Theoretical Medical Sciences Ph.D. Program

The role of D_2 dopamine receptors in the ventral pallidum in rewarding and learning processes in neurotypical and MAM-E17 schizophrenia model rats

Ph.D. Thesis

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1. Introduction

Schizophrenia is a devastating psychiatric disease affecting approximately 1% of the population, characterized by disturbances in mental processes, perceptual distortions and a disruption in the coherence between thoughts, emotions, and behaviors (1). The exact etiology of schizophrenia is unknown; however, the involvement of several central nervous system (CNS) structures and neurotransmitter systems, as well as the significance of genetic and environmental risk factors are assumed (1). The goal of schizophrenia treatment is to reduce symptoms, prevent relapse, and reintegrate the patient into society. This is attempted through pharmacological methods (primarily second-generation, 'atypical' antipsychotics) and non-pharmacological methods (such as psychotherapy). The typically used antipsychotics have dopamine (DA) (primarily D_2) receptor antagonist effects.

A strong correlation can be observed between the antipsychotic efficacy of the drugs and their binding affinity to the D_2 DA receptors (D_2Rs) (2). The complexity of schizophrenia, combined with the adverse side effects of current treatments, means that the management of the disorder remains far from fully resolved. To enhance patients' quality of life, the development of novel medications is essential, which, in turn, necessitates a thorough investigation of the disease and the identification of its underlying causes. Due to the limited opportunities for human studies, the neurochemical and structural changes in the CNS that contribute to the onset of schizophrenia can primarily be examined through post-mortem investigations. Consequently, the use of animal models is indispensable in schizophrenia research. To gain a deeper understanding of the disease's underlying mechanisms and identify new therapeutic targets, a validated animal model is required that replicates the functional and behavioral disruptions observed in individuals with schizophrenia (3).

In our Institute, the MAM-E17 schizophrenia animal model has been used, which is based on the neurodevelopmental theory of the disease. An important advantage of this model is its ability to replicate the disease's dyachronic nature, particularly the emergence of positive symptoms following adolescence. Methylazoxymethanol (MAM) is a methylazoxymethanol β-D-glucoside. Administered at a dose of 25 mg/ml/kg to pregnant rats on day 17 of gestation, MAM is able to cross the placenta and methylate the DNA of rapidly dividing CNS cells in the fetus, thereby inhibiting neuroblast proliferation without affecting glial cell division (4, 5). On day 17 of rat embryogenesis, cell division occurs in regions such as the hippocampus (HPC), striatum (nucleus accumbens), amygdala (AMY), and cortex. As a result of the intervention,

neural defects develop in these regions, which are compensated by the reorganization of corticolimbic circuits (6). These structural changes lead to metabolic activity alterations and DA transmission disturbances in the affected neuronal networks (6). This phenomenon is consistent with the hypothesis that developmental abnormalities in limbic and frontal cortical neural circuits lead to impaired regulation of the basal ganglia and DA systems in schizophrenia (7). MAM-E17 animals display hypersensitivity, hyperreactivity and increased locomotor activity, which are attributed to the hyperactivity of the mesolimbic DA system and correspond to the positive symptoms observed in individuals with schizophrenia (8). The MAM-E17 model animals exhibit several cognitive impairments characteristic of schizophrenia, such as attention deficits (9, 10), as well as disturbances in long-term memory (11) and working memory (11), as well as reduced behavioral flexibility (10). Behavioral inflexibility and perseveration, observed both in MAM-E17 animals and in patients with schizophrenia (5) , are likely the result of combined alterations in various prefrontal regions, including the medial prefrontal cortex (mPFC), insular/perirhinal areas, and the prelimbic/infralimbic regions. Among the negative symptoms, social interaction is practically the only measurable domain in rodents. MAM-E17 animals exhibit a marked reduction in active social behaviors, showing less inclination to initiate contact with unfamiliar conspecifics. In contrast, passive interactions remain unaffected, as they tolerate the presence of an approaching animal (12).

DA is a key neuromodulator and neurotransmitter in the CNS. In various neuropsychiatric disorders, the emergence of symptoms may be influenced by disruptions in the balance between tonic and phasic DA activity (13, 14). In schizophrenia, a reduction in tonic DA release has been reported, despite increased population activity, accompanied by an enhancement of phasic DA release (15). DA plays a central role in regulating behavior by contributing to the rewarding sensations associated with evolutionarily important activities - such as eating, reproduction, and hunting - that support survival and the continuation of the species (16). Rewarding experiences, such as eating or social interactions, elicit an increase in DA levels within the CNS, which enhances an individual's motivation to seek out similar experiences—thereby reinforcing behaviors essential for survival (16). Underlying this process is the reward prediction error (RPE) (17), encoded by mesencephalic DAergic neurons, which reflects the discrepancy between the expected and the actual reward (17). As a result of the phasic-burst activity of DAergic neurons in the ventral tegmental area (VTA), DA is released phasically in the nucleus accumbens (NAC), exerting a rewarding and reinforcing effect both on environmental stimuli and on the animal's motor actions that are contingent upon receiving a reward. It has been shown that the phasic activation of DAergic neurons in the VTA induces

place preference, increasing DA levels in the NAC (18). At the same time, the inhibition of these neurons induces place aversion in the experimental animals (19). DA released in the NAC plays a crucial role in sensorimotor integration, thereby facilitating the development of flexible approach responses (20). Additionally, DA energizes the animal, increasing the vigor of movement and essentially being responsible for "raw" motivation (without hedonistic value) (21). DA, independently of its rewarding effect, plays a fundamental role in learning processes. This is broadly supported by experiments conducted across different brain regions and behavioral paradigms. DA plays a vital role in the long-term consolidation of short-term memories formed through conditioning processes (22). It has been demonstrated in several brain regions that DA and its receptors play a pivotal role in the consolidation processes related to spatial and aversive learning (23-29). According to our current knowledge, during learning processes, changes take place in the brain's synaptic structure, and it is the consolidation and stabilization of these changes that ultimately lead to the formation of memory. DA and its receptors play a fundamental role in modulating synaptic plasticity and its electrophysiological correlates, namely long-term potentiation (LTP) and long-term depression (LTD) (30-34). The former refers to synaptic strengthening, while the latter denotes synaptic weakening.

In schizophrenia, the DAergic system is notably affected. Positron emission tomography (PET) studies have shown altered DA levels in the PFC, the cingulate cortex and the HPC in patients with schizophrenia compared to healthy controls (35). According to more recent data, schizophrenia is not characterized by uniformly increased DA activity, but rather by enhanced DAergic transmission in the mesolimbic regions and reduced DAergic transmission in the PFC (36-38). The negative symptoms of the disorder and the decline in cognitive functions may be linked to decreased D_1R activation in the cortex and HPC, as well as alterations in D_3R expression (39), while the manifestation of positive symptoms is thought to result from increased DA activity in the limbic and subcortical regions (40). An enhanced response to psychostimulants such as amphetamine has been demonstrated in schizophrenia (41), as well as in rodent models of the disorder (12, 42). The positive symptoms of schizophrenia are primarily attributed to the heightened responsiveness of DAergic systems (43). The hyperactivity of the HPC correlates with the presence and severity of psychosis in individuals with schizophrenia (44). In the MAM-E17 rat model, the hyperactivity of the HPC leads to DAergic system hypersensitivity and increased locomotor activity (42), which can be paralleled with the positive symptoms of the disorder (45, 46). Excessive activity of the glutamatergic output neurons in the HPC has been shown to lead to increased DAergic population activity in the VTA region (42) through the NAC-ventral pallidum (VP) axis (47).

D_2Rs are primarily involved in the triggering of the positive symptoms of schizophrenia (43, 48-50), and also play a significant role in cognitive impairments (51), where their elevated expression leads to reduced cognitive flexibility and impaired change of strategy (51, 52). Most current medications used in schizophrenia bind to D_2Rs and reduce aberrant DA transmission (53). Chronic use of D_2R antagonist antipsychotics effectively reduces the number of spontaneously active DAergic neurons via the "depolarization block" mechanism (54-57). MAM-E17 rats respond to D_2R agonist quinpirole with increased DAergic neuronal activity and locomotion, and a significant increase in D_3R mRNA expression in the NAC is observed (D_3R belongs to the D_2R family), suggesting that these receptors are likely responsible for the sensitized locomotor response to quinpirole (58). In addition to the increased VTA DAergic population activity and the elevated number of D_2Rs, an increase in the quantity of DA high-affinity so-called "D_2 High" receptors can also be observed in schizophrenia (59). NAC neurons expressing D_2Rs send GABAergic fibers to the VP, and their stimulation promotes VTA DAergic activity (60). The VP, a key element of the HPC-NAC-VP-VTA axis, plays a central role in regulating the population activity of VTA DAergic neurons (14). However, the involvement of the VP and its DA receptors in the pathomechanism of schizophrenia is still not well understood.

The brain region investigated by us is the VP, situated at the "intersection" of the limbic and extrapyramidal systems, and is a key structure within the basal forebrain (61). Both D_1Rs and D_2Rs can be found in this region (62). Receptors belonging to the D_2R family are primarily located presynaptically in the VP, on GABAergic and glutamatergic fibers coming from the NAC, but a smaller number can also be found postsynaptically on the VP output cells and interneurons (63-65). The VP plays a key role in regulating the population activity of VTA DAergic neurons, as its GABAergic neurons inhibit the firing of VTA neurons (14). The HPC regulates the storage of information in long-term memory through the NAC-VP-VTA axis (66). We have shown that the VP D_2Rs exert a negative regulatory "feedback" effect on both the population and burst activity of VTA DAergic neurons (67). Activation of VP D_2Rs by the agonist quinpirole also influences the behavior of experimental animals: it induces place aversion in a dose-dependent manner (67) and facilitates memory consolidation in passive avoidance (68) and spatial learning (69) processes. However, the impact of VP D_2R inhibition on spatial learning processes, as well as its potential rewarding or even punishing effect is poorly understood. In addition, the role of VP D_2Rs in schizophrenia is largely unknown. However, since the VP is a key component of the circuit regulating VTA DAergic activity, it is likely that the D_2R antagonists, commonly used in the treatment of schizophrenia, exert some

of their effects within the VP. In light of these considerations, our experiments were designed to examine the effects of VP D_2R inhibition on reinforcement and learning processes, as well as to investigate the role of these receptors in the expression of positive symptoms of schizophrenia in healthy/neurotypical and MAM-E17 schizophrenia model rats.

2. Objectives

Based on the data of the literature and our previous findings, it is evident that the activation of VP D_2Rs facilitates memory consolidation processes associated with learning. Based on these, we aimed to investigate how the microinjection of the D_2R antagonist sulpiride into the VP influences spatial learning processes at different doses, and to determine whether VP D_2Rs are essential for spatial learning. The microinjection of quinpirole into the VP proved to have a punishing effect. Therefore, our objective was to investigate whether the inhibition of VP D_2Rs induces place preference (or even place aversion) and how it affects locomotor activity.

The first part of our experiments was conducted on healthy male Wistar rats, dividing them into four groups: a control group, as well as groups treated with 0.1 μg, 1.0 μg, and 4.0 μg of the D_2R antagonist sulpiride, respectively.

1. The Morris water maze (MWM) test was used to investigate the effect of sulpiride microinjected into the VP on spatial learning.
2. The conditioned place preference (CPP) test was used to examine the potential rewarding or aversive effect of the applied doses of the D_2R antagonist. Locomotor activity was monitored throughout the experiments.
3. Finally, a new "spatial" CPP test developed by our research group was used to assess whether the animals can associate the potential rewarding (or aversive) effect of sulpiride with the conditioning zone, based solely on spatial cues.

It is well known that D_2Rs play a crucial role in the development of positive symptoms in schizophrenia. However, the role of VP D_2Rs in schizophrenia remains largely unknown, despite the fact that the VP is a central component of the circuit regulating VTA DAergic activity. Moreover, we have shown that stimulation of VP D_2Rs exerts a negative feedback effect on VTA DAergic activity. This raises the possibility that D_2R antagonists used as antipsychotics exert their effects at least partly through the VP. Our experiments, conducted on

both neurotypical and MAM-E17 animals, aimed to clarify the role of VP D_2Rs in the development of positive symptoms of schizophrenia, as well as to reveal the potential side effects of D_2R antagonist treatment on learning processes. In addition, our goal was to determine whether the effects of D_2R antagonist treatment in the VP differ in neurotypical and MAM-E17 schizophrenia model animals. In the second phase of our experiments, both neurotypical and MAM-E17 rats were used, 4-4 groups per each: a control group and groups treated with 0.1 μg, 1.0 μg, or 4.0 μg of the D_2R antagonist sulpiride, respectively. The following experiments were conducted:

1. Effects of sulpiride microinjections in different doses on learning processes were investigated in MWM test in both neurotypical and MAM-E17 rats. Compared to the protocol used in the first experiments on healthy animals, the number of conditioning trials was increased in order to determine whether it could compensate the learning-impairing effects of sulpiride observed in the first experiments.
2. The potential place preference or place aversion-inducing effect of sulpiride microinjections was examined in traditional CPP test in both neurotypical and MAM-E17 animals. It is well known that the increased locomotor activity in MAM-E17 animals correlates with the positive symptoms of schizophrenia, and therefore, we monitored the effect of sulpiride microinjections into the VP on locomotor activity in both neurotypical and MAM-E17 rats.

3. Materials and methods

3.1. Subjects

In our experiments, we used 124 healthy, 71 neurotypical (these animals are essentially comparable to the healthy ones, but their dams were treated with the MAM vehicle on day 17 of pregnancy, so they can be considered as controls for the MAM-E17 animals), and 73 MAM-E17 male Wistar rats. The hormonal levels of male rats are more stable, thus, to avoid the variability associated with the estrous cycle of females, we decided to use only males in the present experiments. Additionally, using males, our results can be more easily comparable with our previous findings as well as existing data in the literature, ensuring standardization and reproducibility. The animals were housed in separate cages in a climate-controlled animal house of our institute, where the temperature was maintained at 21 ± 2°C and the humidity at 55 ± 10%. The rats had ad libitum access to standard laboratory rodent chow and tap water, and their body weight was continuously monitored throughout the experiments. The body weight of the animals at the start of the experiments ranged from 280 to 320 g. In the room, we applied lighting corresponding to natural day-night cycles: the light period began at 7 AM, and the dark period started at 7 PM. The microinjections were administered to awake, hand-held animals during the experiments, which required prior habituation (i.e., "handling") of the animals. During the surgeries and experiments, we followed the ethical standards of the university (BA02/2000-8/2012, BA02/2000-65/2017, and BA02/2000-64/2017), the national regulations (40/2013. (II. 14.) Hungarian Government Decree), and the international guidelines (European Community Council Directive, 86/609/EEC, 1986, 2010). The MAM-E17 animals and the neurotypical animals were bred in our institute. The estrous cycle of the female animals was monitored, male and female Wistar rats were paired, and the female animals were treated with MAM (MRIGlobal Chemical Carcinogen Repository, Kansas City, Missouri; dissolved in physiological saline at 25 mg/kg) on day 17 of pregnancy. The timing of the treatment and the concentration of the drug are crucial (11, 12). The effect of MAM is selectively exerted on the brain structures that are developing at the time of the treatment, causing long-term anatomical and behavioral changes (5, 70) ,which can be correlated with the symptoms observed in patients with schizophrenia. The duration of pregnancy, offspring number and body weight, as well as reflex functions, are unaffected by the MAM treatment. The offspring of the MAM-treated dams are referred to as MAM-E17 rats, while the offspring of dams treated only with 0.9%

saline (vehicle) were labeled as neurotypical rats. The offsprings were separated from the dams at four weeks of age.

3.2. Stereotaxic surgery

Before the commencement of the experiments, a stereotaxic surgery was performed on the animals, during which stainless steel cannulas (22 gauge) were bilaterally implanted 0.5 mm above the VP using a Narishige micromanipulator. The procedure was conducted under general anesthesia, using a mixture of ketamine and diazepam in a 4:1 ratio (Calypsol, 80 mg/kg body weight, Seduxen, 20 mg/kg body weight, Richter Gedeon Zrt.), administered intraperitoneally at a dose of 2 ml/kg body weight. The coordinates of the target area were determined based on the Paxinos and Watson stereotaxic brain atlas (71), relative to the Bregma point, which is located at the intersection of the coronal and sagittal sutures: ML: ± 2.2 mm, AP: -0.26 mm, DV: -7.1 mm. The guide cannulas were fixed to the skull using screws and a dental acrylic (Duracryl) crown, and then sealed with sterile 27-gauge plugs to prevent clogging. During the surgery, the animals received G-penicillin antibiotic prophylaxis. After the procedure, the animals were given 7 days for wound healing and rehabilitation. Behavioral tests were conducted between 8:00 AM and 6:00 PM.

3.3. Neurological examinations

Prior to the experiments, all animals underwent a neurological examination to confirm the proper functioning of their sensory and motor systems. Both the literature and our findings indicate that MAM-E17 treatment results in an increase in both spontaneous (72, 73) and evoked activity (12, 42). Due to the hyperactivity of the mesolimbic DA system and the hypoactivity of the glutamatergic system (8), MAM-E17 animals exhibit hypersensitivity, hyperreactivity, and increased locomotor activity. The locomotor hyperactivity observed in animal models corresponds to the positive symptoms known to be associated with schizophrenia (3,45, 46). In the course of our experiments, the locomotor activity of animals treated with different doses of sulpiride was measured.

3.4. Materials

In our experiments, we used sulpiride, a substituted benzamide derivative, which belongs to the second-generation (atypical) antipsychotics. It exerts its effects on the mesolimbic DAergic system, and therefore has a minimal potential to cause extrapyramidal side effects. At lower doses, sulpiride primarily exerts a beneficial effect on negative symptoms by enhancing DAergic activity through the blockade of presynaptic D_2Rs. At higher doses, it is effective in alleviating positive symptoms through the inhibition of postsynaptic D_2Rs (74). The D_2R-selective sulpiride *(Sigma-Aldrich Co.: (S)-(-)-Sulpiride, S7771, M = 341.43 g/mol)* was dissolved in 0.1N HCl and titrated back using phosphate buffer and 0.1N NaOH. Sulpiride was administered in three doses: 0.1 μg, 1.0 μg, and 4.0 μg, in a volume of 0.4 μl each bilaterally to the target area (the doses correspond to 0.73 mM, 7.32 mM, and 29.29 mM concentrations, respectively). The doses refer to the microinjection on one side in each case. The control group received only the vehicle in the same volume as the sulpiride microinjections. The doses were determined based on pilot experiments and the effective dose ranges previously used in intracerebral microinjections in other brain regions. The substance administration was performed while holding the animals in hand. The stainless steel injection cannulas were placed into the bilateral implanted guide cannulas. The injection cannulas were connected to 10 μl Hamilton syringes via polyethylene tubing. Using a Cole-Parmer automatic pump (Cole Parmer, IITC, Life Sci. Instruments, California), the solutions were infused bilaterally into the target area for 1 minute, at a volume of 0.4-0.4 μl per side. After the infusion, the injection cannulas were left in the guide cannulas for an additional 60 seconds to allow diffusion into the surrounding tissues and to prevent solution reflux.

3.5. Behavioural experiments

The behavioral experiments were conducted in soundproof and climate-controlled (temperature: 22±2 °C) experimental rooms. The animals' movements were recorded using a camera placed above the apparatus. The data were stored and analyzed using the Noldus EthoVision Basic software (Noldus Information Technology b.v., Wageningen, Netherlands).

3.5.1. Morris water maze test (MWM)

The MWM test is used to investigate spatial learning and memory processes (75). For the experiment, we used a circular pool with a diameter of 150 cm and a height of 60 cm, filled with water at 23±1°C. The water was colored with methylene blue to ensure that the platform was not visible to the animals. The apparatus was virtually divided into four quadrants. In one of these quadrants (i.e. the target quadrant), a 10x10 cm plastic platform was placed 2 cm below the water surface. During the analysis, virtual platforms were marked in the other quadrants at positions identical to the platform's location. Throughout the experiment, the animals were placed in the pool along its wall, facing the wall with their starting positions changing for each session. The rats' spatial navigation was aided by black-and-white images (known as "cues") placed around the apparatus.

On the first day of the experiment, during the habituation phase, the platform was removed from the pool. The animals were allowed to swim for 180 seconds, after which they were divided into four groups based on the distance traveled, ensuring that there were no significant differences in the average distances among the groups. On the second day, in the morning, after placing the platform back in the pool, the animals were made to swim twice, with a 1-minute interval between the two trials (the 1st and 2nd conditioning). The rats were allowed to swim until they found the platform, but if they did not locate it within 180 seconds, the experimenter placed them on the platform. The animals were given one minute on the platform to look around and familiarize themselves with their surroundings. Immediately after the swimming trials, the substances were microinjected into the VP. On the third day, a similar procedure was followed: two swimming trials were conducted, with a one-minute interval between them (the 3rd and 4th conditioning), followed immediately by the substance microinjection. During the conditioning trials, we measured the time it took for the animals to find the platform, which is referred to as the platform finding latency. On the fourth morning, during the test, the platform was removed. The animals were allowed to swim for 180 seconds, and we measured the time it took for them to first cross the location where the platform had been. During both the habituation and the test, we also measured the time spent in the inner zone of the pool (a 60 cm radius circle measured from the center of the pool), as well as the time spent in a 25 cm radius circle surrounding the location of both the platform and the virtual platforms, in order to assess the learning specificity of the spatial location.

In the MWM test conducted with neurotypical and MAM-E17 animals, we extended the above-described paradigm to 5 days. Animals received microinjections also after habituation,

and 3 daily conditioning sessions, with 1-minute intervals between them, were performed for 3 days. The 3rd daily conditioning session was followed by a microinjection of the specified dose of sulpiride or vehicle solution to the target brain area. On the fifth day, a test trial was conducted. During both the habituation and the test trials, in addition to measuring the latency to reach the target, we also recorded the amount of time the animal groups treated with different doses spent in the internal zone of the apparatus (r=60 cm). Furthermore, we measured the time spent within a 25 cm radius surrounding the platform and the virtual platforms (the times for the three virtual platforms were averaged), in order to determine which animal groups displayed spatially specific learning.

3.5.2. Conditioned place preference test (CPP)

The CPP test is used to measure the rewarding/positive reinforcing, or aversive effects of chemical substances, as well as their addiction potential (76). In the open field-based apparatus used in our experiments, it is also possible to monitor the animals' locomotor activity. The experimental apparatus is a circular arena of gray plastic with a diameter of 85 cm and a height of 40 cm, which is virtually divided into four quadrants by thin black lines. These (virtual) quadrants were (indeed) separated during conditioning by removable, transparent Plexiglas panels. External visual landmarks (i.e. "cues") in the apparatus helped differentiate the quadrants and assisted the animals in orientation. The diffuse lighting was provided by a 40 W bulb.

The CPP experiment consisted of habituation (day 1), two pseudo-conditioning and conditioning sessions (days 2-3), and a test (day 4). On the first day, the animals were habituated: after removing the plexiglas panels, they were placed in the center of the apparatus and allowed to move freely throughout the entire area for 15 minutes. During habituation, the distance moved by the animals as well as the time spent in each quadrant were measured. For each animal, we designated a conditioning quadrant as one that proved neutral, meaning the animal spent neither too little nor too much time in that quadrant during habituation. The pseudo-conditioning quadrant was then assigned as the opposite quadrant to the conditioning quadrant for each animal. Based on the time spent in each quadrant, the animals were divided into 4 groups, ensuring that there were no significant differences among the groups in terms of their average values. On days 2 and 3, the quadrants were separated from each other using the Plexiglas panels. In the mornings, during the conditioning sessions, the rats were placed in the conditioning quadrant 5 minutes after the bilateral microinjections, where they remained for 15

minutes. During this time, they were able to associate the effects induced by the administered substance with the location. The rats' orientation was aided by the external visual cues (located on the walls of the apparatus). In the afternoons, we performed a pseudo-conditioning, during which the animals were administered only the vehicle before being placed in the pseudo-conditioning quadrant. The purpose of pseudo-conditioning was to ensure that the animals learned specifically that the rewarding or aversive effects were associated only with the conditioning quadrant, not the entire apparatus. On the fourth day, during the test, the rats were placed in the center of the apparatus without any substance administration and allowed to move freely throughout the entire area for 15 minutes. We measured the time spent in each quadrant as well as the distance traveled by the animals.

The CPP experiment conducted with neurotypical and MAM-E17 animals was performed similarly to the previous experiment: it consisted of a habituation (Day 1), three conditioning and three pseudo-conditioning sessions (Days 2-4), and a test (Day 5). Compared to the previous CPP experiment, the order of conditioning and pseudo-conditioning was reversed to assess whether the potential rewarding or aversive effects of the administered substance in neurotypical animals depend on the order of these sessions. On the first day of the experiment the animals were habituated to the apparatus. On Days 2-4, the quadrants were separated using Plexiglas panels. In the mornings, during the pseudo-conditioning sessions, the rats were placed in the pseudo-conditioning quadrant 5 minutes after receiving vehicle microinjections, where they remained for 15 minutes. In the afternoons, conditioning was performed, during which the animals were placed in the conditioning quadrant after receiving the microinjections. On the fifth day, during the test session, the rats were placed in the center of the apparatus without any substance administration, and they were allowed to freely explore the entire area for 15 minutes. The time spent in each quadrant, as well as the distance traveled by the animals, were measured.

3.5.3. Spatial place preference test

The spatial CPP test developed by our research group is a modified version of the traditional open-field based place preference test, which we designed to examine the spatial specificity of rewarding or aversive effects. For the experiment, we used the same apparatus as in the traditional CPP test, but there was a significant difference between the two experiments during the conditioning and pseudo-conditioning sessions. In the traditional CPP test, the

animals were placed in the conditioning quadrant, where they could memorize its position based on external and internal cues, and they were able to approach the internal cues. In contrast, during the spatial CPP test conditioning, the rats were placed in a 20x20x30 cm square Plexiglas column, which could only be recognized based on its spatial location, similar to the platform in the MWM paradigm. During the spatial CPP test, the apparatus area was virtually divided into four sections. During habituation and the test, the animals were free to move around the entire apparatus. On conditioning days, the square-shaped Plexiglas column was placed eccentrically within the quadrant containing the conditioning zone, not too close to the walls of the apparatus. During the conditioning sessions, five minutes after the substance administration, the animals were placed in the conditioning column, while during the pseudo-conditioning sessions, the animals were placed in the area outside the column of the apparatus, where they were free to move around. This area was referred to as the pseudo-conditioning zone. During the spatial CPP test, we used the same visual cues as in the traditional CPP test; however, in this case, each of these cues could be considered external, as they were located outside the conditioning area. Thus, the animals could only memorize the location of the conditioning based on spatial positioning. The spatial CPP test consisted of four days: habituation, two conditioning days, during which conditioning took place in the morning and pseudo-conditioning in the afternoon, and a test day. During the habituation and test, we measured the time spent in the conditioning zone, as well as the time spent in the virtual pseudo-conditioning zones, which were identical to the conditioning zone but located in the other quadrants (the time spent in the three pseudo-conditioning zones was averaged). To investigate the presence of spatial learning, we compared the time spent in the conditioning zone with the time spent in the virtual pseudo-conditioning zones (the average time spent in the three virtual zones). Additionally, we measured the time spent in the quadrant containing the conditioning zone and the distance moved.

3.6. Histology

After the experiments, the animals were euthanized with urethane (intraperitoneal injection of a 20% urethane solution, 1.4 g/kg body weight), followed by transcardial perfusion with isotonic saline and then with a 10% formaldehyde solution. The removed brains were placed in a buffered, sucrose-containing formalin solution, and after 72 hours of fixation, blocks were cut from them. Frozen sections of 40 μm thickness were prepared using a microtome and stained with cresyl violet. Subsequently, the cannula positions were examined under a light

microscope using the Paxinos and Watson stereotaxic brain atlas (71). Animals in which the cannula was not positioned in the target area were excluded from the statistical analysis.

3.7. Statistical analysis

In the analyses, the time spent in the designated zone for each paradigm was normalized by dividing by the total session time and multiplying by 100, resulting in percentage values. We evaluated our measurement results using one-way and two-way analysis of variance (ANOVA), as well as mixed-design two-way ANOVA, conducted with the "SPSS 20.0 for Windows" software package. In the case of significant differences, a Bonferroni post hoc test was performed to compare the means pairwise. Pearson's correlation test was used to examine potential correlations between variables. A significance level of $p < 0.05$ was considered, and significant values were marked with an asterisk on the graphs. The diagrams represent the mean ± the standard error of the mean (S.E.M.).

4. Results

4.1. Results of the Morris water maze test

During the MWM test, there were no significant differences in platform-finding latency among the groups during the habituation phase. However, during the test phase, the control group located the platform significantly faster than the groups treated with 1.0 μg and 4.0 μg D_2R antagonist. Both the control group and the group treated with 0.1 μg sulpiride found the platform significantly faster during the test compared to the habituation phase.

Based on the analysis of the time spent in the inner zone of the apparatus (r = 60 cm), it was observed that during the test, the groups treated with 1.0 μg and 4.0 μg D_2R antagonist spent significantly less time in the inner zone compared to the control animals and the group treated with 0.1 μg D_2R antagonist. Furthermore, in all treatment groups, it was found that the animals spent significantly more time in the inner zone of the apparatus during the test than during the habituation phase.

To investigate the specificity of spatial learning, we analyzed the percentage of time spent within a 25 cm radius of the platform and the virtual platform during both the habituation and test phases. According to our results, the groups treated with 1.0 μg and 4.0 μg D_2R antagonist spent significantly less time in the zone surrounding the platform compared to the control group. Only the group treated with 4.0 μg D_2R antagonist spent less time in the 25 cm zone surrounding the virtual platform compared to the group treated with 0.1 μg D_2R antagonist. The analysis revealed that only the control animals exhibited spatially specific learning, as they spent significantly more time in the platform zone than in the virtual platform zone.

The animals' locomotor activity was monitored during both the habituation and test trials, and the difference in the distance traveled between the two trials was calculated. No significant differences were found among the groups.

In summary, intra-VP microinjection of sulpiride impaired learning processes in the MWM paradigm in a dose-dependent manner. This impairment is likely due primarily to a disruption of specific spatial learning. In the case of the 1.0 μg and 4.0 μg sulpiride treatments, a complete deficit in spatial learning was observed, along with a deterioration in escape strategy. Throughout the experiment, all groups learned that the platform was located in the inner zone of the apparatus, although the 4.0 μg sulpiride dose also impaired this learning process. Locomotor activity was not significantly affected by any dose of sulpiride treatment.

4.2. Results of the conditioned place preference test

Analysis of the time spent in the conditioning quadrant revealed that there were no significant differences among the groups during the habituation. However, during the test session, animals treated with 4.0 μg D_2R antagonist spent significantly more time in the conditioning quadrant than all other groups. Additionally, the group treated with 4.0 μg sulpiride spent significantly more time in the conditioning quadrant during the test compared to the habituation session.

Analyzing the percentage difference in time spent in the conditioning quadrant between the test and habituation revealed that the group treated with 4.0 μg D_2R antagonist spent significantly more time in the conditioning quadrant compared to the control animals.

Throughout the experiment, the locomotor activity of the animals was continuously monitored. Analysis of the difference in distances moved during the test and habituation

sessions revealed that the group treated with 4.0 μg sulpiride microinjection moved significantly less compared to the control group.

Based on the results of the experiment, it can be concluded that the highest dose of sulpiride microinjection induced a place preference in the animals. Furthermore, sulpiride, in a dose-dependent manner, reduced the distance traveled by the animals 24 hours after the last injection, causing decreased locomotor activity during the test.

4.3. Results of the spatial place preference test

Analysis of the percentage distribution of time spent in the conditioning zone during the habituation and test sessions showed that the group treated with 4.0 μg D_2R antagonist spent significantly more time in the conditioning zone-containing quadrant during the test compared to the other groups. Additionally, this same group spent significantly more time in the conditioning zone-containing quadrant during the test than during the habituation.

Comparing the percentage of time spent in the conditioning zone and the virtual conditioning zone, no significant differences were observed among the groups.

Analyzing the difference in the percentage of time spent in the conditioning zone-containing quadrant during habituation and test phases revealed that the group treated with 4.0 μg D_2R antagonist spent significantly more time in the conditioning zone-containing quadrant compared to the control group.

Analyzing the difference in the distance moved during the test and habituation sessions revealed that the group treated with 4.0 μg D_2R antagonist moved significantly less compared to the control animals.

In summary, during the spatial CPP test, the highest dose of sulpiride microinjection did not induce a place preference for the conditioning zone when the animals had to recognize it solely based on its spatial localization. However, 4.0 μg sulpiride did induce a place preference for the quadrant containing the conditioning zone. A dose-dependent decrease in their locomotor activity was also observed in this paradigm.

4.4. The results of the Morris water maze test conducted with neurotypical and MAM-E17 animals

Analysis of the target-finding latencies in neurotypical animals revealed that each treatment group found the platform location significantly faster during the test trial compared to the habituation trial. Among the MAM-E17 animals, the control group and the group treated with 0.1 μg D_2R antagonist found the platform significantly faster during the test trial than during habituation. During the test trial, the target-finding latency of the group treated with 1.0 μg D_2R antagonist was significantly higher compared to the control and 0.1 μg sulpiride-treated groups.

During the experiment, the time spent in the inner zone of the apparatus (r = 60 cm) was measured. Among the neurotypical animals, all treatment groups spent significantly more time in the inner zone of the apparatus during the test trial compared to the habituation. Among the MAM-E17 animals, the groups treated with 1.0 μg and 4.0 μg D_2R antagonist spent significantly less time in the inner zone during the test trial compared to the control group and the group treated with 0.1 μg D_2R antagonist. The control group and the 0.1 μg D_2R antagonist-treated group spent significantly more time in the inner zone of the apparatus during the test trial than during habituation.

During the MWM test conducted with neurotypical and MAM-E17 animals, we also assessed the average platform-finding latency (in seconds) within the square-shaped zones surrounding the platform and the virtual platform (test–habituation). In neurotypical animals, no significant differences were observed among the various doses of sulpiride treatments; however, there was a significant difference between the platform and the virtual platform zones. In contrast, among the MAM-E17 animals, a significant difference was found between the MAM-E17 control group and the group treated with 1.0 μg of the D_2R antagonist: the platform-finding latency was significantly higher in the 1.0 μg D_2R antagonist-treated group compared to the MAM-E17 controls, for both the platform and the virtual platform zones.

Analysis of the distance moved by the animals during the experiment revealed no significant differences among the groups.

In summary, in the MWM paradigm, sulpiride did not impair learning in neurotypical animals, whereas MAM-E17 animals showed increased sensitivity to sulpiride microinjected into the VP. Among the MAM-E17 animals, the groups treated with 1.0 μg and 4.0 μg D_2R antagonist were unable to learn the location of the platform. No significant differences in

locomotor activity were observed among neurotypical and MAM-E17 animals treated with different doses of sulpiride.

4.5. Results of the conditioned place preference test conducted on neurotypical and MAM-E17 animals

Analysis of the time spent in the conditioning quadrant revealed that neurotypical animals treated with 4.0 μg D_2R antagonist spent significantly more time in the conditioning quadrant during the test session compared to all other groups. Additionally, animals treated with 4.0 μg sulpiride spent significantly more time in the conditioning quadrant during the test than during habituation. In the case of the MAM-E17 animal groups, no significant differences were observed in the time spent in the conditioning quadrant.

Throughout the experiment, the animals' locomotor activity was monitored, and the difference in distance moved between the habituation and test sessions was calculated for both neurotypical and MAM-E17 animals. In the MAM-E17 group, the animals treated with 4.0 μg sulpiride showed a significantly different activity level compared to both the 0.1 μg sulpiride-treated group and the control group. Additionally, the decrease in locomotor activity from habituation to test was significantly smaller in MAM-E17 control animals compared to neurotypical controls. Furthermore, a significant difference was also observed between neurotypical and MAM-E17 animals treated with 4.0 μg sulpiride.

In summary, within the CPP paradigm, the 4.0 μg D_2R antagonist induced place preference in neurotypical animals, whereas no such effect was observed in MAM-E17 animals. Locomotor activity decreased during the test session in neurotypical animals, while it remained largely unchanged in MAM-E17 animals compared to habituation. However, higher doses of sulpiride microinjected into the VP effectively reduced locomotor activity even in MAM-E17 animals.

5. Discussion

5.1. Evaluation of the results of the Morris water maze test

The aim of the MWM test performed on healthy Wistar rats was to examine how sulpiride microinjected into the VP affects learning processes. In the allocentric MWM test (77), animals can find the platform by following primarily two strategies: either by means of spatial learning, acquiring the exact spatial location of the platform, or by learning to swim away from the wall into the inner part of the pool, where they are more likely to find the platform relatively easily. Based on our results, in the first experimental series only the control animals learned specifically the spatial location of the platform, since only this group spent significantly more time on average in the zone surrounding the platform compared to the zone surrounding the virtual platform. However, during the test, all animal groups spent more time in the inner zone of the pool than during habituation. This indicates that all groups learned that the platform was located in the inner zone of the pool, although the higher doses of sulpiride microinjections somewhat impaired this learning process as well.

DA and its receptors play a crucial role in spatial learning and memory consolidation processes across various brain regions. The activation of D_1Rs and D_2Rs in the HPC is essential for effective spatial learning. The D_1R antagonist SCH23390, when microinjected into the gyrus dentatus region of the HPC, impairs spatial learning in the MWM test (78). Furthermore, inhibition of D_2Rs in the CA1 region of the HPC disrupts memory processes related to spatial learning (79). Beyond the HPC, its projection area, the NAC also plays an essential role in memory consolidation in spatial learning paradigms, with the activation of both D_1Rs and D_2Rs being critical for this process (27, 28). In the VP, both D_1R and D_2R activation facilitate the memory consolidation processes in spatial learning. However, as demonstrated by the present and our previous results (69, 80), it is only the activation of VP D_2Rs that constitutes a necessary condition. Based on all these findings, we can conclude that all these structures, where we or others demonstrated that the D_1R or D_2R activation is necessary for spatial learning processes, are the components of that limbic HPC-NAC-VP-VTA axis, which regulates the encoding of information into long-term memory (66).

5.2. Evaluation of the results of traditional and the spatial conditioned place preference test

In our experiments, the highest dose of sulpiride microinjected into the VP induced place preference. A drug can induce place preference in the experimental animals only if it has a positive emotional effect, and this effect temporally coincides with the stimuli of the CPP paradigm in order to become associated with them. Furthermore, it is essential that the short-term memory trace formed in this way becomes stabilized and consolidated within the nervous system to ensure long-term retention (81, 82). If any of these three factors mentioned above is not fulfilled, place preference cannot be observed during the test trial. How is it possible that sulpiride still induced place preference, though it impaired learning in the MWM paradigm. To find a possible solution of this apparent contradiction, we developed the spatial CPP paradigm. One of the key learning strategies that animals can use in the CPP paradigm is spatial learning (83). Based on the results of the spatial CPP paradigm, we can conclude that the animals were not able to associate the rewarding effect of the sulpiride with the environment using purely spatial learning. Therefore, it is likely that during the traditional CPP paradigm, they relied on a different learning strategy to identify the conditioning quadrant. Our results showed that during the test session of the spatial CPP paradigm, animals treated with the highest dose of sulpiride did not spend more time in the conditioning zone itself, but they did spend more time in the quadrant containing it. We can assume - and this is also supported by the results of the MWM test - that animals treated with 4.0 μg of sulpiride were capable of learning, though not based on spatial cues. They learned that something rewarding was present in the spatial CPP apparatus, but their association was imprecise - they were unable to accurately identify the location of the treatment. This likely led to the observed behavior, where animals in this group spent more time near the conditioning zone, but could not precisely locate the treatment zone in space. The traditional CPP test is a suitable paradigm for investigating the rewarding and positive reinforcing effects of chemical substances (76). According to the central theory of reinforcement, substances with positive reinforcing effects facilitate learning and memory processes (84). The significance of our current results lies in drawing attention to an important counterexample, namely that a reinforcing/rewarding substance does not necessarily universally enhance learning.

The rewarding and locomotor activity-enhancing effects of chemical substances in the nervous system are often correlated, but not necessarily so. In our previous experiments, we demonstrated that the D_2R agonist quinpirole microinjected into the VP induces place aversion

and reduces DAergic activity in the VTA (85). In our present experiment, sulpiride had a rewarding effect, inducing place preference, while simultaneously reduced locomotor activity in the animals. However, these two effects did not correlate with each other. This suggests that different mechanisms are likely responsible for each effect. Indeed, the rewarding effect is an acute one, in the sense that the positive emotional impact induced by sulpiride must temporally coincide with the stimuli of the CPP paradigm (86). In contrast, the locomotor effect of sulpiride is a long-run, gradually developing effect, which was still observed one day after the last sulpiride treatment.

5.3. Evaluation of the results of the Morris water maze test conducted on neurotypical and MAM-E17 animals

In healthy animals, sulpiride microinjected into the VP impaired spatial learning processes in a dose-dependent manner; however, this effect was not observed in neurotypical animals. The difference between the two experiments may arise from the fact that more conditionings were performed in the experiment conducted on neurotypical and MAM-E17 animals. We hypothesize that increasing the number of conditioning trials in neurotypical animals may have compensated the negative effects of sulpiride on spatial learning. Another possible explanation can be that the „swimming away the wall" strategy became predominating against the spatial learning strategy by increasing the number of the conditioning trials. Since sulpiride primarily impairs spatial learning, this could explain why we could not observe a deterioration of platform finding latencies in sulpiride-treated neurotypical groups. This is supported by the fact that in our experiments with neurotypical rats - compared to the previous experiments conducted on healthy animals - the control animals did not spend significantly more time during the test trial in the zone surrounding the platform location than in the zone surrounding the virtual platform. The picture is further refined by the fact that spatial learning can be demonstrated in neurotypical animals by using a parameter even more sensitive to spatial learning. Based on all of these, we can accept both possible explanations as true. That is, the number of conditioning trials can indeed at least partially compensate the negative effects of sulpiride on learning, specifically on spatial learning. At the same time, it seems that this also diminishes the use of specific spatial learning strategies in the animals. In the case of MAM-E17 animals, the compensatory effect was not observed. In fact, none of the MAM groups used

spatial learning strategy. Additionally, MAM-E17 animals treated with the higher dose of antagonist were unable to learn the location of the platform, regardless of whether they used a spatial or the „swimming away from the wall" strategy. All of these suggest that MAM-E17 animals are presumably more sensitive to the learning-impairing effects of sulpiride microinjection into the VP than neurotypical animals. Several studies are available in the literature indicating that MAM-E17 model animals exhibit reduced performance in the hidden platform version of the MWM test (87-90). Although in our present experiments the platform finding latencies of the MAM-E17 control animals did not worsen, this is likely mainly due to the fact that the animals did not use the spatial learning strategy. This is supported by the fact that in the visible platform version of the MWM test, MAM-E17 animals did not show a decrease in their learning capabilities (89). In addition to the results obtained in the MWM paradigm, several studies in the literature also support the notion that MAM-E17 rats exhibit weaker learning and generally impaired cognitive abilities (72, 89, 90). MAM-E17 model animals exhibit reduced performance in the radial arm maze test, which assesses spatial working memory associated with the HPC (11). Similar to the schizophrenia animal model, allocentric spatial learning is impaired in patients with schizophrenia (91-94), and they also show reduced performance in virtual mazes designed to assess egocentric spatial learning (95). It has also been shown that patients with schizophrenia perform worse in spatial working memory tests (96). The obtained results therefore can be aligned with human data.

5.4. Evaluation of the results of the conditioned place preference test conducted on neurotypical and MAM-E17 animals

The results of the CPP experiment performed in neurotypical and MAM-E17 animals show that sulpiride induces place preference in a dose-dependent manner in neurotypical animals, thus reproducing our findings in healthy animals. In contrast, none of the sulpiride doses microinjected into the VP of MAM-E17 animals induced place preference. A possible explanation for this phenomenon can be that an intact memory consolidation is necessary for the development of place preference (81), whereas sulpiride, as we observed in the MWM paradigm, generally impairs the learning processes in MAM-E17 animals. The clinical relevance of the CPP studies conducted on MAM-E17 animals lies in the fact that this paradigm, in addition to the examination of rewarding effects of chemical substances, also provides an opportunity to investigate their locomotor effects. The increased locomotor activity observed in MAM-E17 animals can be correlated with the positive symptoms of schizophrenia (45, 46).

According to Kapur's "salience" hypothesis, the positive symptoms of schizophrenic patients may be due to the patient attributing excessive motivational importance ("salience") to environmental events and cues (45), which leads to hyperreactivity to environmental stimuli and, consequently, to psychomotor agitation. The increased locomotor activity observed in MAM-E17 animals may be analogous to the psychomotor agitation described in patients with schizophrenia (46). Therefore, it can be assumed that the animals' heightened locomotor activity at least partially reflects this aberrant salience and hyperreactivity (15, 73). Based on this theory, we would expect the animals - especially when exposed to a novel environment - to show an increased locomotor activity. However, in our current experiment, during the habituation trial when the animals first encountered the CPP apparatus, no significant difference in locomotor activity could be observed between the neurotypical and the MAM-E17 groups. There are findings in the literature indicating that adult male MAM-E17 rats are not hyperactive when first exposed to a novel environment (12, 58, 97, 98). Additionally, there are studies in which the same rats were tested at different ages, making it difficult to determine whether the hyperactivity observed at the end of puberty and in adulthood is specific to those developmental stages - thus reflecting the "salience" hypothesis - or whether it may instead indicate a cross-age habituation deficit (72, 73, 90). According to the latter interpretation, the hyperactivity of MAM-E17 rats only becomes apparent over time, as they are unable to habituate to a novel environment. In contrast, the initial intense exploratory behavior of neurotypical animals gradually subsides as they learn that most environmental stimuli are not motivationally relevant. In our CPP experiments conducted on healthy animals, high-dose sulpiride reduced locomotor activity. Although, we were unable to determine whether this effect was due to the sulpiride accelerating effect on the habituation process, since the control animals did not exhibit habituation to the environment. In the present experiment, however, the increased number of conditioning sessions led to a similar reduction in locomotor activity during the test session across all neurotypical groups, supporting our habituation hypothesis. In contrast, the distance traveled by the MAM-E17 control animals during the test session showed only a minimal decrease, indicating a habituation deficit. Nevertheless, higher doses of sulpiride microinjected into the VP restored the habituation process in the MAM-E17 animals.

How much can we consider a habituation deficit as a positive symptom? What is the difference between Kapur's "salience" theory and the "habituation" theory we propose here? Initially, in a novel environment, numerous environmental cues may gain a transient yet significant motivational value. Over time, however, the impact of these cues becomes inhibited - a process referred to as habituation. In this context, our proposed "habituation" theory can be

viewed as a specific case of Kapur's "salience" theory, in which certain environmental cues acquire an unnecessarily increased and sustained motivational significance due to inadequate habituation learning processes. A similar approach is also suggested by Barkus and colleagues (99). Based on this, habituation deficits could ultimately lead to positive symptoms, and could essentially be one of their important causes. This is particularly significant, as habituation deficits are observed in the early stages of psychosis (100), making it a potential prodromal symptom. Our habituation theory is further supported by the fact that schizophrenic patients exhibit habituation deficits (100-102).

5.5. The presumed mechanism(s) of action of sulpiride microinjected into the VP

There are two main possibilities for the behavioral effects we observed following sulpiride microinjection into the VP: on one hand, sulpiride could locally influence directly or indirectly the activity of the VP neurons, and it can modulate synaptic plasticity within the VP; on the other hand, not independently of the former local effects, it could also affect the activity and synaptic plasticity of other brain regions that are in direct or indirect connection with the VP.

Locally, the D_2R antagonist sulpiride can directly modulate the neuronal function of the VP. In the CNS, D_2Rs generally have an inhibitory effect (103, 104). In the VP, D_2Rs are present presynaptically on GABAergic fibers originating from the NAC (63-65), as well as on glutamatergic fibers coming from the basolateral amygdala (BLA) (105). Microiontophoretic administration of DA in the VP was found to reduce both the inhibitory effect of GABA and the excitatory effect of glutamate (106). In addition, it has been shown that the GABAergic output neurons of the VP also express D_3 receptors, which belong to the D_2R receptor family (107).

In addition to the local acute effect, D_2Rs may also influence long-term processes such as synaptic plasticity. We have shown that D_2R stimulation in the VP inhibits LTP induced by high-frequency stimulation of the BLA - most likely via inhibitory BLA fibers (108) - while it induces LTD, presumably at excitatory terminals (108). Stimulation of D_2Rs alters synaptic plasticity at GABAergic terminals originating from the NAC shell and projecting to the VP, shifting high-frequency stimulation-induced LTD toward LTP (108). There is no direct information available regarding the effects of D_2R antagonists on synaptic plasticity within the

VP. However, Meredith and colleagues demonstrated that systemic administration of the non-selective haloperidol and the selective D_2R antagonist eticlopride over three days induces synaptic degeneration only in the VP - among several brain regions examined - most likely affecting inhibitory synapses (109). This phenomenon may be regarded as the endpoint of synaptic weakening (LTD) induced by D_2R antagonists, and it likely reduces the efficacy of the affected input. The primary inhibitory input to the VP originates from the NAC, which provides dense GABAergic innervation (110). Based on all these findings, we can hypothesize that sulpiride leads to long-term weakening of the NAC-VP synapses, although we cannot completely rule out the possibility that sulpiride may also strengthen the BLA-VP synaptic connection.

In addition to its local effects - and not independently of them - sulpiride administered into the VP may also exert indirect effects on distant brain regions, thereby influencing motivation as well as reinforcement and learning processes. The most plausible candidate for mediating the distant effects of sulpiride microinjected into the VP is one of the main outputs of the VP: the VTA. The VTA serves as a primary source of DA in the limbic system. The VP is known to be a major regulator of population activity of the VTA (14), therefore, by modulating neuronal activity within the VP, sulpiride may indirectly alter the functional output to the VTA. We propose that sulpiride, when microinjected into the VP, induces dose-dependent place preference through immediate local presynaptic and/or postsynaptic mechanisms (as described above), by indirectly activating DAergic neurons in the VTA. This is considered an acute effect that is essential for the development of conditioned place preference, as it requires temporal overlap between the drug's effect and the conditioning environment (81, 82). In contrast, the observed impairments in spatial learning and the reduction in locomotor activity may be explained by the degeneration of D_2R-expressing GABAergic fibers originating from the NAC shell, which in turn could lead to decreased population activity of VTA DAergic neurons. This represents a gradually developing, long-term effect with potentially high clinical relevance, as hyperactivity of VTA DAergic neurons is considered one of the main etiological factors underlying the positive symptoms of schizophrenia (15).

In total, our findings suggest a plausible mechanism by which D_2R antagonist treatment may exert its effects within the limbic system. Moreover, we propose an alternative explanation -complementary to the well-established depolarization block mechanism (57) - how D_2R antagonist antipsychotics may contribute to the alleviation of schizophrenia symptoms. The aim of our future research is to further explore the mechanism of action of sulpiride, with a particular

focus on validating the hypotheses formulated based on the interpretation of our present experimental findings. We hope that our results will contribute to a more comprehensive understanding of the disease's pathomechanism and, in the long run, facilitate the development of novel and more specific therapeutic approaches.

6. Summary

In our experiments, we have confirmed the followings:

1) In the MWM paradigm used for studying spatial learning processes:
   a) the dose-dependent administration of the D_2R antagonist sulpiride into the VP impaired the animals' platform finding latency.
   b) except for the control group, none of the other groups used spatial (specific) learning strategy, indicating that the VP D_2Rs are necessary for spatial learning.
   c) in the groups treated with 1.0 μg and 4.0 μg of sulpiride, the learning strategy of „swimming away from the wall" was also impaired compared to the control animals, although some animals in these groups still learned that the platform was located in the inner zone.

2) In the open field-based CPP paradigm used to measure the rewarding, positive reinforcing effects of the substances and the animals' locomotor activity:
   a) the group treated with the highest dose of sulpiride spent significantly more time in the conditioning quadrant compared to the other groups, indicating the development of place preference. This suggests that the strong inhibition of VP D_2Rs has a rewarding effect.
   b) the highest dose of sulpiride gradually and in the long run reduced the distance traveled by the animals, indicating a decrease in locomotor activity.

3) In the spatial CPP paradigm developed by our research group:
   a) in part, we replicated our findings from the traditional CPP paradigm: the group treated with the highest dose of sulpiride spent significantly more time in the conditioning quadrant containing the conditioned zone.
   b) however, the animals treated with the highest dose of sulpiride were unable to associate the rewarding effect with the conditioning zone based solely on spatial location, indicating a spatial learning deficit.

4) The combined interpretation of the three paradigms emphasizes that a reinforcing/rewarding substance is not necessarily a universal enhancer of learning; in fact, it may antagonize certain forms of learning.

5) In the MWM paradigm conducted on neurotypical and MAM-E17 animals:
   a) increasing the number of conditioning trials compensated the negative effect of the sulpiride microinjected into the VP on spatial learning in neurotypical animals.
   b) in MAM-E17 animals, however, no such compensatory effect could be observed. The groups treated with higher doses of sulpiride, regardless of whether they used the spatial or the „swimming away from the wall" strategy, were unable to locate the platform. Based on this, MAM-E17 animals appear to be more sensitive to the learning-impairing effects of sulpiride microinjected into the VP than neurotypical animals.

6) In the CPP paradigm conducted with neurotypical and MAM-E17 animals:
   a) in the neurotypical animal groups, the highest dose of sulpiride microinjected into the VP induced place preference, thereby replicating the results obtained in the previous CPP experiments.
   b) in MAM-E17 animals, place preference was not developed, likely due to the impairing effects of sulpiride on learning, as well as the increased sensitivity of MAM-E17 animals to this effect.
   c) we demonstrated that the increased locomotor activity observed during the test in MAM-E17 animals can be attributed to a habituation deficit.
   d) sulpiride microinjected into the VP dose-dependently restores the habituation deficit observed in MAM-E17 animals.

7. References

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8. List of publications

8.1. Publications related to the thesis

Dusa D, Ollmann T, Kállai V, Lénárd L, Kertes E, Berta B, Szabó Á, László K, Gálosi R, Zagoracz O, Karádi Z, Péczely L. The antipsychotic drug sulpiride in the ventral pallidum paradoxically impairs learning and induces place preference. Sci Rep. 2022 Nov 10;12(1):19247. doi: 10.1038/s41598-022-23450-z. PMID: 36357539; PMCID: PMC9649625. (IF: 4.600), Q-classification: D1

Péczely L, **Dusa D**, Lénárd L, Ollmann T, Kertes E, Gálosi R, Berta B, Szabó Á, László K, Zagoracz O, Karádi Z, Kállai V. The antipsychotic agent sulpiride microinjected into the ventral pallidum restores positive symptom-like habituation disturbance in MAM-E17 schizophrenia model rats. Sci Rep. 2024 May 29;14(1):12305. doi: 10.1038/s41598-024-63059-y. PMID: 38811614; PMCID: PMC11136981. (IF: 3.800), Q- classification: D1

8.2. Further publications and citable abstracts

Kállai V, Lénárd L, Péczely L, Gálosi R, **Dusa D**, Tóth A, László K, Kertes E, Kovács A, Zagoracz O, Berta B, Karádi Z, Ollmann T. Cognitive performance of the MAM-E17 schizophrenia model rats in different age-periods. Behav Brain Res. 2020 Feb 3;379:112345. doi: 10.1016/j.bbr.2019.112345. Epub 2019 Nov 5. PMID: 31704232. (IF: 3.332), Q- classification: Q2

Zagorácz O, Ollmann T, Péczely L, László K, Kovács A, Berta B, Kállai V, Kertes E, Vörös, **Dusa**, Szábó Á, Lénárd A single injection of neuropeptide QRFP in the lateral hypothalamus decreased food intake. Journal of Psychopharmacology 2025 Mar; 39(3):254-264. doi: 10.1177/02698811241311454. Epub 2025 Feb 8. PMID: 39921588(IF: 4.5), Q- classification: D1

8.3. Presentations and conference abstracts

Zagorácz, O ; Ollmann, T ; Péczely, L ; László, K ; Kovács, A ; Berta, B ; Kállai, V ; Kertes, E ; Vörös, D ; **Dusa, D** et al. Neuropeptide QRFP enhances memory in passive avoidance paradigm. In: International Neuroscience Conference, Pécs 2024: Abstract book, Pécs, Magyarország (2024) 302 p. p. 195 Paper: P6.08

Dusa, DA ; Kállai, V ; Ollmann, T ; László, K ; Kertes, E ; Marosné, BB ; Gálosi, R ; Zagoracz, O ; Lénárd, L ; Péczely, LZ The effects of sulpirid on spatial learning in healthy and MAM E-17 schizophrenia model rats. In: IBRO Workshop (2020) Paper: 46

Trencséni, E ; Szabó, Á ; Hegedűs, DÁ ; Mokbel, T ; Péczely, L ; Ollmann, T ; **Dusa**, **D** ; Kállai, V ; Lénárd, L, Examination of learning capabilities on MAM-E17 schizophrenia rat model in prepuberty, puberty, and adulthood.In: 14th Young European Scientist Meeting (2019) p. 50

Kállai, V ; Ollmann, T ; Péczely, L ; Gálosi, R ; Tóth, A ; Kovács, A ; **Dusa, D** ; Berta, B ; Kertes, E ; László, K et al. A MAM-E17 skizofrénia patkánymodell: kognitív képességek vizsgálata 3 különböző életkorban. In: Magyar Élettani Társaság 2018. évi Vándorgyűlése: előadás és poszter absztraktok (2018) 132 p. Paper: P2.12

Ollmann, T ; Péczely, L ; Kállai, V ; **Dusa, D** ; László, K ; Berta, B ; Kovács, A ; Kertes, E ; Gálosi, R ; Zagoracz, O et al. Role of ventral pallidal dopamine-neurotensin interactions in the regulation of reward and anxiety. In: Magyar Élettani Társaság 2018. évi Vándorgyűlése: előadás és poszter absztraktok (2018) 132 p. Paper: PP1.52

Péczely, L ; Ollmann, T ; Kállai, V ; **Dusa, D** ; László, K ; Berta, B ; Kovács, A ; Kertes, E ; Gálosi, R ; Zagoracz, O et al. A ventralis pallidumba injektált szulpirid hatása a tanulási folyamatokra Morris-féle úsztatási tesztben egészséges és MAM-E17 skizofrénia modell állatokon. In: Magyar Élettani Társaság 2018. évi Vándorgyűlése: előadás és poszter absztraktok (2018) 132 p. Paper: P2.11

Tóth-Pál, Zs ; Szabó, Á ; Trencséni, E ; Rinfel, A ; Péczely, L ; Ollmann, T ; **Dusa, D**; Kállai, V ; Lénárd, L, General activity and sensorimotor gating in the MAM-E17 schizophrenia rat model.In: The Association of Medical Schools in Europe (AMSE) 2018 Regular Confer-ence & General Assembly: Best Practice for Research Teaching in Medical Schools in WHO Europe (2018) pp. 39-40. , 2 p.

Trencséni, E ; Szabó, Á; Rinfel, A; Tóth-Pál, Zs; Péczely, L ; Ollmann, T ; **Dusa, D**; Kállai, V; Lénárd, L, Examination of cognitive performance on MAM-E17 schizophrenia rat model in different age-periods. In: Recoop, HST Association (szerk.) RECOOP 9th Annual Project Review Meeting (2018) 140 p. p. 54