Research report

The COGITAT holeboard system as a valuable tool to assess learning, memory and activity in mice

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ABSTRACT

The comprehensive and stress-free assessment of various aspects of learning and memory is a prerequisite to evaluate mouse models for neuropsychiatric disorders such as Alzheimer’s disease or attention deficit/hyperactivity disorder (ADHD). COGITAT is an automated holeboard system allowing simultaneous assessment of spatial working and reference-memory performance which we have adapted in this study to enable its usage with mice. The holeboard apparatus consists of an open-field chamber with a 25-hole floor insert, each hole being monitored by infrared light beams, located on three different levels, allowing the distinction between visits of holes, i.e. the animal reaches the bottom of the hole, or inspections, which means only superficial exploration of the hole. Across trials, animals learn a pattern of five baited holes. Here, we show that C57BL/6 mice readily acquire this task within 5 days when submitted to six trials per day. A number of individual parameters – overall exploratory activity, number of visits into or inspections of holes, number of baited, unbaited, or previously baited holes visited or inspected, reinspections of or revisits into any holes, number of pellets eaten, time to find pellets, and reference and working memory errors—are obtained simultaneously and results are immediately available after the end of each experiment. The muscarinic antagonist scopolamine impaired task performance, while the cognitive enhancer metrifonate (an acetylcholinesterase inhibitor) reduced error rates. Overall, our data indicate that this spatial learning task will be useful to characterize spatial memory in various genetic or pharmacological mouse models.

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1. Introduction

Learning and memory processes are thought to underlie a variety of human psychiatric disorders, including neurodegenerative disorders such as Alzheimer’s disease but also attention deficit/hyperactivity disorder (ADHD). A number of behavioral paradigms have been developed over the past decades to assess spatial learning and memory performance in rodents. Commonly used in this regard are mazes, including the Y-Maze, radial-arm maze and water-maze [1–3]. These paradigms have provided many insights into the neurobiological mechanisms and the neuroanatomical substrates of learning and memory. However, the results are often inconsistent from one experiment to another [4] and from one laboratory to another [5]. Furthermore, different testing procedures have had to be used to measure various cognitive and motor capabilities, working and reference memory and serial learning. These sequential studies interfere with each other and are bound to involve different circumstances and environmental conditions, which will affect the animals’ behavior in different ways and hamper a strictly comparable and complete view of the animals’ memory function.

Holeboard learning paradigms for rats have been described previously [6–8]. Previous studies indicated that mice are able to learn to retrieve one food pellet located in a holeboard task setup [9,10]. However, the simplicity of the task used precludes direct assessment of reference- and working-memory performances. Kuc et al. adapted a protocol similar to the one published by Van der Stay et al. [8,11]. However, their setup is limited in picturing the animals’ behavior in detail, as it is unable to distinguish between superficial head dips and deep inspections of a visited hole. In an attempt to overcome these problems, we have modified an elaborated holeboard system for rats, named COGITAT system [6]. Our goal was to modify and validate this system to be suitable for mice, and allow-
**Table 1**

**Variable** | **Definition/explanation**
--- | ---
**Activity** |  
Total distance | Total distance traveled in the course of one trial (cm)
Overall inspections | The sum of inspected and visited holes, with and without food collection
Upper light beam disruptions | The total number of disruptions of upper light beams in one trial
Lower light beam disruptions | The total number of disruptions of lower light beams in one trial
**Acquisition** |  
Time to find pellets | The time it took to find the pellets within a time limit
Pellets eaten | The number of pellets eaten
**Errors** |  
Reference memory errors, total | The percentage of the sum of inspections and visits to previously baited holes in relation to the total number of holes inspected and visited
Working memory errors, total | The percentage of the sum of inspections and visits to unbaited holes in relation to the total number of holes inspected and visited
completed as soon as the animal had collected and eaten all of the pellets within the allotted time span of 240 s or when the time span had elapsed, whichever came first. Spatial cues available for the animals inside the holeboard enclosure were the entrance with the starting box, the four corners of the enclosure, the upper edges of the 25 holes, and the side walls for orientation with the vibrissae (thigmotaxis) as tactile stimuli. Outside the enclosure, distal cues visible through the transparent Plexiglas walls were, besides the experimenter (who maintained the same position throughout the experiments), a wall, a window, a black curtain and a rack with the cages of the remaining experimental animals. In the center above the hole board the video camera (VideoMot2, TSE Systems, Bad Homburg, Germany) was attached. Each animal was subjected to six trials per day. During the first experimental session, a trial duration of 240 s provided the animals with the opportunity to explore the hole board and to find the five hidden pellets. 

2.5. Statistical procedure

For statistical evaluation a repeated measures analysis of variance (ANOVA), with Greenhouse-Geisser adjustments (groups and trials as factors), was used for each variable. Scheffé tests served as post-hoc-analyses of between group differences. The results are displayed as means ± SEM of the individual trials of the corresponding experimental periods.

3. Results

During the animals’ acquisition of this learning task, the COGITAT system automatically provides 23 different parameters about the animals’ overall exploratory behavior and their learning and memory capabilities in a comparison between vehicle, scopolamine and metrifonate pretreated animals. Additionally, we measured the total distance moved by the animals by means of video tracking software (VideoMot2, TSE Systems, Bad Homburg, Germany). From all available parameters, we chose 8 variables to distinguish between three different behavioral domains: Activity, Acquisition and Errors. The Activity domain comprises the variables total distance moved, overall inspections as well as the frequency of the disruptions of the upper and lower lightbeams (ulb, lib, respectively). For the Acquisition domain, the variables time to find pellets and pellets eaten were taken. Finally, for the Error domain we looked at the reference memory error (rme) as well as at the working memory error (wme). The reference memory error is defined as the number of visits of unbaited holes in relation to the cumulative number of upper light beam disruptions. The working memory error is defined as the number of visits of previously baited holes in relation to the cumulative number of upper light beam disruptions.

3.1. Activity

Fig. 2 shows the general activity of the mice during the task. All three groups readily searched for the hidden pellets. Fig. 2a shows the total distance moved by the animals while searching for the food pellets. The data reveal significant effects for trial (F(2, 36) = 1.794, p < 0.001) and group (F(2, 36) = 8.902, p = 0.009) with scopolamine treated animals moving longer distances as compared to metrifonate treated animals (p = 0.012). No between group differences can be seen in the latency to enter the holeboard (F(2, 36) = 0.086, p = 0.919, data not shown).

Repeated measures ANOVA of overall inspections (Fig. 2b) revealed significant effects for trial (within-subject effect: F(2, 36) = 26.562, p < 0.001) and trial x group interaction (F(2, 36) = 3.520, p < 0.001) as well as significant differences between groups (Fig. 1a, F(2, 36) = 25.275, p < 0.001): scopolamine treated animals showed increased levels of activity when compared to metrifonate (p < 0.001) and vehicle treated (p < 0.001) animals.

When looking at inspections of the holes at the upper lightbeam stage (Fig. 2c), we also found significant effects for trial (within-subject effect) (F(2, 36) = 23.060, p < 0.001) and trial x group interaction (F(2, 36) = 6.156, p < 0.001) as well as significant between-group differences (F(2, 36) = 7.571, p = 0.002). Metrifonate treated animals exhibit fewer hole visits at the ulb stage as compared to vehicle controls (p = 0.014) and scopolamine treated animals (p = 0.004). There were no differences between scopolamine and vehicle treated animals.

In contrast, at the lower lightbeam stage (Fig. 2d) the within-subject effect for trial (F(2, 36) = 15.304, p < 0.001) and the trial x group interaction (F(2, 36) = 5.294, p < 0.001) are significant, but no between-group differences could be observed (F(2, 36) = 2.844, n.s.). Consequently, animals from the three pharmacological groups did not differ for this variable.

3.2. Acquisition

Fig. 3 shows the general ability of mice to learn the task (Fig. 3a displays the time needed to complete the task, while Fig. 3b shows the number of pellets found by the animals). A training or learning effect is present in all three groups over the time course for both acquisition related variables [main effect for trial (within-subject effect), time to find pellets (F(2,36) = 39.732, p < 0.001) and pellets eaten (F(2,36) = 44.615), p < 0.001]. There were also significant trial x group interactions for both variables [trial x group, time to find pellets (F(2, 36) = 7.037, p < 0.001) and pellets eaten (F(2, 36) = 4.526), p < 0.001]. In addition, we found significant between-group differences for both variables [main effect for group (between subjects effect), time to find pellets (F(2, 36) = 16.576), p < 0.001] and pellets eaten (F(2, 36) = 16.798), p < 0.001).

Post Hoc Scheffé tests revealed significant differences between the scopolamine treated and the vehicle groups for both variables with scopolamine treated mice taking more time to find pellets (p = 0.003) and eating fewer pellets (p = 0.001) compared to vehicle treated mice. There was also a significant difference between scopolamine and metrifonate treated mice for both variables, with metrifonate treated mice taking less time to find pellets (p < 0.001) and eating more pellets (p < 0.001) than scopolamine treated mice. No difference between metrifonate and vehicle treated mice was observed.

3.3. Errors

Repeated measures ANOVA for reference memory error (rme) revealed a significant main effect for trial (within-subject effect rme (F(2, 36) = 24.182), p < 0.001) and a significant trial x group interaction: group rme (F(2, 36) = 6.908), p < 0.001), shown in Fig. 4a. There also is a significant main effect for group (between-subjects effect rme(F(2, 36) = 4.728), p = 0.021) with vehicle treated animals making more reference memory errors than metrifonate treated animals (p = 0.025).

The repeated measures ANOVA for working memory error (Fig. 4, wme) also reveals a significant main effect for trial (inner subject effect wme (F(2, 36) = 10.039), p < 0.001, a significant trial x group interaction (trial x group wme (F(2, 36) = 7.977), p < 0.001) and a significant main effect for group (between-subjects effect wme (F(2, 36) = 46.488), p < 0.001). All of the 3 wme Post Hoc Scheffé tests reveal significant effects: metrifonate treated animals commit fewer working memory errors than vehicle treated animals (p < 0.001), and vehicle treated animals commit fewer working memory errors than scopolamine treated animals (p < 0.001). Finally, scopolamine treated animals commit more working memory errors than metrifonate treated animals (p < 0.001).

4. Discussion

Using the novel modified COGITAT holeboard/videotracking system, we were able to detect changes in behavioral measures related to exploration and memory. While some of the obtained
parameters can also be obtained by other methods, such as the water maze or the radial arm maze, it is essential to stress that there is a fundamental difference between the water and radial arm mazes on one and the hole board on the other side. In the water maze, stress forces the animals to use the best strategy enabling the animal to escape from the water as quickly as possible [2,16]. In contrast, both the radial arm maze and the hole board offer the animals the opportunity of exploring a new environment and finding food. On the holeboard in particular, mice display exploratory [11,17] as well as food-searching behavior [11] without being forced to. Importantly, the system described here differs from conventional holeboards in that infrared beams are positioned on three levels of the holes. This provides the possibility to determine precisely whether mice explore parts of the board only superficially, perform deep inspections or eat the hidden pellet. However, there are big differences between rats [6] and mice in terms of motivation to enter the holeboard and actively search for the pellets, which was a crucial point when establishing this task. A restricted daily feeding procedure during the experiments had to be established to motivate the animals to perform the task. In contrast to rats, mice still engaged in the search for pellets if they expected to receive their entire daily food ration after the trials. In contrast, rats act the opposite way, as they do not search for pellets when their daily ration of food is given at the end of the trials [6]. Therefore, the COGITAT procedure, which has been previously only used in rats [6], had to be modified slightly to meet the specific requirements of mice. These modifications included the above mentioned changes in the feeding procedure of the animals. However, when the animals were motivated to search for the hidden food pellets, it was not only possible to measure differences in working memory (which can also be done in the water maze), but simultaneously also in reference memory. It has to be mentioned that food restriction can be seen as a stressful factor that might influence behavioral testing, especially when it is as complex as a learning task. However, there are studies reporting better performance in comparable tasks (radial maze) in mice that were calorically restricted in a manner similar to our food restriction paradigm (however this was even done over a longer period of time as compared to our study) as opposed to mice that were allowed to feed ad libitum [28]. This data suggests that the more ecologically valid way of offering food in a restricted manner improves cognitive abilities in mice.

As C57BL/6 mice are generally viewed as “good performers” in spatial learning tasks [18], and as they are a common background for genetic modification and thus are often used in behavioral paradigms, we decided to use this mouse strain for our validation experiments. To disrupt learning, we used the anticholinergic compound scopolamine, known to cause cognitive impairment [12,13], and the acetylcholinesterase inhibitor metrifonate as a cognitive enhancer [14,15]. The effective periods for both compounds encompassed at least the approximately 180 min of testing per day [19,20]. Scopolamine, a non-selective muscarinic antagonist, has been shown to induce performance impairments in a variety of learning and memory tasks in rodents [11,21,22]. In this study, daily pretreatment with doses of 0.1, 0.3 and 1.0 mg/kg of scopolamine resulted in significant performance deficits in C57BL/6 mice. The highest dose, however, also induced a significant decrease in activity during training (distance travelled, not shown) which is consistent with previously reported results [11,23]. Hence, we opted to use the lowest dose of 0.1 mg/kg, as this dose resulted
Fig. 3. Data on the Acquisition behavioral domain. These panels show the general ability of mice to learn the task. (A) Shows the time taken to find all hidden food pellets in the given time of 240 s per trial, whereas (B) illustrates how many of the 5 hidden pellets were found within the 240 s time span. Full circle, metrifonate 50 mg/kg i.p.; triangles, scopolamine. 0.1 mg/kg s.c.; open circle, vehicle.

in spatial learning impairments without inducing any motor side effects.

In contrast to scopolamine, daily pretreatment with metrifonate enhanced the holeboard performance of C57Bl/6 mice. As can be seen in Fig. 2a, metrifonate and vehicle treated animals had a significantly decreased total distance moved on the holeboard, as they found more hidden pellets (Fig. 3b) in a shorter period of time (Fig. 3a), indicating more effective search strategies. This assumption is backed up by the overall inspections of a hole variable, as the scopolamine treated animals inspected holes significantly more often than the two other groups (Fig. 2b–d). Increasing the number of inspections increased their chances of finding pellets, although this required increased effort and lacked a sophisticated search strategy. This increase in speed diminished their attention to the task and to the pattern and resulted in a higher rate of inspections of unbaited holes. As a consequence, obvious reference memory deficits are present (see Fig. 4) in scopolamine treated animals, for which they are, however, able to compensate by an increased locomotor and exploratory activity, as can be seen in Fig. 2a. However, this compensation does not correlate to learning success—measured as the number of pellets consumed—as compared to vehicle or metrifonate treated animals (Fig. 3a). These findings correspond well to a similar setup used by Kuc and associates [11]. With respect to working memory error, scopolamine treated animals performed poorer compared to vehicle treated animals. In contrast, metrifonate treated animals performed superior on days 4 and 5 (Fig. 4a) arguing for the importance to measure reference memory performance. It could be argued that the weaker performance of scopolamine treated animals might be a motivational problem resulting from an attenuated feeling of hunger. However, scopolamine treated mice not only searched longer (which could be an argument for being less hungry) but also made more mistakes and were more active which clearly suggests that they were trying to find the pellets. In the end, also scopolamine treated mice were able to complete the task almost as well as the two other groups, which again suggests that the problem does not result from decreased motivation. Taken together, this set of experiments demonstrate that our system is able to disen-
Fig. 4. Data on the Errors behavioral domain. Two forms of errors are depicted. (A) Shows the percentage of the sum of inspections and visits to unbaited holes in relation to the total number of holes inspected and visited (reference memory errors), whereas (B) shows the percentage of the sum of inspections and visits to previously baited holes in relation to the total number of holes inspected and visited (working memory errors). Full circle, metrifonate 50 mg/kg i.p.; triangles, scopolamine 0.1 mg/kg s.c.; open circle, vehicle.

tagle various aspects of learning and memory not accessible by more simplistic paradigms, thereby allowing better interpretation of the animals’ behavior especially important in mouse models of cognitive disorders.

When discussing the results of behavioral tests, housing and lighting conditions during testing should always be considered as influencing variables, especially as we conducted the experiments presented here during the light (and therefore inactive) phase of the day. We have however shown in a previous study [24] that the time of day of the behavioral testing does not have a large effect on results. Furthermore, many studies (see for example [25]) demonstrated that learning and memory can reliably be tested in the light phase, and thus we are confident that our results are consistent. As for the housing conditions, mice were singly housed for the experiments, and thus it could be argued that this isolation causes cognitive deficits. However, one study comparing housing effects on the forced swim test and the tail suspension test found higher occurrence of depression-like behavior in mice that were housed in groups and as a consequence were subjected to social stressors (as opposed to single housed mice) [26]. Moreover, group housing increases phenotypic variance [27] which can be a problem in behavioral testing.

5. Conclusion

In summary, the COGITAT holeboard appears to be well suited for testing mice and allows a direct assessment of reference- and working-memory performance simultaneously. Here, we used established pharmacological interventions to depict the ability of c57Bl/6 mice to complete a learning task. The automatic and computerized simultaneous analysis of a variety of behavioral parameters, together with their statistical and graphical presenta-
tion, offers an objective characterization of cognitive, memory, and learning capabilities. It also enables to measure learning and activity parameters in a single experiment, substantially saving time and simplifying experimental protocols.

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References