Theoretical Medical Sciences Ph.D. Program

The role of dopamine receptors of the central nervous system in memory consolidation processes

Ph.D. Thesis

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Pécs, 2014

1. Introduction

Basically, adaptation to the enviromental changes can be realised via reflex-regulatory and behavioural responses. If the reflex responses are not sufficient to compensate the effect of the enviromental changes, then the learning processes are those which can result in a new, enviromental effect compensating, stable reflex-regulatory and/or behavioural response. The learning process can be devided into acquisition and consolidation phases. The former means the response formation, while the latter is the stabilization of responses. In the present experiments we focused mainly on the investigation of the consolidation processes.

The dopamine (DA) is one of the most important neurotransmitters and neuromodulators in the central nervous system. The main sources of DA in the brain are the mesolimbic, mesocortical and nigrostriatal DA-ergic pathways originating from the DA-ergic neurons of the ventral tegmental area (VTA), substantia nigra (SN) and retrorubral area. According to our present knowledge five different DA receptors are known (D1-5), which can be grouped in two major families (i.e. D1-subtype and D2-subtype, respectively) [1]. In different brain regions the role of DA and its receptors has been confirmed in motor-regulation [2, 3], motivation [3-6], attentional and perceptual processes [3, 5, 7], furthermore rewarding effect of DA has been demonstrated [8-10]. Besides these processes DA has an outstanding importance in learning and memory. Based on the firing pattern of the mesencephalic DA-ergic neurons the phasic activity reflects the prediction error between the actual and the expected reward, thereby forming the biological signal of learning [11]. The role of DA in learning processes has been proven in various brain regions, in different behavioural paradigms. [12-20]. The role of DA in memory formation has been confirmed not only on the behavioural level, but on the structural level as well. It has been shown in numerous brain regions that DA receptors are necessary for the formation of the long term potentitation (LTP) and long term depression (LTD) which are the electrophysiological correlates of synaptic plasticity. The activation of the D1 DA receptors plays an important role in the hippocampal (HPC) LTP formation [12], and modulates it in the striatum and in the prefrontal cortex (PFC) [21, 22]. In the striatum both the D1 and the D2 DA receptor activation are necessary for the formation of LTP and LTD [23].

The venral pallidum (VP) is a basal forebrain structure which was originally described by Heimer and Wilson in 1975 as the ventral, subcommissural extension of the globus pallidus [24]. The VP is in connection with several brain regions involved in learning

processes such as the nucleus accumbens (NAC) [25, 26], the amygdala (AMY) [27] or the PFC [28, 29]. The VP is in reciprocal connection with the mesencephalic DA-ergic nuclei, its DA-ergic afferentation is originating mostly from the VTA and to a lesser extent from the SN [30]. In the VP both D1 and D2 DA receptor subtypes have been detected using autoradiography [31, 32] and immunohistochemistry [33, 34]. The role of the ventral pallidal DA is known in motor and motivational processes, and its role can be supposed in positive reinforcement (reward) as well. It has been shown that electrical self-stimulation can be developed in the VP [35], and it can be attenuated by the systemic application of DA antagonists [36]. The amphetamine induced place preference (acquisition) can be prevented by the excitotoxic lesion of the VP, however, the already formed place preference is not affected by it [37]. Place preference can be induced by the administration of the indirect DA agonists cocaine or amphetamine into the VP [38]. The formation of the cocaine-induced place preference can be blocked by 6-hidroxidopamine (6-OHDA) lesion of the VP [39]. It has been demonstrated that cocaine remarkably increases the DA level in the VP [39].

2. Objectives

Based on the above information, DA has a key role in learning, and especially in the consolidation processes as it was confirmed in the PFC, AMY, dorsal striatum (CPU), HPC and NAC as well. All of these brain areas have connections directly or indirectly with the VP, which is densely innervated by the DA-ergic neurons of the VTA, mainly via the fibers of the MLDR. In the VP both D1 and D2 DA receptor subtypes can be found. Until now these receptors have been investigated only in the regulation of motor/locomotor activity and in certain motivational processes, however, less is known about their role in learning and memory processes. In our present experiments we strove to clarify the role of the VP DA receptors in memory consolidation and in the stability of the formed memory, and therefore, the following experiments were performed:

1. In Morris water maze test effects of DA agonists on spatial learnig related memory consolidation and on stability of the formed memory against extinction were investigated with:
   a) the D1 DA receptor agonist SKF38393 and
   b) the D2 DA receptor agonist Quinpirole
   microinjected into the VP.

2. In one-trial inhibitory avoidance paradigm effects of DA agonists on negative reinforcement related memory consolidation and on long-term stability of the formed memory were investigated with:
   a) the D1 DA receptor agonist SKF38393 and
   b) the D2 DA receptor agonist Quinpirole
   microinjected into the VP.
3. In both paradigms selective DA receptor antagonists were used to confirm that the effects of D1 DA receptor agonist SKF38393 were mediated via the D1 DA receptors, while the effects of D2 DA receptor agonist Quinpirole were the consequence of D2 DA receptor activation. In the former case the D1 selective SCH23390, while in the latter the D2 selective Sulpiride was applied.
4. The basic condition of the investigation of memory consolidation is that short-term memory has to be formed in the experimental animals, which can be stabilised in the long run in the nervous system via the memory consolidation processes. In the Morris water maze test the formation of the short-term memory could be observed after one trial due to the schedule of the experiment, however in the one-trial inhibitory avoidance task a separate experiment was planned to demonstrate the development of short-term memory.
5. It is well-known that the indirect DA agonist cocaine and amphetamine microinjected into the VP can induce place preference, however, little information is available about the DA or its direct agonist. For the better interpretation of our experimental results, it was important to clarify whether the applied agonists have direct rewarding or punishing effects besides the memory consolidation influencing impact. Therefore, effects of both agonists were investigated in conditioned place preference test.

3. Materials and methods

3.1. Subjects

In our experiments 430 male Wistar rats weighing 280–320 g at the beginning of the experiments were used (LATI, Gödöllő). Animals were kept in a light and temperature controlled room (12:12 h light–dark cyclewith lights on at 06:00 a.m., 22 ± 2◦C). Tap water and standard laboratory food pellets (CRLT/N standard rodent food pellet, CharlesRiver Laboratories, Budapest) were available ad libitum. Food and water consumption and body

weight were measured daily. During the microinjections, awake, well-handled rats were gently held in hand. Rats were housed individually and cared for in accordance with institutional (BA02/2000-8/2012), national (Hungarian Government Decree,40/2013. II. 14) and international standards (European Community Council Directive,86/609/EEC, 1986, 2010).

3.2. Surgery

By means of stereotaxic technique stainless steel bilateral guide tubes (22 gauge diameter, 0.64 mm) were stereotaxically implanted 0.5 mm above the VP. Coordinates of the target structure relative to the Bregma (the crossing point of the sutura coronalis and saggittalis) were ML.: ± 2.2 mm, AP.: - 0.26 mm DV.: - 7.1 mm according to the stereotaxic atlas of Paxinos and Watson [40].

3.3. Materials

In our experiments the D1 DA receptor agonist SKF38393 (Sigma-Aldrich Co.: R-(+)-SKF-38393 hydrochloride, S101, molecular weight 291.77 g/mol) was applied in three different doses: 0.1 μg (0.85 mM), 1.0 μg (8.56 mM) and 5.0 μg (42.84 mM), as well as the D2 DA receptor agonist Quinpirole (Sigma-Aldrich Co.: Quinpirole hydrochlride, Q102, molecular weight 255.79 g/mol) in three different doses: 0.1 μg (0.98 mM), 1.0 μg (9.77 mM) and 5.0 μg (48.89 mM) . Both agonists were dissolved in physiological saline (vehicle). Control animals received the vehicle in equal volume to that used for the agonist microinjections.

The 5.0 μg (38.55 mM) dose of the selective D1 DA receptor antagonist SCH23393 (Sigma-Aldrich Co.: (R)-(+)-SCH-23390 hydrochloride, D054, molecular weight 324.24 g/mol) was used to investigate the specificity of the D1 DA receptor agonist SKF38393, while 4.0 μg (29.29 mM) or 0.4 μg (2.93 mM) dose of the D2 DA receptor antagonist Sulpiride (Sigma-Aldrich Co.: (S)-(-)-Sulpiride, S7771, molecular weight 341.43 g/mol) was applied to investigate the specificity of the D2 DA receptor agonist Quinpirole. Antagonists were dissolved in physiological saline (vehicle), and this vehicle solution was microinjected to the animlas of the corresponding control group in equal volume to that used for the antagonist microinjections. In all cases the drugs were microinjected bilaterally in a volume of 0.4 μl into

the target area. The antagonists or vehicle were microinjected 15 minutes prior to the administration of the agonist or vehicle. All microinjections were delivered into the target area by Hamilton syringe and Cole-Parmer programmable perfusion pump.

3.4. Behavioural experiments

Behavioural tests were carried out in sound-proofed and air-conditioned (temperature: 22±2 °C) experimental rooms. Behaviour of animals was recorded by a video camera and recorder, furthermore data analysis was made by means of EthoVision Basic software (Noldus Information Technology B.V., Wageningen, The Netherlands).

3.4.1. Morris water maze test

The Morris water maze test is applied to investigate the spatial learnig and memory processes. In the experiments a circular pool (1.5 meter in diameter and 60 cm in height)l was filled with water (23±1 °C) up to 40 cm. The circular pool was devided virtually into four quadrants, from which in one quadrant (the target quadrant) a transparent plastic platform was placed under the level of the water with 2 cm. The place of the platform was constant throughout the whole experiment. Water was coloured with methylene blue to prevent the visibility of the platform for the rats. Rats were placed into the water maze at randomly assigned but predetermined locations. The pool was surrounded with simple black and white geometric shapes representing external cues, which helped the orientation of the animals.

One day before the start of training (0. day), rats were habituated to the pool by allowing them to perform swimming for 90 sec without platform. During the habituation the distanced moved by the rats was measured. Thereafter the animals were divided into four groups in a way that the groups had similar means concerning the distance moved. In the morning of the first day, after the replacement of the platform, two trials for spatial learning were performed, the two trials were separated by one minute interval (first and second conditioning trial). This was followed by the microinjection of the solutions. The short intertrial interval ensured the possibility to observe in the second trial the short-term memory formed during the first trial. In the morning of the second day the schedule of the first day was repeated, rats were placed into the water maze for two trials (third and fouth conditioning) with one minute intertrial interval, and the second trial was immediately followed by the microinjections. During the conditionings (conditioning 1.-4.) the latency to

find the hidden platform (escape latency) was measured. Rats remained in the pool as long as they found the platform. After finding the platform, the rats were allowed to remain there for 60 s to map the enviroment. Animals failing to find the platform in 180 sec, were placed on the platform and were allowed to rest for 60 sec. In the morning of the third day the platform was removed (extinction or trial without platform), and animals were allowed to swim for 180 sec. In this trial the latency to the first crossing of the removed platform's place was measured. In addition to the first crossing, three other parameters were measured: the time spent in the target quadrant, the number of the entries into the target quadrant (where the platform was previously placed) and number of crossings at the place of the removed hidden platform. Obviously, the increased preference for the place of the platform and its environment can be reflected, but only weakly, by the first two parameters compared to the latter one. In the afternoon of the third day, the test trial was carried out: the platform was replaced and the latency to finding the safe platform was measured again. During the experiments, in each trial the mean swimming velocities of the animals were measured.

3.4.2. Inhibitory avoidance test

As a classic learning model for negative reinforcement, the one trial step-through inhibitory avoidance paradigm was used. The experimental apparatus consisted of a large (60×60×60 cm), square based, well illuminated chamber with light-gray walls and attached to a small box (15×15×15 cm) equipped with a removable roof, painted black, and having metal-grid floor for the delivery of electrical shocks. The two compartments (the small black and the big gray) were separated by a guillotine door. In each trial the rats were placed in the center of the larger well illuminated chamber, and the latency of entering the dark box - called step-through latency- was recorded. Each trial lasted maximum 180 sec.

Prior to the conditioning, the rats were habituated to the experimental apparatus (0. day), during which they were allowed to explore the whole apparatus. In this trial the latency of the first entering to the dark box was measured. After habituation the animals were divided into four groups having similar means of step-through latency. In the conditioning trial the rats were placed into the large illuminated chamber and after entering the dark compartment the door was closed and an unescapable electric foot shock (0.5 mA, 1 sec) was applied through the floor grid 3 times. The conditioning trial was immedialtely followed by the microinjections. The tests were performed 24 hours (2. day), 1 week (8. day) and 2 weeks (15. days) after conditioning (Test 1, Test 2, Test 3; respectively). During the tests animals did not

receive any shock or microinjection after entering the dark compartment. In the habituation the measurement was terminated after 180 sec, and in the conditioning and in the test trials if the animal fully entered with its four extremities into the dark chamber or if the 180 sec has elapsed.

To demonstrate the formation of the short-term memory in case of the inhibitory avoidance paradigm an additional experiment was performed. Animals were habituated to the apparatus similarly to the above experiments. After the habituation animals were divided in two groups. In case of both groups the experiment was similarly performed like in the above experiments up to the shocking. After the shocking the animals of the first group were microinjected with physiological saline into the VP (this group was similar to the simple control group), while the animals of the second group were replaced into the apparatus after 1 minute (replaced controls), and the step-through latencies were measured (test after 1 minute). Thereafter the replaced animals were also microinjected with physiological saline. After 24 hours both groups were tested (corresponding to the Test 1).

3.4.3. Place preference test

The place preference paradigm is applied to investigate the positive or negative reinforcing (rewarding or punishing) effects of different chemical substances. The open field-based place preference test used in our experiments correnspons to that elaborated by Hasenohrl and Huston [41]. Our corral apparatus consisted of a circular open field, with a diameter of 85 cm and 40 cm high wall. Black lines divided the floor into four quadrants of equal size. External visual cues in the surroundings helped the animals' spatial orientation inside the apparatus and these were in constant position during the experiments.

The place preference test was performed for four consecutive days. The first day of the experiments animals were habituated, all animals were placed in the center of the apparatus. Thereafter the rats had free access to all parts of the apparatus for 900 sec. During the habituation the distance moved by the rats, the time spent in the treatment quadrant and the number of entries into the quadrants were measured. Before the administration of the drugs neither preference nor aversion were observed in the animals, i.e. significant differences were not found among the times spent in the different quadrants. Treatment quadrant (target quadrant) was determined to be one of the four quadrants in which the animal had spent neither the longest nor the shortest time during habituation. After the habituation the animals were divided into four groups based on the time spent in the quadrants in a way that the

groups had similar means. The distribution of the treatment quadrants was balanced within each groups, the animals were randomly assigned to the different groups. During conditioning trials the quadrants were physically separated by each other by means of a plexiglass barrier. After the drug microinjections animals were placed into the treatment quadrant. The rats were restricted to the treatment quadrant for 15 minutes, during this time the rats could associate the effect induced by the drug to the treatment quadrant. The animals could see the external visual cues during conditioning, which facilitated their orientation. Both conditioning days the same process was applied. On the fourth day the plexi barrier has been removed. The animals were placed in the center of the apparatus, and they had free access to all parts of the apparatus for 15 minutes. During the test the time spent in the each quadrants - including in the treatment quadrant -, distance moved and the number of entries into the quadrants - including into the treatment quadrant – were measured.

3.5. Data analysis

3.5.1. Statistics

Data were evaluated by one-way and two-way ANOVA, or by paired t-test using the SPSS data analysis program (SPSS 20.0 for Windows). To check the homogeneity of the samples F-test was applied. Comparison of the groups was carried out with Tukey post hoc analysis. Statistical significance was established at $p < 0.05$.

3.5.2. Histology

At the end of the experiments animals were anaesthetised with 20% solution of urethane and were perfused transcardially with physiological saline followed by 10% formalin solution. Brains were sliced with a freezing microtome in 40 μm sections. Sections were stained with cresyl violet, and the injection sites were reconstructed by means of light microscope and according to a stereotaxic atlas [40]. Animals with incorrect cannula placements were excluded from the statistical evaluation.

4. Results

4.1. Histological results

Histological examination showed that the cannulae were precisely and symmetrically tipped into the target area (i.e. VP) in 382 of the altogether 430 rats, while the cannulae of the remaining rats were mistargetted (n = 40) or their acrylate "headpiece" was damaged or came off (n = 8). The rats with incorrect cannula position or damaged "headpiece" were excluded from the statistical analysis.

4.2. Results of the Morris water maze test

4.2.1. Effects of activation of the D1 dopamine receptor

Effects of SKF38393 treatment:

In this experiment effect of the D1 DA receptor agonist SKF38393 treatment on escape latency was investigated in Morris water maze test. Our results showed that there was no significant difference among the groups in the first two conditioning trials. In the first conditioning trial the rats found randomly the hidden platform, correspondingly their latency was a relatively high value. However, one minute after the first conditioning trial, in the second conditioning trial, all groups found significantly faster the platform compared to the first conditioning trial, i.e. their escape latencies significantly decreased. These findings confirmed the formation of the short-term memory. *The second conditioning was immediately followed by the first microinjection of the solutions. After 24 hours the results of the third conditioning trial indicated that the 0.1 and 1.0 μg agonist treated groups could recall the place of the platform which was already learned in the previous day, while the control and the 5.0 μg agonist treated groups* - although their escape latencies were somewhat reduced relative to that of the first conditioning - *almost completely forgot it.* In the fourth conditioning trial, one minute after the third conditioning trial, repeatedly all groups found the platform signinficantly faster compared to the first conditioning trial. In the morning of the third day, in the trial without the platform (which was an extinction trial as well) latencies of all groups were significantly shorter relative to the first conditioning trial. After the platform was replaced, *in the test trial there was a significant difference again among the groups*: *the*

0.1 and 1.0 µg agonist treated groups found significantly faster the hidden platform compared to the control group whose escape latency was similar to that measured on the third conditioning trial.

In the extinction trial - in addition to the first crossing of the removed platform's place - the number of entries into the target quadrant (where the platform was previously placed), the time spent in the target quadrant and the number of crossings at the place of the removed hidden platform were measured. Comparision of the means showed that there was no significant difference among groups in any parameter.

In all trials the mean velocities of the animals were measured. Significant differences were not revealed by statistical analysis in any trial.

Effects of SCH23390 treatment:

In order to clarify whether the effect of the D1 DA receptor agonist SKF38393 was specific to the D1 DA receptors, the selective D1 DA receptor antagonist SCH23390 was applied.

Our results demonstrated that the effects of the agonist were mediated via the D1 DA receptors, since the antagonist pretreatment eliminated the effects of the 1.0 µg agonist treatment: animals of the antagonist + agonist treated group behaved similarly to that of the control group, their latencies did not differ significantly from that of the control animals in any trial. Effect of the antagonist applied by itself was similar to that of the vehicle, although, escape latencies of the antagonist treated animals were sligly shorter compared to those of controls in the third conditioning trial and in the test trial when the platform was re-placed.

In the extinction trial the number of entries into the target quadrant, the time spent in the target quadrant and the number of crossings at the place of the removed hidden platform were measured. *The means of the antagonist treated group proved to be significantly shorter to the means of the 1.0 µg agonist treated group in case of all the parameters, while to the means of the control group only in the time spent in the target quadrant.*

There were no significant differences among the mean velocities of the groups in any trial.

4.2.2. Effects of activation of the D2 dopamine receptors

Efects of Quinpirole treatment:

In this experiment effect of the D2 DA receptor agonist Quinpirole treatment on escape latencies of the rats was investigated in Morris water maze test. In case of all groups the formation of the short-term memory was confirmed by the results of the first two conditioning trials similar to the experiments with the D1 DA agonist. *The second conditioning was immediately followed by the first microinjection of the solutions. After 24 hours the results of the third conditioning trial indicated that the 1.0 and 5.0 μg agonist treated groups could recall the place of the platform which was already learned in the previous day, while the means of the controls and of the 0.1 μg agonist treated group* - although their escape latencies were somewhat reduced relative to that of the first conditioning - *statistically were similar to those of the first conditioning trial.* In the fourth conditioning trial and in the extinction trial there were no significant differences among the groups, all of those found the platform or the place of the removed platform significantly faster compared to the first conditioning trial. *When the platform was replaced the 1.0 and 5.0 μg agonist treated groups found the platform with a significantly shorter latency than the control group,* whose escape latencies were similar to the results of the third conditioning trial.

In the extinction trial - in addition to the first crossing of the removed platform's place - the number of entries into the target quadrant (where the platform was previously placed), the time spent in the target quadrant and the number of crossings at the place of the removed hidden platform were measured. Comparision of the means showed that there were no significant differences among groups in any parameter.

In all trials the mean velocities of the animals were measured. Significant differences were not revealed by statistical analysis in any trial.

Effects of Sulpiride treatment:

The D2 DA receptor antagonist Sulpiride was applied to clarify whether the effects induced by the Quinpirole developed via the acivation the D2 DA receptors. *Our results demonstrated that the agonist had its effect through the D2 DA receptors, because the antagonist pretreatment eliminated the effects of the 1.0 μg agonist treatment.* Means of the antagonist + agonist treated group did not differ significantly from those of the control group in the third conditioning trial and in the test trial when the platform was replaced. The

formation of the short-term memory in the first two conditioning trials was demonstrated in these experiments as well. Important finding was that *the microinjection of the antagonist + agonist or the antagonist by itself immediately after the second conditioning trial impaired the learning functions*, which was confirmed by the fact that the latencies of these groups after the second conditioning trial were not significantly different from the latencies of the first conditioning trial.

In the trial without the platform (extinction trial) in addition to the first crossing of the removed platform's place other parameters were measured as well (see above). The results of the analysis revealed that *the mean of the antagonist + agonist treated group was significantly lower compared to those of the controls and the 1.0 μg agonist treated group considering the number of crossings at the place of the removed platform*.

The mean velocities of the groups were compared statistically to each other in each trial: significant differences were not revealed.

4.3. Results of the inhibitory avoidance test

4.3.1. Effects of activation of the D1 dopamine receptors

Effects of SKF38393 treatment:

The effect of the different doses of the D1 DA receptor agonist SKF38393 on step-through latency was investigated in inhibitory avoidance test. In the conditioning trial significant difference was not found among the groups. 24 hours after the conditioning trial, in the first test latencies of the *1.0 μg and 5.0 μg agonist treated groups were significantly longer relative to that of the control group, furthermore both doses of the agonist enhanced the retention one and two weeks* (i.e. in the second and in the third test) *after conditioning.*

Effects of SCH23390 treatment:

The selective D1 DA receptor antagonist SCH23390 was used to clarify whether the effect of the SKF38393 developed via the D1 DA receptors. The antagonist was applied 15 minutes prior to the agonist treatment or by itself. Based on the statistical analysis within each trial, in the conditioning trial there was no significant difference among the groups. On the other hand, *the 1.0 μg dose of the agonist increased the step-through latency compared to the*

latencies of all other groups in the first, in the second and in the third test trial, as well. These findings confirmed the effects of SKF38393 were mediated via D1 DA receptors.

4.3.2. Effects of activation of the D2 dopamine receptors

Effects of Quinpirole treatment:

The effect of the D2 DA receptor agonist Quinpirole was investigated on the step-through latency of the rats. In the conditioning trial there was no significant difference among the groups. *24 hours after the conditioning trial, in the first test the 0.1 μg agonist treatment increased significantly the step-through latency* compared to the control, the 1.0 μg and the 5.0 μg agonist treated groups. Moreover the 0.1 μg dose of the agonist *enhanced the retention* one and two weeks after conditioning.

Effects of Sulpiride treatment:

Based on the results of the experiments with the D2 DA receptor agonist Quinpirole, the D2 DA receptor antagonist Sulpiride was applied to reveal the receptor specificity of the Quinpirole's effects. The analysis within the trials demonstrated that *the 0.1 μg dose of the D2 DA agonist increased the step-through latency in the first and second test compared to the control, the antagonist + agonist and the antagonist treated groups.* However, in the third test the 0.1 μg dose of the agonist increased latency compared only to the control and the antagonist + agonist treated groups. In summary our findings proved that the effects of the *Quinpirole were mediated via the activation of the D2 DA receptors*.

4.3.3. Verification of the formation of the short-term memory in inhibitory avoidance paradigm

Akin to the Morris water maze paradigm, in the inhibitory avoidance paradigm it was also necessary to demonstrate the formation of the short-term memory whose consolidation could be enhanced by the appropriate doses of the agonists. In this experiment to confirm the short-term memory formation, two physiological saline treated groups were applied: one group was replaced into the apparatus only 24 hours after the conditioning, while the other group - similarly to the schedule of the Morris water maze paradigm - was replaced one

minute and 24 hours (corresponding to the first test) after the conditioning. The means of the single sessions were compared to each other within both groups. In case of the first group (replaced once) there was no significant difference between the means of the conditioning trial and the first test (after 24 hours). *In case of the second group (replaced twice) the animals entered into the dark compartment significantly earlier relative to the conditioning trial*, however after 24 hours, in the test trial they behaved as in the conditioning trial. These findings proved the formation of the short-term memory in the inhibitory avoidance paradigm.

4.4. Results of the place preference test

4.4.1. Effects of SKF38393 treatment in place preference test

Effect of the D1 DA receptor agonist SKF38393 on the time spent in the treatment quadrant was investigated in conditioned place preference paradigm. Significant differences were not revealed among the groups by the statistical analysis within the trials.

In addition to the time spent in the treatment quadrant, the distance moved, the number of entries into the treatment quadrant and the percentage between the number of entries into the treatment quadrant and into the all quadrants (included the treatment quadrant) were measured. *Significant differences were not revealed among the groups in the habituation or in the test session.*

4.4.2. Effects of Quinpirole treatment in place preference test

Effect of the D2 DA receptor agonist Quinpirole on the time spent in the treatment quadrant was investigated in conditioned place preference paradigm. Significant differences were not revealed among the groups by the statistical analysis within the trials.

In addition to the time spent in the treatment quadrant, the distance moved, the number of entries into the treatment quadrant and the percentage between the number of entries into the treatment quadrant and into the all different quadrants were measured. Significant difference was not revealed among the groups in the habituation session. However *in the test session the 1.0 μg D2 agonist Quinpirole treatment significantly increased the distance moved and the number of entries into the treatment quadrant* compared to the controls and the 0.1 μg agonist treated groups. Considering the ratio (percentual evaluation)

between the number of entries into the treatment quadrant and into the all different quadrants, there was no statistical difference among the groups in the test session.

5. Discussion

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

The Morris wate maze test is a widely used method to investigate the spatial learning and memory processes [42-44]. In our swimming experiments the formation of short-term memory was confirmed. According to our interpretation the differences among the groups during the third conditioning trial could be due to the memory consolidation enhancing effect of the 0.1 μg and 1.0 μg D1 DA receptor agonist SKF38393, as well as, of the 1.0 μg and 5.0 μg D2 DA receptor agonist Quinpirole. This is supported by the fact that the latencies of these groups were very similar to those of the second conditioning trial, i.e. the short-term memory has been consolidated by the effective doses of the agonists. The second microinjection of the agonists did not affect significantly the consolidation processes.

The role of the D1 and D2 DA receptors in memory consolidation has been confirmed in the NAC [13, 45], the HPC [46-48] and in the AMY [18, 46] by means of direct and indirect DA agonist, as well as by DA antagonist. Further evidences are provided by genetical manipulations and chemical lesions: in genetically modified D1 DA receptor deficient mouse spatial learning deficiency can be observed [12, 49], furthermore the 6-OHDA lesion of the HPC and the NAC shell region can result in learning disturbances in Morris water maze test [50, 51]. Interestingly, the 6-OHDA lesion of the CPU impairs the spatial, i.e. allocentric learning besides the egocentric one [52], which is contradictory to other findigs showing that DA release in the dorsal striatum does not play an essential role in spatial learning [18, 46, 48]. One reason of the latter result can be that the lesion was performed before the conditioning, which could influence not only the consolidation processes, but the acquisition or motivational processes as well. Another possible explanation is that in the absence of the DMI pretreatment the 6-OHDA lesion affected not only the DA-ergic, but also the noradrenergic terminal elements [52].

An additional important finding is provided by our experiments. In the third day morning the platform was removed, i.e. an extinction was performed with the experimental animals. The same day, afternoon, the platform was replaced to investigate the effect of the

previous/morning extinction on memory. Results of the test confirmed that the 0.1 μg and 1.0 μg SKF38393 as well as the 1.0 μg and 5.0 μg Quinpirole significantly decreased the latencies to find the platform compared to the control group. There are several possible explanations of the observed phenomenon. On the one hand (and we prefer this explanation), the effective doses of the agonists increase the stability of the formed memory against extinction. On the other hand, the differences observed during the test can be due to perseverative behaviour of the rats evoked by that certain doses of the agonists. This latter alternative has to be considered because it is supported by several experimental data. Namely, the stimulation of the DA receptors reduces behavioural flexibility, impairs strategy changing ability and increases the number of perseverative responses [53-56]. Consequently, it cannot be excluded that DA receptors of the VP may play a role in the induction of perseverative behaviour. In order to clarify this question, in the extinction trial - in addition to the latency to the first crossing of the removed platform's place - the number of entries into the target quadrant, the time spent in the target quadrant and the number of crossings at the place of the removed hidden platform were measured. Neither in case of the experiments with D1, nor with the D2 DA receptor agonist was significant difference among groups analysing any of these parameters. Both the control and the agonist treated groups (including the non effective doses) searched the platform at its original place, than - after not finding it there - they started to follow the general searching strategy. The results of the extinction trial support the view of that the attractive characteristic of the platform's place is not reduced by one extinction trial, but the stability of the memory related to the place decreases. This latter means that during the test trial it is more difficult to find the platform for the control rat compared to the animal treated with the effective dose. Based on these findings our original hypothesis concerning that both agonists increase the memory stability can be maintained.

The D1 DA receptor antagonist SCH23390 and the D2 DA receptor antagonist Sulpiride pretreatment proved that the SKF38393 activated the D1 DA receptors, while the Quinpirole the D2 DA receptors. The SCH23390 applied by itself did not influence significantly the learning processes, but the extinction was somewhat enhanced. To prove this latter, further experiments are required, however. In contrast to the effect of the D1 DA antagonist the Sulpiride applied by itself or in combination with the agonist resulted in that these animals found relatively slowly, or they did not found the hidden platform after the second conditioning trial. This effect of the antagonist is probably due to the general and long-term impairment of learning processes. It is well known that D2 DA antagonists may have an extinction-like effect, i.e. to their effects the motivational value of the reward, reward

related signals and actions decreases [57-60]. Therefore, it was important to exclude that the antagonist treatment influenced permanently the motivational processes of the animals. During the trial, when the platform was removed, the searching behaviour of the antagonist treated rats was very similar to those of controls, however the former animals found the platform slower than the latters. This confirmed that the antagonist does not influence the motivational processes, but impairs the learning of the animals.

The role of D2 DA receptors in consolidation processes is supported by other experimental findigs as well, which have demonstrated that the D2 DA receptors of the NAC (the main input structure of the VP) and of the HPC are necessary for the consolidation processes of the spatial learning [13, 45, 48].

We can conclude that the activation of the VP D1 and D2 DA receptors accelerates the memory consolidation in spatial learning, and increases the stability of the formed memory against extinction. Furthermore, activation of the VP D2 DA receptors is the necessary condition of these processes, the block of these receptors leads to the general imparment of the spatial learning processes.

5.2. Evaluation of the results of the inhibitory avoidance test

The inhibitory avoidance test is a widely used paradigm to investigate the negative reinforcement (punishment-learning). In our experiments the D1 DA receptor agonist SKF38393 dose-dependently, furthermore, the D2 DA receptor agonist in 0.1 μg dose proved to be effective, that is improved learning and the retention. In a separate experiment it was demonstrated that the short-term memory formation in rats is also observable in the inhibitory avoidance paradigm akin to the Morris water maze test. According to our interpretation, the effective doses of the agonists enhanced the consolidation of this short-term memory. The role of DA and its receptors in inhibitory avoidance learning and in the related memory consolidation processes have been generally proven in different brain areas. In mouse, microinjection of the D1 DA receptor antagonist SCH23390 and the D2 DA receptor antagonista Sulpiride in the core region of the NAC dose-dependently prevented the memory consolidation, while in the shell region of the NAC only the Sulpiride had a similar effect [16]. The memory consolidation in inhibitory avoidance learning requires the co-activation of the DA receptors of the NAC shell region and of the basolateral AMY [15]. The SCH23390, but not the Sulpiride microinjected into the chick CPU impairs the inhibitory avoidance

learning and memory processes [61]. In inhibitory avoidance learning paradigm the D1 DA receptor antagonist SCH23390 microinjected into the anterolateral prefrontal and the posteroparietal cortices, as well as, into the CA1 region of the HPC before conditioning inhibits the acquisition [62]. After conditioning - the same substance - applied in the CA1 region of the HPC enhances the formation of the short-term memory, however, in the anterolateral prefrontal and the entorhinal cortices impaires the long-term memory [62]. In the same paradigm the SCH23390 in the anteromedial precentral PFC prevents the memory consolidation [14]. Additional findigs, partly inconsistent with the previous results, are that the 6-OHDA lesion of the AMY impairs the active avoidace learnig, but interestingly slightly improves the passive (inhibitory) form of it [63]. Furthermore, DA administered into the NAC before conditioning impairs the inhibitory avoidance learning [64]. Our criticism against these two latter experiments are identical with those decribed in the discussion of the results of the Morris water maze test. Accordingly, in case of the absent DMI pretreatment the 6-OHDA lesion is not specific to the DA-ergic terminals, namely it will affect the noradrenergic fibers as well, and DA administration prior to the conditioning can influence both the motivational and motor processes interfering with the learning/memory consolidation effects.

The D1 DA receptor antagonist SCH23390 and the D2 DA receptor antagonist Sulpiride pretreatment confirmed that the SKF38393 activated the D1 DA receptors, while the Quinpirole the D2 DA receptors. The antagonists applied by itself did not affect significantly the memory consolidation and its retention.

In summary the activation of the VP D1 and D2 DA receptors enhances the memory consolidation and the long-term stability of the formed memory in inhibitory avoidance learning.

5.3. Evaluation of the results of the place preference test

The place preference test is applied to detect the reinforcing (rewarding or punishing) effect of different chemical substances [65, 66], which makes it important to investigate the addictive effect of different drugs. In our experiments neither the D1, nor the D2 DA receptor agonist treatment evoked place preference. It is well known that cocaine microinjected into the VP induces place preference [38] by significantly increasing the DA level in the VP. The cocaine-induced place preference can be eliminated by 6-OHDA lesion of the VP [39], and similar result can be obtained by application of the opiate antagonist naloxone [67]. Based on

these findings the place preference inducing effect of cocaine is realised via DA-ergic and opiate-ergic mechanisms in a way that none of these systems' activity is satisfactory in itself, but both are necessary for the formation of the place preference. This latter requires the fulfillment of two conditions in the experimental animals: the administered substance 1.) has to possess rewarding/positive reinforcing effect and 2.) this effect has to be consolidated in the central nervous system. Both in the Morris water maze test and in the open field based place preference paradigm the spatial learning of the experimental animals is necessary to find the platform in the former or the treatment quadrant in the latter. Therefore, it can be supposed that certain doses of the agonists fulfilled the consolidation condition in the place preference test, similarly to the Morris water maze test, but the place preference did not develop because of the absent rewarding effect of the agonists. It is well confirmed by experimental data that 1.) the primary site of the DA rewarding effect is the NAC [10, 68-72], 2.) the rewarding effect of cocaine develops not, or only partly via the DA system [73-76]. Separability of the rewarding and the consolidation effects has been shown by the experiments, in which the influence of D1 and D2 DA receptors on morfine induced place preference was investigated in the AMY. In both cases place preference was not induced by the activation of the DA receptors in itself, however, DA receptor activation amplified the morphine induced place preference [77, 78].

In addition to the classic parameter of the place preference - the time spent in the treatment quadrant - the number of the entries into the treatment quadrant was measured, which can reflect the orientation response for the signals of the treatment quadrant. Our results showed that in case of the D1 DA receptor agonist there was no significant difference among the groups, while the 1.0 μg dose of the D2 DA agonist increased the number of the entries. Based on the analysis of the locomotor activity this elevation was not due to the treatment quadrant-specific orientation, but rather to the increased locomotor activity. It was assumed that the observed increase in locomotor activity during the test is identical with the phenomenon of the conditioned locomotor activity [65, 79]. To justify this assumption the locomotor activity of the rats was measured during the conditioning trials, (although the results obtained in open field paradigm by Gong and Neil were reproduced considering the effect of the Quinpirole [80]) but the results did not support our previous assumption. The 1.0 μg dose of the agonist did not produce an increased locomotor activity, so this effect could not be consolidated in the nervous sytem of the rats. So this option had to be rejected, the interpretation of the increased locomotor activity during the test caused by the 1.0 μg Quinpirole treatment requires data of further experiments.

In summary neither the D1 DA receptor agonist SKF38393, nor the D2 DA agonist Quinpirole microinjected into the VP induce place preference.

6. Summary

The followings were confirmed in our experiments:

1. In the Morris water maze paradigm investigating the spatial learing processes
   a) the 0.1 and 1.0 μg doses of the D1 DA receptor agonist SKF38393, as well as
   b) the 1.0 and 5.0 μg doses of the D2 DA receptor agonist Quinpirole
   microinjected into the VP accelerate the memory consolidation and enhance the stability of the formed memory against extinction.

2. In the inhibitory avoidance paradigm investigating the learning processes related to the negative reinforcement
   a) the D1 DA receptor agonist SKF38393 dose-dependently, as well as
   b) the 0.1 μg dose of the D2 DA receptor agonist Quinpirole
   microinjected into the VP enhance the memory consolidation and the long-term stability of the formed memory.

3. In both paradigms it was confirmed that the
   a) effect of SKF38393 is eliminated by the D1 DA receptor selective antagonist SCH23390 pretreatment, and
   b) effect of Quinpirole is eliminated by the D2 DA receptor selective antagonist Sulpiride pretreatment
   in the VP.

4. The SCH23390 applied by itself was ineffective in both paradigms, however the Sulpiride applied by itself prevented the learning processes in a long-run and general way in the Morris water maze paradigm, while in the inhibitory avoidance paradigm it was ineffective.

5. In both paradigms formation of the short-term memory was demonstrated, which could be consolidated by the agonists microinjected into the VP.

6. Neither the D1 DA receptor agonist SKF38393, nor the D2 DA receptor agonist Quinpirole microinjected into the VP induce place preference.

7. References

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8. List of publications

8.1. Publications related to the thesis

Péczely, L., T. Ollmann, K. László, A. Kovács, R. Gálosi, Á. Szabó, Z. Karádi, L. Lénárd: Effects of ventral pallidal D1 dopamine receptor activation on memory consolidation in morris water maze test. Behavioural Brain Research, 274: 211-218, 2014. (http://dx.doi.org/10.1016/j.bbr.2014.07.031) (**IF: 3,391**)

Péczely, L., T. Ollmann, K. László, A. Kovács, R. Gálosi, Á. Szabó, Z. Karádi, L. Lénárd: Role of D1 dopamine receptors of the ventral pallidum in inhibitory avoidance learning. Behavioural Brain Research, 270: 131-136, 2014. (http://dx.doi.org/10.16/j.bbr.2014.04.054) (**IF: 3,391**)

8.2. Other publications with impact factors

László, K., K. Tóth, E. Kertes, **L. Péczely**, L. Lénárd: The role of neurotensin in positive reinforcement in the rat central nucleus of amygdala. Behavioural Brain Research, 208(2): 430-435, 2010. (doi:10.1016-j.bbr.2009.12.022) (**IF: 3,393**)

László, K., K. Tóth, E. Kertes, **L. Péczely**, T. Ollmann and L. Lénárd: Effects of neurotensin in amygdaloid spatial learning mechanisms. Behavioural Brain Research, 210(2): 280-283, 2010. (doi:10.1016/j.bbr.2010.02.038) (**IF: 3,393**)

Kovács, A., K. László, R. Gálosi, K. Tóth, T. Ollmann, **L. Péczely**, L. Lénárd: Microinjections of RFRP-1 in the central nucleus of amygdala decreases food intake in the rat. Brain Research Bulletin, 88(6): 589-595, 2012. (doi: 10.1016/j.brainresbull.2012.06.001) (**IF: 3,327**)

László, K., K. Tóth, E. Kertes**, L. Péczely**, T. Ollmann, A. Madarasy-Szűcs, L. Lénárd: The role of neurotensin ín passive avoidance learning ín the rat central nucleus of amygdala. Behavioural Brain Research, 226: 597-600, 2012. (doi: 10.1016/j.bbr.2011.08.041) (**IF: 3,327**)

Kovács, A., K. László, R. Gálosi, T. Ollmann, **L. Péczely**, O. Zagoracz, N. Bence, L. Lénárd: Intraamygdaloid microinjection of RFamide-related peptide-3 decreases food intake in rats. Brain Research Bulletin, 107: 61-68, 2014. (http://dx.doi.org/10.1016/j.brainresbull.2014.07.002) (**IF: 2,974**)

Lénárd, L., A. Kovács, T. Ollmann, **L. Péczely**, O. Zagoracz, R. Gálosi, K. László: Positive reinforcing effects of RFamide-related peptide-1 ín the rat central nucleus of amygdala. Behavioral Brain Research, 275: 101-106, 2014. (http://dx.doiorg/10.1016/j.bbr.2014.08.051) (**IF: 3,391**)

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8.3. Further publications and citable abstracts

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