Spatial working memory is one of the most complicated processes of the central nervous system. The organism needs to select and coordinate the relevant sensory inputs in order to survive in a constantly changing environment. In many neurological diseases, such as Alzheimer's disease and Parkinson's disease, spatial working memory is altered. Therefore, it is essential to understand the mechanisms of spatial working memory in various memory tasks. Although there are few useful methods, which have previously been identified to evaluate the effects of drugs in working memory, avoidance-based visuospatial working memory tasks for understanding the cognitive functions, are very limited in the literature. In the present study, we designed a novel avoidance-based visuospatial working memory task. The main goal in this method is to force the mouse for using his peripheral and central sensory inputs and motor skills against an aversive stimulus. In order to validate this task, we administered the following standard reference drugs (scopolamine, piracetam and physostigmine) at their effective doses. Pretreatment with scopolamine did not change the acquisition time, but it increased the retention time and retention errors. On the other hand, pretreatment with piracetam interestingly increased the acquisition and retention time, but it decreased the retention errors when compared to scopolamine group. Furthermore, pretreatment with physostigmine alone decreased the acquisition and retention time, as well as the retention errors. In scopolamine + physostigmine group, the drug combination did not change the acquisition time, but physostigmine decreased the retention time and retention errors-induced by scopolamine. Our results clearly show that the ladder avoidance task can be used in visuospatial working memory studies in rodents.

Keywords: Visuospatial Working Memory; Physostigmine; Scopolamine; Piracetam; Mice.

Introduction

Spatial working memory is one of the most complicated processes of the central nervous system. The organism needs to select and coordinate the relevant sensory inputs in order to survive in a constantly changing environment [1]. Processes begin when the organism collects and stores sensory cues from the environment and manipulates these information for a variety of cognitive and remembrance tasks. Those sensory cues are usually visual information in most common maze studies [2, 3]. Such as in the Morris water maze, which is one of the most common preclinical models for spatial working memory, the animal tries to find a submerged invisible escape platform in a circular water tank located at a fixed place within the laboratory (environment) [4-7]. Moreover, in the radial arm maze, another commonly used preclinical model, the animals which were previously put on dietary restriction are expected to find the reinforcements (such as peanut) at the ends of the eight arms radiating out from a central hub by using objects placed around the laboratory for reference. The goal of this task is to enter all eight arms and eat the reinforcements without re-entering an arm, which also requires an intact and healthy hippocampus [8-10]. Unlike the previous methods, the Y-maze is much simpler task for determination of spatial recognition based on the ability of rodents to explore novel environments where the Y-shape arms have different cues [11]. Since then, investigators have modified these tasks, i.e. the paddling pool escape task, where the animals are expected to find the correct tubing as a means of escape back to their home cage [12], and radial arm maze in the water tank [13].

As it has been previously described, spatial working memory is a system that involves an immediate processing of the environmental cues which are distinguished or sensed and analyzed by an individual when he/she interferes with a spatial task [14]. Moreover, in many neurological diseases, such as Alzheimer's disease, Parkinson's disease and schizophrenia, spatial working memory is altered [15–18], and some of the drugs used in such disorders could help to treat the impairment of working memory [19]. Therefore, it is essential to understand the mechanisms of spatial working memory in...
various memory tasks as well as in various neurological disorders.

Recently, it has been shown that repeated exposure to sensory stimuli, with or without reward may induce stimulus-specific modifications of learning and memory which can be altered by visual, auditory, somatosensory and olfactory systems in vertebrates [20]. Moreover, Takatsu-Coleman et al. [21] reported the effects of a mild and acute stimulus, such as foot shock, combined with environmental cues could improve spatial memory in the plus-maze discriminative avoidance task.

In the present study, we designed and developed a novel avoidance-based visuospatial working memory task, the ladder avoidance task. The main goal of this method is to force the mouse to use his peripheral and central sensory inputs and motor skills by combining the environmental cues against those mild stimuli.

**Materials and methods**

**Animals**

Male albino mice weighing 25-30 g obtained from Cukurova University Medical Sciences Research Center were used in the experiments. They were housed (5 mice per cage) in an environmentally controlled vivarium under 12 h light-dark cycle, and provided with commercially available food and water ad libitum. Mice were allowed 1 week to adapt to the laboratory before use. Experiments were conducted between 09:00 to 11:00 h in a semi-soundproof laboratory. All procedures conformed to NIH guidelines and were approved by the University of Cukurova Animal Care and Use Committee.

**Drugs**

Following drugs were chosen as reference drugs in this task. Scopolamine HBr (S-1875, Sigma), piracetam (P-5295, Sigma) and physostigmine salicylate (58523, MacFarlan Smith) were used in the experiments. Scopolamine (1 mg/kg) and physostigmine (0.05 mg/kg) were dissolved in isotonic saline and injected intraperitoneally in a volume of 0.1 mL / 10 g body weight. Piracetam (2 g/kg) was also dissolved in isotonic saline and given orally in a volume of 0.3 mL / 10 g body weight. These doses were chosen according to our preliminary studies [22, 23]. Scopolamine and physostigmine were given 30 min before the acquisition trial, and piracetam was given 60 min before the trial. In scopolamine-physostigmine combination group, scopolamine was injected 10 minutes prior to physostigmine. If the scopolamine was injected in the left abdomen, piracetam was injected in the right abdomen, or vice versa. Control animals were received saline injections.

**Evaluation of visuospatial working memory: the ladder avoidance task**

The ladder avoidance task was designed and developed by Dr. Inan. The apparatus is a transparent Plexiglas cubic chamber (30 cm x 30 cm x 30 cm) which has an open top and an open bottom (Fig. 1A). Four conductive ladders with three steps each were mounted in the middle of the sidewalls of the apparatus (Fig. 1B). The black triangle on the top of the 4th ladder is a visual cue for the escape route (Fig. 1A-B). The chamber was then placed on to a hot-plate which provides a mild and stressful stimulus. The temperature of the hot-plate was set to 45.0 ± 1.0 °C, and a continuous electric current (50 Hz, 1 mA) was delivered to each ladder by an isolated stimulator, except the escape ladder. On the habituation session, each mouse was gently handled and placed into the task individually and allowed for 10 minutes to explore the apparatus. During the habituation session, no heat and no current were delivered. The apparatus was cleaned with 5% ethanol solution after each trial. Twenty-four hours after the habituation session, each mouse was

Figure 1. A transparent Plexiglas cubic chamber (30 cm x 30 cm x 30 cm) which have an open top and an open bottom (A). Four conductive ladders with three steps each were mounted in the middle of the side walls of the apparatus (B). The triangle on the top of the 4th ladder is a cue for the escape route (escape ladder).
then placed into the apparatus and they were expected to find and climb the escape ladder. This session was called as acquisition trial which the animals experienced the aversive stimulus by approaching the false ladders. During this session, the animals also learned the escape ladder's spatial location by getting information from the environment. The time (seconds), taken for the mouse to climb the escape ladder was recorded as acquisition time. After the acquisition session, each mouse was then placed into its home cage for 30 minutes. During this resting period, the chamber was relocated by turning it to 180 degrees clockwise in order to change the location of the escape ladder and visual cue. In other words, if the escape ladder were on the west, it would be on east by changing the location. Then, each mouse was again placed into the chamber individually and the time, taken for the mouse to climb the escape ladder was recorded as memory retention time. During this session, the stimulus was given. In addition, false approaches to the other ladders were also recorded as the memory retention errors.

Statistical Analysis

Results are presented as means ± S.E.M. Statistical analyses were performed with SPSS statistical software (Version 14.0, SPSS Inc., Chicago, Il). Behavioural data were analyzed by One-way analysis of variance (ANOVA) followed by a post-hoc Tukey HSD test. The minimum level of statistical significance was set at P<0.05.

Results

The effects of saline (N=8), scopolamine (N=7), piracetam (N=7), physostigmine (N=8) and scopolamine+physostigmine (N=8) on acquisition time, retention time and retention errors are shown in Figure 2, 3 and 4 respectively. Pretreatment with scopolamine (1 mg/kg) did not change the acquisition time (P>0.05), but it significantly increased the retention time and retention errors when compared to saline group (P<0.05). On the other hand, pretreatment with piracetam (2 g/kg) interestingly increased the acquisition and retention time (P<0.05; piracetam group vs. saline group, and piracetam group vs. scopolamine group), but it decreased the retention errors when compared to scopolamine group (P<0.05), and increased them when compared to saline group (P<0.05). Furthermore, pretreatment with physostigmine (0.05 mg/kg) alone decreased the acquisition and retention time, as well as the retention errors (P<0.05; physostigmine group vs. saline group, and physostigmine group vs. scopolamine group). In scopolamine+physostigmine group, the drug combination did not change the acquisition time (P>0.05), but physostigmine in this combination significantly decreased the retention time and retention errors-induced by scopolamine when compared to both saline and scopolamine groups (P<0.05).
Discussion

Working memory, usually refers to a system of limited capacity which temporarily keeps information, such as a telephone number or a location, in mind and process that information for an incoming event [24, 25]. Moreover, it is very important to have the ability to process a visual information when that information are changed place in space [26]. During the processing of the information, working memory may also retrieve past events and/or experiences from long-term memory in order to solve a cognitive task [27]. In the mean time, the organism collects and stores sensory cues from the environment, which usually are visual or verbal information [2, 3].

The term visuospatial working memory has been first attributed by Boller [28] in patients with Parkinson’s disease [29]. Since then, it has been shown that hippocampus and prefrontal cortex are the main structures which play significant role in the mechanisms of visuospatial working memory [30, 31].

In the present study, we demonstrated a unique visuospatial working memory task, the ladder avoidance task, in mice by applying two different aversive stimuli, the heat and the electric current. During the habituation period, mice explored the new environment of the task without receiving any stimulus. Twenty-four hours later, the acquisition trial, they experienced and learned how the task works by receiving the aversive stimulus. In addition, they also learned the escape ladder’s location, by getting information from the environment. That information, namely the cue, was a black triangle on the top of the escape ladder. The first aversive mild stimulus, the heat, might actually be alike as light in the passive avoidance test [33] which forces the animal to move in a secure compartment. In other words, it stimulates the first action. In addition, the second aversive mild stimulus in our task, the electric current on the three ladders, leads the animal to choose the correct ladder to escape. Thus, each cue in this task represents a different parameter, such as visual information, spatial location and working memory, and allows the animals to process all available data for successful performances.

In order to validate our task, we administered standard reference drugs which have learning and memory altering potency in rodents [22]. As it is discussed in the results section, pretreatment with scopolamine, a well known amnesic and anticholinergic agent [32], did not change the acquisition time, but it significantly increased the retention time and retention errors. On the other hand, pretreatment with piracetam, a nootropic agent increased the acquisition and retention time. This effect might be task specific, since piracetam and its analogues have been found to be effective in most common maze studies [33, 34], but ineffective when given alone in spatial memory related tasks [35, 36]. In the present study, piracetam decreased the retention errors when compared to scopolamine group. The fact is, piracetam seems to have variable properties and most probably not a good choice of drug in working memory related studies. This is because, piracetam has recently been found ineffective in the acquisition phase (day 1, 2 and 3) of Morris water maze [37], one of the most reliable working memory assessing tasks in rodents, and learning and memory performances in the passageway water maze test [38] and step-down passive avoidance test [38, 39] in SAMP8 mice which have been using in aging and age-associated diseases. Furthermore, pretreatment with physostigmine, an acetylcholinesterase inhibitor decreased the acquisition and retention time, as well as the retention errors. In scopolamine+physostigmine group, the drug combination did not change the acquisition time, but physostigmine in this combination significantly decreased the retention time and retention errors-induced by scopolamine. Taken together, our data from scopolamine, physostigmine and scopolamine+physostigmine groups are consistent with previous results related to spatial working memory [40-43], which also proves the validation of our novel ladder avoidance task.

In conclusion, the ladder avoidance task can be used in visuospatial working memory studies in rodents as an alternative task to common mazes, such as radial arm maze or water maze.

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