이학석사학위논문

마우스를 위한 터치스크린 기반 paired associates learning (PAL) task의 최적화와 이 task의 배측 해마에 대한 의존성

Optimization of the touchscreen-based paired associates learning (PAL) task for mice and the dorsal hippocampal dependency of the task

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생명과학부

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Optimization of the touchscreen-based paired associates learning (PAL) task for mice and the dorsal hippocampal dependency of the task

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A dissertation submitted to the Graduate Faculty of Seould National University in partial fulfillment of the requirement for the Degree of Master of Science

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Abstract

Optimization of the touchscreen-based paired associates learning (PAL) task for mice and the dorsal hippocampal dependency of the task

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Two versions of the touchscreen paired-associate learning (PAL) task have been developed for rodents: same PAL (sPAL) and different PAL (dPAL). These tasks have been used to test object-location memory in different studies, and are also crucial in studying murine models of Alzheimer's disease and schizophrenia. However, the relatively long time needed for the tasks (approx. 50 days for mice) limits their widespread use. By giving training that was more intensive with a higher

number of trials, we shortened the time required for learning saturation in sPAL and dPAL to about one third of the time required for the generally used protocol. Furthermore, we developed a new simplified version of sPAL, termed 2-object sPAL by applying a reduced number of objects and trial types for sPAL. In this task, mice could reach learning saturation fast in 6 days. Our pharmacological experiments indicate that the function of the dorsal hippocampal CA1 region is necessary for the performance of the two PAL tasks with the new conditions and the new 2-object sPAL. This work has considerably improved the usefulness of the touchscreen PAL tasks to increase the learning rate, but they remain highly hippocampus-dependent object-location memory tasks.

Keywords: Touchscreen, operant conditioning, paired-associate learning, object-location memory, mouse, hippocampus, cognition

Student Number: 2014-25004

Introduction

Among many neuropsychological tests in the Cambridge Neuropsychological Test Automated Battery (CANTAB), the object-location paired-associate learning (PAL) test was shown to be the best test for classifying individuals as patients of Alzheimer's disease or healthy control individuals (Blackwell et al., 2004; Swainson et al., 2001). In addition, patients with schizophrenia also show impairments in performing this task (Barnett et al., 2005). To study these diseases more thoroughly with animal models, the PAL task has been adapted into a touchscreen-based behavioral task for rodents. The touchscreen testing method was developed as a novel automated behavioral procedure for assessing various cognitive functions in rodents (Brigman et al., 2013; Bussey et al., 1997a; Bussey et al., 1997b; Bussey et al., 1994; Bussey et al., 2008; Bussey et al., 2001; McTighe et al., 2013) and the PAL task is one of the most frequently used tasks in this system. Because the PAL task is performed in this automated system, many of the advantages of this system are those of the PAL task. It produces more reliable data, does not put much stress on experimental animals, and the findings from this task can be easily translated to humans because this task can be performed with humans as well (Bussey et al., 2012; Horner et al., 2013;

Nithianantharajah et al., 2015).

Two versions of the touchscreen PAL task, same PAL (sPAL) and different PAL (dPAL), have been developed recently to test object-location memory (Talpos et al., 2009) and used widely in rodents. First, these tasks were applied in rats in some studies (Kumar et al., 2015; Talpos et al., 2014). The dPAL task was shown to be sensitive to pharmacological inhibition of hippocampal activity (Talpos et al., 2009). In addition, this task was used to test the effect of antagonism of various N-methyl-D-aspartate (NMDA) receptor subtypes in rats (Kumar et al., 2015). The PAL task has also been used in mice in various studies (Bartko et al., 2011b; Delotterie et al., 2015; Kim et al., 2015). To test if hippocampal neurogenesis is important to perform a spatial task, the PAL task was performed in mice with ablated neurogenesis (Clelland et al., 2009). This automated task was also tested with transgenic mice, in which some genes related to psychiatric disorders were knocked out (Bartko et al., 2011a; Nithianantharajah et al., 2013).

Nevertheless, compared with other conventional memory tasks for rodents, like the Morris water maze test, the touchscreen PAL tasks have not been widely used. The long time

required to perform sPAL and dPAL may be one reason why the usage of these tasks has been limited. In typical cases, for the main task itself, it takes 50 days to finish the experiment because mice reached learning saturation after performing 50 sessions (one session per one day) (Clelland et al., 2009; Delotterie et al., 2014; Horner et al., 2013; Nithianantharajah et al., 2013). In these studies, mice performed 36-trial sessions for the PAL training. In the present study, in order to shorten the experimental period and improve the usefulness of PAL tasks, we firstly introduced training that was more intensive with 60 trials per session for a more rapid acquisition rate. Furthermore, for the cases in which faster and simpler PAL tests may be more suitable, we developed a simplified form of the sPAL task. Our goal in doing this was to make a touchscreen PAL task that requires a similar period of time as the Morris water maze test, in which about 12 days are needed to perform the whole task, including the handling procedures (D'Hooge and De Deyn, 2001; Lee et al., 2014; Vorhees and Williams, 2006).

Finally, we tested whether the PAL tasks with the new conditions are hippocampus-dependent. The dPAL task has previously been shown to rely on hippocampal function in rats

and mice (Delotterie et al., 2015; Kim et al., 2015; Talpos et al., 2009). In these studies, rats or mice learned the task first, and were then tested again following hippocampal lesioning or intrahippocampal infusion of various drugs that inhibited hippocampal activity. Similarly, we also tested whether the hippocampus is required to perform the more intensive dPAL task with a greater number of trials. In the case of sPAL, the hippocampal dependency had previously been tested in rats; however, the performance was not affected by pharmacological manipulation of the hippocampus (Talpos et al., 2009). Therefore, we tested whether the function of hippocampus is crucial in the sPAL task in mice. To our knowledge, this is the first attempt to test the involvement of the hippocampus in the sPAL task in mice. In addition, we also tested the hippocampal dependency of the newly developed 2-object sPAL.

Methods

Animals

Eight- to ten-week-old male C57BL/6N mice were used (Orient Co., Korea). After their arrival, they were caged in groups of four, and food and water were available *ad libitum*. The mice were maintained on a 12- h light/dark cycle. Food restriction started 2 days before the beginning of pretraining for touch screen testing. The weights of the mice were maintained at around 85% of their initial weights throughout the behavioral experiments.

Apparatus

We used the Bussey-Saksida touch screen operant chamber (#80614) for touch screen testing (Campden Instruments Ltd., UK), with previously reported chamber settings (Horner et al., 2013). In order to operate the touchscreen system and collect data, we used the Whisker server and ABET software, which were provided by Campden Instruments. Stimuli of the same size and images (flower, spider, and airplane) were used in the sPAL, dPAL, and 2-object sPAL tasks. Because the 2-object sPAL has only two trial choices, the flower and airplane images, and not the spider image, were used. For liquid reward, liquid condensed milk (Seoulmilk, Korea) mixed with an equal volume

of tap water was used.

Touchscreen pretraining

After starting food restriction at the age of 9-10 weeks, mice were handled for 2 consecutive days. The mice were then habituated to the touchscreen operant chambers in a 10-minute session without any signals for 1 day. The next day, mice were habituated to the operant chambers with access to the liquid reward. The liquid reward (8 μl) was given every time the mouse's nose poked the illuminated reward magazine. This second habituation protocol continued for 40 minutes. After the habituation protocols, touchscreen pretraining was started in order to progressively train mice to touch visual stimuli on the screen and receive the reward. The pretraining comprised of three stages, initial touch, must touch, and incorrect punishment.

In the initial touch training, mice gained the liquid reward (8 μl) by touching stimuli of various shapes appearing on one of the three windows of the screen. The liquid reward was given alongside delivery of an auditory tone. If mice did not touch the presented stimulus for 30 seconds, then the liquid reward was given with the disappearance of the stimulus. After mice consumed the liquid reward, another trial was

started automatically after 20 seconds. The initial touch stage was performed for only 1 day for all mice.

Next, in the must touch training, a trial began when the mouse's nose poked the illuminated reward magazine. Then, a square stimulus was displayed on one window, and the liquid reward was given only when mice touched the stimulus. There was an inter-trial interval (ITI) of 15 seconds after each trial. When a mouse finished 60 trials within 60 minutes for 2 out of 3 days, the final pretraining stage, incorrect punishment, was started. Mice performed the must touch training for at least for 3 days.

In the last stage of pretraining, incorrect punishment, two elements were added to the must touch training. Firstly, if the mice touched a window with no stimulus (blank), a time-out was given for 5 seconds. Secondly, when the mice touched the blank window, a correction trial was started after the time-out and ITI. In the correction trial, the square stimulus was displayed in the same position as the previous one. If the mice showed a correct response in the correction trial, another normal trial was administered. Only the responses in the normal trials were counted for the calculation of percentage of correct responses. A session finished when each

mouse completed 30 trials or 60 minutes had elapsed. When mice attained 75% accuracy for 2 out of 3 days, the mice went on to the PAL tasks.

sPAL and dPAL

The basic rules for these two tasks were the same as previously described (Talpos et al., 2009). Just as in the must touch training, a trial started in these tasks when the mouse's nose poked the illuminated magazine. In each task, six different combinations of stimuli were presented. In these combinations, the flower on the left window, the spider on the middle, and the airplane on the right were the correct stimuli. When mice touched these correct stimuli, they received the liquid reward, and an ITI (15 seconds) was introduced. When the nose poked incorrect stimuli, a time-out (5 seconds) was given, and the ITI followed before the starting of the next trial. Just as in the incorrect punishment training, a correction trial was performed after an incorrect response. A session ended when a mouse completed 60 trials or 60 minutes had elapsed. Four mice were trained for dPAL and another four mice were used for the sPAL task.

2-object sPAL

For this task, the handling procedure was skipped and pretraining was performed after the food restriction started. The general conditions were the same as the sPAL task with a few modifications to reduce the time required for learning saturation. First, only two images (flower and airplane) were used as visual stimuli in this task. Additionally, the number of combinations was also reduced to two. Two flowers or two airplanes were presented, with the left flower and the right airplane used as the correct stimuli. At the first attempt, two adjacent windows were used to display stimuli and there were 30 trials in one session. At the second attempt, because it seemed that mice were likely to make more mistakes due to the proximity of the two windows, we used two distant windows with one excluded window in the middle. In this attempt, a session ended when a mouse finished 60 trials or 60 minutes had elapsed. Fourteen mice were trained and performed this task (the first attempt [N=7] and the second attempt [N=7]).

Hippocampal cannula implantation

After each group of mice finished the PAL task training, bilateral hippocampal cannulation surgery was performed under stereotaxic guidance. Each mouse was anesthetized with an intraperitoneal injection of ketamine. Firstly, bilateral

craniotomy was performed with a drill bit at -1.8 mm anteroposterior (AP), ±1.5 mm mediolateral (ML) for the insertion of a double-guide cannula. The guide cannula was then slowly targeted to the hippocampal CA1 area (-1.8 mm AP, ±1.5 mm ML, -1.2 mm dorsoventral [DV]). After the infusion cannula was placed precisely at the target site, dental cement was applied to fix the infusion cannula firmly and seal the surgical site. Mice trained in sPAL and dPAL were given a recovery period of 10 days after the surgery. In the case of the 2-object sPAL, the mice were allowed to recover for a longer period of 20 days after the surgery. During the recovery period, food and water were provided *ad libitum* and food restriction was resumed 1 or 2 days before the relearning stage started.

Hippocampal dependency test

For each PAL task, we first trained the mice again until they reached the same level of performance as prior to receiving the cannulation surgery. In sPAL and dPAL, the mice were trained again for 7 sessions in each task and showed an accuracy rate of around 80%. The next day, each mouse was infused bilaterally with a vehicle solution (saline) and tested. During the infusion process, the mice were

anesthetized with isoflurane. At first, the dummy cannula covering the guide cannula was removed and an injector cannula (0.5 mm longer than the guide cannula) connected to two Hamilton syringes was inserted into the guide cannula for infusion. After the insertion, saline was infused at a regular speed for 2 minutes (0.5 μl/side) and the insertion cannula was replaced by the dummy cannula 1.5 minutes after the end of the infusion. The mice were returned to the home cage and given 25 minutes to recover from anesthesia. Subsequently, each mouse was put into the touchscreen chamber and tested in their respective PAL task.

The following day, in order to inactivate the hippocampal CA1 region, a solution of CNQX (3 mM) and TTX (20 μM) were infused bilaterally (0.5 μl/side) into each mouse, and the mice were tested again. The concentration of the drug solution was determined according to a method described in a previous study (Goshen et al., 2011). The whole procedure for infusion and testing in the respective PAL task was the same as that for the vehicle session. Lastly, to see if the drug affected the function of hippocampus temporarily and did not damage the hippocampal tissues permanently, we tested mice in their respective PAL task on the next day without any

pharmacological manipulations. One mouse in dPAL and two mice in the 2-object sPAL were excluded because the guide cannula detached from the head. One other mouse in the 2-object sPAL group was also excluded because it could not learn the task and showed around 50% correct responses for all six sessions. Therefore, the final number of mice that underwent the hippocampal dependency testing for each task was as follows: sPAL (N=4), dPAL (N=3), and 2-object sPAL (N=4).

Results

Fast acquisition of the sPAL and dPAL tasks with overlearning

In most previous studies for mice using sPAL or dPAL, a single session consisted of 36 trials. With this widely used condition, mice reached an accuracy of about 80% in sPAL after 40 sessions and 70% in dPAL after 50 sessions (Clelland et al., 2009; Delotterie et al., 2014; Nithianantharajah et al., 2013). In order to allow the mice to learn the PAL tasks faster, we applied a new condition of overlearning, in which mice were required to finish 60 trials per session. In the sPAL task, mice reached an average accuracy over 70% by the 11th session and showed an accuracy higher than 75% continuously from session 13 to session 15 **(Fig. 1A)**. In the case of the dPAL task, mice attained an average accuracy over 70% by the 12th session and over 75% in sessions 12, 13, and 15 **(Fig. 1B)**. There was no significant difference between the acquisition rate of sPAL and dPAL (2-way ANOVA with repeated measures on time, p=0.38, N=4). These data indicate that when an intensive learning protocol of 60 trials per session is used, the acquisition rate shows approximately three-fold increase compared with that of the previously used protocol (36 trials

per session). This greatly shortens the experimental time needed for the PAL tasks.

Development and optimization of the 2-object sPAL task

We tried to formulate a simpler task, which requires less time for acquisition but still involves hippocampus-dependent object-location memory. At first, we simply reduced the number of objects and windows to two and the number of trials per session to 30 **(Fig. 2A)**. It took 11 sessions for mice to reach 66.2% average accuracy **(Fig. 2B)**. The learning rate of mice shown in this protocol was not that fast and quite similar to that of sPAL or dPAL in this study **(Fig. 1A, B)**. For the second attempt, we tried 60 trials per session to add to overlearning effects. In addition, we inserted an empty window between the two object-presenting windows in order to reduce unwanted errors due to the close proximity of the two target objects **(Fig. 3A)**. In this new protocol, mice attained 75.2% accuracy in only 6 sessions **(Fig. 3B)**. The percentage correct remained almost the same until session 4 and increased rapidly starting from session 5. Including the touchscreen pretraining for approximately 8 days, the whole experiment required only 14 days. These results suggest that mice can

learn the 2-object sPAL task in a short period and this task can be used as a new simple task for testing object-location memory in mice.

Hippocampal dependency of the two PAL tasks

To determine if the two PAL tasks require the function of the dorsal hippocampus (dHP), we investigated the effect of inactivating the dHP after acquisition of the two tasks. First, vehicle infusion into the dHP had no effect on the performance in the two PAL tasks **(Fig. 6)**. When a solution of the AMPAR antagonist, CNQX, and the sodium channel blocker, TTX, was infused into the dHP 25 minutes before testing, the percentage correct decreased significantly to 65.1% in the sPAL task (vehicle: 80, TTX+CNQX: 65.089, paired t-test, $p<0.05$, N=4) **(Fig. 5A, 6)**. The effect was larger in dPAL performance, where the accuracy decreased to 48.9% (vehicle: 78.889, TTX+CNQX: 48.912, paired t-test, $p<0.05$, N=3) **(Fig. 5B, 6)**. The dPAL task is considered to be very highly hippocampus-dependent given that the accuracy rate went below the level of chance. One day after the test with CNQX and TTX infusion, both PAL tasks were tested again without any treatment and the performance of the mice was similar to that of the vehicle session **(Fig. 6)**. This

proves that CNQX and TTX infusion inactivated the dHP transiently and did not damage the function of the dHP. Overall, both of the PAL tasks were shown to require the normal function of the dorsal hippocampus for proper performance. In particular, dPAL can be thought of as a highly hippocampus-dependent object-location memory task.

Hippocampal dependency of the 2-object sPAL task

Since we had lowered the level of difficulty in creating a 2-object sPAL task, it is possible that this task can be performed well without the function of the dHP. Therefore, we tested whether this task also requires the function of the dHP by using the same protocol as for the sPAL hippocampal dependency test. First, saline infusion into the dHP did not affect the percentage correct in the 2-object sPAL task **(Fig. 8, 9)**. Inactivating the dHP with CNQX and TTX infusion significantly reduced the accuracy rate to 65.8% (vehicle: 82.084, TTX+CNQX: 65.834, paired t-test, $p<0.05$, N=4) **(Fig. 8, 9)**. The extent of reduction in the percentage correct was quite similar to that of sPAL task, in which the accuracy was 65.1% after the inactivation of the dHP **(Fig. 6)**. This shows that the the 2-object sPAL task still requires the

function of the dHP despite its relative simplicity. Similar to the case of the sPAL task, the performance recovered to around 80% on the next day in the 2-object sPAL task **(Fig. 9)**.

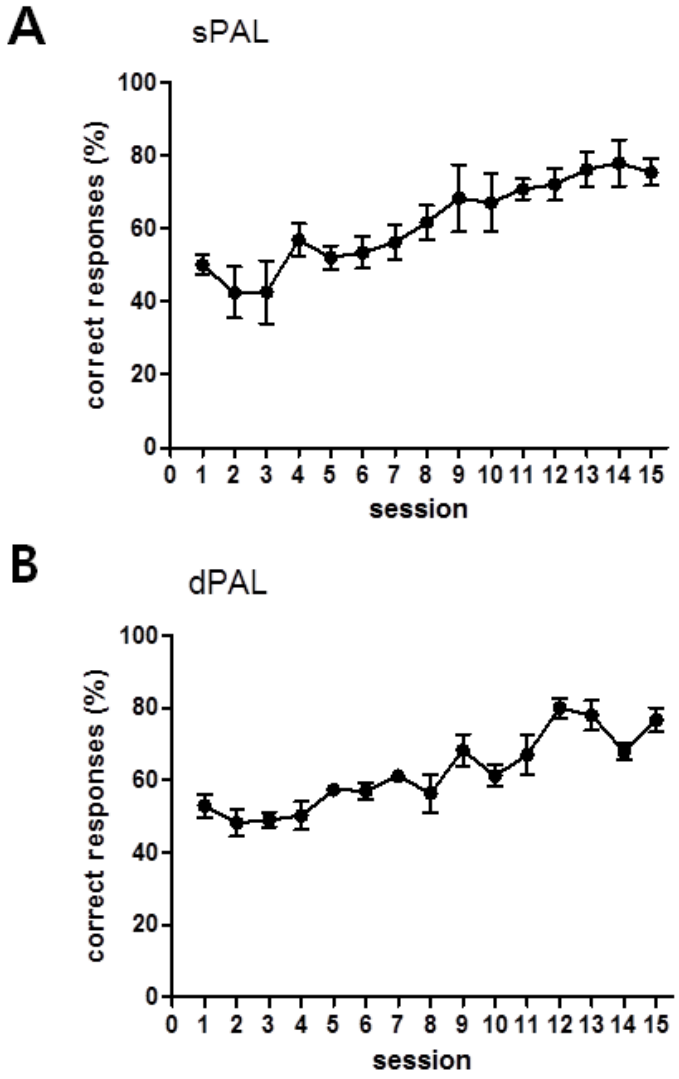
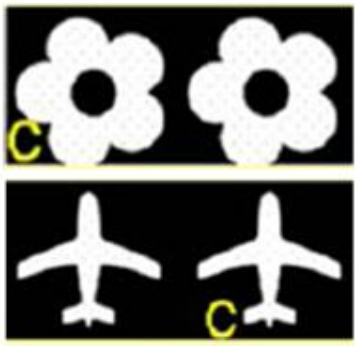

A
sPAL
correct responses (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
session
B
dPAL
correct responses (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
session

Figure 1. The rapid learning curve of the PAL tasks with overlearning in mice. (A) Acquisition curve of the same PAL (sPAL) task. Sixty trials were performed per session. N=4. (B) Acquisition curve of the different PAL (dPAL) task. N=4.

A


C
C

B

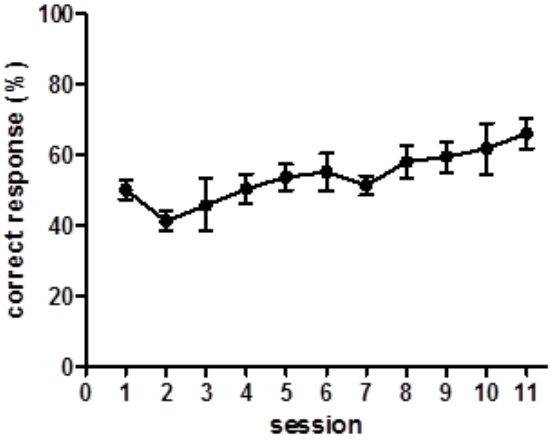
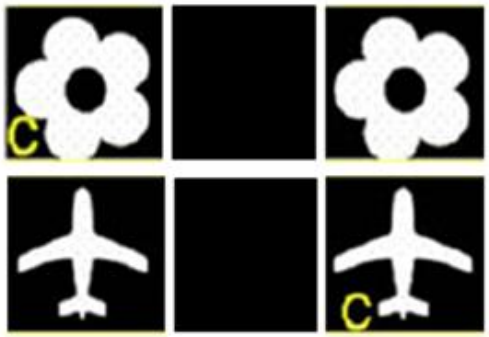

100
80
60
40
20
0
correct response (%)
0 1 2 3 4 5 6 7 8 9 10 11
session

Figure 2. Development of the 2-object same PAL task. (A) Trial types presented in the first attempt with two windows. (B) The % correct in the first attempt. Thirty trials per session were performed. N=7.

A



B

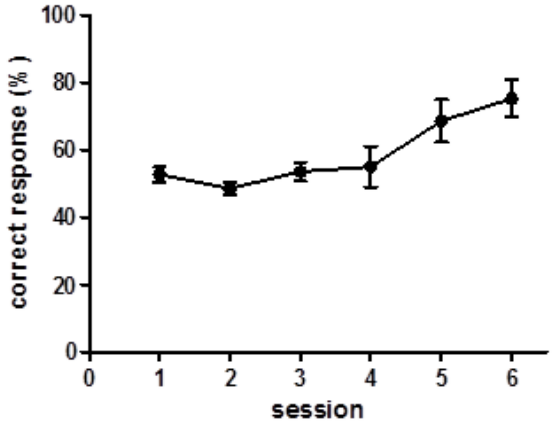


Figure 3. Optimization of the 2-object same PAL task. (A) Trial types given in the second attempt with one excluded window in the middle (B) The % correct in the second attempt. Mice were required to finish 60 trials per session in 60 minutes. N=7.

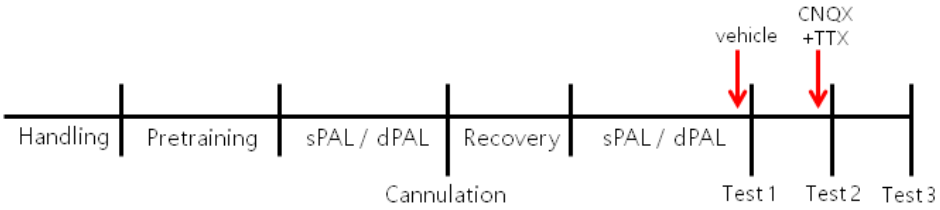

vehicle
CNQX
+TTX
Handling
Pretraining
sPAL / dPAL
Recovery
sPAL / dPAL
Cannulation
Test 1
Test 2
Test 3

Figure 4. The overall experimental protocol for the hippocampal dependency test of the same and different PAL tasks. After hippocampal cannula implantation, mice were trained again for 7 days. Test 1, test 2, and test 3 were performed in one session respectively.

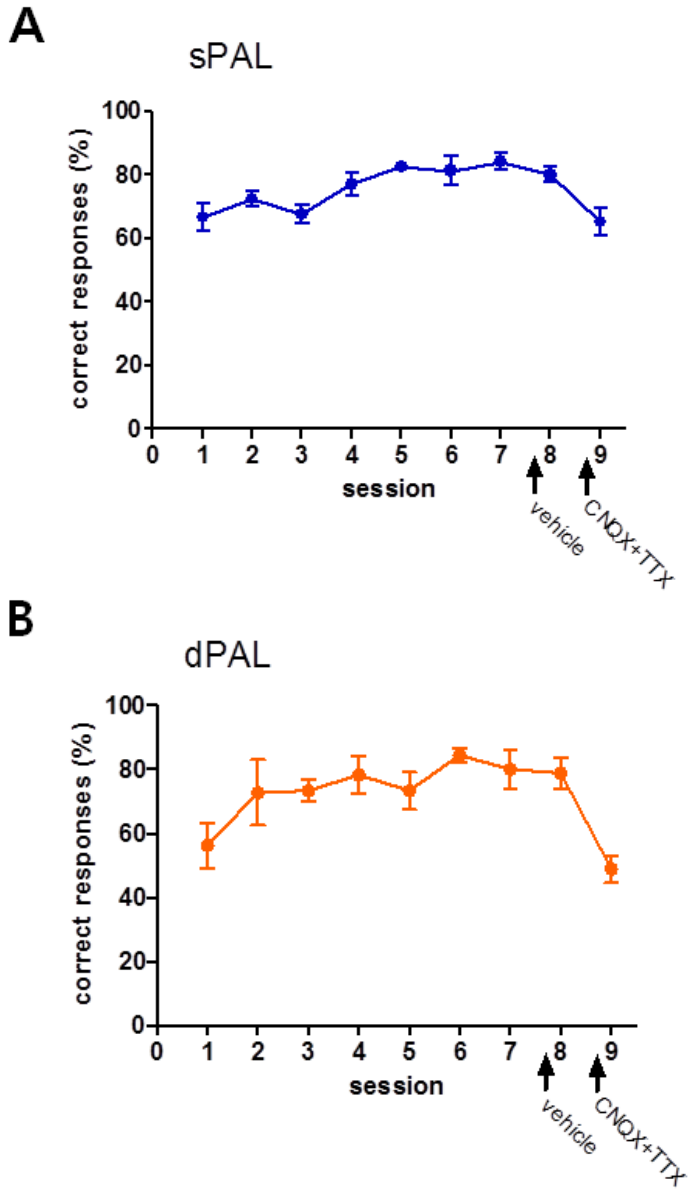

A
sPAL
correct responses (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9
session
vehicle
CNQX+TTX
B
dPAL
correct responses (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9
session
vehicle
CNQX+TTX

Figure 5. Percentage correct responses during the relearning and testing in the sPAL and dPAL task. (A) % correct of the sPAL task. Mice could reach the fully learned level again within 7 sessions. (B) % correct of the dPAL task.

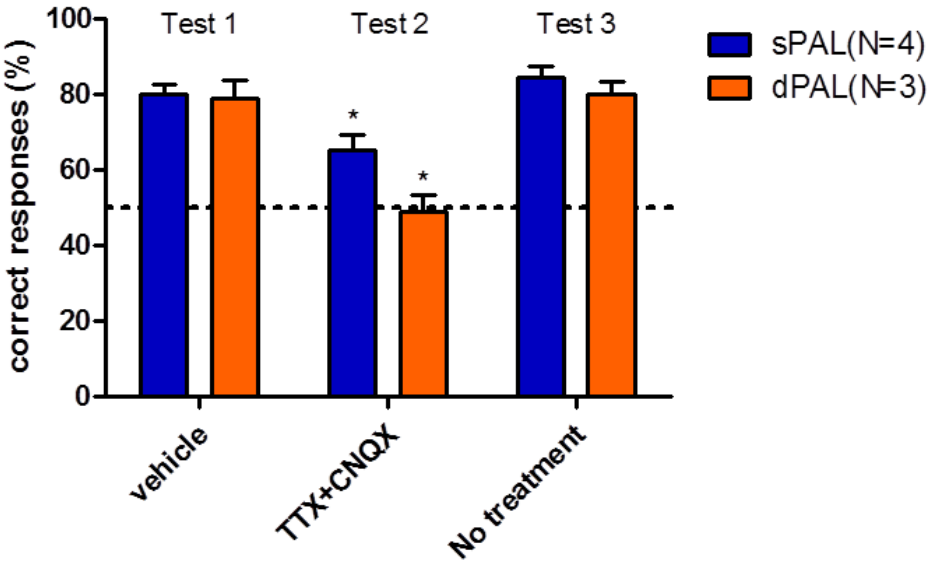

Test 1
Test 2
Test 3
sPAL(N=4)
dPAL(N=3)
correct responses (%)
100
80
60
40
20
0
*
*
vehicle
TTX+CNQX
No treatment

Figure 6. Hippocampal dependency of sPAL and dPAL. Saline was used as a vehicle in test 1. A 0.5 μl solution of TTX (20 μM) + CNQX (3 mM) was infused into the dorsal hippocamal CA1 region before test 2. Test 3 was performed without any pharmacological manipulations. N=4 in sPAL and N=3 in dPAL. Data are presented as mean±standard error of the mean (SEM). *p<0.05 vs. vehicle.

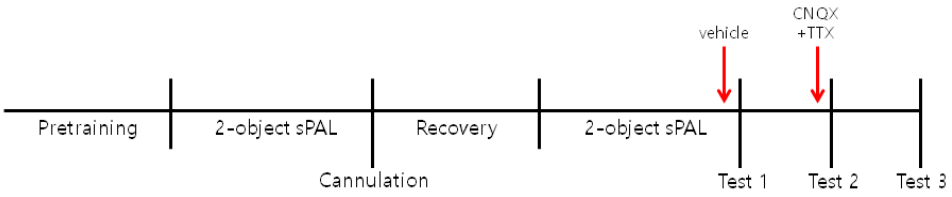

vehicle
CNQX
+TTX
Pretraining
2-object sPAL
Recovery
2-object sPAL
Cannulation
Test 1
Test 2
Test 3

Figure 7. The overall experimental protocol for the hippocampal dependency test of the 2-object sPAL task. After hippocampal cannula implantation, mice went through the relearning phase for 6 days. Test 1, test 2, and test 3 were performed in one session respectively.

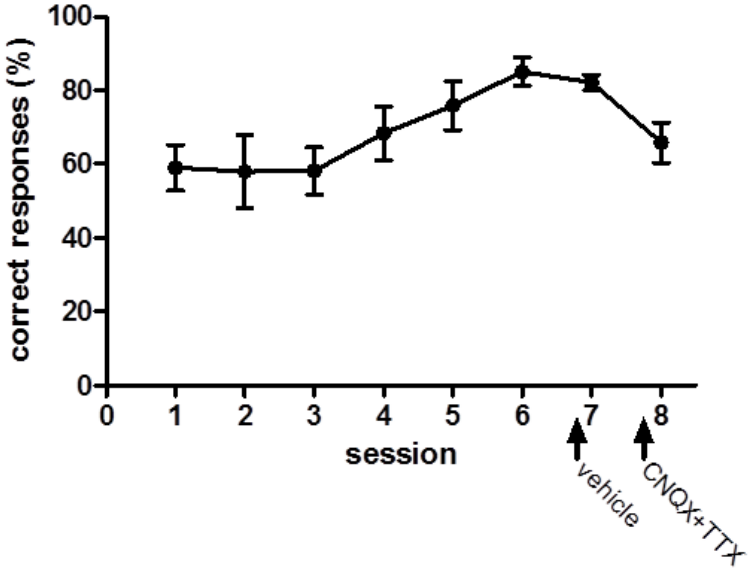

correct responses (%)
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
session
vehicle
CNQX+TTX

Figure 8. The learning curve during the relearning phase and percentage correct in the testing phase in the 2-object sPAL task. Mice reached the fully learned level again at session 6 and then were tested with pharmacological manipulations.

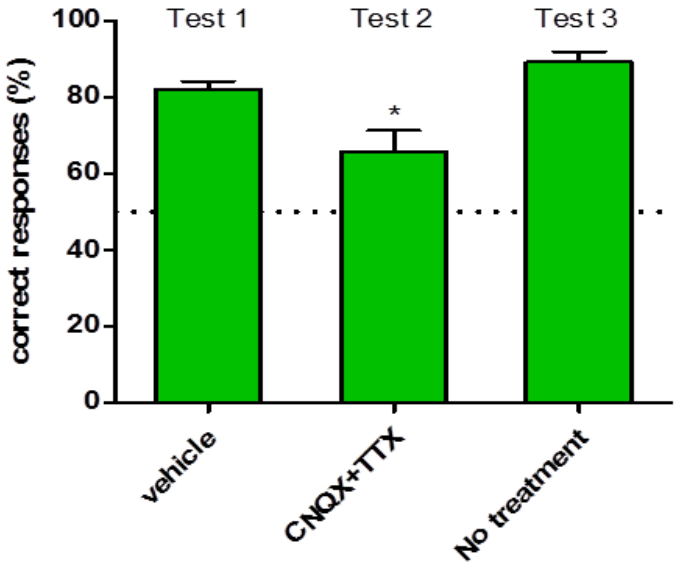

Test 1
Test 2
Test 3
100
80
60
40
20
0
correct responses (%)
*
vehicle
CNQX+TTX
No treatment

Figure 9. Hippocampal dependency of the 2-object sPAL. Overall procedure was the same as in the hippocampal dependency test of sPAL and dPAL. N=4. Data are presented as mean±standard error of the mean (SEM). *p<0.05 vs. vehicle.

Discussion

In this study, we tried to enhance the usefulness of the touch screen PAL task by optimizing various experimental parameters. First, by introducing a new protocol with a higher number of trials (about two-fold the number used in previous studies), we could shorten the experimental period needed for learning saturation in sPAL and dPAL to about 15 sessions. Therefore, the acquisition rate of the tasks increased about three-fold compared with that of the general protocol used in previous studies. Second, by reducing the number of objects and trial types in sPAL, we developed a simplified version of sPAL, which takes much less time for learning saturation. In this 2-object sPAL task, the necessary experimental period for learning saturation is about 6 days. Third, we have shown that sPAL and dPAL protocols with the new parameters still require the function of the dorsal hippocampal CA1 region. Moreover, 2-object sPAL also showed a similar result, in which the average accuracy of mice decreased to approximately 65.8% by the inactivation of the dHP.

Typically, one session comprises of 36 trials for the sPAL and dPAL tasks (Clelland et al., 2009; Horner et al., 2013; Nithianantharajah et al., 2013). Under this protocol,

mice require about 40 days to attain an average accuracy of 80% in sPAL and 50 days to reach 70% average accuracy in dPAL. In order to improve the experimental efficiency of both tasks, we tried to reduce the time required for learning saturation by applying more intensive PAL training with a greater number of trials. This was based on reports that overlearning enhanced long-term retention in human (Driskell et al., 1992; Rohrer et al., 2005). At the very first session, the average number of trials completed was 40.75 in sPAL and 44.25 in dPAL. This indicates that mice experienced overlearning in both tasks from the very first session compared with the previous protocol, in which only 36 trials were performed in one session. The average number of trials performed increased gradually, and reached 60 by the session 7 of sPAL and session 6 of dPAL. From these sessions, mice experienced about two-fold overlearning continually in each session. Continual overlearning appeared to increase the learning rate of mice in both PAL tasks. As a result, mice showed an average accuracy of 75% at around session 13 and reached learning saturation in about 15 sessions **(Fig. 1A, B)**. This greatly reduces the experimental time required for these tasks. Furthermore, because all mice finished 60 trials in one session from session 6 onwards almost every time, it may be possible to

introduce stronger overlearning effects, for example, by using one hundred trials per session, to increase the learning rate further.

We also developed a new 2-object sPAL task. In order to develop a simplified version of the sPAL task to allow easier testing, we reduced the degree of difficulty of the task. Generally, in sPAL, three objects are used and six types of trials are presented (Delotterie et al., 2014; Talpos et al., 2009). In the 2-object sPAL task, we used two objects and two types of trials. Moreover, we introduced an excluded window between the two active windows and performed 60-trial sessions for training. Consequently, mice could learn the task at a rapid pace and reached 75.2% average accuracy by session 6. This experimental period is quite short, even when compared with that of a widely used spatial memory task, the Morris water maze test, which takes about 7 days (D'Hooge and De Deyn, 2001; Lee et al., 2014; Vorhees and Williams, 2006). Therefore, this simplified task can be used as a timesaving touchscreen PAL task to assess object-location memory in a simple manner. Of course, it is not always better to use a simplified and rapidly learned task rather than a more complex and slowly learned task. In some cases, it may be more suitable to use a

slowly learned PAL task to detect subtle differences in the cognitive functions of two groups. According to the various needs of the study using the PAL tasks, experimenters can choose the original, or new protocols with overlearning or simplification as presented in this study.

Finally, we tested the hippocampal dependency of three PAL tasks. As mentioned in the introduction, the different PAL task has been already shown to be hippocampus-dependent in rats and mice (Delotterie et al., 2015; Kim et al., 2015; Talpos et al., 2009). We found that the new protocol with a reduced experimental period because of overlearning did not affect the hippocampal dependency of this task. The dPAL task with this protocol seemed to be almost completely hippocampus-dependent, as indicated by the sharp decrease of the accuracy rate. In addition, to our knowledge, we showed for the first time that the function of dorsal hippocampus in mice is crucial in performing the sPAL task as well. However, the reduction in percentage correct because of dHP inactivation was smaller than it was in dPAL. This was in line with a previous study, in which various results suggested that dPAL is more sensitive to hippocampal disruption than sPAL in rats (Talpos et al., 2009). This might be because sPAL has

relatively lower complexity than dPAL in terms of the combinations of object and location. On the other hand, in the 2-object sPAL task, the decrease in the accuracy rate was similar to that in the sPAL task despite the simplicity of the 2-object sPAL task. This may be because the basic rule concerning the relationship between object and location in this task was the same as that in sPAL and the dHP is needed to learn that rule.

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국문 초록

마우스를 위한 터치스크린 기반 paired associates learning (PAL) task의 최적화와 이 task의 배측 해마에 대한 의존성

김 명 원

설치류를 위한 두 가지 버전의 터치스크린 paired-associate learning (PAL) task가 개발되었다. 이는 same PAL (sPAL)과 different PAL (dPAL)이다. 이 task들은 다양한 연구에서 사물-위치 기억을 테스트하기 위해 사용되었고 또한 알츠하이머병과 조현병의 마우스 모델을 연구하는 데 있어서도 중요하다. 그러나 이 task들은 상대적으로 긴 시간(대략적으로 마우스의 경우 50일)이 소요되기 때문에 널리 사용되지 못하고 있다. 이 연구에서는 더 많은 수의 trial을 포함한 더 강도 높은 training을 제공함으로써 sPAL과 dPAL에서 완전히 학습하는데 요구되는 시간을

단축시켰다. 구체적으로는 일반적으로 통용되는 protocol을 사용한 경우의 1/3 정도로 기간을 단축시켰다. 더 나아가서 sPAL task의 간소화된 새로운 버전인 2-object sPAL task를 개발하였다. 이 task에서는 마우스들이 6일만에 빠르게 완전한 학습에 도달할 수 있었다. 그리고 약물학적 실험을 통해서 배측 해마 CA1 영역의 기능이 새로운 조건의 두 가지 PAL task들과 새로운 2-object sPAL을 수행하는 데에 필수적이라는 것을 보였다. 이 연구는 학습 속도를 향상시킴으로써 터치스크린 PAL task들의 유용성을 크게 향상시켰으며, 이 task들이 새로운 조건에서도 여전히 해마에 상당히 의존적인 사물-위치 기억 task들로 사용될 수 있음을 보였다.