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Effects of Non-Spatial Pre-Training on Learning and Memory Impairment Detection in the Morris Water Maze

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Abstract

One of the most popular learning and memory tests is the Morris water maze. The Morris water maze is a circular pool filled with water with a hidden platform under the water surface. The test is appropriate for rodents, especially rats and mice. The testing protocol comprises 2 parts that evaluate learning ability and memory retention. When animals are placed in the pool, they experience stress, which is the driving force for discovery of a strategy to leave the water. In the experiment, animals use environmental cues to find the location of the hidden platform in the pool. After consecutive training days, animals can more quickly locate the hidden platform. The last day of the task involves a memory test without the platform. It shows a limitation of the test in mild learning and memory deficit models such as 2-vessel occlusion. Differences between the normal and memory impairment models are expressed only in a narrow range. So, we tried to modify the original protocol for mild learning and memory impairment models. We used an albino rat strain for the experiment. A pre-training strategy of 3 days of swimming in the pool with a visible platform prior to the ordinary task was used. The results suggest that this pre-training strategy improved learning and memory in the rat model. When compared to normal rats and 2-vessel occlusion rats (a rat model for vascular dementia), those that participated in the pre-training strategy showed an increase in the percent difference of area under the curve for learning trials. In conclusion, the pre-training strategy increases ability to discriminate learning and memory impairment in the rat model, especially for the mild learning and memory deficit models.

Keywords: Pre-training strategy, Morris water maze, mild cognitive impairment

Introduction

The Morris water maze is one of the most popular methods of behavioral testing for rodents. This test comprises 2 sessions: a learning or acquisition trial and a probe trial. Spatial learning is measured from the learning trial and reference memory is evaluated from the probe trial. Normally, the learning trial tests ability to find a fixed, hidden platform in relation to extra-maze cues. Trials with a visible platform are used to evaluate visual performance, sensorimotor performance, and motivation [1]. The hippocampus is the important brain area responsible for learning ability during the hidden-platform session [2]. Due to its high reliability and robustness, the water maze is extensively used in learning and memory studies, assessment of neurocognitive disorder models, and research examining the treatment of these disorders [3]. However, in mild cognitive impairment models such as unilateral common carotid

artery occlusion or permanent bilateral common carotid artery occlusion (2VO), the ordinary protocol may detect no differences in learning and memory impairment. Differences between the normal and memory impairment model are expressed only in an insignificant narrow range for the original swimming procedure. Therefore, it may be beneficial to increase the sensitivity of the test for detecting mild cognitive impairment.

Chronic cerebral hypoperfusion is a condition found in vascular dementia, Alzheimer's disease, or even normal aging [4,5]. This condition alters metabolism, biochemistry, histology, and behavior. Cognitive impairment in terms of learning and memory impairment is detected in this condition. Because of low levels of cerebral blood flow reduction, learning and memory impairment occurring in the model is mild and occasionally undetected. Cerebral blood flow (CBF) is reduced to approximately 35 - 45 % of the control level in the cortical white matter area and approximately 60 % of the control level in the hippocampus. The CBF was progressively recovered at 1 week after 2VO induction. The recovery of blood flow was slightly reduced or virtually normal after 8 weeks and 3 months in different studies. Neuronal damage was found in 55 % of 2VO rats at 4 weeks and 67 % at 8 to 13 weeks after 2VO induction [6]. To get better discriminative power of Morris water maze, we tried to detailed modify this behavioral testing protocol that increase the power of the task for mild cognitive impairment detection. We found that non-spatial learning can increase the power of the task to detect learning and memory impairment in the rat model.

Materials and methods

Animals

Eight-week-old male Spraque Dawley rats were used in this experiment. All animals were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhon Pathom, Thailand. The animals were housed in ventilated isolation cages under natural light/dark cycle with a constant room temperature of 25 °C and were given food and water ad libitum. Experimental procedures were approved by Siriraj Animal Care and Use Committee, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand. 2VO was conducted as previously described. For this procedure, a cervical incision was made, and the common carotid arteries were exposed. The vagus nerves were separated from the common carotid arteries, and the vessels were ligated using suture thread [7].

Experimental design

The 34 rats were randomly allocated into 5 groups as outlined in **Table 1**. Groups 1 and 3 were subjected to the normal strategy and Groups 2 and 4 were subjected to the pre-training strategy. To compare the effects of swimming time and task difficulty, we designated Group 5 as 4-month-old 2VO rats with 90-seconds swimming time and a double-size platform. For the normal strategy, the rats were tested with a hidden-platform session, a probe test, and a visible-platform session, in this order. The pre-training strategy was sequenced as a visible-platform session, hidden-platform session, and probe test (**Figure 1**).

Table 1 Group allocation.

Group1	Normal rats with normal strategy
Group2	Normal rats with pre-training strategy
Group3	8 week-2VO rats with normal strategy
Group4	8 week-2VO rats with pre-training strategy
Group5	4 month-2VO rats with 90-seconds swimming time

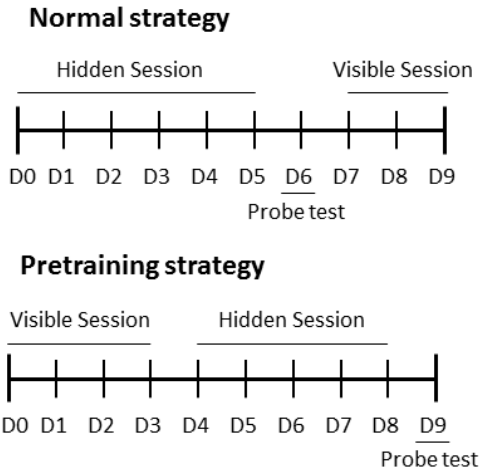

Figure 1 Swimming protocol for normal and pre-training strategies.

Morris water maze

Our procedure was slightly modified from previous studies [1,3]. The water maze was a 2-metercircular swimming pool located in a room with various extra-maze cues. The pool was filled with water to a level of 25 cm. A glass platform was submerged 2 cm below the water surface and located at the center of a fixed quadrant. Video was recorded with a ceiling-mounted camera controlled by a computer. The hidden-platform session and probe test are the conventional Morris water maze protocols used to evaluate learning and memory. In the hidden-platform session and learning trial, the rats were allowed to swim for 120 s to find the hidden platform. Latency of each rat to find the hidden platform was measured for 4 trials per day for 5 days. The location of the hidden platform was always the same. The probe test was performed on the sixth day. During the probe test, the platform was removed from the maze, and the rats swam for 120 s. The time spent in the target quadrant in which the platform was previously located in the hidden-platform session was recorded.

The visible-platform session was conducted for 3 days beginning on the seventh day for the normal strategy groups and before the hidden-platform session for the pre-training groups. The platform was raised 2 cm higher than the water surface and marked with two 40-millimeter balls to provide visible cues as to its location. Other extra-maze cues were excluded, and the maze was covered with a curtain as described in [8]. The swimming procedures were tested in the same way as for the hidden-platform session, but the platform location was changed to a different quadrant for each trial. Briefly, rats were allowed to swim for 4 trials a day for 3 days. Only escape latencies were measured before and after the hidden session. The area under the curve was used for determination of spatial learning as previously described in [9].

Statistical analysis

Results were expressed as mean ± SEM. The differences in mean escape latencies from the Morris water maze test and the probe tests were analyzed using an unpaired Student's t-test to determine the differences between groups. ANOVA followed by Tukey's post-hoc test was used for comparison of discrimination capability. Statistical significance was at $p < 0.05$.

Results

Effect of pre-training strategy on learning ability

In normal rats, naive and pre-trained rats showed improvement in escape latency within each group along the training period, which indicates improved learning performance from day 1 to day 5. Pre-trained rats showed significant decreases in escape latency compared with naive rats on days 1 through 5. This was expressed at $p < 0.001$ on days 1, 2, and 3 and $p < 0.05$ on days 4 and 5 (**Figure 2**). In 8-week-old 2VO rats, both naive and pre-trained rats showed improved learning performance during the task as shown in the decrease in escape latency after training for each day. Pre-trained rats showed better improvement in learning ability compared with naive rats. Pre-trained rats spent less time than naive rats, with $p < 0.001$ on days 1 and 5 and $p < 0.05$ on days 2 and 4 (**Figure 3**).

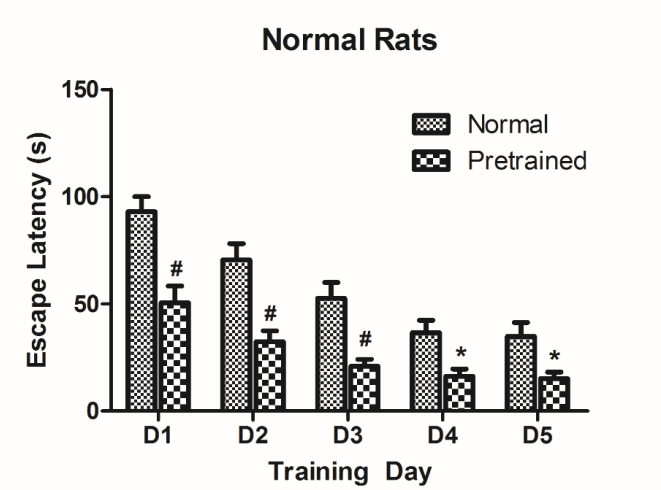

Figure 2 Mean escape latency of naive and pre-trained normal rats from training days 1 to 5. # $p < 0.001$, * $p < 0.05$, $n = 8$.

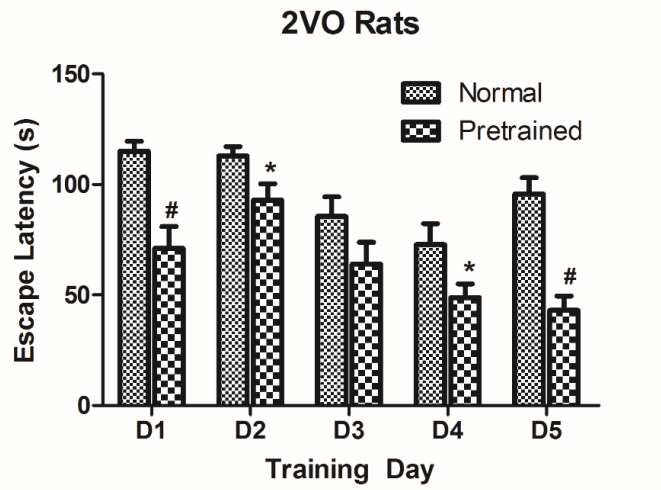

Figure 3 Mean escape latency of naive and pre-trained 2VO rats on training days 1 to 5. # $p < 0.001$, * $p < 0.05$, $n = 5$.

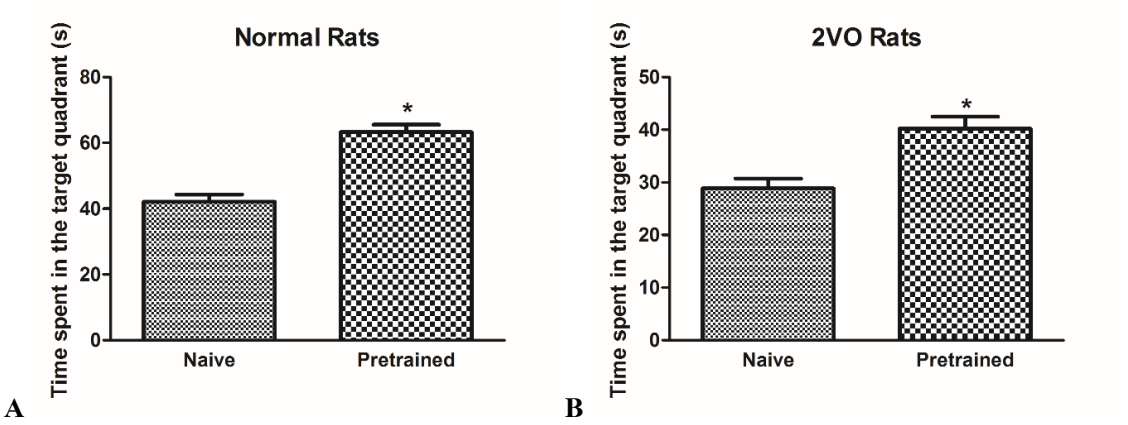

Figure 4 Average time spent in the target quadrant for normal rats (A) and 2VO rats (B). * $p < 0.05$, $n = 8$ for normal rats and $n = 5$ for 2VO rats.

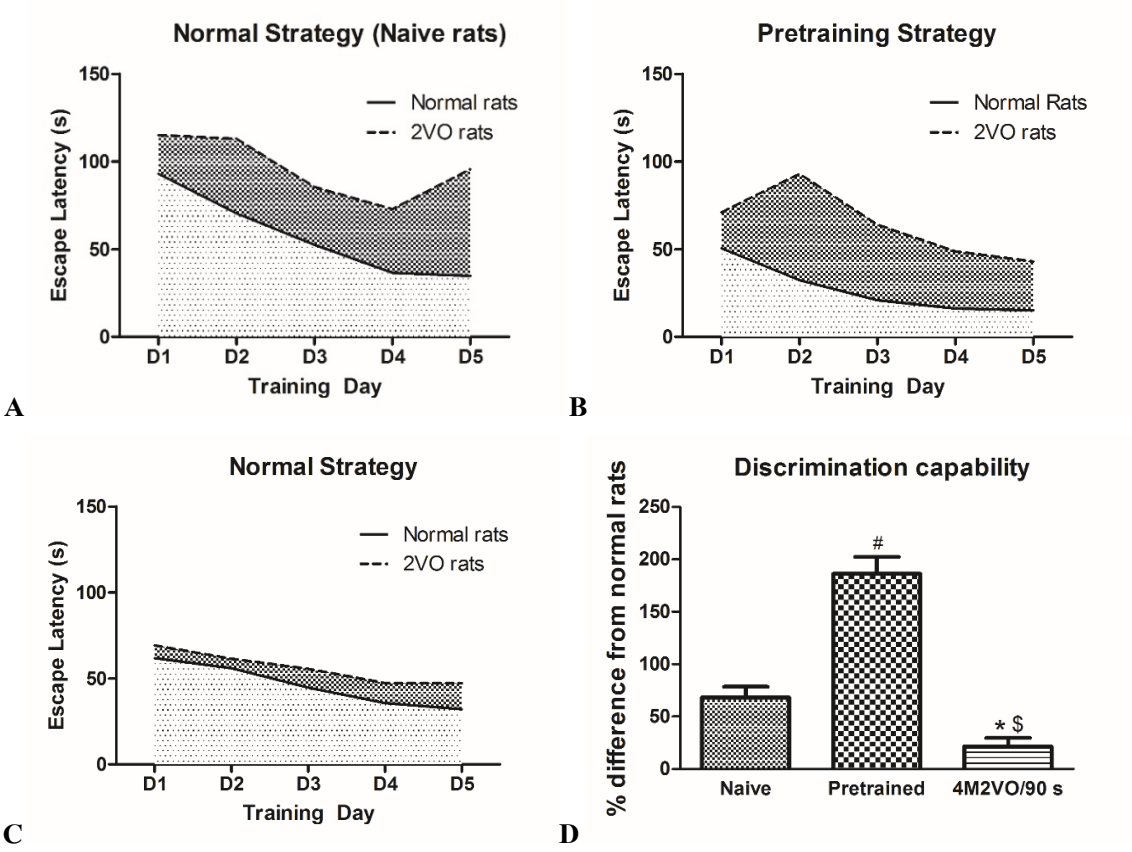

Figure 5 Area under the curve comparing normal and 8-week-old 2VO rats for the normal (A) and pre-training strategies (B) and 4-month 2VO rats with 90-seconds swimming time (C). Discrimination capability is shown as percent difference from normal rats for normal and pre-trained 8-week-old 2VO and 4-month-old 2VO rats with 90-seconds swimming time (D). # $p < 0.001$, * $p < 0.05$ compared with naïve rats and $ $p < 0.001$ compared with pre-trained rats.

Effect of pre-training strategy on reference memory

The pre-training strategy increased memory retention compared with naive rats for both normal and 2VO rats. For normal rats, pre-trained rats showed a statistically significant increase in time spent in the target quadrant compared with naive rats, which implies more memory retention. Eight-week-old 2VO rats also spent more time in the target quadrant compared with naive rats, which indicates more memory retention (**Figure 4**).

Pre-training strategy and ability to discriminate learning impairment

To clearly display the difference between normal and 2VO rats for the normal and pre-training strategies (**Figures 5A - 5B**), we used the area under the curve normalized with normal rats as previously described [8]. The percent differences for the normal and pre-training strategies were 68.15 and 186.00, respectively (**Figure 5D**), while the percent difference for 4-month-old 2VO rats with 90-seconds swimming time and a larger platform was 21.42 (**Figure 5C**).

Effect of testing sequence on the visible-platform session

The learning task used in the pre-training strategy was a visible-platform training session for 3 days. We also analyzed the area under the curve for the visible-platform session before and after the hidden-platform session by group. Percent differences for normal rats for the visible-platform session before and after the hidden-platform session were 69.71and 221.60, respectively (**Figure 6**).

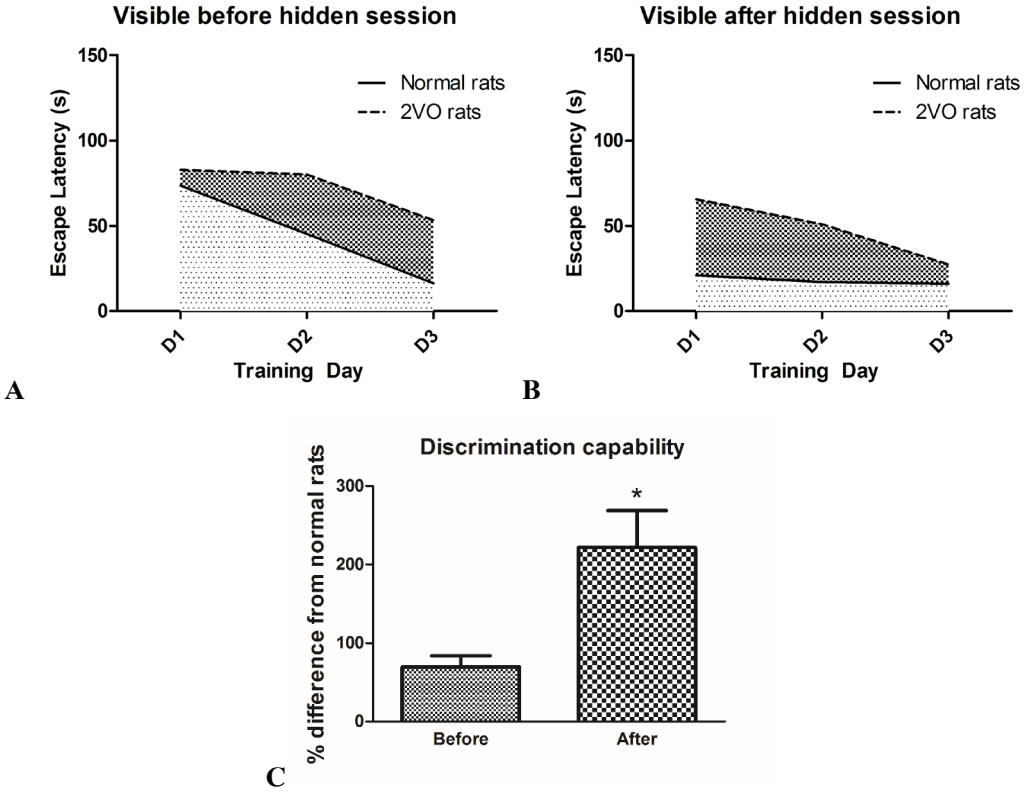

Figure 6 Area under the curve comparing normal and 8-week-old 2VO rats with the visible-platform session before the hidden-platform session (A) and after the hidden-platform session (B), and discrimination capability between the visible-platform session before and after the hidden-platform session shown as percent difference from normal rats (C). * $p < 0.05$ compared with hidden-platform session before the visible-platform session.

Discussion

Spatial learning and memory impairment can sometimes be only scarcely observed in mild cognitive impairment models. Normally, spatial learning and memory impairment can be detected if pyramidal cell density in the hippocampus decreases more than 30 % [10,11]. Modifications can be made to the training protocol to increase sensitivity of the maze or further combine it with other mazes (e.g., the 8-arm radial water maze) [12]. In this study, we used a visible-platform trial for non-spatial learning before learning and probe trials. In the visible trial, the maze was surrounded by a curtain with no distal cues, and the platform was changed for each trial to prevent spatial learning as previously described [13]. Pre-trained rats showed improvement in escape latency in the hidden-platform session for both normal and 8-week-old 2VO rats. The rats learned to escape from the water in the visible-platform trial using only the visible platform and visual cues at the platform without extra-maze cues. Experience from non-spatial learning can decrease escape latency in the learning trial of the hidden-platform session and increase the rate of learning acquisition. Our result agrees with others that both spatial and non-spatial pre-training strategies can improve performance for standard water-maze procedures [14,15]. We hypothesize that non-spatial learning shares some common escape-strategy aspects with the learning trial of the hidden-platform session. Stated more simply, escaping from the water comprises 2 components, namely, finding the platform and discovering how to use the platform. The later component is likely the same for the visible-platform session (non-spatial strategy) and hidden-platform session (spatial strategy), which both involve non-spatial components for acquisition of knowledge of the water maze. These components are learning to find the platform, discovering what to do with it and learning how to use it as a shelter [16]. Few studies have offered explanations about the effects of prior learning. As mentioned in Morris' study, prior learning may have endocrinological and other stress effects on this type of learning [1]. Corticosterone levels have been observed as related with pre-training. Decreased levels were previously found in pre-training groups [14,15,17,18]. Mineralocorticoid and glucocorticoid receptors are expressed in the hippocampus and amygdala. It shows inverted-U shape effects on cognition with stress hormones. Normally, 80 - 90 % of mineralocorticoid receptor and 10 - 15 % of glucocorticoid receptors are found among these areas, however, high levels of glucocorticoid will cause memory impairment [19,20]. The sensorimotor disturbance hypothesis is another explanation of the impact of pre-training [21]. During the daily progression of training trials, the rats showed decreased sensorimotor disturbance as observed from the reduction of some behaviors. The behaviors that tended to be eliminated were periphery swimming, swim-overs, and excessive jump-offs [2]. Moreover, pre-training can reduce drug-induced learning deficit in the second task [22]. The rats also showed improvement in escape latency in the visible-platform trial as training progressed, which means the rats understand strategies regarding how to find the platform and what to do with it. As observed in the learning trial after the visible-platform session, escape latency also improved in the visible-platform trial preceded by the learning trial and the probe trial. These results suggest that provided a learning task has the same strategy as the subsequent learning task, there is an improvement in the subsequent learning task. This results in an increase in not only spatial learning but also time spent in the target quadrant after pre-training in both normal and 2VO rats. This suggests an increase in memory retention in the transfer test. The area under the curve was used to illustrate the overall performance in the learning trial [9]. We compared normal rats and 2VO rats in the standard protocol (normal strategy) and a protocol with a preceding visible session (pre-training strategy) and found that percent differences between 2VO rats and normal rats increased when using the pre-training strategy. This strategy provides greater ability to detect spatial learning deficit and increased differences in retention memory for 73 % of rats. The use of 2 different training strategies may be beneficial due to differences in learning flexibility between groups that can be detected in a reversal trial. The visible trial also showed that first performing a learning trial for the hidden-platform session and a probe trial increased the percent difference from normal rats. The results imply that non-spatial and spatial training affect each other. The procedural simplicity of the task may depend on the interaction of different cerebral areas [8]. In addition, task difficulty plays an essential part in acquisition [13]. We found that 4-month-old 2VO rats exhibited less percent difference from normal rats because of the larger size of the submerged platform. The time allowed for swimming is important for detecting learning and

memory impairment in mild cognitive deficit models because the pathology occurs in an uneven state as noted in the 2VO model [6].

Taken together, the slight modification in swimming protocol can increase sensitivity of the maze for detecting learning and memory deficit without adjustment of the hardware. If non-spatial training shares aspects of spatial training, this increases the percent differences between control groups and models in terms of spatial learning and memory. On the other hand, spatial training preceding non-spatial training can also enhance the percent difference between groups in non-spatial training.

Conclusions

Pretraining can increase the possibility to detect mild early impairments in learning and memory which may not be detected by the original protocol. The training shows the same effects in both hidden and visible sessions without any modifications of hardware.

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