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Prostorová kognice u potkanů:

zpracování informace o pozici vzdálených objektů

Spatial cognition in rats:

processing of information about the position of distant objects

Disertační práce

Školitel:
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Abstrakt

Prostorové chování je široce studováno pro pochopení kognitivních funkcí a objasnění jejich neurofyziologického substrátu. Hipokampus hraje klíčovou roli v mnoha prostorových úlohách. Stále není jasné, zda je funkční hipokampus nutný pro rozeznávání pozice vzdálených objektů umístěných v nepřístupném prostoru. Abychom odpověděli na tuto otázku, vyvinuli jsme nový operantní test, ve kterém potkani rozeznávají pozici objektu umístěného v nepřístupném prostoru. Roli dorzálního hipokampu v této úloze jsme studovali pomocí zablokování jeho aktivity muscimolem. Naše výsledky ukázaly, že intaktní potkani používají dorzální hipokampus pro rozeznávání pozice vzdálených objektů umístěných v nepřístupných částech prostředí. Navíc jsme prokázali, že kognitivní výkonnost v této úloze není ovlivněná změnou motorické aktivity způsobenou aplikací prazosinu.

Klíčová slova: prostorová kognice, operantní podmiňování, hipokampus, muscimol, prazosin

Abstract

Spatial behavior is widely studied to understand cognition and its neurophysiological substrate. Hippocampus plays a crucial role in many spatial tasks. It is unclear whether hippocampus is necessary for recognizing position of distant objects located in inaccessible space. To address this question we developed a novel operant-conditioning task in which rats recognize position of an object located in an inaccessible space. We assessed the role of the dorsal hippocampus in the task by blocking its activity with muscimol. Our results showed that intact rats use the dorsal hippocampus for recognizing position of the distant object located in the inaccessible part of the environment. In addition, we showed that the cognitive performance in the task is not affected by the changes in motor activity induced by prazosin.

Key words: spatial cognition, operant conditioning, hippocampus, muscimol, prazosin

I Introduction

1 Spatial behavior

The survival of animals depends on their ability to memorize locations (places), and to use behavioral strategies to navigate efficiently between their home base and other places of interest (Save and Poucet, 2005). Navigation is the process of determining and maintaining a course or trajectory from one place to another (Gallistel, 1990).

1.1 Route and mapping navigation

O'Keefe and Nadel (1978) proposed that animals can use either routes or maps to navigate. The route navigation utilizes a chain of associations between a number of motor responses that link relevant external stimuli to navigate. For example, the animal reaches a goal by following a list of instruction such as: turn right at the tree, go towards the well and then turn left etc. (Jeffery, 2003). The map navigation is linked to cognitive map hypothesis which is based on the idea that some animals dispose of an internal mental representation of the environment (Tolman, 1948). This representation encodes the geometrical relationships between landmarks and places in the environment and is not dependent on the current position of the animal.

The route and the mapping navigation are distinguished in relation to neural structures which were responsible for these processes. The route navigation relies on extra-hippocampal structures while the map navigation is hippocampal-dependent. Both navigations are at least in part independent.

The route navigation generates routes which can be viewed as lists of guidances and orientations. The guidances serve as landmarks to be approached or

followed by any available behavior and the orientations specifying a particular movement to be made in the presence of a particular cue. The route navigation is inflexible and allows leading an animal from one point to another. This type of navigation can be easily disrupted by alterations of relevant spatial cues (O'Keefe and Nadel, 1978).

The map navigation is based on spatial relationships in an environment which are contained in the hippocampal cognitive map. A map can be described as a set of connected places which offers to an animal many possible paths between any two points in the environment. The map navigation is very flexible and relatively invulnerable to changes in the environment because it does not rely on a particular cue (O'Keefe and Nadel, 1978).

1.2 Piloting and dead reckoning

The forms of navigation can be classified also in relation to available categories of cues. The two main classes of spatial cues are allothetic and idiothetic. Allothetic cues are provided by the environment and include visual, olfactory and auditory information which are located in the near and distant surroundings of an animal. Idiothetic cues are generated during the animal's own active or passive movements. Thus, some external motion-related information such as optic flow fall also into idiothetic cues. Typical idiothetic information is provided by the vestibular, proprioceptive and somatosensory systems (efferent copies of movement commands) (Save and Poucet, 2005).

There are also two different categories of spatial reference frames, i.e. systems of coordinates: allocentric and egocentric. To avoid any confusion these terms are not related, in contrast to allothetic and idiothetic, to the category of sources of spatial

information. The egocentric frame of reference specifies location and orientation of a spatial cue with respect to a body part of an animal. The allocentric frame of reference specifies location and orientation with respect to elements and features of the environment independently of the animal's position (Thomas, 2010).

The two navigational strategies which utilize the different categories of available cues are piloting and dead reckoning (Gallistel, 1990). These categories of navigation do not rely on totally different neural structures in contrast to the classification into the route navigation and the mapping navigation. Piloting and allothetic navigation are equivalent terms as well as dead reckoning and idiothetic navigation. Piloting requires the use of the relationships between relatively stable external cues whereas dead reckoning requires the integration of cues generated by self-movement (Whishaw and Gorny, 2009).

Piloting utilizes the allothetic cues for both guidance and for constructing internal representation of the environment (O'Keefe and Nadel, 1978; Morris, 1981; Sutherland & Dyck, 1984; Redish, 1999). In contrast, dead reckoning utilizes the idiothetic cues and generates the representation of the geometric relation between the position where the dead reckoning started and the current position of the animal (Gallistel, 1990). Thus, dead reckoning integrates self-motion cues to allow the animal to return to a starting point (Mittelstaedt and Mittelstaedt, 1980; Seguinot et al., 1993; Whishaw and Gorny, 1999).

2 Operant conditioning

Conditioning is a behavioral process during which a response becomes more frequent or more predictable in a given environment as a result of delivery of reinforcers. A reinforcer may be defined as any event that will increase or maintain the frequency of a pattern of behavior with which it is associated (Blackman, 1974).

Operant behavior is a tool to achieve a goal. This type of learning is conditioned by internal needs of a subject. Operant conditioning in principle leads a subject to fix the consequences of its behavior and act upon them in the future. The subject actively learns to perform an action (motor response) in order to avoid an unpleasant stimulus (e.g. electric shock) or to obtain a pleasant stimulus (e.g. food reward). Development of a successful behavioral strategy is usually achieved by trial-and-error method.

The procedure that leads to an increase or maintenance of frequency of behavioral pattern that is followed by a reward or by avoiding a punishment is called reinforcement (Blackman, 1974). If a response is not reinforced for longer time, it gradually fades away from behavioral repertoire because it is not necessary to perform patterns of behavior which do not produce positive feedback.

Positive reinforcement is a procedure by means of which a positive link between a behavioral pattern and a pleasant stimulus is established. If a test subject produces the behavioral pattern, it receives a reward. Otherwise, no reward is delivered. The frequency of the particular behavior is increased in this way.

Negative reinforcement is strengthening of behavior that allows an animal to avoid punishment. If the animal carries out a particular behavioral pattern, an unpleasant stimulus is terminated or does not occur.

The classic test apparatus for evaluating operant behavior is the Skinner box, an automated test apparatus first devised and developed by B. F. Skinner when analyzing the behavior of rats responding to obtain food reward (Skinner, 1938). In the classical case, it is a small experimental chamber with a lever inside. Near the lever is a feeder into which food is delivered when an animal executes an operant response. Discriminative stimuli are provided by a variety of other ancillary devices which are different in relation to the type of experiment.

Operant chamber paradigms enable far more precise control of the factors which determine the behavior than can be achieved by conventional observation. Using different stimuli to signal the class of responses that will be reinforced, it is possible to determine the nature of the sensory discriminations that an animal can make, and subsequently its performance on cognitive tasks (Döbrössy et al., 2009).

Frequently used operant behavior in laboratory experiments is lever pressing. This kind of operant responses has two big advantages. The first advantage is that an animal can emit this operant behavior in accordance to its intrinsic motivation and to experimental design. Lever pressing can be performed very often or very rarely or not at all. Well trained animal can press the lever up to 100 times per minute. Another reason for selecting this type of operant behavior is that the experimenter can use a computer to easily monitor the responses and evaluate data obtained during the session. In addition, a reward can be delivered immediately after the operant response is emitted.

2.1 Schedules of reinforcement

Schedules of reinforcement are a set of rules relating to the likelihood and timing of delivery of a reward to an experimental animal in relation to its patterns of

behavior. Schedules of reinforcement allow monitoring of a time strategy of the animal and also enable designing experiments in order to optimize responses of the animal in relation to presented stimuli. In addition, schedules of reinforcement are used to eliminate the time strategy. Thus, the animal has to rely only on external sensory stimuli during solving a behavioral task. There are several basic types of schedules of reinforcement.

Continuous reinforcement means that an animal receives a reward immediately after each operant response (e.g. lever press). Other types of schedules of reinforcement are also called intermittent because not all the operant responses are rewarded (Hintzman, 1978).

During fixed ratio every *n*-th emitted response is rewarded. For variable ratio is typical that the actual number of responses needed for obtaining reward follows some likelihood distribution, e.g. geometric or uniform, with a mean value *n*. Variable ratio is then specified by the average number of responses that an animal has to emit in order to receive a reinforcer (Blackman, 1974).

2.2 Stimulus control

Operant behavior can be controlled by variety of different stimuli. An animal emits operant responses with high frequency only when a particular stimulus or condition is presented. Stimulus control allows studying perception and discrimination of colors, contrast, brightness, tones, shapes, context etc. (Blackman, 1974). Moreover, operant behavior can be controlled by the position of the subject in an environment (Klement and Bures, 2000; Pastalkova et al., 2003) or by configuration of objects (Nekovarova and Klement, 2006). In addition, Experiment I presented in this

PhD thesis showed that the position of a stationary or moving distant object can also be used for stimulus control (Klement et al., 2010).

The combination of intermittent reinforcement schedule and stimulus control provides a powerful and unique technique for studying spatial cognition in non-moving subjects.

3 Behavioral tasks for studying spatial cognition in rats

The main idea of behavioral spatial tasks is to study spatial cognition. These tasks make it possible to test whether an animal recognizes its own position in an environment, and whether it is able to plan and carry out a path to a goal location. Spatial tasks are also used to study neural mechanisms involved in navigation. The apparatuses, in which are laboratory experiments carried out, are called mazes. When solving spatial tasks the animal can navigate using spatial cues that serve as landmarks. These spatial cues are divided into proximal, which are part of the maze (intramaze), and distal, which are located outside the maze (extramaze) (Jeffery, 2003). The mazes are commonly used because they allow the experimenter to control the types of information that the animal has available to solve the task.

Behavioral tasks for studying spatial cognition in rats, which are described in the PhD thesis, are divided into two main parts: a) tasks presented in a real environment and b) tasks utilizing computer screen for stimuli presentation.

3.1 Tasks presented in a real environment

In the first part, I mention few tasks which use real stimuli as spatial cues and are situated in the real environment, and which were crucial in the history of the research of spatial cognition.

3.1.1 Navigational tasks

This kind of behavioral tasks tests the ability of rats to navigate between different places. In most of them, the rats navigate to a hidden goal, whose position can be found in relation to distal (extramaze) spatial cues.

Complex mazes were frequently used in the beginnings of research of spatial behavior. Nowadays they almost disappeared from common use. In this task, animals have to learn their path to the goal by remembering their turns at each junction. The difficulty with this test is that it is unclear how the animal solves the task. One rat can reach the goal because it remembers the sequence of body turns (left, right, right, left, etc.), while another may remember the general direction of the hidden goal. Additionally, if the animal makes a mistake and gets lost in the maze, it is not clear how to collect additional data from this experiment.

T-maze can be described as a fragment of the complex mazes. It is the result of the effort to reduce the complex mazes to only one spatial choice. An animal is placed at the end of the stem arm. The animal should choose between the left or the right arm at the junction. Only one choice is considered correct and is rewarded (Fig. 1). T-maze may serve as a test of working memory (short-term and temporary memory for recent events), if the reward is positioned alternately in both arms and the animals must remember where the reward was found in the previous trial. Or it can be used to test reference memory (long-term memory for unchanging aspects of the task). In this case, the reward is constantly placed in the same arm and the time between trials can be extended.

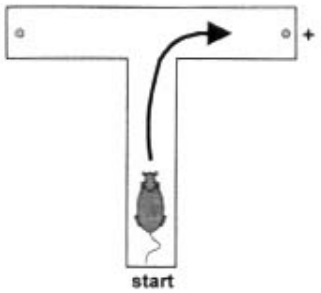

Fig. 1: Scheme of the T-maze. The reward arm is signified by the +. Reproduced from Save and Poucet (2005).

Radial mazes are geometrically simpler than the complex mazes because all arms originate in one central area (Fig. 2). The radial maze was invented by Olton (1977) and usually has 4, 8 or 12 elevated arms. Performance is scored according to the number of correctly and incorrectly visited arms. The standard radial maze enables to test different cognitive abilities. The task is used to test working memory. The animal obtains reward at each arm of the maze but only during the first visit of the arm. Thus, it should remember the visited arms. Somewhat surprisingly, animals do not solve the task by visiting various arms in succession. In the forced-choice task the reward is located at the end of all arms but the animal is allowed to visit only certain arms. After this forced selection the previously closed arms are opened and the animal is allowed to collect remaining reward. By this, stereotyped strategies are disrupted and the animal must rely on its spatial memory. The second most common version of the radial maze is the 4/8 task in which food reward is available only at the end of half of the arms. Thus, the animal has to remember which arms contain reward and which are empty. This is a test of reference as well of working memory because the animal has to learn not only the position of arms, which contain the food reward, but also which arms it has already visited during an ongoing trial.

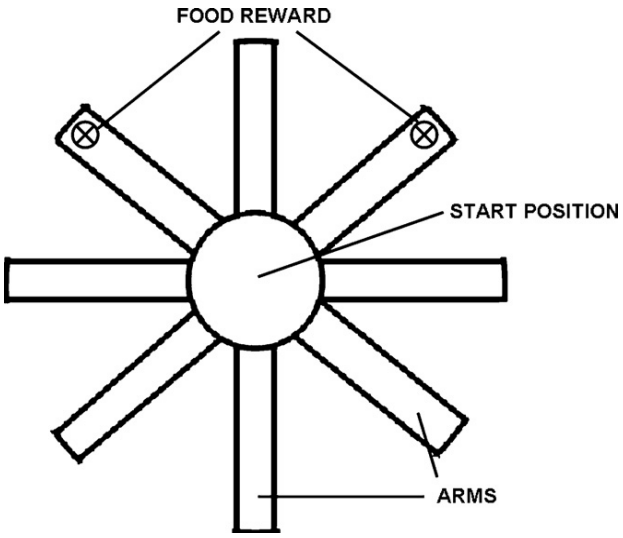

Fig. 2: Scheme of the 8-arm radial maze. Reproduced from Paul et al. (2009).

Radial maze has one major disadvantage because in this task it is still difficult to determine which type of information the animal uses to solve the task. For example, the rat can remember which arm has to choose based on a list of sensory characteristics (e.g. odors) typical of each arm. This aspect can be reduced by cleaning the maze but not always is the experimenter able to absolutely rule out this strategy.

For this reason there was a need to develop a task in which proximal cues, namely odors, will be eliminated. Such task was invented by Morris (1981). In so called Morris water maze a rat is placed in a circular pool (usually about 2 m in diameter) and it should search for an escape platform. The platform is either visible (cue version of the task) or it is submerged (place version of the task)(Fig. 3). The cue version is used to determine if a drug or other experimental manipulation causes crude alterations in visual acuity that might confound the analyses of data from the place version of the task (Terry, 2009). In the place version the rat initially randomly searches the hidden platform. In the following trials, it gradually learns to swim to the platform directly exploiting distal visual cues in the experimental room. If the location of the platform varies from one experiment to another, the rat never learns to swim directly to the platform which confirms that the platform cannot be found directly. The performance of the rats is most commonly evaluated by the trajectory of the animal and/or on account of the time which is spent by the animal to find the hidden platform. When the rats learned the task a probe trial can be carried out. In the probe trial, the platform is removed from the pool to measure spatial preference for the previous platform location. This is accomplished by measuring the percentage of time spent (and distance swam) in the previous target quadrant as well as the number of crossings over the previous platform location. These assessments provide a second estimate of

the strength and accuracy of the memory of the previous platform location (Terry, 2009).

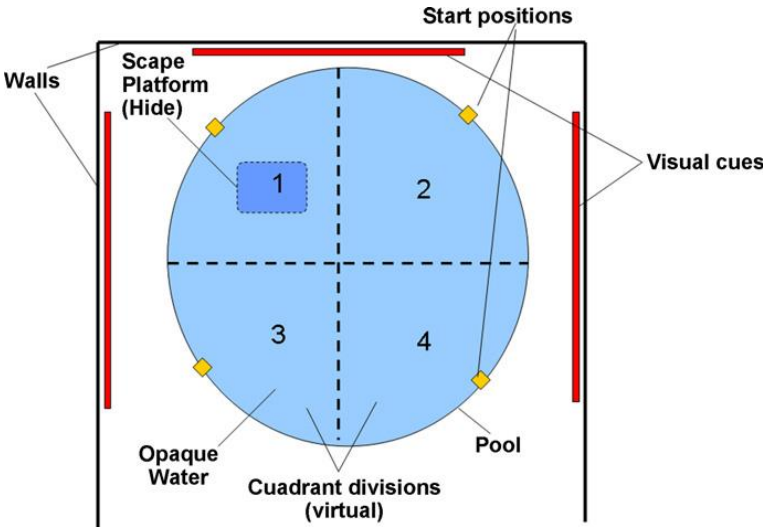

Fig. 3: Scheme of the Morris water maze. Reproduced from Paul et al. (2009).

3.1.2 Place recognition tasks

These tasks test the ability of animals to determine their own position in an environment. Therefore, the animals have to recognize if a particular place has been previously visited by them or not. There are only few tasks which were designed to test this ability in rodents. Two of them (Klement and Bures, 2000; Pastalkova et al., 2003) were developed in our laboratory (Dpt. of Neurophysiology of Memory, Institute of Physiology, AS CR).

In the place recognition task introduced by Klement and Bures (2000) was a rat placed in an operant chamber with transparent walls which was equipped with a feeder and a lever, and was passively transported over a circular trajectory (Fig. 4). The lever presses were rewarded only when the rat passed across a 60°-wide sector of the trajectory. This sector was recognizable in relation to distal visual cues (tables, windows, door, shelves) which were in the experimental room. The responding rate of

lever presses increased when the rat was approaching the reward sector and had a peak at the entrance into the reward sector. In contrast, the responding rate rapidly declined to zero at the exit from the reward sector. To prevent the rats from utilizing a time strategy in solving the task the direction of rotation was changed at pseudorandom intervals. Therefore, the rats, which were passively transported through the environment, were able to recognize their own position in this environment with reference to distal visual cues.

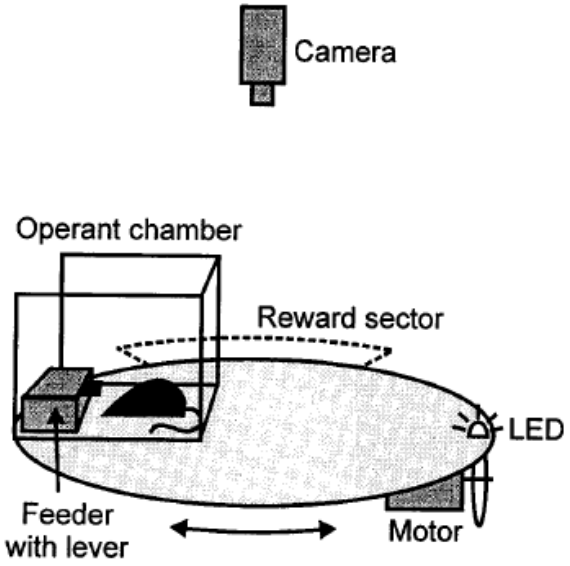

Fig. 4: Apparatus for testing place recognition in rats. The camera detected an infrared light-emitting diode on the perimeter of the circular rotating arena to monitor the position of the operant chamber. Reproduced from Klement and Bures (2000).

The task developed by Pastalkova and her colleagues (2003) was designed to assess the ability of rats to recognize and anticipate their position relative to movable objects. The rats were placed in an operant chamber equipped with a feeder and a lever. The chamber had only the front wall transparent (the other walls were non-transparent) and through this front wall could the rats observe movable objects fixed on a rotating circular arena which was surrounded by an immobile black cylinder

(rotating scene place recognition task, Fig. 5A). There were either two adjacent objects (a blue-white striped cylinder and an adjacent green box) or a planar cue (a piece of red paper cut in the shape of a 60° arena sector). The lever presses were only rewarded when a radius separating the two adjacent objects or dividing the planar cue into two halves (pointing radius) entered a reward sector. The reward sector was a 60°-wide sector of the circular trajectory and it was recognizable with respect to the stationary operant chamber. The rats increased the responding rate when the pointing radius was approaching the reward sector. The same results were obtained when the operant chamber with the rats was passively transported around the circular arena (rotating observer place recognition task, Fig. 5B). Thus, the rats were able to recognize their own position relative to objects rotating on an inaccessible platform.

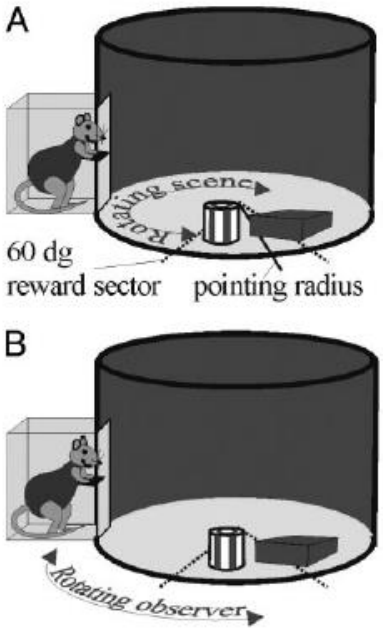

Fig. 5: (A) Rotating scene place recognition task. (B) Rotating observer place recognition task. Reproduced from Pastalkova et al. (2003).

The two previous tasks both utilized operant behavior and the rats had not to move through an environment but made their spatial responses via lever presses in the

operant chamber. The last place recognition task which will be mentioned is completely different.

Hollup and his colleagues (2001) trained rats to swim in an annular water maze with a remotely controlled escape platform at a constant location in the corridor. The platform remained deeply submerged until the rat had swum at least one full lap in the corridor. Thereafter, the platform was raised to the accessible position and the rat could reach it when it was swimming across it. Every fourth trial was a probe trial, in which the platform remained deeply submerged for the first 60 s. The swimming speed of rats was monitored during the probe trial. Well trained rats decreased their swimming speed near the location of the submerged platform which indicated that the rats recognized the target place in relation to multiple visual cues in the experimental room.

3.1.3 Object-position recognition tasks

Rodents do not recognize only their own position in an environment but also the position of objects located in their visual field.

The most common experiments for testing object-place recognition in rats are based on novelty-preference paradigm. Rats naturally tend to approach and explore novel objects which are assumed to have no natural significance to the animal and which have never been paired with a reinforcing stimulus (Dere et al., 2007). This paradigm was for the first time utilized in a behavioral task for rats in the one-trial object recognition task (Ennaceur and Delacour, 1988). The one-trial object recognition task consists of a sample phase and a test phase. During the sample phase, the rats are exposed to two identical objects in a familiar arena. After a delay, the rats underwent the test phase in which two dissimilar objects are presented: a familiar,

which has been already presented in the sample phase, and a novel one. The rats spend more time exploring the novel object suggesting that the familiar object was recognized (Ennaceur and Delacour, 1988; Dix and Aggleton, 1999).

The one-trial object–place recognition task is a modification of the novelty-preference paradigm which allows measurement of memory for spatial locations of distant objects. This task is very similar to the one-trial object recognition test. The only difference is that in the test phase is one of the two familiar objects presented in the sample phase shifted to a novel location instead of replacing of one of the familiar objects by a new one (Fig. 6). Successful recognition is displayed by the rat spending a greater amount of time exploring the object in the novel location during the test phase (Ennaceur et al., 1997; Dix and Aggleton, 1999).

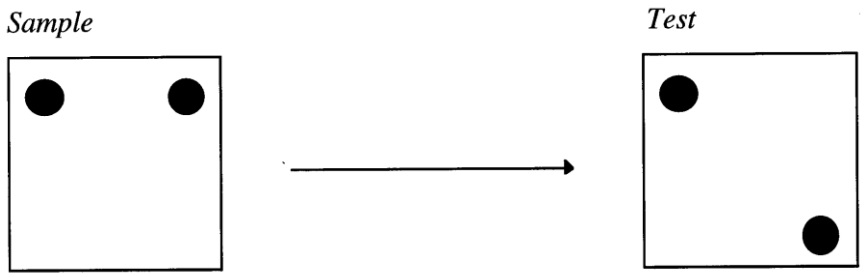

Fig. 6: One-trial object-place recognition task. Pairs of identical objects are represented by pairs of black circles. Reproduced from Dix and Aggleton (1999).

Long and Kesner developed a couple of different tasks which assessed the ability of rats to recognize the position of objects located on a dry arena. Namely, to study the memory for allocentric distance (Long and Kesner, 1996) and for egocentric distance (Long and Kesner, 1998).

In the first study, rats were trained on a go/no-go task in which they have to remember the distance separating two identical objects (Long and Kesner, 1996).

Each trial consisted of two phases: a study and a test phase. In the study phase, the rats could explore a couple of objects placed on a dry arena. The objects were either 2 cm or 7 cm apart and a food reward was located under both of them in the study phase because this was the to-be remembered allocentric distance. In the test phase, the rats were presented with the couple of same objects which were separated by a distance that was either the same as in the study phase or was not (2 cm or 7 cm). If the rats displaced objects that were separated by a distance that matched the distance in the study phase, they received the food reward. In the opposite case, no reward was available for displacing the objects. In each phase (study or test) only one allocentric distance was presented. The performance of the rats was evaluated by means of the latencies from the start of presentation of the objects in the test phase until the rat moved one of the objects. Shorter latencies referred to higher preference for the particular distance between the objects. Trained rats showed in the test phase higher preference for the distance that matched the distance in the study phase (reward distance). Therefore, the rats were able to recognize the allocentric distance.

In the second study, rats were trained in a delayed matching-to-sample (go/no-go) task in which they have to remember the distance separating them from an object (Long and Kesner, 1998). The complete trial again consisted of a study phase and a test phase and the experimental design was quite identical to the previous task. However, only one object was presented in both phases and crucial variable was the distance of the object from the door that separated the rat from the object. The distance could be either 40 cm or 80 cm. Because the distance traveled by the rats for any given trial was not constant and varied between 40 cm and 80 cm running speed was used for evaluating the performance of the rats as the dependent measure. Well trained rats

ran faster to the object placed in a reward distance from them in the test phase. Thus, the rats recognized its egocentric distance from the object.

Moreover, within this study the rats were tested in a matching-to-sample task that measured memory for a single spatial location (Long and Kesner, 1998). In the study phase, the rats were presented with an object at one of the four possible spatial locations. In the test phase, the rats received a food reward only when the object was placed at the same location as in the sample phase. The rats were able to learn the task and to remember the single spatial location.

Other tasks that assess the ability of rats to recognize the position of objects are described in detail in next part of this chapter because computer screens or touchscreens are used for stimuli presentation in their experimental design (Nekovarova and Klement, 2006; Talpos et al., 2009; Talpos et al., 2010).

3.2 Tasks utilizing computer screen for stimuli presentation

Since all experiments, which are presented in the section "Results", were done in apparatuses that utilize computer screen for stimuli presentation, a special part of this chapter is dedicated to this type of behavioral tasks. The early tasks for studying cognition in rats, which used computer screen for the presentation of sensory stimuli, were introduced at the beginning of the 90^{th} years of the last century. This methodology provides many advantages. The computer screen allows presenting a wide variety of stimuli and environments, therefore, countless different tasks can be developed. The manipulation of a large number of variables (stimuli) would be very difficult, expensive or even impossible in a real environment, is easy and user-friendly. Moreover, this experimental approach allows comparisons between different species. A big attention is currently devoted to the development of these tasks. Recent

studies showed that the same neural structures in rats are involved during solving the spatial tasks utilizing computer screen for stimuli presentation as in the spatial tasks performed in the real environment (e.g. McTighe et al. 2009, Talpos et al. 2009, Talpos et al. 2010).

The next two tasks exploited non-spatial stimuli (Gaffan and Eacott, 1995; Prusky et al., 2004). They demonstrate the ability of rats to perceive the visual stimuli presented on a computer screen.

The computer-controlled Y-maze developed by Gaffan and Eacott (1995) allowed automated testing of rats' learning and memory with visual stimuli. Two closely adjacent monochromatic computer screens were placed at the end of each of the three arms of the maze (Fig. 7). A feeder was positioned between each pair of monitors. After the rat approached the pair of monitors on which were displayed the correct stimuli, a few food pellets were delivered into the trays placed near the feeder as a reward. The rat's location in the maze was monitored by infrared beam photodetectors. Two classes of stimuli were presented on the computer screens: scenes (internally complex patterns with varying numbers of foreground shapes distributed across contrasted backgrounds) and objects (internally homogeneous single figures, confined to the central part of the display). The rats efficiently discriminated both classes of visual stimuli. Therefore, the rats were able to perceive visual information displayed on the computer screens and utilize it for solving the task.

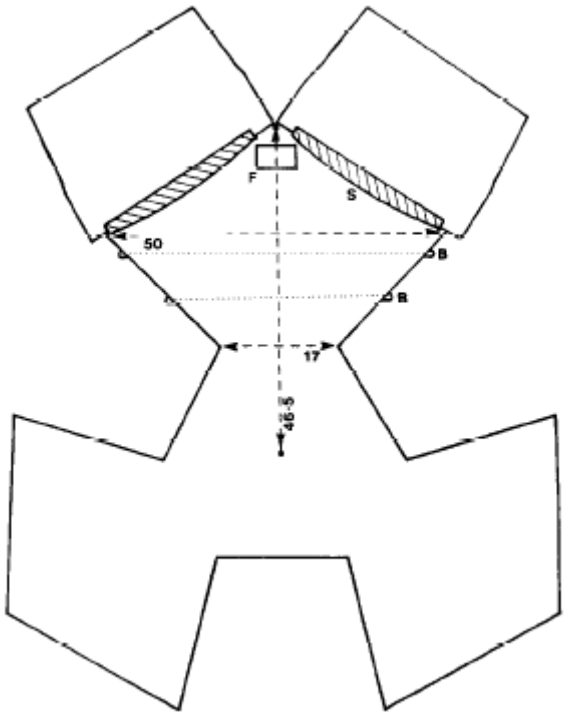

Fig. 7: Scheme of the computer-controlled Y-maze. (F) Feeder. (S) Monitor screen. (B) Photobeam transmitters. Dashed lines show dimensions in centimetres. Dotted lines show the location of the beams. Reproduced from Gaffan and Eacott (1995).

Prusky and his colleagues (2004) developed a non-spatial, picture-based, trial-unique, delayed matching-to-sample task for rats. The task also utilizes computer screens for stimuli presentation. Rats were trained to discriminate black-and-white pictures, which were displayed on computer monitors as visual stimuli. The monitors were placed at the end of a trapeizoidal-shaped tank filled with water (Fig. 8). Rats chose the path to one of the two monitors at a choice point (46 cm apart from the monitors). If they discriminated the correct stimulus from the incorrect one, they would swim to a hidden platform submerged in front of the monitor with the correct stimulus and could escape from the water. Even though the position of the correct stimuli on each monitor varied randomly between sessions the rats chose the right path with high accuracy. This study confirmed that the rats are able to use sensory information displayed on the monitor to solve behavioral task.

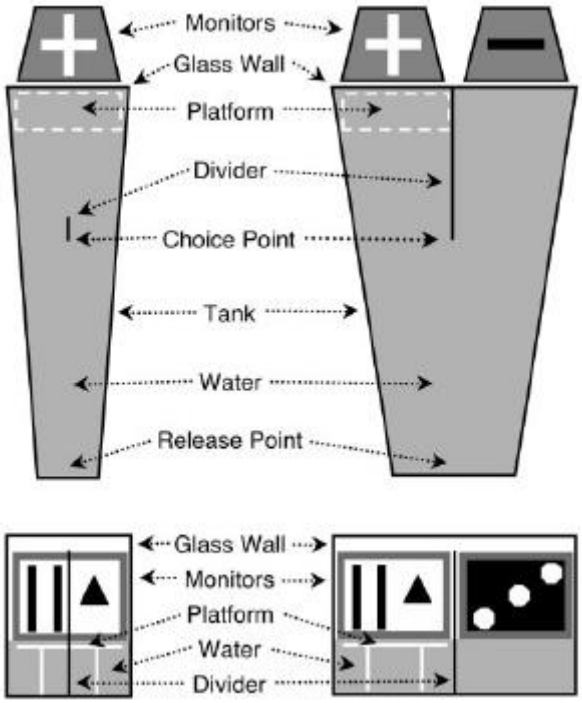

*Fig. 8: Schematic representation Prusky's apparatus. (*left*) Sample pool. (*right*) Choice pool. (*upper*) Top view. (*lower*) Front view. Choice pool: The sample picture (+) and a novel picture (-) were each displayed on monitors facing into the end of a trapezoidal-shaped tank. The hidden platform was placed only in front of the monitor with correct (sample) picture. Adapted from Prusky et al. (2004).*

The studies mentioned above showed that rats are able to perceive and discriminate visual stimuli presented on the computer screens in tasks assessing non-spatial behavior. However, can they utilize these stimuli also in spatial tasks? Talpos and his colleagues developed a paired-associate learning task (2009) and a trial-unique nonmatching-to-location task (2010). Both tasks were carried out in a computer-automated testing apparatus using touchscreen for visual stimuli presentation.

The first task was designed to study object-in-place paired-associative learning. Rats were trained to discriminate the position of an object displayed on the touchscreen. The reward and the non-reward object were the same and differing only in their location on the touchscreen. Two duplicates of one of three possible objects were displayed on every trial (Fig. 9). A response at the correct location would lead to a delivery of a reward food pellet. The rats acquired the task with high accuracy (over

80 %). Therefore, they are able to use sensory stimuli presented on the touchscreen for discrimination of object's position.

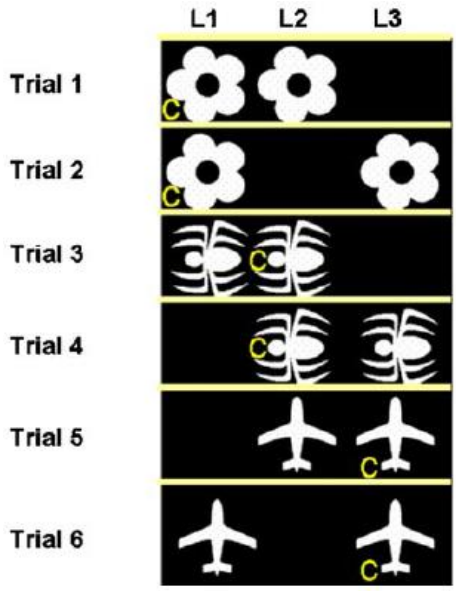

Fig. 9: Examples of different trials and the correct pairing between objects and their locations (L1 – L3) in the object-in-place paired-associative learning task. The reward location for each stimulus is signified by the C. Adapted from Talpos et al. (2009).

In the second task, rats were trained in an operant chamber to touch a reward area on the monitor (Fig. 10). In the sample phase, a visual stimulus appeared in a sample location. In the following test phase, two locations were illuminated: the previous sample location (non-reward) and a new location (reward). If the rat correctly selected the new (reward) location, a reward food pellet would be delivered. The separation between the sample and the new location varied. In condition of a maximum stimulus separation the rats achieved high levels of accuracy even when the delay between the sample phase and the test phase was 6 s. However, in case of the minimum stimulus separation, when the sample location and the new location were adjacent, the performance of rats dropped to a chance level even when the delay between the sample phase and the test phase was only 1 s. Nevertheless, this study

showed that rats are able to utilize visual stimuli displayed on the touchscreen for spatial pattern separation (the ability to disambiguate similar spatial locations) (Talpos et al., 2010).

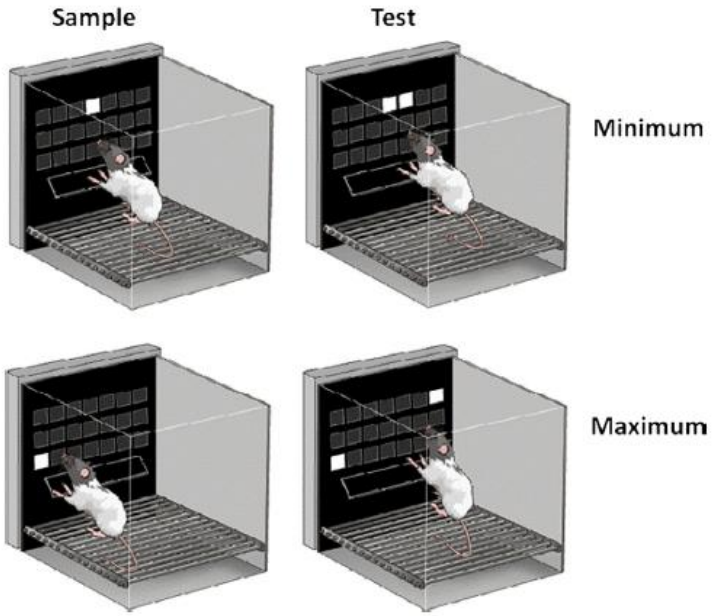

*Fig. 10: Touchscreen apparatus for studying spatial pattern separation in rats. (*upper*) Low separation trial. (*lower*) High separation trial. (*left*) Sample phase. (*right*) Choice phase. Reproduced from Talpos et al. (2010).*

Hölscher and his team (2005) first demonstrated that not only humans and primates are able to navigate in a 3-D virtual environment as has been shown before (Rieser et al., 1990; Leighty and Fragaszy, 2003). They developed a virtual reality set-up that covers a large part of the rat's visual field (360° of azimuth, –20° to +60° of elevation). It was combined with a treadmill in which the animal runs on top of an air-cushioned polystyrene sphere (Fig. 11). Any translational movement of the animal led to a rotation of the sphere which was monitored by the computer as a trajectory of the rat. The computer subsequently generated corresponding changes in the virtual environment through a beamer and via several mirrors. The rats were trained in a task in which a square array of cylinders with 0.5·m diameter and 2 m distance to each other was presented in the virtual environment. The cylinders were covered with

vertical black and white stripes. When the rat entered the area below a cylinder it was rewarded with a drop of sugar water. The water was delivered to a thin tube through an oral intubation. To prevent the rats to return simply to the same cylinder to get another reward they defined an outer radius. The rat had to cross the radius before it could get another reward at the same cylinder. The rats were capable of finding of the cylinders. Thus, they were able to navigate in the virtual environment.

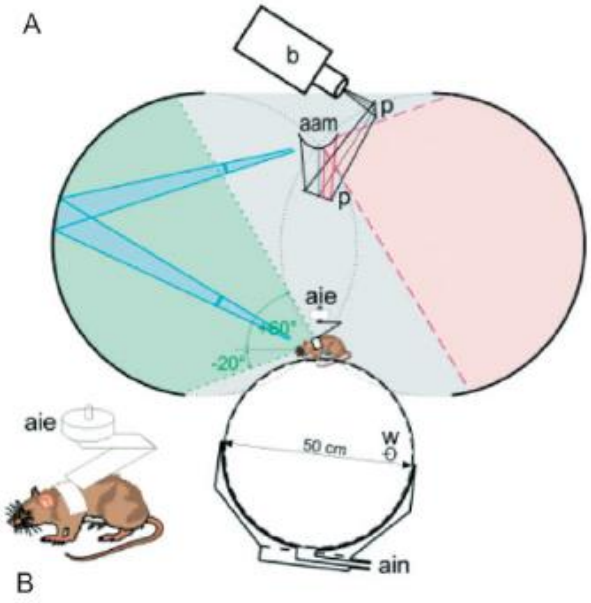

Fig. 11: The apparatus for testing navigation of rats in the 3-D virtual environment. (A) General view (cross-section). (B) Attachment of the rat on the top of the air-cushioned polystyrene sphere. (w) Wheels that provide the rotation of the sphere. (b) Beamer. (p) Plane mirrors. (AAM) Angular amplification mirror. (AIE) Angular incremental encoder (to measure the body orientation). Reproduced from Hölscher et al. (2005).

The following task was developed in our laboratory (Dpt. of Neurophysiology of Memory, Institute of Physiology, AS CR). The study done by Nekovarova and Klement (2006) preceded the experiments which are part of this PhD thesis and were done in similar apparatus. Nekovarova and Klement trained rats to recognize the configuration of objects displayed on a distant computer screen (Fig. 12). The scene

consisted of a white moving bar and a white stationary rectangle presented on a black background. Food-deprived rats were placed in a modified Skinner box located 36 cm apart from the computer screen and were trained to press a lever to obtain food reward. Lever presses were rewarded only when the bar touched the rectangle. Rats expected reward when the moving bar was closer to the stationary rectangle and they increased the responding rate of lever presses with the decreasing distance between the two objects. Therefore, the rats were able to recognize the configuration of these two objects displayed on the computer screen.

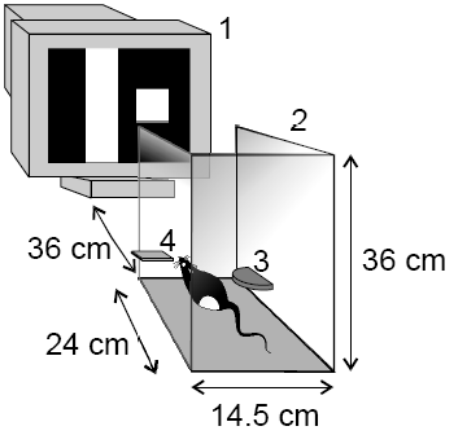

Fig. 12: Scheme of the modified Skinner box for testing recognition of the configuration of objects on the computer screen. (1) Computer screen. (2) Operant chamber. (3) Hopper. (4) Lever. Reproduced from Nekovarova and Klement (2006).

4 Neural substrate of spatial behavior

A large number of studies showed a crucial role of hippocampus, a part of a mammalian limbic system, in spatial memory. In the research of spatial behavior is a big attention focused also on several types of neurons. Particularly on place cells, head-direction cells and grid cells.

4.1 Hippocampal formation

Hippocampus is a part of hippocampal formation (Fig. 13). The hippocampus proper has three subdivisions: CA1, CA2, and CA3 (CA means cornu ammonis). The other regions of the hippocampal formation include the dentate gyrus, subiculum, presubiculum, parasubiculum, and entorhinal cortex (Amaral and Lavenex, 2007).

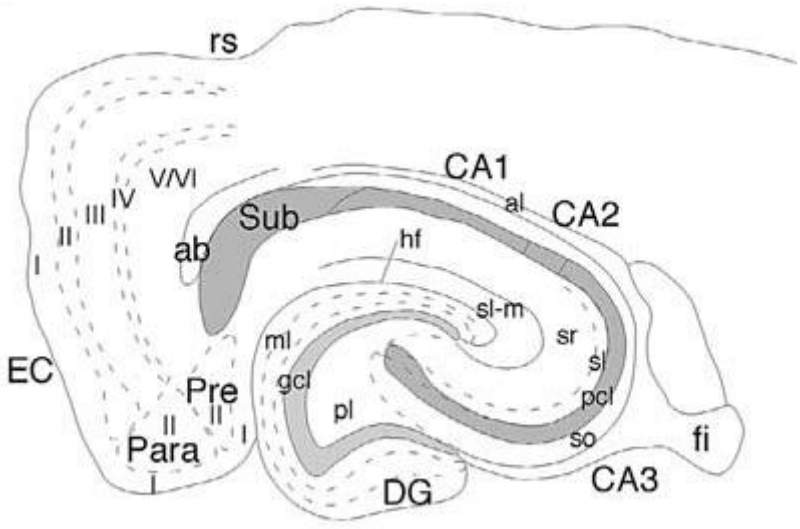

Fig. 13: Horizontal section through the hippocampal formation of a rat. (CA1-CA3) Cornu ammonis fields of the hippocampus. (DG) Dentate gyrus. (Sub) Subiculum. (Pre) Presubiculum. (Para) Parasubiculum. (EC) Enthorinal cortex. Reproduced from Amaral and Lavanex (2007).

The hippocampal formation consists of three types of principal neurons: granule cells in the dentate gyrus, CA1 pyramidal neurons and CA3 pyramidal cells (Bischofberger et al., 2006). The interconnections between these cells form the trysynaptic circuit (Fig. 14). Perforant path is responsible for the excitatory input from layer 2 of pyramidal cells of the entorhinal cortex to dentate gyrus and to CA3 pyramidal cells. Neurons in layer 3 of the entorhinal cortex project to the CA1 pyramidal neurons and the subiculum. The granule cells of the dentate gyrus project through mossy fibers to the CA3 pyramidal cells. Pyramidal neurons in the CA3 field project to CA1 via Schaffer collaterals and pyramidal cells in CA1 project to the subiculum and back to the deep layers of the entorhinal cortex. Subiculum projects to enthorinal cortex as well.

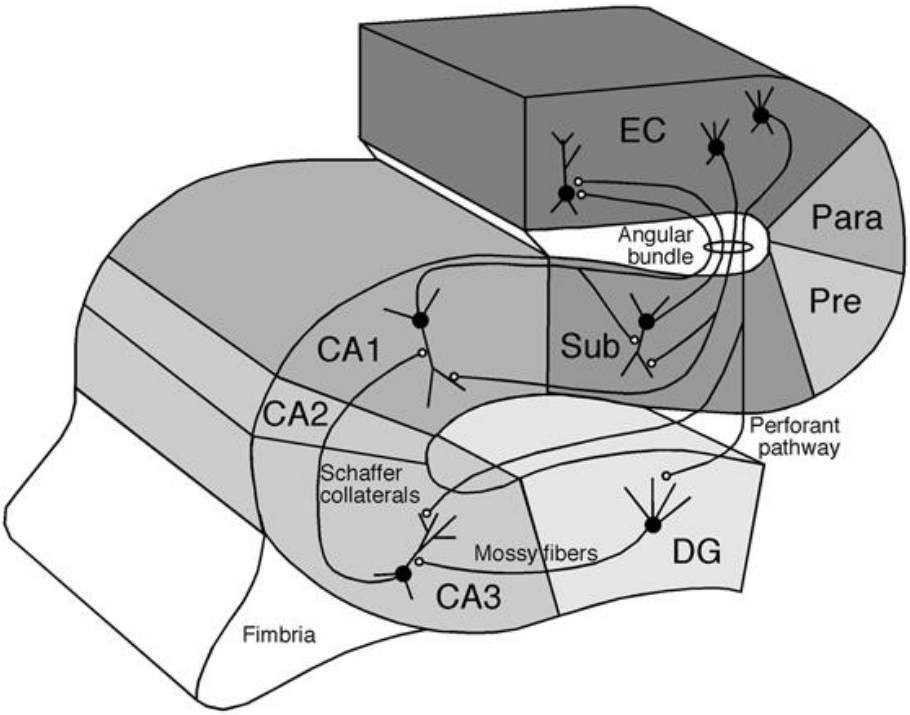

Fig. 14: Scheme of the trysynaptic circuit. (CA1-CA3) Cornu ammonis fields of the hippocampus. (DG) Dentate gyrus. (Sub) Subiculum. (Pre) Presubiculum. (Para) Parasubiculum. (EC) Enthorinal cortex. Reproduced from Amaral and Lavanex (2007).

The rat hippocampus can be divided into dorsal and ventral part. The dorsal and ventral parts of the hippocampus may process qualitatively different kinds of information. The dorsal half of the hippocampus is more important for spatial learning than the ventral half (Moser et al, 1993).

4.2 Place cells

Place cells were first discovered in the rat hippocampus by O'Keefe and Dostrovsky (1971). From the anatomical point of view they are pyramidal cells in the hippocampus, especially in its dorsal part, but they were also detected in the ventral hippocampus (Jung et al., 1994; Poucet et al., 1994) and in the entorhinal cortex (Frank et al., 2000). Functionally, the place cells are characterized by their location-specific activity (Fig. 15A). A particular place cell is intensely active only when the rat's head is in a certain part of the environment called the cell's firing field or place field (Muller, 1996).

In a stable environment, each place cell has its own stable firing field. This stability lasts for months (Muller et al., 1987). This fact indicates that the representation of the environment persists and it is not constructed de novo whenever an animal enters the environment. Moreover, it has been demonstrated that several different environments are represented by the place cells (Muller and Kubie, 1987).

In an open environment, activity of a particular place cell is not dependent on the direction in which the rat is looking. It is true even if the rat sees different scenes depending on the direction of its head. On the other hand, place cells show directionally selective activity when the animal moves along a linear path (McNaughton et al., 1983).

Estimation of the distance by place cells probably depends on visual information. However, it has been shown that place cells can also use the information generated during active or passive movement of the animal (Quirk et al., 1990; Jeffery et al., 1997). Therefore, place cells may use both allothetic and idiothetic information for distance estimation. The firing field of a place cell remains unchanged also after orientation cues in the environment are hidden by darkness (Quirk et al., 1990).

4.3 Head direction cells

Head direction cells were first discovered by Ranck in postsubiculum (1985). Later they were found in several other brain parts including thalamic nuclei (Mizumori and Williams, 1993; Taube, 1995), areas of retrosplenial and extra-striate cortex (Chen et al., 1994), lateral mammillary nuclei (Stackman and Taube, 1998) and the dorsal striatum (Wiener, 1993).

A typical head direction cell is strongly active only when the rat's head points in specific direction (the preferred direction) (Fig. 15B). The activity of head direction cells is not affected by the position of the animal in the environment. Different head direction cells show activity at different preferred directions and altogether cover the entire compass (Muller et al. 1996).

4.4 Grid cells

Grid cells are a type of neurons that were identified in medial entorhinal cortex and generate action potentials when an animal is within a certain area in the environment (Hafting et al., 2005). In contrast to place cells, grid cells have multiple circular firing fields which tile the floor of an environment in a hexagonal array that extends horizontally in all directions (Marozzi and Jeffery, 2012) (Fig. 15C).

Individual firing fields of grid cells are spaced with constant spacing and the distance between two firing fields ranges from 30 to 60 cm depending on the location of the grid cell in entorhinal cortex (Jeffery and Burgess, 2006). This regular array of firing fields is suggestive of an intrinsic distance-measuring process which may mediate metric information to place cells and allow them to position their place fields accurately in space (Jeffery, 2011).

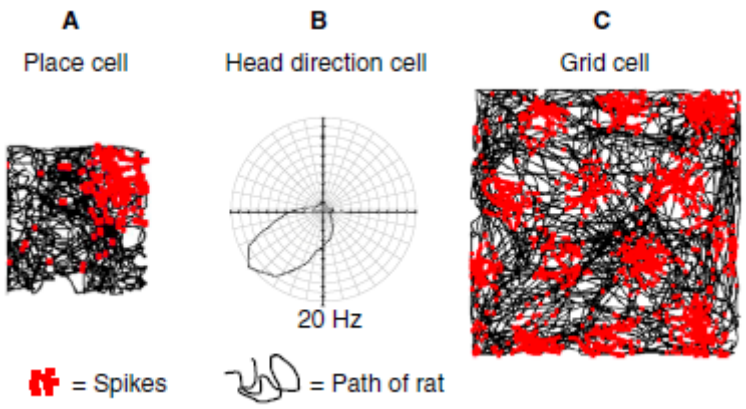

Fig. 15: Typical firing patterns of a place cell (A), a head direction cell (B) and a grid cell (C). (A, C) The neuronal action potentials (red squares) are superimposed on the path of the rat (black line) at the place where the rat was when the cell fired. (B) The firing is shown in the form of firing rate (distance from origin) as a function of head direction. Adapted from Marozzi and Jeffery (2012).

5 Lesions and inactivations of brain structures

Permanent lesion or reversible inactivation of a particular neural structure is widely used technique in the study of its role in brain functions. Both of these methods have their shortcomings but they are still essential and irreplaceable techniques in neuroscience research because some experimental questions cannot be answered without them.

5.1 Permanent lesions

The advantage of permanent lesions is that their extension can be verified by histological analysis. On the contrary, the main drawback is that the nervous system can compensate the effect of the lesion with its reorganization in response to tissue damage.

Several permanent lesion techniques are used. The oldest technique to study the role of a particular brain structure is tissue removal which can be done by using aspiration or resection method.

Permanent chemical lesions are used to selectively remove very small parts of the brain. The most commonly used method in permanent lesion chemical technique are the microinjections of neurotoxins, especially of ibotenic or kainic acid (Jarrard, 1983; Jarrard, 1989). Another possibility is the microapplication of NMDA (N-methyl-D-aspartate) which leads to excitotoxicity in target cells. This method is often used for lesions of the dorsal hippocampus (e.g. Ferbinteanu and McDonald, 2001; Quinn et al., 2002; Ito et al., 2005; Otto and Poon, 2006).

Electrolytic lesions are also used to study the function of deep brain structures as hippocampus (e.g. Cassel et al., 1998; Galani et al., 2002; Mogensen et al., 2004). The connection between hippocampus and subcortical structures is interrupted by

fimbria-fornix lesion. However, the connection between hippocampus and cortical areas is unaffected after fimbria-fornix lesion.

5.2 Reversible inactivations

The main advantage of reversible inactivation is that its effect on behavior of an animal can be compared with the performance of the same animal in the task before and after the inactivation. In addition, short-term effect of inactivation does not allow the nervous system of the animal to recover its function and the animal does not have enough time to adopt an alternative strategy. Thus, reversible inactivation is used to test the function of a particular brain structure at the time of its inactivation. A major drawback in comparison to permanent lesions is that the extension of reversible inactivation cannot be verified precisely by histological analysis.

Another advantage of reversible inactivations is that each animal serves as its own control. Therefore, fewer animals can be used in a particular experiment which is beneficial not only for work ethic but also for the credibility of the results (Lomber, 1999).

5.2.1 Chemical techniques

Chemical techniques are used to inactivate both surface and deep brain structures. The damage to surroundings brain areas is reduced to minimum because inactivating agents are delivered to a particular brain area through implanted cannulae with very small diameter. This allows inactivating of really tiny parts of nervous tissue such as individual laminae in the cortex or thalamus (Malpeli, 1983; Malpeli, 1999).

Several drugs are used as inactivating agents but the most common drugs used for hippocampal inactivation are muscimol and tetrodotoxin.

Muscimol is a potent $GABA_A$-receptor agonist that causes rapid and reversible suppression of neurophysiological activity (Allen et al., 2008). Muscimol does not block the transmission of action potentials along axons. Thus, its effect is more limited to the structure into which it has been injected. The maximum physiological effect of muscimol occurs within 40 min after its application and lasts for several hours (Mao and Robinson, 1998). The most frequently used dose of muscimol for blocking activity of dorsal hippocampus is 0.5 μg per one side (e.g., Corcoran et al., 2005; Czerniawski et al., 2009; Iordanova et al., 2011).

Tetrodotoxin inhibits voltage-gated sodium channels in a highly potent and selective manner without effects on any other receptor and ion channel systems (Narahashi, 2008). This prevents all affected neurons from generation and propagation of action potentials. The duration of physiological effects of tetrodotoxin is similar to muscimol. Tetrodotoxin blockade is maximal between 30 and 120 min after administration, decays exponentially, and generally vanishes within 24 h (Zhuravin and Bures, 1991). In most studies 5 ng of tetrodotoxin is dissolved in 1 μl of saline and injected to the dorsal hippocampus for its inactivation (e.g. Zhuravin and Bures, 1991; Fenton and Bures, 1993; Cimadevilla et al., 2001; Klement et al., 2005; Wesierska et al., 2005).

5.3 Effect of hippocampal lesions and inactivations

Because of the massive interconnection of parts of the brain it is difficult to unambiguously interpret the results of studies with lesions or inactivations. Effect of these techniques can never be certainly ascribed to lesioned or inactivated structure. However, many of studies and experiments show that hippocampal animals (i.e.

animals without functional hippocampus) are impaired in spatial orientation and navigation.

There are several different explanations and theories of this phenomenon. O'Keefe and Nadel (1978) proposed that the hippocampus mediates a neural representation of physical space, that is, a cognitive map. Some scientists thought that the hippocampal system is critical to normal learning and memory because of its function as the central part of a configural association system (Sutherland and Rudy, 1989). This view was subsequently updated that the critical neural system for configural associations is in cortical circuitry outside the hippocampus, however, the output from the hippocampal formation contributes to configural processing by selectively enhancing cortical units representing stimulus conjunctions (Rudy and Sutherland, 1995).

Another possible explanation is that the hippocampus is responsible for the ability to learn relations between stimuli (Eichenbaum, 1996). This is reflected in activity associated with conjunctions of cues according to their temporal order, similarity, or spatial arrangement, as well as relations of cues to their significance and responses made to them, i.e. virtually any relationship worth remembering (Eichenbaum et al., 1999). Others suggest, as O'Keefe and Nadel, that hippocampus has a specific role in spatial memory (Burgess et al., 2002).

5.3.1 Hippocampus and navigational tasks

According to O'Keefe and Nadel (1978), the mapping navigation depends on intact hippocampal formation while the route navigation does not.

Rats without functional hippocampus show impairment in the T-maze (e.g. Dudchenko, 2001; Lalonde, 2002) and in the radial maze (Olton et al., 1978). The

place version of the Morris water maze is also hippocampal dependent while the cue version of the task, i.e. the navigation to a visible platform, does not require intact hippocampus (Morris et al, 1982).

Most of the studies in rodents showed that hippocampus is necessary also for dead reckoning (Maaswinkel et al., 1999; Save et al., 2001; Whishaw et al., 2001; Kim et al., 2013; but see Alyan and McNaughton, 1999).

5.3.2 Hippocampus and place recognition tasks

Some studies claimed that rats are able to recognize their position without functional hippocampus (Whishaw and Jarrard, 1996; Dudchenko et al., 2000), however, other studies brought evidence that this ability depends on hippocampus (Hollup et al., 2001; Klement et al., 2005).

Hippocampal lesions caused a severe deficit in the identification of a location in an annular water maze (Hollup et al., 2001). Rats with inactivated hippocampus were not able to recognize their own position in an environment with reference to distal visual cues when they were passively transported through the environment (Klement et al., 2005). In contrast, Whishaw and Jarrard (1996) demonstrated that the hippocampus is not essential for navigation and place recognition if rats were extensively trained to swim to a visible platform in the Morris water maze and then given probe trials on which the visible platform was removed. Moreover, Dudchenko and his colleagues (2000) reported that hippocampal rats can discriminate between two distant locations in a non-matching to position task.

The discrepancy of results can be explained with the difference in the behavioral training. If two distant places are associated with different stimuli then an alternative strategy can be employed. Another possibility is to provide views

containing both distant orientation cues the visible goal location. In this case associations between the views and the movements toward the goal location can be formed by an extensive training in which incremental learning takes place (Klement et al., 2005).

5.3.3 Hippocampus and object-position recognition tasks

The role of hippocampus in recognition of position of objects has been studied in several behavioral tasks. Since the main aims of this PhD thesis are to develop such a task and to study the role of hippocampus in it, the object-position recognition tasks are discussed in more detail in the section "Discussion".

Hippocampal rats are impaired in the one-trial object-position recognition task (e.g. Ennaceur et al., 1997; Mumby et al, 2002; Barker and Warburton, 2011). Intact hippocampus is involved in the memory but not the perception of allocentric distance information (Long and Kesner, 1996). Rats with hippocampal lesion were also impaired relative to controls in the delayed matching-to-sample task for egocentric distance (Long and Kesner, 1998). In addition, the hippocampus plays a role in the retrieval of previously learned object-place associations (Gilbert and Kesner, 2004).

Experiments performed on apparatuses which utilize computer screen for stimuli presentation showed that hippocampus is crucial both for object-in-place paired-associative learning (Talpos et al., 2009) and the ability to disambiguate similar spatial locations (McTighe et al., 2009; Talpos et al, 2010).

The role of hippocampus in recognition of position of objects located in an inaccessible space is analyzed in next parts of this PhD thesis.

II Aims of the thesis

6.1 Development of the object-position recognition task

Animals do not only determine their own position in an environment but they also determine positions of other objects. Moreover, they perceive objects not only at places that they can visit but also at places that are inaccessible. The first aim was to develop a behavioral task in which rats recognize position of an object located in an inaccessible space. We call the task "the object-position recognition task". Two versions of such a task are presented. Both versions utilize computer screen for stimuli presentation. In the first version the object is stationary except the moments when it jumps from one position to another. In the second version the object moves continuously across the computer screen. The task is presented in Experiment I.

6.2 Assessing the role of hippocampus in the object-position recognition task

In rodents, hippocampus plays crucial role in various spatial tasks. For example, it is necessary for navigation to target places according to distal cues and for recognition of these places. Rat hippocampus is also necessary for the recognition of positions of objects that can be explored. Thus, a subject can learn the object's position by associating its own location with the object at that location. However, it is unclear whether the hippocampus is involved in recognition of position of inaccessible objects. To address this question, we trained rats in the object-position recognition task. The role of the dorsal hippocampus was assessed by blocking its activity with muscimol and it is described in Experiment II.

6.3 Pharmacological validation of the object-position recognition task

Application of several drugs that affect central nervous system also affects motor activity. Thus, spatial tasks with minimal demands on locomotion can be useful in behavioral pharmacology to study spatial cognition after the application of drugs that affect motor activity of animals. The object-position recognition task is such a non-locomotor task because motor activity of the animals is reduced to lever pressing. The last aim of the PhD thesis was to validate the object-position recognition task with prazosin, a drug with known pharmacological effects on behavior. Prazosin has depressant effect on motor activity and no effect on spatial cognition. The effect of prazosin on behavior in the object-position recognition task is described in Experiment III.

III Methods

7.1 Subjects

The subjects (Experiment I: n = 13; Experiment II: n = 12; Experiment III: n = 16) were male Long-Evans rats (3-months old at the beginning of the experiment). The rats were obtained from the breeding colony of the Institute of Physiology, Academy of Sciences of the Czech Republic, and housed in groups of two or three per cage in a temperature-controlled room (21 ºC) with a regular 12/12 light/dark cycle. Water was freely available but access to food was restricted to maintain the rats at 90% of their free feeding weight (380-450 g). All procedures were in accordance with Animal Protection Code of Czech Republic, EU directive 86/609/EEC and National Institute of Health guidelines.

7.2 Apparatus

The apparatus consisted of an operant chamber, a feeder, a LCD monitor (19" screen size in Experiment I; 24" screen size in Experiment II and III), and a computer (Fig. 16). The operant chamber (length x width x height: 24 cm x 14 cm x 36 cm) had opaque walls. The front wall was only 4 cm high allowing direct view at the monitor located 37 cm in front of the chamber. The operant chamber and the monitor were standing on two separated 75 cm high pedestals. This prevented rats from escaping over the front wall. The operant chamber was equipped with a horizontal lever (size: 2.5 cm 3 2.5 cm) and with a semicircular hopper (diameter: 4 cm). The lever was on the left wall 14 cm above the floor and 4.5 cm from the front wall. The semicircular hopper was located on the right wall 5.5 cm above the floor and 4 cm from the front

wall. If activated, the feeder delivered one to three 20 mg pasta pellets to the hopper. The computer registered lever presses, activated feeder and displayed graphics on the computer screen. The software was written by Daniel Klement in Quick Basic 7 and used 640 pxl x 480 pxl resolution for the graphical output. To shorten the time necessary for the experiments, the rats were trained in two identical apparatuses (A and B) located in a dimly illuminated experimental room.

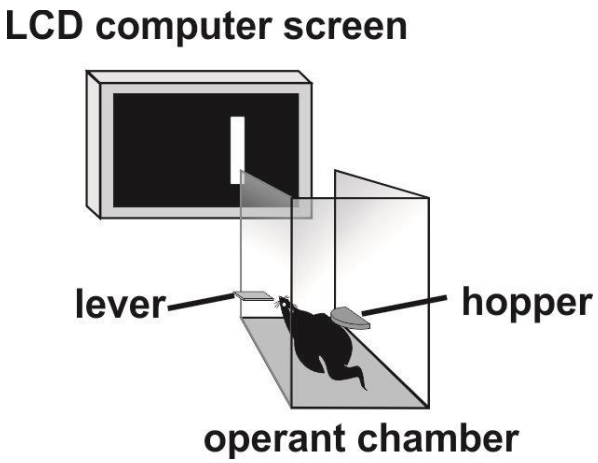

Fig. 16: Scheme of the experimental apparatus. Adapted from Levcik et al. (2013b).

7.3 Experiment I

7.3.1 Pretraining

Food-deprived rats were trained to press the lever in the operant chamber for food reward under the continuous reinforcement schedule. The rats required from three to seven sessions lasting approximately 30 min to learn the operant behavior. During the training a white rectangle (width × height: 80 × 150 pxl) was displayed at position 339 pxl (Fig. 17, Phase 1). This position is called "reward position". We refer to the 2-dimensional rectangle as to an object. Each rat was randomly assigned to one of the two apparatuses and it was trained there only.

7.3.2 Object-position recognition task (version 1 - stationary object)

Rats were trained to discriminate the reward position (339 pxl) of the object on the screen from two other positions: left (0 pxl) and right (559 pxl) (Fig. 17, Phase 1). The rats were rewarded only if they pressed the lever when the object was in the reward position. At the beginning of a session the object was displayed in the reward position. The session started after a rat pressed the lever. Since this moment the object changed its position every 135 s in a pseudorandom order. The sequence was: Rew, L, R, L, Rew, R, L, Rew, R, Rew, R, L, R, Rew, L, R, Rew, L for the apparatus A and Rew, L, Rew, R, L, Rew, R, L, R, Rew, R, Rew, L, R, Rew, L, R, L for the apparatus B, where Rew denotes the reward position, L the left position and R the right position. These sequences repeated three times during the session. We used different sequences for each apparatus to prevent possible synchronization of the reward periods between the apparatuses. If the reward periods were synchronized then a rat in one apparatus

could detect reward periods by hearing the sound of activated feeder from the other apparatus.

The rats were rewarded for each correct response in the beginning of the training. This continuous reinforcement schedule was changed to variable ratio schedule after the rats had started to preferentially respond during the reward periods. A subject should emit several responses to get a single reward and this number changed randomly after each reward. The average number of responses necessary for activating the feeder gradually increased during the training. Individual rats reached different values. They were between 2.5 and 5.5.

7.3.3 Test of stimulus generalization

After the rats reached asymptotic performance we carried out a stimulus generalization test session. In the test session the object was presented in six positions: in three familiar positions (0 pxl, 339 pxl and 559 pxl) and in three new positions (112 pxl, 225 pxl and 449 pxl) (Fig. 17, Phase 1 - Test). Each new position was presented nine times for 15 s (three times after each familiar position). Responses in the new positions were not rewarded. The test session was carried out four times. These sessions were interspersed among 41 standard sessions. Two test sessions were carried out shortly after the rats reached stable level of performance and two before the beginning of training in version 2 with moving object.

This test session was carried out to see whether the rats perceived a single object displayed at different positions (the responding rate would be inversely related to the distance of the object to the reward position and directly related to the distance to the nearest non-reward position) or whether they perceived distinct pictures without any spatial relationship (the responding rate in the novel positions would be equal).

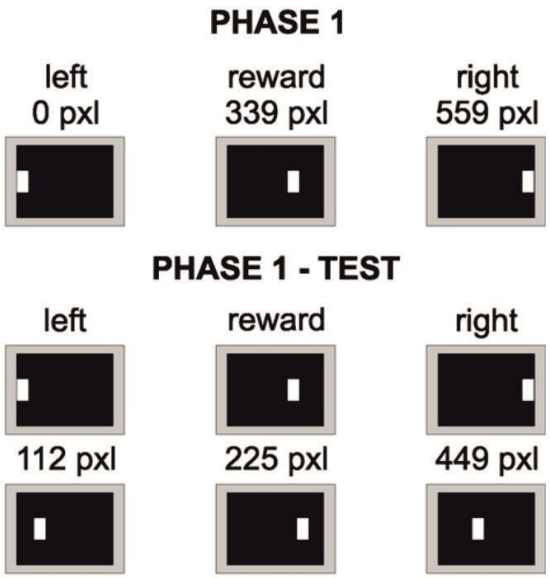

Fig. 17: Stimuli presented on the computer screen in version 1 of the object-position recognition task (Phase 1) and in the stimulus generalization session (Phase 1 - test). Adapted from Klement et al. (2010).

7.3.4 Object-position recognition task (version 2 - moving object)

Twelve rats trained in version 1 continued in training in version 2. In version 2 the object moved continuously across the screen. It shuttled between the left and the right sides of the screen. The rats were rewarded if they pressed the lever when the object moved through a reward region. The reward region occupied 2/7 of the screen. It was situated between 260 pxl and 419 pxl (Fig. 18, Phase 2), around the reward position defined in version 1. The object moved either slowly (10 pxl/s) or fast (20 pxl/s). The speed changed only at the sides of the screen. The object started from the left position in apparatus A and from the right position in apparatus B. Then it moved between the two sides of the screen with following speeds: slow, slow, fast, slow, fast, fast, slow, fast. This sequence repeated six times. Due to the two speeds the reinforced periods lasted either 8 s or 16 s. Non-reinforced periods ranged from 14 s to 39 s (average 30 s) in Apparatus A and from 21 s to 52 s (average 30 s) in Apparatus B. Continuous reinforcement schedule was used.

7.3.5 Test with invisible object

After the rats reached asymptotic performance we tested how their performance depended on position of the object (session 44). In this test session the object was invisible but the time schedule remained the same (Fig. 18, Phase 2 - Test).

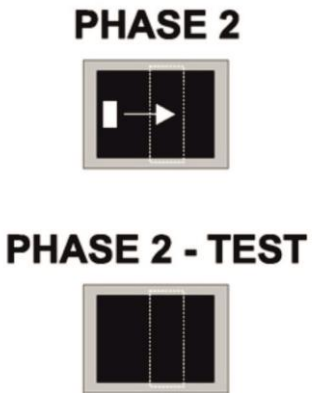

Fig. 18: Moving object presented on the computer screen in version 2. The reward region is situated between 260 pxl and 419 pxl (dotted lines). No stimulus was presented in the test session (Phase 2 - test). Adapted from Klement et al. (2010).

7.3.6 Data analysis

To find out how the rats solved the task we analyzed responding rate as a function of object position and/or of time. In version 2 we also analyzed percentage of rewarded responses. Results are reported as mean ± SEM.

Statistical tests were done with software R. Data were analyzed either by linear mixed effect models and interpreted as ANOVA or by repeated t-tests. In the first case, Tukey multiple comparison test was used as a post hoc test. The level of significance was set to 0.05. In the second case, Holm-Bonferroni correction for alpha values was used to keep level of significance of the multiple t-tests below 0.05.

7.4 Experiment II

7.4.1 Pretraining

Food-deprived rats were trained to press the lever in the operant chamber for food reward under the continuous reinforcement schedule. The rats required from three to six sessions lasting approximately 30 min to learn the operant behavior. During the training a white rectangle (width × height: 40 × 300 pxl) was displayed at position 220 pxl (Fig. 19). This position is called “reward position”. We refer to the 2-dimensional rectangle as to an object. Each rat was randomly assigned to one of the two apparatuses and it was trained there only.

7.4.2 Object-position recognition task

The rats were conditioned to press the lever for food reward when the white rectangle was displayed in the reward position and not to press when it was displayed in the two non-reward positions (Fig. 19). Half of the rats were trained for 54 sessions and the other half for 28 sessions (as it is shown in section ‘‘Results’’, the two groups had learned the task equally well, therefore, they were pooled together for further analyses). The durations and the number of presentations of stimuli changed during the training but it was fixed for the last 11 sessions. The training sessions started with the rectangle in the reward position. The rectangle changed its positions every 30 s in a pseudorandom order. Each apparatus had its own pseudorandom sequence in order to prevent possible synchronization of the reward periods between the apparatuses. Using this method, we eliminated the strategy by which a rat in one apparatus could detect or exclude the reward periods by hearing the sound of activated feeder in the other apparatus. The pseudorandom sequence of positions was the same as in Experiment I.

The sequence was repeated two times during the training sessions, thus, the sessions lasted 18 min. Initially, the rats were rewarded for each correct response. Later, when they preferentially responded to the reward stimulus, the continuous reinforcement schedule was replaced by the variable ratio schedule with geometric distribution of the number of presses necessary for getting the reward. The average number of responses necessary for activating the feeder was gradually increased to three.

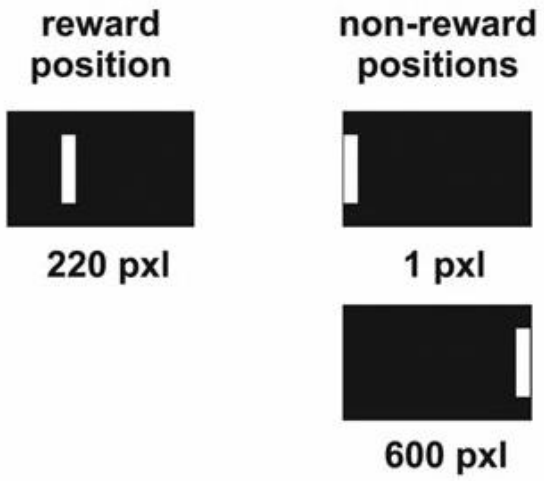

Fig. 19: Stimuli presented on the computer screen in the object-position recognition task. Adapted from Levcik et al. (2013).

7.4.3 Test of stimulus generalization

In the stimulus generalization session, the object was displayed not only in the familiar positions (1, 220, and 600 pxl) but also in three novel positions (110, 347, and 474 pxl). Each novel position was displayed nine times (three times after each familiar position). Operant responses were not reinforced during the presentations of the novel stimuli. The presentations were shortened to 15 s in order to decrease the likelihood of learning the reward contingency of these stimuli. The presentation of the familiar stimuli lasted 30 s, and their reward contingencies were unchanged with respect to the standard training sessions.

7.4.4 Test of the role of hippocampus in the object-position recognition task

After the surgery, the rats were retrained in the object-position recognition task for 17 sessions. Then they received a habituation bilateral infusion of muscimol into the dorsal hippocampus and were left in their homecages until the next day. The inactivation session, in which muscimol was applied into the dorsal hippocampus, was carried out after two standard sessions following the habituation infusion of muscimol. The control session, in which saline was applied into the hippocampus, was carried out after two standard sessions following the inactivation session.

7.4.5 Brightness discrimination task

Eleven of the twelve rats were subsequently trained to discriminate bright and dark stimulus (one rat was excluded, because its guide cannula was damaged). The reward stimulus was bright screen, and the non-reward stimulus was dark screen (Fig. 20). The pseudorandom sequence of reward and non-reward periods as well as the duration of the periods was the same as in the object-position recognition task. Because there was only one non-reward stimulus in the brightness discrimination task, unlike two stimuli in the object-position recognition task, we indicated the transition between two non-reward periods (dark screen to dark screen transition) by a short light glimpse (100 ms).

Fig. 20: Stimuli presented on the computer screen in the brightness discrimination task. Adapted from Levcik et al. (2013).

7.4.6 Test of the role of hippocampus in the brightness discrimination task

When the rats had reached asymptotic performance in the brightness discrimination task (seven to nine training sessions), an inactivation session was carried out to test the role of hippocampus in this task. The inactivation session was followed by a control session after another two standard training sessions. The infusion of muscimol in the inactivation session and saline in the control session was done under the same protocol used in the object-position recognition task.

7.4.7 Surgery

The rats were anesthetized with ketamine (85 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and mounted in a stereotaxic apparatus (TSE Systems). Administration of atropine (0.2 mg/kg, i.p.) prevented bradycardia, bronchospasm, and salivary secretion during anesthesia. The skull was exposed, and two small holes (1.2 mm in diameter) were drilled into it (AP -4.0 mm and L ±2.5 mm with respect to bregma). Guide cannulae (outer diameter: 0.7 mm, length: 11.5 mm) were inserted through the holes in the skull so that their lower tips were 2.5 mm below dura. Two bone screws were attached to the skull. The guide cannulae were fixed to the skull and to the bone screws with dental cement. After the surgery, the rats were allowed to recover for 10 days.

7.4.8 Inactivation procedure

Either muscimol (0.3 μg in 0.3 μl saline) or saline (0.3 μl) was slowly infused into both dorsal hippocampi 40 min before behavioral testing. The rats were then tested at the time of the maximum physiological effect of muscimol which occurs within 40 min and lasts for several hours (Mao and Robinson, 1998). The infusion

procedure was done by means of an infusion cannula (outer diameter: 0.45 mm), attached to a 1-µl Hamilton syringe by polyethylene tubing. The infusion cannula was inserted into the guide cannula, so that its tip protruded 0.5 mm beyond the tip of the guide cannula. The administration of the solution lasted 1 min. The infusion cannula was left in the place for 1 min before and for 1 min after the infusion. The sessions following the infusion of muscimol are referred to as ‘‘inactivation sessions,’’ and the sessions following the saline infusion are referred to as ‘‘control sessions.’’

7.4.9 Histology

After completion of all behavioral procedures, the rats were anesthetized with sublethal dose of ketamine and xylazine. A small amount of black ink dissolved in 0.3 µl of saline was administered into both hippocampi in the same way as muscimol or saline in the inactivation and control sessions respectively. The rats were perfused transcardially with saline (250 ml) followed by 4% paraformaldehyde solution (250 ml). The brains were removed and placed in 4% paraformaldehyde solution and afterward in 30% sacharose for 24 h. Subsequently, the brains were frozen, cut into 50-µm slices, and stained with cresyl violet.

7.4.10 Data analysis

We analyzed the responding rate during the stimuli presentations. The data analysis was restricted to those periods of stimuli presentation which were preceded by the non-reward periods (the reasons are given in the section ‘‘Object-position recognition task’’ in ‘‘Results’’). The dependence of the responding rate on the distance between the current object’s position and the reward position (Test of stimulus generalization) was expressed by the slope of linear regression. Two slopes

were calculated for each rat: one for the non-reward positions on the left from the reward position (positions 1 and 110 pxl) and the other for the non-reward positions on the right from the reward position (positions 347, 474, and 600 pxl). The results are reported as mean ± SEM. Statistical tests were done with software R. Group means were compared by means of the ANOVA with repeated measures or by the paired or unpaired t-tests. If appropriate, post hoc tests were conducted by Tukey multiple comparison. P-values of repeated t-tests were adjusted according to Holm (1979). The level of significance was set to 0.05.

7.5 Experiment III

7.5.1 Pretraining

Food-deprived rats were trained to press the lever in the operant chamber for food reward under the continuous reinforcement schedule. The rats required from three to nine sessions lasting approximately 30 min to learn the operant behavior. During the training a white rectangle (width × height: 40 × 300 pxl) was displayed at position 380 pxl (Fig. 21). This position is called "reward position". We refer to the 2-dimensional rectangle as to an object. Each rat was randomly assigned to one of the two apparatuses and it was trained there only.

7.5.2 Object-position recognition task

The white rectangle was displayed on the screen during the whole session. The rats were conditioned to press the lever for food reward when the rectangle was displayed in the reward position and not to press when it was displayed in the two non-reward positions (Fig. 21). The rectangle was displayed in the reward position at the beginning of the training session. It changed its position every 35 s. The order of presented positions was pseudorandom. The rats were trained for 34 sessions. The duration of the presentation of the rectangle in one position changed during the training but it was fixed to the 35 s mentioned above in the last 16 training sessions. Each apparatus had its own pseudorandom sequence same as in Experiment I and in Experiment II. The sequence was repeated three times during the training sessions, thus, the sessions lasted 31.5 min. Initially, the rats were rewarded for each correct response. Later, when they preferentially responded to the reward stimulus, the continuous reinforcement schedule was replaced by the variable ratio schedule with

geometric distribution of the number of presses necessary for getting the reward. The average number of responses necessary for activating the feeder was gradually increased to four. The first response after the change of the stimulus was never rewarded.

Fig. 21: Stimuli presented on the computer screen in the object-position recognition task. Adapted from Levcik et al. (2013b).

7.5.3 Test of the effect of prazosin in the object-position recognition task

After 34 standard sessions, when all the rats had reached an asymptotic performance, they were assigned to the 2 mg/kg and 3 mg/kg groups to match their cognitive performance. Thereafter, the rats received a habituation intraperitoneal injection of 2 mg/kg (n = 8) or 3 mg/kg of prazosin (n = 8) and were left in their homecages until the next day. Then the rats underwent the control session (saline application) after two standard sessions following the habituation infusion of prazosin and the test session (2 mg/kg or 3 mg/kg prazosin application) the next day.

7.5.4 Drug application

Prazosin (Sigma-Aldrich, Czech Republic) was dissolved in distilled water at a concentration of 0.5 mg/ml and injected intraperitoneally 20 min prior to behavioral

testing at the dose of 2 mg/kg or 3 mg/kg in the test session. The same volume of saline (0.9% solution of NaCl) was injected in the same way in the control session. The doses of prazosin were chosen on the basis of previous experiments in our laboratory, which were done in the active place avoidance task (Stuchlik and Vales 2008).

7.5.5 Data analysis

We analyzed the overall responding rate (number of presses per second; expressed in Hz) and the cognitive efficiency (ratio of reward and non-reward presses) of rats. The responding rate was analyzed during the whole session. However, the data analysis of the cognitive efficiency was restricted to those periods of stimuli presentation which were preceded by the non-reward periods and only to the first 15 seconds of these periods. This restriction was introduced in order to decrease the effect of the reaction of the feeder on behavior. For example, an animal may keep responding not because it sees the reward stimulus on the screen but because its immediately preceding responses were reinforced (for detailed information see the section ‘‘Object-position recognition task’’ in ‘‘Results’’ in Experiment II). One rat was excluded from the analysis of the cognitive efficiency because it pressed the lever only once in the test session (2 mg/kg of prazosin) and this response was not made in the first 15 seconds of the stimulus presentation. The results are reported as means ± S.E.M. Statistical tests were done with R software. Group means were compared by the Wilcoxon signed rank tests. The level of significance was set to 0.05. Holm-Bonferroni correction was used to keep the level of significance of the multiple comparisons 0.05 (Holm 1979).

IV Results

8.1 Experiment I

8.1.1 Object-position recognition task (version 1 - stationary object)

We averaged performance of each rat across four sessions. These sessions were taken from the later phase of training when the number of responses as well as the percentage of correct responses was stable between consecutive sessions. These evaluated sessions preceded four test sessions described below. The averaging across four sessions was done to reveal differences in responding in the two non-reward positions where the overall responding rate was low.

Responding rate as a function of time elapsed since the object changed its position of trained rats is shown in Fig. 22A. The rats responded with the highest rate when the object was in the reward position and this preference lasted during the whole 135 s period. The responding rate at the two non-reward positions was much lower. However, at the beginning of the 135 s period it was higher at the right position than at the left position (Fig. 22A). The right position was closer to the reward position than the left position. Below we show that this difference was significant.

The preferential responding during the reward period does not necessarily mean that the rats were paying attention to the object on the screen. For example their responding could be based on the outcomes of their previous responses. This strategy would be effective because the duration of the periods was long (135 s). It could be further improved by checking the reinforcement conditions only after the object jumped from one position to another. The jump produced salient flash stimulus and

thus no spatial information was necessary to recognize beginnings of the periods. To see how the rats responded to the jumps, we plotted the responding rate before and after the object changed its position (Fig. 22B). Before the change the responding rate depended on the reinforcement schedule. It was high in the reward position and low in both non-reward positions. Immediately after the change the responding rate reflected the previous reinforcement schedule, however, few seconds later the responding rate was different in each position. It was highest in the reward position followed by the right position and then by the left position. The difference between the left and the right positions was most apparent between 5 s and 10 s after the change.

Fig. 22B indicates that the responding rate during 5-10 s after a jump did not depend on the object position before the jump but only on the current position of the object. In other words the rats did not use the knowledge that the reward periods were always followed by the non-reward periods while the non-reward periods were followed by both types of periods with equal probability. If they used this knowledge then it would be expected that the responding rate after a reward period would be always low independently of whether the object jumped to the left or to the right position.

Fig. 22C shows responding rates in the three object positions during the 5-10 s interval after the change. It stresses the negative relationship between the responding rate during the evaluated interval and the distance between the object and the reward position. This relationship indicates that the rats estimated distance between the object and the reward position at least shortly after the object changed its position.

We tested the differences among the responding rates in the three positions during the last 5 s before the change (interval 130-135 s) and during the interval 5-10 s after the change. In order to make the data more similar to normal distribution and of

similar variance in the three locations we used log2 of frequencies in the statistical tests. Because some of the frequencies were zero we added 1/135 to all the frequencies before applying the logarithm. Since the overall responding rate of the rats trained in Apparatus B seemed to be lower than the responding rate of the rats trained in Apparatus A, we added apparatus as a factor into the statistical model.

Mixed effect ANOVA with "object position" as within subject factor and "apparatus" as between subject factor confirmed that there was an effect of "object position" on the responding rate during the last 5 s of the periods ($F(2, 22) = 13.3289$, $p = 0.0002$) but no effect of the "apparatus" ($F(1, 11) = 0.0782$, $p = 0.7850$) and no effect of the interaction ($F(2, 22) = 0.2894$, $p = 0.7516$). Tukey multiple comparison test showed that the responding rate at the reward position was different than the responding rate at the left position (reward-left: $z = 2.861$, $p = 0.0117$) and also at the right position (reward-right: $z = 3.151$, $p = 0.0046$). The responding rate at the left and at the right positions were not different (right-left: $z = -0.290$, $p = 0.9548$).

The same analysis applied on the interval 5-10 s after the beginning of the periods also showed an effect of "object position" on the responding rate ($F(2, 22) = 77.70697$, $p < 0.0001$) but no effect of the "apparatus" ($F(1, 11) = 2.74416$, $p = 0.1258$) and no effect of the interaction ($F(2, 22) = 0.36259$, $p = 0.7000$). Tukey multiple comparison test showed that there were differences among responding rates at all the three positions (reward-left: $z = 9.448$, $p < 10\text{-}4$; reward-right: $z = 5.341$, $p < 10\text{-}4$; right-left: $z = 4.107$, $p = 0.000135$).

The interval 5-10 s after the object changed its position was chosen after we analyzed the four sessions. For this reason we repeated the statistical tests with a different set of four sessions. These sessions immediately preceded the analyzed sessions. The statistical results were identical.

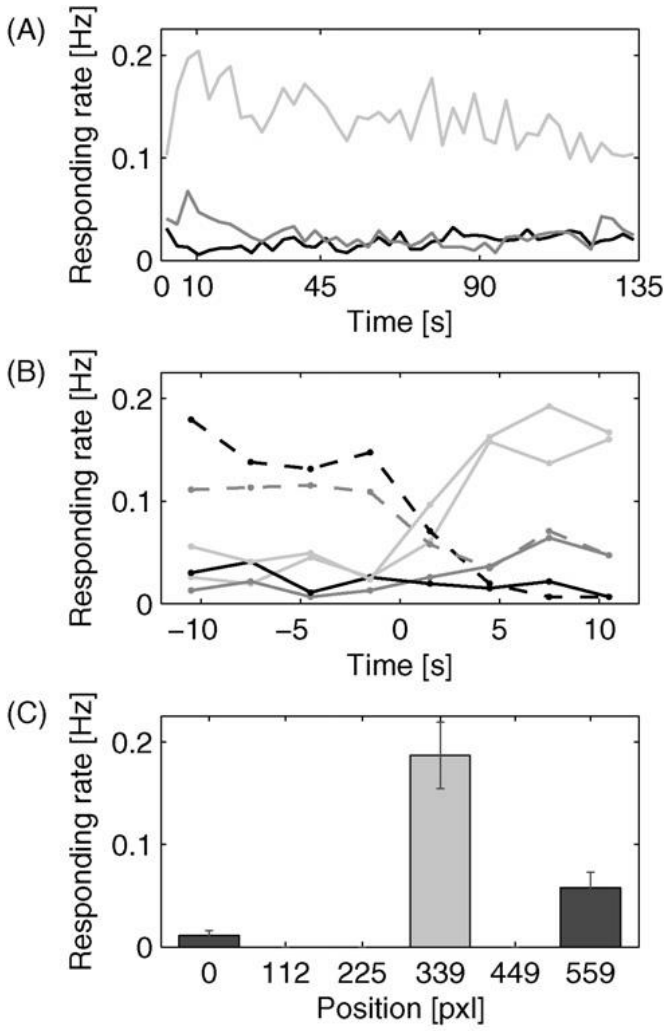

Fig. 22: Version 1 – stationary object. (A) Responding rates in the three positions of the object as a function of time elapsed since the object changed its position (black line - left position, gray line - right position, pale gray line - reward position). Bin width is 3 s. (B) Responding rate before and after the object changed its position. The change occurred at time zero. Bin width is 3 s. The shades of the lines denote newly acquired positions (black line - left position, gray line - right position, pale gray line - reward position). The dash lines denote that before the change the object was in the reward position, the full lines denotes that before the change the object was in one of the non-reward positions. (C) Responding rate (average ± SEM) during the interval 5-10 s after the object changed its position as a function of object's position. Adapted from Klement et al. (2010).

8.1.2 Test of stimulus generalization

According to the above results, shortly after the object changed its position responding rates at the two non-reward positions depended on the distance to the reward position. We tested this hypothesis by modifying the standard session. We

added short periods (15 s) during which the object was displayed at three new positions. Each new position was presented nine times during the test session (three times after each familiar position). The rats were not rewarded during these periods.

Fig. 23 shows the responding rate at all the six positions during 5-10 s after the object changed its position. The responding rate increased with decreasing distance between the object and the reward position. The maximum was reached in position 225 pxl. This position was just beside the reward position on its left side. The responding rate in the reward position is decreased by the presence of reward.

Mixed effect ANOVA with "object position" as within subject factor and "apparatus" as between subject factor was used to test effects of object's position and of the apparatus on the responding rate during the period 5-10 s after the change. There was an effect of "object position" ($F(2, 22) = 18.68058$, $p < 0.0001$) but no effect of the "apparatus" ($F(1, 11) = 0.15760$, $p = 0.6990$) and no effect of the interaction ($F(2, 22) = 2.12816$, $p = 0.1429$). Tukey multiple comparison test showed that the responding rate at the reward position (339 pxl) was different from the responding rate at the left (0 pxl) and at the right (559 pxl) positions (339-0: $z = 4.663$, $p < 0.001$; 339-559: $z = 3.139$, $p = 0.02102$), responding rate at position 225 pxl was different from positions 0 pxl, 112 pxl and 559 pxl (225-0: $z = 6.161$, $p < 0.001$; 225-112: $z = 3.361$, $p = 0.01017$; 225-559: $z = -4.637$, $p < 0.001$). Position 449 pxl was different from position 0 pxl (449-0: $z = 3.443$, $p = 0.00752$). No other differences were found.

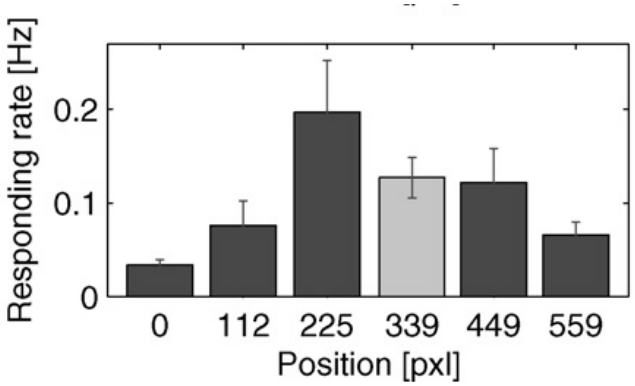

Fig. 23: Test of stimulus generalization (test sessions with new positions of the object): responding rate (average ± SEM) during the interval 5-10 s after the object changed its position as a function of object's position. Adapted from Klement et al. (2010).

8.1.3 Object-position recognition task (version 2 - moving object)

Well trained rats markedly increased their responding frequency before the object entered the reward region (Fig. 24, session 43). Fig. 24 shows responding rate as a function of object's position and velocity in the beginning of the training (session 1), before reaching asymptotic performance (session 5), at the asymptotic performance (session 43) and in a test session in which the rats did not see the object (sessions 44).

The responding rate decreased inside the reward region and remained low until the object reached the opposite side of the screen. In the case the object moved from the reward region toward the left side, the already low responding rate decreased even more as the distance between the object and the reward region increased (see 3rd and 4th graphs in Fig. 24). Many features of this pattern were present in the first and in the fifth sessions, however, they were less pronounced.

The object was invisible in the test session (session 44). In this session the responding rate gradually increased after the object left the reward region until maximum frequency was reached. The steep increase in responding rate before the reward region was not present. Consumption of reward decreased the responding rate in the reward region. We evaluated two variables reflecting the spatial performance of

the rats: percentage of rewarded presses and distribution of non-rewarded presses emitted when the object moved toward the reward region.

The percentage of rewarded presses increased during the training from 21.2 ± 2.1% (session 1) to 30.0 ± 2.9 (session 43) in the rats trained in Apparatus A and from 24.6 ± 2.4 (session 1) to 35.2 ± 2.2 (session 43) in the rats trained in Apparatus B. In the test session the percentage of rewarded presses was 20.0 ± 1.6% in Apparatus A and 23.9 ± 0.8% in Apparatus B. Mixed effects ANOVA with "session" as within subject factor and "apparatus" as between subject factor showed an effect of "session" ($F(2, 20) = 20.9784$, $p < 0.0001$) but no effect of "apparatus" ($F(1, 10) = 3.9774$, $p = 0.0741$) and no effect of the interaction ($F(2, 20) = 0.1319$, $p = 0.8772$). Tukey multiple comparison showed that session 43 (asymptotic performance) was different from session 1 (43-1: $z = 3.428$, $p = 0.0018$) and from the test session (44-43: $z = -3.875$, $p < 0.001$).

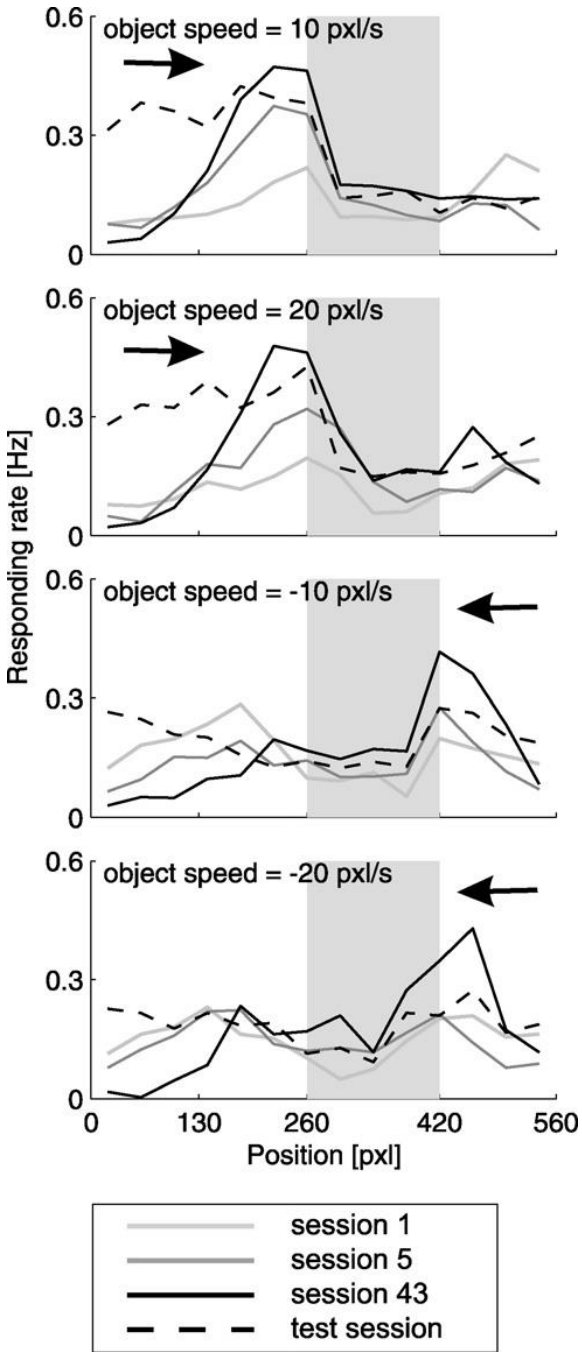

Fig. 24: Version 2 – moving object. Responding rate as a function of position in four sessions showing the development of behavior during the training and in the test session: session 1 (pale gray line), session 5 (gray line), session 43 (black line) and the test session (session 44) (dash black line). The object was not visible in the test session but all the other experimental conditions were unchanged. Bin width is 40 pxl. Positive speed indicates left-to-right movement and negative speed indicates right-to-left movement of the object. Reproduced from Klement et al. (2010).

The accumulation of non-rewarded responses before the reward region (Fig. 24) also indicates that the rats recognized the position of the reward region. For

statistical testing we represented this accumulation by an "average responding position". The average responding position was an average of object positions in the moments in which responses were emitted. The average responding position was calculated only from non-rewarded responses emitted when the object moved toward the reward region. We calculated the average responding position for each direction and for each speed of object's movement.

Random responding during the left-to-right movement of the object would result in average responding position around 130 pxl. Higher values with the limit at 259 pxl would indicate accumulation of responses before the reward region. The average responding position increased during the training. It was 163 pxl ± 11 pxl in session 1, 182 pxl ± 5 pxl in session 5, 197 pxl ± 5 pxl in session 43 for the rats trained in Apparatus A. Lower values were observed in the rats trained in Apparatus B. They were 142 pxl ± 11 pxl in session 1, 165 pxl ± 7 pxl in session 5 and 181 pxl ± 8 pxl in session 43. When the rats did not see the object (session 44) the average responding position was close to 130 pxl. It was 139 pxl ± 4 pxl in the rats trained in Apparatus A and 134 pxl ± 3 pxl in the rats trained in Apparatus B.

We tested whether the average responding position during the left-to-right movement of the object was different from the expected position of random responding (130 pxl) by using a separate t-test for each apparatus (A and B), for each object speed (slow and fast) and for each of the three sessions (1, 43 and 44-test session). Together it was 12 t-tests. To keep the level of significance below 0.05 we used Holm-Bonferroni correction for alpha values. Results are shown in Table 1 in columns "Left-to-right movement of the object". When the rats were on their stable level of performance, the average responding position was closer to the reward region than the theoretical average position of random responding. This difference was

significant for the rats in both apparatuses and for both speeds of the object. When the object was invisible (test session), the average responding position was not different from the expected position of random responding (130 pxl).

We also compared the average responding positions between the apparatuses, between the two speeds of the object and among sessions 1, 43 and 44 (test session). Mixed effects ANOVA with "session" and "object speed" as within subject factors and "apparatus" as between subject factor showed an effect of "session" ($F(2, 20) = 43.873$, $p < 0.0001$), but no effect of "apparatus" ($F(1, 10) = 9.279$, $p = 0.0123$), no effect of "speed" ($F(1, 30) = 0.265$, $p = 0.6107$) and no effects of all the interactions ("session" and "speed": $F(2, 30) = 2.531$, $p = 0.0964$; "session" and "apparatus" $F(2, 20) = 1.009$, $p = 0.3824$; "speed" and "apparatus": $F(1, 30) = 0.167$, $p = 0.6854$; "session" and "speed" and "apparatus": $F(2, 30) = 0.812$, $p = 0.4534$). Tukey multiple comparison showed that the test session (session 44) was different from the other sessions (44-1: $z = -3.437$, $p = 0.00174$; 44–43: $z = -5.430$, $p < 0.001$). No other differences were found.

Random responding during the right-to-left movement of the object would result in average responding position around 490 pxl. Accumulation of responses before the reward region would result in lower values with the limit 420 pxl. The average position decreased during training. It was 487 pxl ± 4 pxl in session 1, 471 pxl ± 5 pxl in session 5, 465 pxl ± 3 pxl in session 43 for the rats trained in the left apparatus and 480 pxl ± 6 pxl in session 1, 478 pxl ± 5 pxl pxl in session 5, 475 pxl ± 5 pxl in session 43 for the rats trained in the right apparatus. In the test session (session 44) the position was close to 490 pxl. It was 486 pxl ± 5 pxl for the rats in the left apparatus and 487 pxl ± 5 pxl for the rats trained in the right apparatus.

We repeated both statistical tests concerning the average responding position for the right-to-left movement of the object.

Comparisons of the average responding position with the theoretical expected position of random responding (490 pxl) is shown in Table 1 (columns "Right-to-left movement of the object"). The rats trained in Apparatus A accumulated their responses before the reward region in session 43 (asymptotic performance) during both speeds of the object. On the contrary, the average responding position of the rats trained in Apparatus B was not different from the expected position of random responding. There were also no differences between the observed and the theoretical expected position of random responding in the first session and in the test session.

Session	Apparatus A			
	Left-to-right movement of the object		Right-to-left movement of the object	
	Slow	Fast	Slow	Fast
1	—	+	—	—
43	+	+	+	+
44 (test)	—	—	—	—
Session	Apparatus B			
	Left-to-right movement of the object		Right-to-left movement of the object	
	Slow	Fast	Slow	Fast
1	—	—	—	—
43	+	+	—	—
44 (test)	—	—	—	—

Table 1: Results of multiple t-tests with Holm–Bonferroni correction for alpha values in which the average responding position was tested against the expected position of random responding (130 pxl for the left-to-right movement and 490 pxl for the right-to-left movement of the object). The symbol "+" denotes significant difference at level 0.05. The symbol "—" denotes no difference on level 0.05.

The comparisons of apparatuses and of sessions 1, 43 and 44 (test session) and of the two speeds of the object were as follows. Mixed effects ANOVA with “session” and “object speed” as within subject factors and “apparatus” as between subject factor showed an effect of “session” ($F(2, 20) = 13.19$, $p = 0.0002$) but no effect of “apparatus” ($F(1, 10) = 0.43$, $p = 0.5285$), no effect of “speed” ($F(1, 30) = 0.40$, $p = 0.5316$) and no effects of all the interactions (“session” and “speed”: $F(2, 30) = 1.41$, $p = 0.2606$; “session” and “apparatus” $F(2, 20) = 2.29$, $p = 0.0773$; “speed” and “apparatus”: $F(1, 30) = 1.04$, $p = 0.3158$; “session” and “speed” and “apparatus”: $F(2, 30) = 0.99$, $p = 0.3830$). Tukey multiple comparison showed that session 43 was different from sessions 1 and 44 (43-1: $z = -3.119$, $p = 0.00517$; 44-43: $z = 2.624$, $p = 0.02364$). No other differences were found.

The above statistical results confirmed that the well trained rats (session 43) accumulated non-rewarded responses before the reward region, these responses were not influenced by the speed of the object (thus they reflected distance between the object and the reward region rather than the time remaining to the entrance into the reward region) and that the accumulation depended on the visual stimuli on the screen. The rats trained in Apparatus A concentrated their non-rewarded responses closer to the reward region than the rats trained in Apparatus B. There was a suspicion that both groups of rats (Apparatus A, Apparatus B) used different behavioral strategies to solve the task. More detailed analysis is given in our publication Klement et al. (2010).

8.2 Experiment II

8.2.1 Histology

The locations of the places, where muscimol and saline were administered, were verified by histology. All the infusion sites except one were located in dorsal hippocampi (Fig. 25). In one rat, the right infusion site (Fig. 25, the bottom brain in the third column) was at the lateral edge of the dorsal hippocampus. For this reason, the rat was excluded from the analyses of the experiments in which muscimol or saline was infused in the brain. Nevertheless, the behavioral effect of muscimol infusion in this rat was not different from the other rats.

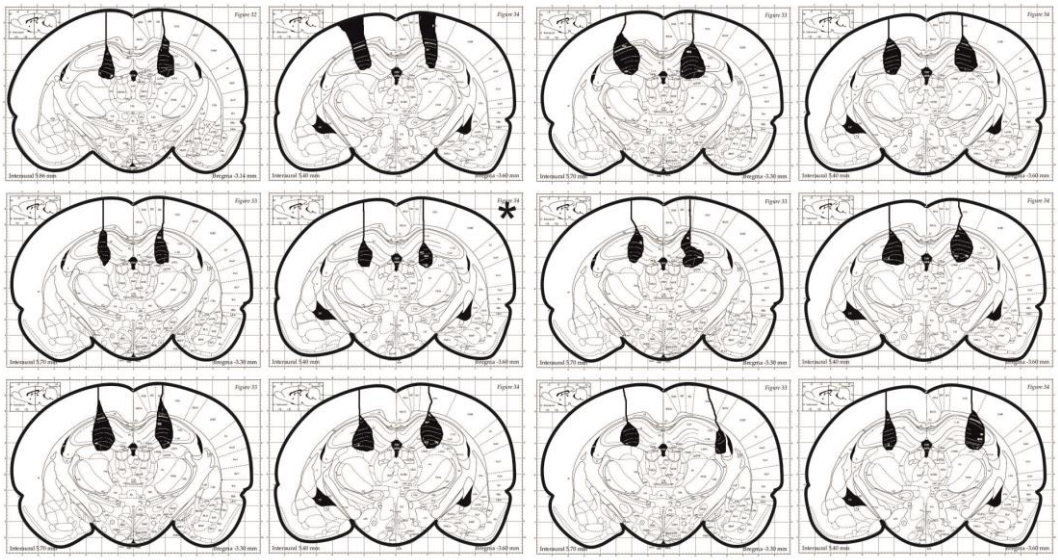

Fig. 25: Spreads of black ink injected at the infusion sites after the completion of the behavioral experiments. The black star identifies the rat that was not trained in the brightness discrimination task. Coronary sections (AP coordinates with respect to the bregma range from -3.14 to -3.60 mm) were adapted from Paxinos and Watson (1998). Adapted from Levcik et al. (2013).

8.2.2 Object-position recognition task

All the rats (n = 12) learned to preferentially respond when the object was displayed at the reward position. The percentage of correct responses across the last

five training sessions ranged from 51% ± 2% in the worst performing rat to 76% ± 5% in the best performing rat. All rats performed better than the chance level of 33.3% (repeated t-tests with Holm's adjustment of p-values: all adjusted p-values < 0.0109).

The rats trained for 28 sessions reached the same level of performance as the rats trained for 54 sessions (t_{10} = -0.5088, p = 0.622). Both groups were also not different in the overall responding rate (t_{10} = -0.1232, p = 0.9044); therefore, they were merged together for the further analyses.

The lever pressing of trained rats reflected not only the stimulus on the screen but also the rule in the sequence of stimuli that the reward stimulus was always followed by the non-reward stimuli, whereas a non-reward stimulus was followed by both types of stimuli (reward and non-reward) with equal probability. As the result, the responding rate at the two non-reward positions was two times lower if the preceding stimulus was the reward stimulus (0.06 ± 0.01 Hz) than if the preceding stimulus was the other non-reward stimulus (0.11 ± 0.02 Hz; t-test: t_{11} = -3.3151, p = 0.0069). Therefore, in the rest of Experiment II, we analyze and present the responding rates only for those presentations of stimuli which were preceded by the non-reward stimuli. However, the major conclusions of Experiment II are the same regardless of whether this data restriction is used or not. We also restricted the analyses of the responding rates to the first 15 s and/or to only the first 5 s of stimuli presentation. This restriction was introduced in order to decrease the effect of the reaction of the feeder on behavior. For example, an animal may keep responding not because it sees the reward stimulus on the screen but because its immediately preceding responses were reinforced.

Performance of the trained rats is shown in Fig. 26 (last training session). During the first 15 s of stimuli presentation, the rats responded with higher frequency

when the object was displayed at the reward position than in the two non-reward positions (one-way ANOVA with repeated measures: ($F(2,22) = 17.3133$, $p < 10^{-4}$; Tukey multiple comparison test: 1-220 pxl: $z = -4.483$, $p < 10^{-4}$, 600-220 pxl: $z = -5.295$, $p < 10^{-4}$, 600-1 pxl: $z = -0.744$, $p = 0.737$).

8.2.3 Test of stimulus generalization

We tested whether the rats interpreted the stimuli on the screen as three distinct pictures without any spatial relationship or whether they saw a single object at three different positions. The object was presented in the three familiar positions (1, 220, and 600 pxl) and also in three novel positions (110, 347, and 474 pxl). The responses at all the positions, except in the reward position (220 pxl), were not reinforced. The expectation was that if the rats interpreted the stimuli as distinct pictures without spatial relationship among them, then the responding rate in the novel positions would be equal; however, if they saw a single object displayed at different positions, then the responding rate would decline as the distance to the reward position increases and the distance to the nearest non-reward position decreases. Fig. 26 (stimulus generalization) shows that the later possibility was the case. The responding rates were different in all the adjacent non-reward positions including the novel positions ($F(4,44) = 15.9863$, $p < 10^{-4}$; Tukey multiple comparison test: 110-1 pxl: $z = 2.972$, $p = 0.0246$, 347-110 pxl: $z = 3.099$, $p = 0.0166$, 474-347 pxl: $z = -3.225$, $p = 0.0110$, 600-474 pxl: $z = -3.984$, $p < 0.001$). The responding rate declined from the reward position on the left site at rate -0.74 ± 0.20 mHz/pxl (t-test: $t_{11} = -3.6953$, $p = 0.0035$) and on the right site at rate -0.76 ± 0.18 mHz/pxl (t-test: $t_{11} = -4.2581$, $p = 0.0013$). The responding rate at the reward position was lower than expected from a presumed ‘‘hill’’-shaped gradient of

the stimulus generalization (Fig. 26, stimulus generalization). It was due to reward consumption after occasional delivery of food reward at this position.

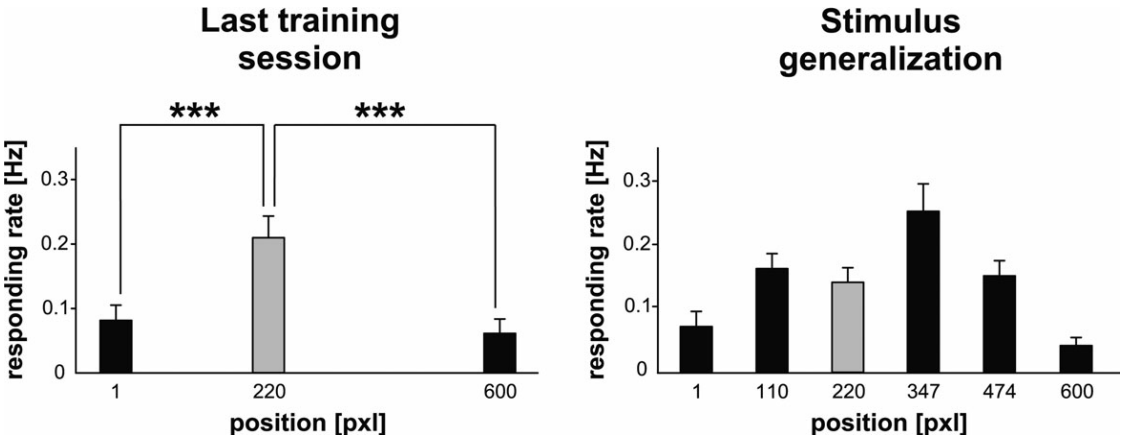

*Fig. 26: Asymptotic performance in the object-position recognition task (*left*) and the test of stimulus generalization (*right*). The responding rates (mean, SEM) during the first 15 sec of stimuli presentation. The responding rates were calculated from those stimuli presentations that were preceded by the non-reward familiar stimuli. The gray color indicates the reward stimulus and the black color indicates the non-reward stimuli. The object's positions 1, 220, and 600 pxl were familiar, whereas the positions 110, 347, and 474 pxl were novel for the rats. The three stars indicate significant difference at the level of 0.001. Reproduced from Levcik et al. (2013).*

8.2.4 Test of the role of hippocampus in the object-position recognition task

After the surgery, the rats were retrained to their previous level of performance and then tested in one session with inactivated hippocampus. During the first 15 s of object's presentation, the rats with inactivated hippocampus (n = 11) responded equally to all three positions (Fig. 27, Muscimol). The one-way ANOVA with repeated measures was not significant ($F(2,20) = 2.7144$, $p = 0.0906$]. Statistically, the same results were obtained for shorter intervals, for example, for the first 5 s ($F(2,20) = 0.8346$, $p = 0.4486$).

In the control session, when saline was administered in the hippocampus, the same rats responded with higher frequency when the object was displayed in the reward position than in the two non-reward positions where the responding rates were

equal (Fig. 27, saline). The differences were significant for the first 15 s of stimuli presentation ($F(2,20) = 10.2465$, $p = 0.0009$; Tukey multiple comparison test: 1-220 pxl: $z = -3.745$, $p = 0.0005$, 600-220 pxl: $z = -3.989$, $p = 0.0002$, 600-1 pxl: $z = -0.236$, $p = 0.9698$) as well as for the first five seconds ($F(2,20) = 7.8294$, $p = 0.0031$; Tukey multiple comparison test: 1-220 pxl: $z = -3.089$, $p = 0.0057$, 600-220 pxl: $z = -3.553$, $p = 0.0011$, 600-1 pxl: $z = -0.433$, $p = 0.9017$).

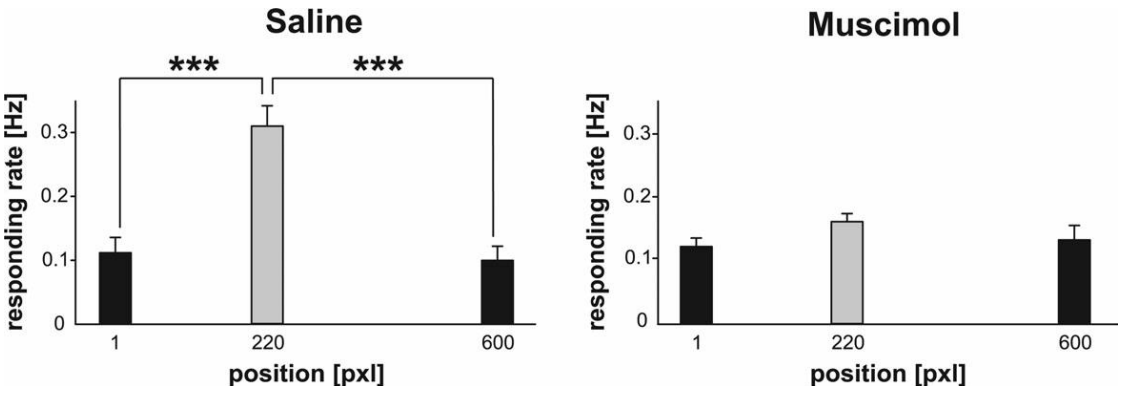

Fig. 27: The effect of hippocampal inactivation on the performance in the object-position recognition task. The responding rates (mean, SEM) during the first 15 sec of stimuli presentation in the control (saline) and the inactivation (muscimol) sessions. The responding rates were calculated from those stimuli presentations which were preceded by the non-reward stimuli. The gray color indicates the reward stimulus, and the black color indicates the non-reward stimuli. The three stars indicate significant difference at the level of 0.001. Reproduced from Levcik et al. (2013).

8.2.5 Brightness discrimination task

To test whether the hippocampal inactivation by muscimol altered the operant behavior, 11 of the 12 rats were further trained to discriminate the light screen (reward stimulus) from the dark screen (non-reward stimulus). One rat was not trained in this task because its guide cannula was damaged (Fig. 25, the second slice in the second column), and one rat was excluded from the analysis after the histological verification of infusion sites (see the section ‘‘Histology’’).

8.2.6 Test of the role of hippocampus in the brightness discrimination task

After the rats had reached a stable level of performance, the role of hippocampus in the brightness discrimination task was tested in the same way as in the object-position recognition task. In accordance with the data analysis mentioned earlier, the responding rates were calculated only for those presentations of stimuli, which were preceded by the non-reward stimulus. The rats with inactivated hippocampus discriminated the bright and dark conditions (Fig. 28, Muscimol). The difference was significant for the first 15 s of stimuli presentation (paired t-test: t_9 = 3.9853, p = 0.0032) as well as for the first 5 s (paired t-test: t_9 = 4.0384, p = 0.0029).

The rats discriminated the light and dark conditions and also in the control session when saline was infused into the hippocampus (Fig. 28, Saline). The paired t-tests were significant for the first 15 s of stimuli presentation (t_9 = 5.9705, p = 0.0002) as well as for the first 5 s (t_9 = 4.6963, p = 0.0011).

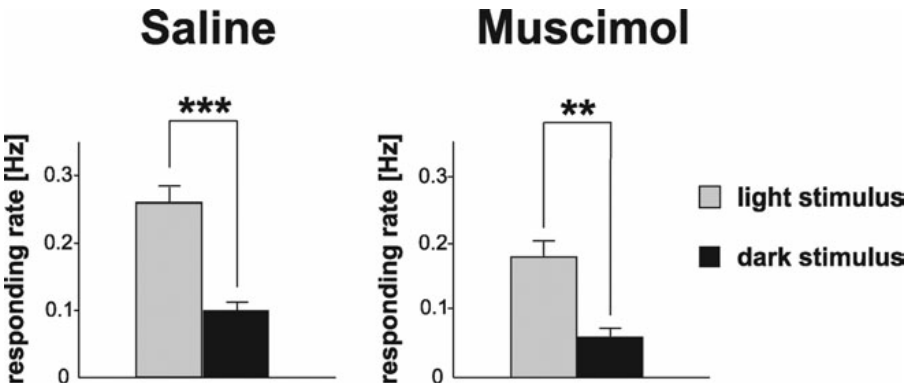

Fig. 28: The effect of hippocampal inactivation on the performance in the brightness discrimination task. The responding rates (mean, SEM) during the first 15 sec after the stimuli presentation in the control (saline) and inactivation (muscimol) sessions. The responding rates were calculated from those stimuli presentations which were preceded by the non-reward stimuli. The gray color indicates the reward stimulus, and the black color indicates the non-reward stimulus. The two and three stars indicate significant differences at the level of 0.01 and 0.001, respectively. Reproduced from Levcik et al. (2013).

8.3 Experiment III

8.3.1 Test of the effect of prazosin in the object-position recognition task

The assignment of the rats to the 2 mg/kg and 3 mg/kg groups was done to match their cognitive efficiency in the last standard session before the habituation infusion (2 mg/kg group: 0.83 ± 0.05; 3 mg/kg group: 0.79 ± 0.04; Wilcoxon rank sum test: W = 36.5, p = 0.6742). The overall responding rate tent to be lower in the 2 mg/kg group, although the difference was not significant (2 mg/kg group: 0.11 ± 0.04 Hz; 3 mg/kg group: 0.14 ± 0.02; Wilcoxon rank sum test: W = 15, p = 0.083).

The analysis of the overall responding rate showed no effect of the dose of 2 mg/kg of prazosin on motor activity (Fig. 29, upper left). The overall responding rate of rats was 0.10 ± 0.03 Hz in the control session and 0.07 ± 0.03 Hz after the application of 2 mg/kg of prazosin (Wilcoxon signed rank test: V = 6, p-adjusted = 0.1094). The dose of 3 mg/kg decreased the responding rate to 55 ± 5 % of control (Fig. 29, upper right). The overall responding rate of rats was 0.14 ± 0.02 Hz in the control session and 0.08 ± 0.01 Hz after the application of 3 mg/kg of prazosin (Wilcoxon signed rank test: V = 6, p-adjusted = 0.0156). The reduction of the lever-pressing activity was observed in all rats in the test session with the dose of 3 mg/kg.

The dose of 2 mg/kg had no effect on cognitive performance in the object-position recognition task (Fig. 29, lower left). The ratio of reward and non-reward presses was 0.73 ± 0.01 in the control session and 0.77 ± 0.01 after the application of 2 mg/kg of prazosin (Wilcoxon signed rank test: V = 17, p-adjusted = 0.6875). Injection of the dose of 3 mg/kg also did not alter the cognitive efficiency (Fig. 29, lower right). The ratio of reward and non-reward presses was 0.81 ± 0.03 in the control session and

0.87 ± 0.03 after the application of 3 mg/kg of prazosin (Wilcoxon signed rank test: V = 6, p-adjusted = 0.2968).

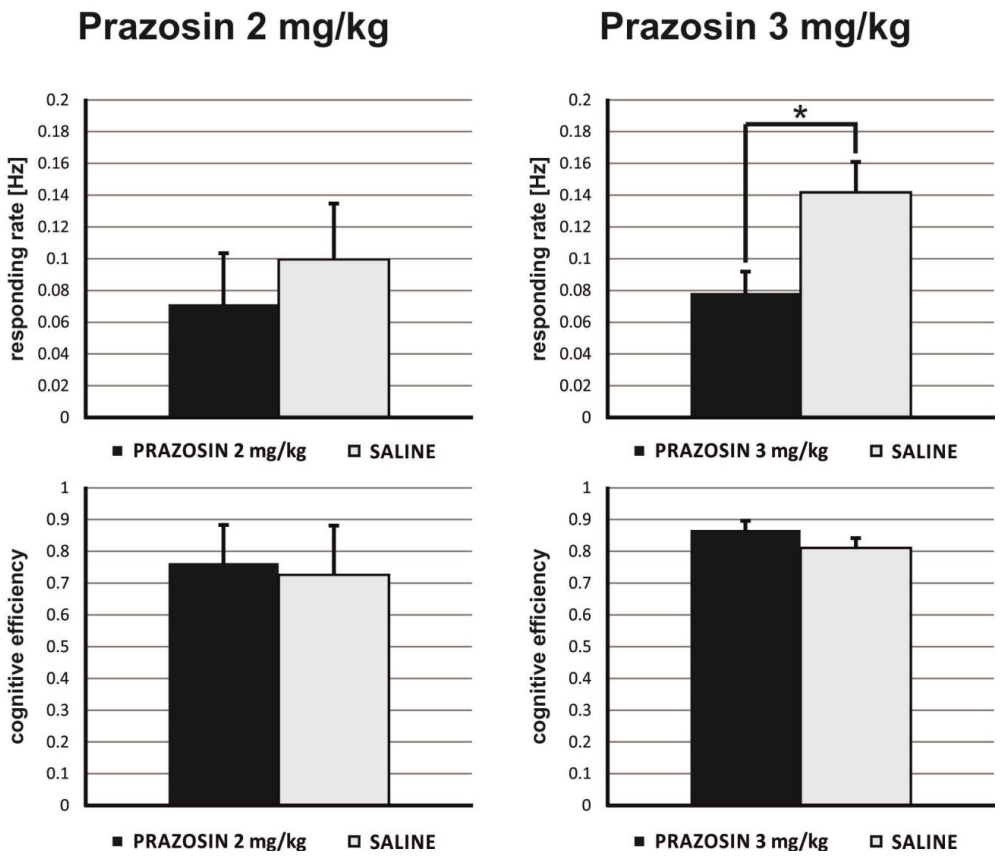

*Fig. 29: Overall responding rate of lever presses (*upper graphs*) and cognitive efficiency (*lower graphs*) in the test sessions and in the control sessions. Cognitive efficiency represents the ratio of reward and non-reward presses emitted during the first 15 s after the onset of stimuli presentation (for further details see the section "Data analysis"). The black color indicates the application of prazosin (2 mg/kg or 3 mg/kg, i.p.) and the grey color indicates the application of saline. Data are mean ± SEM. The one star indicates significant difference at the level of 0.05. Reproduced from Levcik et al. (2013b).*

V Discussion

9.1 Object-position recognition task

We present two versions of behavioral task for rats in which visual stimuli were presented on a computer screen. In the first version of the task the rats were discriminating a particular position of an object from two other positions. In the second version of the task the rats were recognizing when a moving object was passing through a particular region. The to-be-recognized position in the first version of the task as well as the to-be-recognized region in the second version had to be recognized with respect to surrounding orientation cues such as the frame of the screen.

The task was designed in the way that the rats could easily distinguish the visual stimuli presented on the screen. According to Prusky et al. (2004), Long-Evans rats similarly to other pigmented rats have visual acuity around 1 cycle per degree. In the present tasks the rats kept their head close to the lever or to the hopper during the sessions. It was approximately 41cm from the screen. From this distance the rats saw the object at angle 6.5 º in the horizontally plane and 11.9 º in the vertical plane (if the object was located at the sides of the screen the viewing angles were approximately about 0.1 º smaller). In the first version of the task the separation angle between the left and the reward position was 25.8 º and the separation angle between the right and the reward position was 16.5 º. All these angular distances were highly above the rat's discrimination ability. The rats could see the whole screen under angle 49.2 º.

We claim that the rats solved both tasks spatially even though non-spatial strategies were theoretically possible. For example, in the first version of the task the rats could preferentially respond during the reward period without paying attention to

the stimuli on the screen. They could keep their responding rate high in the case their responses were frequently rewarded and low when no rewards were delivered. In addition, the jump of the object from one position to another indicated that the reinforcement schedule might change. The rats could react to this salient stimulus by emitting several responses to find out what was the new reinforcement schedule without paying attention to the position of the object.

An argument against these strategies comes from differential responding in the left and in the right positions after the object jumped to these positions. The rats responded at a higher rate when the object jumped to the right position which was closer to the reward position than the left position (Fig. 22C). Thus the operant responding was influenced by the distance between the object and the reward position in the beginning of the periods. This view was confirmed by the test sessions in which the object was displayed in three unfamiliar positions. The responding rate increased with decreasing distance between the object and the reward position also in the unfamiliar positions (Fig. 23). It indicated that the rats were estimating distance between the object and the hidden reward position.

The stimulus generalization test session ruled out the possibility that the rats reacted to the direction of the jumps of the object. In the standard sessions the right position was always displayed after the left-to-right jumps while the left position after right-to-left jumps. Thus, the higher initial responding in the right position compared to the left position could be explained by reaction to the direction of the jumps. This was not the case because in the test session the responding rate was highest when the object was displayed in position 225 pxl (Fig. 23). The object was displayed in this unfamiliar position two times more often after the right-to-left jump (from the reward and from the right positions) than after the left-to-right jump (from the left position). If

the rats increased their responding after left-to-right jump then the activity at this position should be low and not the highest.

The gradual increase in responding rate with increasing similarity between the object and the reward position is in agreement with data obtained on other animal species (e.g. rats, rabbits, horses and pigeons). Animals generalized rewarded stimuli (visual, auditory, somatosensory or gustatory) as they responded to similar stimuli as well (Blackman, 1974; Richardson et al., 1984; Dougherty and Lewis, 1991; Dougherty and Lewis, 1993; Ohyama et al., 2003).

In the second version of the task the rats recognized position of the moving object on the screen. The rats increased responding frequency before the object entered into the reward region (Fig. 24). This increase was visible in the first session and became more prominent during the training. The accumulation of presses before the reward region depended on the visual stimuli displayed on the screen. This was shown in the test session in which the object on the screen was invisible but all the other aspects of the task were unchanged. The rats compensated for the inability to effectively determine reward periods by increasing their overall activity and the accumulation of responses before the reward region disappeared (Fig. 24).

The responding rate depended on the distance of the object from the reward region rather than on the time to the entrance into the reward region. This can be seen in similar distributions of non-rewarded presses before the reward region during slow and fast movement of the object. There was no statistical difference between these two. Another argument against the temporal anticipation of reward comes from the gradual increase in responding rate with decreasing distance between the object and the reward position in the first task. In the first version of the task the object remained

in the same position for 135 s. Therefore independently whether it was close to or far from the reward position the time remaining to the next reward was always long.

The high responding before the reward region cannot be explained by the imprecise estimation of object position only. In the study done by Nekovarova and Klement (2006), which preceded this experiment, the reward region was directly marked by a visual cue on a screen. Similarly to the present task, the rats increased their activity with decreasing distance between the moving object and the visual cue. The increased activity started when the distance between the two objects was several times greater than visual acuity of the rats (Long-Evans strain).

The difference between the present design of the tasks and the previous design (Nekovarova and Klement, 2006) is that the reward position was directly marked by an object and the moving object stopped in the reward position. Therefore, rats could discriminate reward and non-reward periods by means of several strategies. Some of them were non-spatial, e.g. the rats could recognize whether the object is moving (non-reward periods) or whether it is stationary (reward periods) and/or whether there was a gap between the moving object and the cue (non-reward periods) or whether there was no gap (reward periods). Despite the results indicated that the rats recognized position of the moving object with respect to the cue at the goal location the alternative strategies should be always excluded. In the present tasks the goal location is not marked by a visual cue and the object does not change its behavior when it arrives to the goal location. This eliminates the non-spatial strategies potentially present in the study done by Nekovarova and Klement (2006).

The present task is different from the other behavioral tasks testing recognition of object's position. The commonly used tasks utilize a modification of the novelty-preference paradigm which takes advantage of the rodents' natural tendency to

approach and explore objects in novel positions longer than objects in familiar positions (Ennaceur et al., 1997; Dix and Aggleton, 1999). In other tasks rats have to remember the allocentric or egocentric distance of objects (Long and Kesner, 1996; Long and Kesner; 1998) or learn association between objects and locations (Talpos et al., 2009; Talpos et al., 2010). All these tasks are described in detail in the section "Introduction".

In the present task rats do not approach the object but they remain in the same place during the whole experiment. This can be potentially useful for dissociating neural activity representing subject's position from activity representing object's position. In addition, the experimental design allows to study recognition of position of both moving and stationary object. To our knowledge our object-position recognition task is the first task for rats addressing the recognition of position of a moving object. The continuous movement of the object corresponds to the situation when an animal sees a moving classmate, prey or predator. Due to the tendency of the rats to increase responding rate with decreasing distance between the object and the goal location, the present task also gives information about distance estimation.

Both versions of the present task are suitable for testing recognition of position of a distant object with respect to a hidden location in rats. It completes other tasks in which the subject should determine its own position relative to a hidden goal location (Klement and Bures, 2000; Pastalkova et al., 2003; Kelemen et al., 2005; Terrazas et al., 2005). The presentation of stimuli on a computer screen gives high flexibility for modifying both tasks. It lines up these tasks to an increasing number of rodent behavioral tasks employing computer screen for stimuli presentation (Sun et al., 1992; Sahgal and Steckler, 1994; Gaffan and Eacott; 1995; Keller et al., 2000; Bussey et al., 2001; Prusky et al., 2004; Nekovarova and Bures, 2006; Nekovarova and Klement,

2006; Bussey et al., 2008; Talpos et al., 2008; McTighe et al., 2009; Talpos et al., 2009; Talpos et al., 2010; Ward et al., 2013).

9.2 Role of hippocampus in the object-position recognition task

The finding of Experiment II is that the hippocampal inactivation impaired performance in the object-position recognition task (Fig. 27) but it did not impair performance in the brightness discrimination task (Fig. 28). We argue below that this finding demonstrates that intact rats use hippocampus for recognizing position of objects located in an inaccessible part of the environment.

We blocked the hippocampus by a $GABA_A$-receptor agonist, muscimol. The spared performance after the administration of saline indicated that the impairment after the administration of muscimol was caused by muscimol and not by the stress or mechanical stimulation of the hippocampus during inactivation. The most frequently used dose of muscimol for blocking activity of dorsal hippocampus is 0.5 μg per one side (e.g., Corcoran et al., 2005; Czerniawski et al., 2009; Iordanova et al., 2011). This dose disrupted the operant behavior in our task. For this reason, we tried a lower dose of 0.3 μg per one side of hippocampus. This dose was the highest dose that does not significantly impair the performance in the spontaneous alternation task (Krebs-Kraft and Parent, 2008); however, it was much higher than the dose of 0.07 μg, which blocked retrieval of reference spatial memory in Morris water maze (Moser and Moser, 1998) and which affected the performance in an operant delayed alternation task of long delay (Maruki et al., 2001).

In Experiment II, the rats with inactivated hippocampus were able to discriminate dark and light conditions in the brightness discrimination task (Fig. 28) as demonstrated previously (e.g., Klement et al., 2005). The purpose of the brightness

discrimination task was to rule out various explanations of the impairment in the object-position recognition task. Both tasks were as similar to each other as possible. They were carried out on the same apparatuses. The durations of the reward periods and the non-reward periods as well as their sequences were also identical. The results from the brightness discrimination task showed that the hippocampal inactivation by muscimol did not disrupt operant behavior and that the operant behavior was still under stimulus control. The inactivation did not considerably changed motivation of the rats to obtain the reward, and it did not lead to perseverative behavior. Thus, the impairment in the object-position recognition task after hippocampal blockage can be attributed to the inability of the rats to discriminate and process stimuli on the screen.

The crucial question is whether the rats interpreted the stimuli as spatially unrelated pictures or whether they perceived a single object in different positions. Arguments for the later possibility are mentioned in the section "Test of stimulus generalization" in Experiment I. The rats presented in Experiment II also showed the same distance-responding relationship in the test of stimulus generalization (Fig. 26, Stimulus generalization). Thus, we conclude that the impairment in the object-position recognition task after hippocampal inactivation was due to the inability of the rats to recognize position of the object displayed on the computer screen.

We do not claim that rats without hippocampus are not able to efficiently solve the object-position recognition task. Our results showed that if rats are trained with the hippocampus, then their strategy requires the hippocampus. It is possible that rats trained without the hippocampus would find an alternative strategy based on different neural circuitry as it was shown in other cognitive tasks (Maren et al., 1997; Gaskin et al., 2003; Driscoll et al., 2005). Long and Kesner (1998) showed that rats with permanent hippocampal lesion can learn to recognize one of four possible object's

positions within a rectangular maze. This result suggests that hippocampal rats could possibly learn the present object-position recognition task if trained without hippocampus.

Experiment II extents previously published experiments demonstrating that hippocampus is necessary for recognizing positions of objects located within the accessible part of the environment (Long and Kesner, 1996; Gilbert et al., 1998; Mumby et al., 2002; Gilbert and Kesner, 2004; McTighe et al., 2009; Talpos et al., 2009, 2010, Barker and Warburton, 2011). In these experiments, rats made contacts with the objects at least during the learning phase. The position of the objects can be learned by associating rat position indicated by activity of hippocampal neurons with the object located at that place. This explanation fell when the object is located at a place the rat has never visited unless the hippocampal neurons code not only the position of the subject but also other positions where the subject is currently not present or even never could be present. Ho et al. (2008) recorded hippocampal neurons while rats were chasing a moving object. The authors reported that the neurons exhibited standard subject-position specific activity which was modulated by various features of the movement of the object and by the mutual spatial relationship between the object and the subject. No neuron-coding position of the object was found. On the other hand, D. Lopez-Pigozzi and his colleagues (unpublished observations) measured the activity of hippocampal pyramidal neurons in rats located in an operant chamber with transparent walls. The rats observed a moving object outside the accessible space. They should turn either left or right depending on whether the object located outside of the chamber moved leftward or rightward. They reported that hippocampal neurons exhibited object-position specific activity similar to the subject-position specific activity found in many previous studies. Thus, it is possible that the hippocampal

neurons provide signal carrying information about position of objects in inaccessible space with which other relevant signals, for example, representation of an object and reward, could be associated.

9.3 Pharmacological validation of the object-position recognition task

We have demonstrated that α1-adrenoceptor antagonist prazosin (3 mg/kg, i.p.) decreased the overall motor activity without affecting the cognitive performance in the object-position recognition task. The lower dose (2 mg/kg, i.p.) had no effect on the responding rate nor on the cognitive efficiency (Fig. 29).

The absence of the effect of prazosin on the responding rate at the 2 mg/kg dose might be due to the low responding rate in the corresponding control session. The rats assigned to this 2 mg/kg group tent to in general respond at lower rate than the rats assigned to the 3 mg/kg group.

Other studies investigated effects of prazosin in operant tasks. Overwhelming majority of these tasks assessed its effect on the responding rate and motivation. For instance, prazosin (0.5 mg/kg, i.p.) decreased responding rate (lever-pressing) in food self-administration operant tasks (Dwoskin and Sparber 1983, Zhang and Kosten 2005). However, the application of this drug at similar or higher doses (0.25-2 mg/kg, i.p.) did not reduce food self-administration in other studies (Forget et al. 2010, Lê et al. 2011). These dissimilar results could be explained by different schedules of reinforcement used in the studies mentioned above. The effect of prazosin on lever-pressing in operant food self-administration tasks was distinguishable only in experiments that applied higher fixed ratio (e.g. FR-15) in their experimental protocol. Prazosin also affects the rewarding effects of several drugs, e.g. nicotine, alcohol, cocaine and heroin (Zhang and Kosten 2005, Wee et al. 2008, Greenwell et al. 2009,

Forget et al. 2010, Lê et al. 2011, Verplaetse et al. 2012). Although the motivational processes for food-seeking and drug-seeking are not the same, the effect of prazosin on motivation is evident. In Experiment III, prazosin (3 mg/kg, i.p.) decreased the responding rate (to 55 ± 5 % of control; Fig. 29, upper right) which is in agreement with the general depressant effect of this drug on motivation and/or motor activity.

Several studies showed that prazosin do not alter spatial cognition in common behavioral tasks. Prazosin (0.5 mg/kg or 5 mg/kg, i.p.) did not impair cognitive performance in place and/or cue version of the radial arm maze, while the high dose increased the time to complete the cue task (Liao et al. 2002). This drug (at doses 0.1, 0.3, 1 and 2 mg/kg, i.p.) also did not induce cognitive deficit in retention of the hidden platform version of the Morris water maze, although the highest dose decreased swimming speed (Riekkinen et al. 1996). In agreement, we showed that prazosin had no effect on spatially-driven cognition although it decreased the motor activity in the object-position recognition task.

In a few studies, a non-specific effect of prazosin on performance in behavioral tasks was observed. Hahn and Stolerman (2005) reported that prazosin (1 mg/kg, s.c.) facilitated improvement in response accuracy induced by nicotine in the five-choice serial reaction time task. This could indicate positive effect of prazosin on visuospatial attention. However, the same dose decreased anticipatory responding (criterion that appears to be modulated by motivational processes) in this task. The authors explained this observation as an example of response-depressant effects of a pharmacological manipulation causing an “artificial” increase in accuracy. Therefore, better performance in the five-choice serial reaction time task after the application of prazosin in the presence of nicotine was caused by the negative effect on motivation and it cannot be assigned to the enhancement of visuospatial attention. Prazosin also

impaired performance in the active place avoidance task (Stuchlik and Vales 2008). The drug at the dose 4 mg/kg (i.p.) decreased locomotion of the rats as well as all behavioral measures of spatial cognition. The authors proposed that the impairment of cognitive performance was caused by altered motor activity rather than by impaired spatial cognition.

According to these findings, we could expect altered cognitive efficiency after the application of the dose of prazosin that affects responding rate in the object-position recognition task. However, the spatial performance of the rats in the present task was not significantly influenced by decreased motor activity induced by prazosin.

VI Conclusions

1) In Experiment I, we presented two versions of a behavioral task utilizing computer screen for stimuli presentation in which rats recognize position of an object located in an inaccessible space (object-position recognition task). In the first version of the task the object was stationary, in the second version it moved across the computer screen. We demonstrated that the rats solved both versions of the task using spatial information, i.e. position of the object.

2) In Experiment II, we showed that rats with inactivated dorsal hippocampus are impaired in the object-position recognition task while their performance in the brightness discrimination task is unaffected. Therefore, intact rats use hippocampus for recognizing position of a distant object located in the inaccessible part of the environment.

3) In experiment III, we validated the object-position recognition task with a drug with known pharmacological effects on spatial behavior and showed that prazosin has no effect on cognitive performance also in the present hippocampal-dependent object-position recognition task despite it decreased the responding rate.

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List of publications

impacted journals:

a) publications directly relevant to the thesis

Levcik, D., Stuchlik, A., Klement, D., 2013. Effect of block of α1-adrenoceptors on overall motor activity but not on spatial cognition in the object-position recognition task. Physiol Res, ACCEPTED. **IF = 1.531** (2012).

Levcik, D., Nekovarova, T.,Stuchlik, A., Klement, D., 2013. Rats use hippocampus to recognize positions of objects located in an inaccessible space. Hippocampus 23 (2), 153-161. **IF = 5.492** (2012).

Klement, D., **Levcik, D.**, Duskova, L., Nekovarova, T., 2010. Spatial task for rats testing position recognition of an object displayed on a computer screen. Behav Brain Res 207 (2), 480-489. **IF = 3.327** (2012).

b) publications not directly relevant to the thesis:

Fajnerova, I., Rodriguez Manchola, M., Brom, C., Bubenikova-Valesova, V., **Levcik, D.**, Svoboda, J., Horacek, J., Stuchlik, A., Vlcek, K. Spatial memory protocols in virtual human Morris water maze: a tool for translational approach. UNDER REVIEW.

non-impacted journals:

Vlček, K., **Levčík, D.**, Nedělská, Z., Laczó, J., Vyhnálek, M., Hort, J., 2011. Prostorová navigace jako kognitivní doména v diagnostice mírné kognitivní poruchy. [Spatial navigation as a cognitive domain in the diagnosis of mild cognitive impairment.] Psychiatrie 15 (2), 23-27.

Levčík, D., Klement, D., Nekovářová, T., Valeš, K., Stuchlík, A., 2010. Antagonista NMDA-receptorů MK-801 narušuje rozeznávání pozice vzdáleného objektu. [The NMDA-receptor antagonist MK-801 impairs recognition of position of a distant object.] Psychiatrie 14 (2), 15-18.

Levčík, D., Klement, D., Nekovářová, T., Petrásek, T., Stuchlík, A., 2009. Vliv antagonistů noradrenergních receptorů na rozeznávání pozice pohybujících se objektů. [The effect of noradrenergic receptor antagonists on recognition of position of a moving objects.] Psychiatrie 13 (4), 176-180.