerability to SCZ, resulting in the onset of the illness (Jones et al., 2007). It has been found that the HPA axis plays its role in SCZ through having knock-on effects on other neural systems, specifically the dopaminergic neurotransmission system. Higher baseline levels of cortisol were found in SCZ patients compared with healthy controls. Furthermore, drugs that raised cortisol levels aggravated the symptoms of SCZ; while neuroleptics significantly blunted HPA activity and decreased cortisol levels (Walker et al., 1997). The concentration of liquor CRH was slightly, but significantly higher in SCZ patients than in control subjects (Banki et al.,

1987). In SCZ dexamethasone suppression test (DST) shows non-suppression: cortisol release is not inhibited by dexamethasone via feedback inhibition of the HPA axis and it is associated with negative and cognitive symptoms (Newcomer et al., 1991). The mean rate of non-suppression to the DST in SCZ approximately 20%, which is much lower than that described in depression but still higher than in the normal population (Yeap et al., 2005). Perhaps the increased AVP release into portal vessels causes the non-suppression.

In our present study haloperidol, risperidone and aripiprazole increased ACTH and corticosterone levels (clozapine and olanzapine also, but only at the high, sedative doses). A human study showed that haloperidol administration can increase cortisol levels (Murburg et al., 1986), but basically most clinical studies have found that antipsychotics reduce stress hormone levels or do not influence them (Piriu et al., 2015; Handley et al., 2016). However, there is no data for perinatal age.

All in all, with these studies face and predictive validity of this animal model of SCZ has been confirmed. However, further studies needed to map the regulation of MS-USV during the early postnatal age. USV deficit in Brattleboro pups seems to be a reliable tool for preclinical screening of antipsychotics, as it requires a very minor amount of drug.

6.2 Role of vasopressin receptor subtypes in maternal separation-induced ultrasonic vocalization

Pharmacological analysis showed that high dose (30 mg/kg) of V1aR antagonist and all studied doses of V1bR antagonist reduced MS-USV, while V2R antagonist elevated it in the smallest studied dose (3 mg/kg). The MS-USV levels correlated positively with ACTH levels in the case of V1bR antagonist only, similarly to Brattleboro rats. None of the studied intervention influenced the latency of righting and negative geotaxis suggesting that they are without any sedative side-effects.

As anxiety is a stress-related disorder, drugs influencing the HPA axis was in the focus of interest for its treatment. Indeed, the first selective and orally active non-peptide antagonist of V1bRs, SSR149415, reduced stress-related behavior inducing anxiolytic and antidepressant-like effects in adult rodent models (Griebel et al., 2002). We found a strong anxiolytic effect of the V1bR antagonist also in rat pups with all studied doses (3-10-30 mg/kg), which confirms our previous results with 10 (Varga et al., 2015) and 30

mg/kg (Zelena, 2018). In a previous experiment the same V1bR antagonist in the same doses showed only a tendency to reduce USV (Iijima et al., 2005). However, they used 9-11-day-old animals (for age-dependent MS-USV see Fig.1 in (Varga et al., 2015)) and 5 min measurement, which might be responsible for the reduced sensitivity of their assay. Similarly, the tendency seen with another V1bR antagonist, TASP0233278 (Iijima et al., 2014) might be also attributed to their less sensitive assay, as they also used 5 min measurement and older animals (21-30 g in contrast to our 16-20 g animals). The shorter recording time (5 min) might have been also covered any possible difference between wild-type (WT) and V1bR KO mice as well (Scattoni et al., 2008). However, in these V1bR KO animals the repeated MS was not able to induce any potentiation in their MS-USV in contrast to their WT littermates, supporting some anxiolytic role of this receptor subtype.

In our hands the positive correlation between USV and stress-hormones in the case of V1bR antagonist treatment confirmed that this receptor-subtype is able to influence anxiety through regulation of stress-hormones. Interestingly, not the end-hormone of the axis (in rodents, corticosterone), but the pituitary component, ACTH was implicated in this phenomenon. It is in line with the results found in Brattleboro rats (see **Figure 18. C, D** and (Varga et al., 2015; Zelena, 2018)), where ACTH levels did not go parallel with the corticosterone levels. Although it is hard to separate the effect of ACTH from the effect of its downstream molecules (e.g. glucocorticoids, mineralocorticoids or adrenal androgens produced in the adrenal cortex), based upon ACTH administration many extra-adrenal effects of ACTH have been suggested e.g. cardiovascular, metabolic, motivational, memory influencing (Zelena, 2012c). In regard to stress-related psychiatric disorders chronic ACTH administration in rats mimicked the symptom of treatment-resistant depression (Kitamura et al., 2008). ACTH-producing tumors were also associated with mood swings (McCaughey et al., 1987), however, in this case the role of other, downstream factors cannot be entirely closed out. Additionally, other pathways may also contribute to the V1bR-directed anxiogenesis as previous studies showed altered V1bR protein levels in the rat hypothalamus in connection with anxiolytic treatment (Kokras et al., 2011).

On the other hand, several data speak in favor of V1aRs in anxiety (Ebner et al., 1999). In adult rodents both genetic (KO mice) and pharmacological (receptor antagonism)

blockade of the V1aRs showed anxiolytic effects (Egashira et al., 2007). Our data confirmed the anxiolytic effect of V1aR antagonism in pups, however, only in the highest dose (30 mg/kg). A 10 min observation was sufficient to reveal the anxiolytic role of another V1aR antagonist, JNJ-17308616, in 11-day-old CD rat pups, too (Bleickardt et al., 2009). Interestingly, this was also detected only in the highest (100 mg/kg) dose, when it may influence also the V2Rs. As SSR49059 cannot cross the blood-brain-barrier in adults (Tribollet et al., 1999), we might assume that in pups the increased permeability of the blood-brain-barrier made its effect possible. We might suppose a central effect of V1aR antagonists as at the periphery the vasoconstrictory role of V1aR is dominant. Indeed, V1aR KO mice have lower blood pressure (Koshimizu et al., 2006). As a drop in the blood pressure might be stressful, it was not surprising, that the highest dose of the V1aR antagonist (30 mg/kg) stimulated the HPA axis. However, the stress could hardly explain the anxiolysis.

The effect of combined V1aR and V1bR antagonism were slightly stronger than any antagonist alone (more close to the effect of the V1bR antagonist), without any correlation with stress hormones. Thus, it seems that during the early postnatal period both V1b- and V1aRs are involved in the development of anxiety, most probably through a central brain target other than the HPA axis.

We did not truly expect any anxiolytic effect of the V2R antagonist, as in 8-9-day-old Sprague Dawley rat pups the AVP administration-induced MS-USV was antagonized by V1R influencing drugs (Winslow et al., 1993a). In our hands 3 mg/kg of V2R antagonist was even anxiogenic, while 30 mg/kg was highly stressful. As V2Rs are important in salt-water homeostasis, their antagonism may induce an imbalance leading to appearance of anxiety and high-levels of stress-hormones. As mentioned earlier, the HPA axis parameters were clearly separated from the MS-USV behavioral measure.

6.3 Function of central vasopressin expression on social behavior

It is of note that despite the fact that there was no difference between +/+ and di/di animals in both social investigatory and aggressive behavior, AVP-AAV treated di/di animals investigated conspecific juveniles significantly longer and showed a lower level

of aggression than +/+ rats (**Figure 26. B**). This suggests that AVP, originating in the SON may have signaled on MeA neurons to promote non-aggressive social interactions.

This confirms a rather old hypothesis of a specific contribution of intra-SON AVP to social behavior (Engelmann et al., 1994b). Indeed, it was suggested that AVP may differently regulate social behavior, acting on different brain regions (Fodor et al., 2014). For instance, higher AVP levels within the lateral septum stimulated aggressive behavior, while within the bed nucleus of stria terminalis inhibited it (Veenema et al., 2010). Similar discrepancy was observed for endogenous AVP release in the septum *versus* amygdala for the control of coping behavior during forced swimming(Ebner et al., 1999; Ebner et al., 2002). This could explain how breaking a balance of extrahypothalamic AVP signalling in the brain of the di/di rat by rescuing AVP synthesis in the SON might have unmasked the role of this nucleus in social behavior (**Figure 26. A**). The change in social interactions was not the consequence of a general increase in investigatory curiosity, because during a novel object recognition test di/di-AVP animals showed an even lower object investigation than +/+ rats (data not shown). This implies that AVP signalling in the SON may selectively affect social curiosity and may contribute to fine-tuned social behavior, in concert with other neurotransmitters/neuromodulators. In this respect, AVP signalling in the MeA might be especially important, as this area is sensitive to chemosensory signals, particularly non-volatile ones (Keshavarzi et al., 2014; Westberry et al., 2017). That might be the background for its involvement in social recognition (Noack et al., 2015). Indeed, MeA lesion in mice reduced aggressive behavior either permanently or temporarily (Wang et al., 2013b) and decreased social investigation (Noack et al., 2015). Interestingly, intra-MeA (but not i.c.v.) administration of a V1a antagonist significantly impaired maternal recognition (measured by maternal behavior latencies) (Nephew et al., 2008). This supports not only the hypothesis of the importance of AVP signalling within the MeA via V1a receptors for social behavior, but also our idea that systemic manipulation of AVP signalling (i.e. by i.c.v. injection or in a general knockout animal) may trigger both inhibitory and excitatory effects compensating each other and, therefore, resulting in unchanged behavior. However, further studies have to evaluate the physiological impact of AVP originating in the SON on information processing in the MeA, since in normal, non AVP-deficient animals locally synthetized AVP might dominate the interaction with intra-MeA AVP receptors (Csikota et al., 2016).

It is worth mentioning that the role of AVP might depend on the strain as well as on sex (Fodor et al., 2016b; Terranova et al., 2017). Moreover, cellular morphology of the MeA is sexually dimorphic (Cooke et al., 2005). Thus, further studies should emphasise the sex differences.

6.4 Epigenetic changes in vasopressin-deficient Brattleboro rats

The acetylation of a single lysine residue (AcH3K9) was studied in our experiment, because decreased level of AcH3K9 in PFC was found in a pharmacological model of SCZ (PCP in mouse) (Aoyama et al., 2014). Moreover, previous observation found a decrease of AcH3K9 in human SCZ patients (Gavin et al., 2008). Our results in di/di rats were in good agreement both with the pharmacological model and human data.

Both in human patients and animal models of SCZ dysfunction of the dopaminergic system was observed (Deutch, 1992) and as it mentioned before, the currently used antipsychotics antagonize dopamine receptors (Harrison, 1999). In the PCP model of SCZ low AcH3K9 caused dysfunction of the dopaminergic systems and an atypical antipsychotic, clozapine ameliorated the AcH3K9 level mainly on D1 receptor positive cells in the PFC (Aoyama et al., 2014). It is also known, that D1 signaling regulates histone modification (Schroeder et al., 2008) and we also have found significant positive correlation (**see Table 10**) between the number of AcH3K9 positive cells in the PFC and elements of the dopaminergic system (DP, Acb, LS). Although we did not measure significant alteration in the mRNA level of dopamine degrading enzyme COMT neither in FB nor in HC, we can hypothesize that in Brattleboro rats the lack of AVP induces epigenetic modification leading to disturbances of the dopaminergic system, which contributes to the SCZ-like behavioral alterations. Indeed, higher dopamine content (Williams et al., 1985; Feenstra et al., 1990) and upregulated dopamine receptors (Shilling et al., 2006) were reported in di/di animals.

In the following we have examined those brain areas, which are thought to be involved in the development of SCZ. We have found significant decrease in the number of AcH3K9 positive cells in the DP of di/di rats. The role of DP is less known and investigated than the neighboring IL and PrL despite their structural similarities (Zelena, 2012a).

Ventral part of the PFC specifically responsible for a flexible shifting to new strategies related to spatial cues, furthermore, on the basis of its connections with autonomic centers, for the integration of internal physiological states with salient environmental cues for the guidance of behavior (Heidbreder et al., 2003). DP shares projections relevant to reward circuitry such as glutamatergic afferents to the ventral tegmental area (Geisler et al., 2007). Furthermore, the IL and DP both project heavily to the AcbS (Brog et al., 1993).

There are lots of evidences that the Acb (because its dopamine content) is involved in the pathology of SCZ (Csernansky et al., 1998; Moore et al., 1999). Although alteration of dopamine receptors in the Acb was found in di/di rats (Shilling et al., 2006), we did not find significant genotype difference in the number of AcH3K9 labeled cells. It seems that in Brattleboro rat, AcH3K9 alteration is not involved in the development of symptoms, but changes at other lysine residues might have some importance.

LSV plays an important role in various behavioral processes (Sheehan et al., 2004), integrates sensory stimuli conveying this information to responsible brain areas to direct motivation behaviors. LSV, showing significantly lower AcH3K9 reactivity in di/di than control rats, was activated (measured by c-Fos immunochemistry) during PPI (van Luijtelaar et al., 2001). Medial PFC sends glutamatergic projections to LS (Sheehan et al., 2004), thus, the lower cell activity of DP induced by decreased acetylation might lead to reduced acetylation/activation of LSV neurons.

LS is strongly interconnected with the HC predominantly by inhibitory GABAergic neurons (Burjanadze et al., 2015), which connection has an established role in learning and memory (Roland et al., 2014). Moreover, the septo-hippocampal (CA1) GABAergic neurons were activated during locomotion and salient sensory event in behaving mice (Kaifosh et al., 2013). Thus, it is not surprising, that reduced inhibitory GABAergic tone in the HC lead to enhanced AcH3K9 level in the CA1 of di/di rats compared to control animals. These data, along with postmortem analyses (Benes et al., 2007; Sharma et al., 2008), suggested that histone modifications in the HC might also contribute to the behavioral alteration in SCZ through aberrant regulation of one or more genes. Indeed, epigenetic mechanisms were initially described for their ability to promote differentiation including neurogenesis and HC is one of the two regions in which generation of new

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functional neurons from neural stem cells occurs throughout the adult life. Adult hippocampal neurogenesis contributes to learning and memory, core features of SCZ (van Praag et al., 2002; Deng et al., 2010).

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7 Conclusions

Our studies have extensively examined AVP-deficient Brattleboro rat as a possible model of SCZ from several perspectives to confirm its suitability for preclinical research.

We confirmed that clinically effective antipsychotics alleviated SCZ-like symptoms in adult, male AVP-deficient Brattleboro rat. Thus, therapeutic response of the strain to medication is similar that in human SCZ patients. Therefore, (additional to its *face* and *construct* validity, see introduction) based upon its *predictive validity* this animal might be a good preclinical model of SCZ. Although PPI was assumed to model cognitive deficit, it may resemble also positive and negative symptoms (Braff et al., 1999). Additionally, SD models also negative symptoms. As new drugs should target the presently untreatable negative and cognitive symptoms (Sanchez et al., 2017), this might be a special strength of this model as di/di Brattleboro rats showed deficit presumably in these domains.

Besides, 7-8-day-old di/di Brattleboro rat pup can be a good model for the communication deficit of SCZ. This animal model might be an excellent opportunity to test newly developed drugs: all antipsychotics used in our experiment restored low MS-USV in AVP-deficient animals at lower doses. Furthermore, MS-USV of pharmacologically produced AVP-effect deficient Wistar rat pups was also reduced compared to controls and it positively correlated with pituitary AVP level, confirming the role of this neuropeptide in separation-induced vocalization.

AVP originating from the SON might contribute to the fine-tuning of social behavior neuronal network of rodents, possibly via a pathway involving the MeA.

All experienced behavioral deficits in di/di Brattleboro rats are possibly occurred through epigenetic changes, which also might reinforce the validity of di/di rat as a model of SCZ. However, further studies are needed to investigate this possible connection.

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8 Summary

Due to its various function vasopressin (AVP) has been associated with many psychiatric disorders, including schizophrenia (SCZ). Our previous study confirmed that - based upon its face validity - AVP-deficient (di/di) Brattleboro rat can be a good genetic model for SCZ.

Our present aim was to confirm the role of centrally released AVP in the development of SCZ-like behavior and to investigate possible epigenetic changes using di/di rat.

We demonstrated that antipsychotic treatment normalized SCZ-like behavioral alterations in adult animals similar to that in human. Maternal separation-induced ultrasonic vocalization (MS-USV) was reduced in di/di rat pups, which was successfully restored with smaller, non-sedative doses of atypical antipsychotics. With the help of pharmacological AVP deficiency, AVP receptor subtypes' role was studied on MS-USV of Wistar rat pups. Interestingly, both V1a and V1b receptors have been shown to participate in this behavioral alteration.

To investigate possible molecular mechanisms, contribution of magnocellularly released AVP from the supraoptic nucleus of the hypothalamus was studied. Stress induced c-Fos activation was normalized in the nearby medial amygdala, but not in remote brain areas (e.g. central or basolateral amygdala, lateral hypothalamus). This phenomenon might contribute to the SON AVP involvement in the fine-tuning of social behavior.

Epigenetic changes (histone AcH3K9 modification, which might occur in human SCZ patients as well) were also found in di/di rat brain with differences in frontal region (dorsal peduncular cortex and ventral part of the lateral septum) and hippocampus compared to control.

The findings with antipsychotic treatment further support the predictive validity of di/di rats as SCZ models. Moreover, the role of centrally released AVP in the development of SCZ-like behavior was confirmed. As another possible mechanism, epigenetic changes might contribute to the appearance of SCZ-like behavior in di/di rats. All in all, AVP-deficient Brattleboro rat might be a good preclinical model of SCZ: it may improve the success of drug development for negative and cognitive symptoms.

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9 Összefoglalás

Számos funkciója miatt a vazopresszin (AVP) több pszichiátriai rendellenességhez is köthető, például a skizofréniához (SCZ). Korábbi tanulmányaink megerősítették, hogy az AVP-hiányos (di/di) Brattleboro patkány jó genetikai modellje lehet a skizofréniának.

Disszertációmban ismertetett kutatásaink célja az volt, hogy megerősítsük a centrálisan felszabaduló AVP szerepét az SCZ-szerű viselkedés kialakulásában, és megvizsgáljuk az ehhez társuló lehetséges epigenetikai változásokat di/di patkányban.

Kimutattuk, hogy antipszichotikum kezelés hatására normalizálódtak a SCZ-szerű viselkedésbeli változások felnőtt állatokban, hasonlóan az emberhez. Az anyai elválasztás-indukálta ultrahang vokalizáció (MS-USV) csökkenést mutat di/di kispatkányokban, amelyet kisebb, nem szedatív dózisú atipikus antipszichotikumok sikeresen helyreállítottak. Emellett farmakológiai AVP-hiány előidézésével az AVP-receptor altípusok szerepét vizsgáltuk Wistar patkánykölykök MS-USV-jében. Érdekes módon azt találtuk, hogy mind a V1a, mind a V1b receptorok részt vesznek e viselkedési változás kialakulásában.

A lehetséges centrális mechanizmusok vizsgálatához a szupraoptikus magok (SON) magnocellulárisan felszabadult AVP-jének szerepét tanulmányoztuk. A stressz okozta c-Fos aktiváció a közeli mediális amigdala területén normalizálódott, de a távolabb fekvő agyterületeken (pl. centrális, bazolaterális amigdala, laterális hipotalamusz) nem. Ez a jelenség hozzájárulhat ahhoz, hogy a SON-ból felszabaduló AVP részt vesz a társas viselkedések finomhangolásában.

Az epigenetikai változásokat (hiszton AcH3K9, amely előfordulhat SCZ betegeknél is) szintén vizsgáltunk di/di patkányban, és különbséget találtunk a frontális régióban és hippocampusban a kontrollhoz képest.

Az antipszichotikum kezeléssel kapott eredmények megerősítik a di/di patkányok, mint SCZ modellek prediktív validitását. A központilag felszabadult AVP, illetve a leírt epigenetikai változások hozzájárulhatnak a SCZ-szerű viselkedés megjelenéséhez di/di patkányokban. Összességében elmondható, hogy az AVP-hiányos Brattleboro patkány jó preklinikai modellje lehet az SCZ-nak: javíthatja a negatív és kognitív tünetekre irányuló gyógyszerfejlesztések sikerét.

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11 List of own publications

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