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Article

Post training reward is beneficial to the behavior paradigm building

Xianji Pan

Precise Genome Engineering Center, School of Life Sciences, Guangzhou University, Guangzhou 510006, China; kidd.pan@qq.com

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Abstract: The behavior paradigm building of laboratory animals is a common method in biological research. Training animals according to different paradigms can often be used to explore some characteristics of animals. Conditional training is the starting point and foundation of each paradigm training. Goal behavior training is the main content of paradigm building. Effective paradigm training can save a lot of time and improve the state of experimental animals. This research results showed that proper post training reward could effectively improve the training motivation and training efficiency of mice. These behaviors will also benefit the effect of behavior paradigm building. Therefore, we suggest that an appropriate and effective post training reward scheme should be design for each behavior paradigm building.

Keywords: behavior paradigm; post training reward; conditional training; goal behavior training

1. Introduction

Behavior paradigm is a very common phrase in the scientific community, which is commonly used in psychology, sociology, biology and other fields [1]. Behavior paradigm is considered to be the combination of individual thought and action, which refers to the behavior stereotype of an individual with a specified cognitive. Behavioral paradigms are not unique to human beings, and they also exist widely in animal kingdom. Psychology and sociology mainly focus on the behavior paradigms unique to human society, while biology work hard on the behavior paradigms that all creatures can possess. In biological research, we often establish a specific behavior paradigm for experimental animals to simulate some behavior habits in animal society or even human society. Through the research on the experimental animals that have established behavior paradigm or originally have, we can explore a specific behavior.

There are many kinds of experimental animals used in biological experiments, the most common should be mice or rats [2,3]. These experimental animals play an indispensable role in biological research. A lot of basic research cannot do without experimental animals. When we need to study some common behavior habits of human society, such as cooperation and so on, we need to train experimental animals to establish behavior paradigms [4]. Different paradigms will have different training items and training requirements, which need researchers to design according to the research purpose. Behavior paradigms generally have reward type and punishment type, which means the training is motivated by reward or punishment. Reward type behavior paradigm refers to that experimental animals are rewarded when they meet the conditions, while the punishment type behavior paradigm refers to that experimental animals are punished when they fail to meet the conditions. But no matter what kind of behavior paradigm, establishment is generally having two stages:

conditional training and goal behavior training. The purpose of conditional training stage is to train experimental animals to acquire a certain ability. All behavioral paradigms need conditional training to enable experimental animals to acquire reflective behavior. As for the goal behavior training stage, the researchers designed it according to their own experimental goals, in order to let the experimental animals learn the specified behavior paradigm. Goal behavior training stage is the main content of behavior paradigm establishment, while conditional training stage is the premise.

Post training reward refers to offering appropriate reward after each training. As long as the appropriate dosage is controlled, the reward will not have a negative impact on training. In many behavioral paradigms, post training reward is not common [5,6]. Many researchers do not realize the importance of post training reward. However, we confirm that post training reward has a positive effect on the training of behavior paradigm building. Post training reward should be paid attention to in the establishment of behavior paradigm.

2. Method

2.1. Experimental subjects

Experiments were carried out with male C57BL/6 mice (3months old, 250–300 g at the beginning of the experiments) provided by South China Normal University. Animals were housed in pairs in cages until the end of the experiments. Mice were randomly paired and were kept on a 12-h light/dark cycle with constant ambient temperature (21.5 ± 1 °C) and humidity (55 ± 8%). All the experimental process met the requirements of animal ethics.

2.2. Behavioral procedures

All experiments were carried out in a Skinner box (**Figure 1A**) reformed by our team. Skinner box comes from Lafayette Instrument Company (Skinner Box, P.O. Box5729•Lafayette, IN47903USA), and uses ABETII software to record data. A pressure lever is arranged in the Skinner box, and a reward trough (feeder) is arranged on the opposite side. Each time the feeder is activated, it will provide 2-5uL of syrup as reward. There were no obstacles in the whole environment, and the mice could clearly see the pressure lever device and the feeder. The mice were deprived of water from the day before training until to the end of training. Then according to the experimental requirements begin the following experimental training (**Figure 1B**):

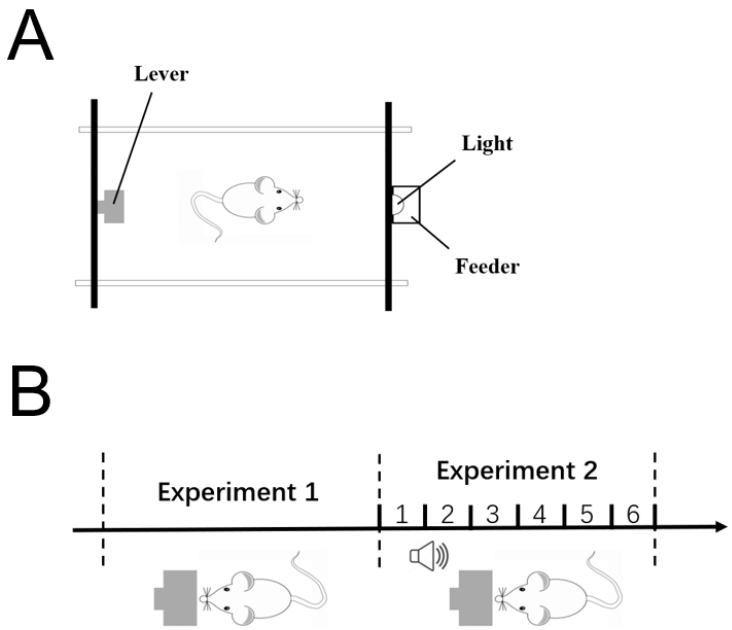

Figure 1. Experimental design. A, schematic diagram of Skinner box, which contains pressure lever, indicator light and feeder. B, the experiment process includes experiment 1 and experiment 2, and experiment 2 is carried out after experiment 1.

Experiment 1 is a simple conditional learning training, which is very common in the establishment of paradigm, and it is the most basic beginning of experimental animal behavior training. After the mice pressed down the pressure lever, the prompt light in the feeder would be on, and the reward would be provided in the feeder. The valid data were recorded after the mice pressed the lever and touched the trough to get food. If the mice only pressing the lever or touching the feeder was not be counted. The mice were trained once a day for 40 minutes, and offer or not offer appropriate rewards after training. The appropriate reward is let the mice drink water freely for two minutes. When the mice can complete 60 valid training in a 40-minute training, the experiment 1 ended.

Experiment 2 was carried out after experiment 1, and the listening training was added. The specific training is similar to experiment 1, but the environment will regularly play the same sound for a short time. Only when the sound is played, the mouse can get the reward by pressing the lever, but not at other times. Experiment 2 included 6 days, the first three days were training days, and the last three days were data recording days. The average value of the three-day data recorded represents the behavior training results of the mice. The requirement of data recording is the same as experiment Similarly, after each training, the mice were given an appropriate reward. In fact, the purpose of Experiment 2 is to simulate the specific behavior learning in the actual experimental paradigm. Combined with the data of Experiment 1 and Experiment 2, we can explore the effect of post training reward.

3. Results

3.1. Post training reward can improve the efficiency of conditional training

As described in Methods, a total of twenty mice were successfully trained along two successive experiments. Twenty mice were divided into two groups with ten mice in each group. After training, one group of mice could get appropriate reward, while the other group did not. The results of Experiment 1 show that the conditioned learning of mice was in line with common sense, and the learning trend was fitted by sigmoid curve (**Figure 2A**). Of course, we also found that the learning efficiency of mice with post training reward was significantly higher than that of mice without reward. The mice with post training reward started learning earlier (starting from the day 4) and had better learning efficiency (reaching the learning requirements on the day 10). In contrast, mice without reward started learning on day 7 and completed the experiment on day 13. That already close to 30% improvement. However, it should be noted that even if there is a post training reward, the learning time of mice does not seem to be shortened (It took 6 days for the mice with post training reward, and 7 days for the mice without reward). In other words, post training reward can significantly change the state and efficiency of learning but not the time or process of learning and training.

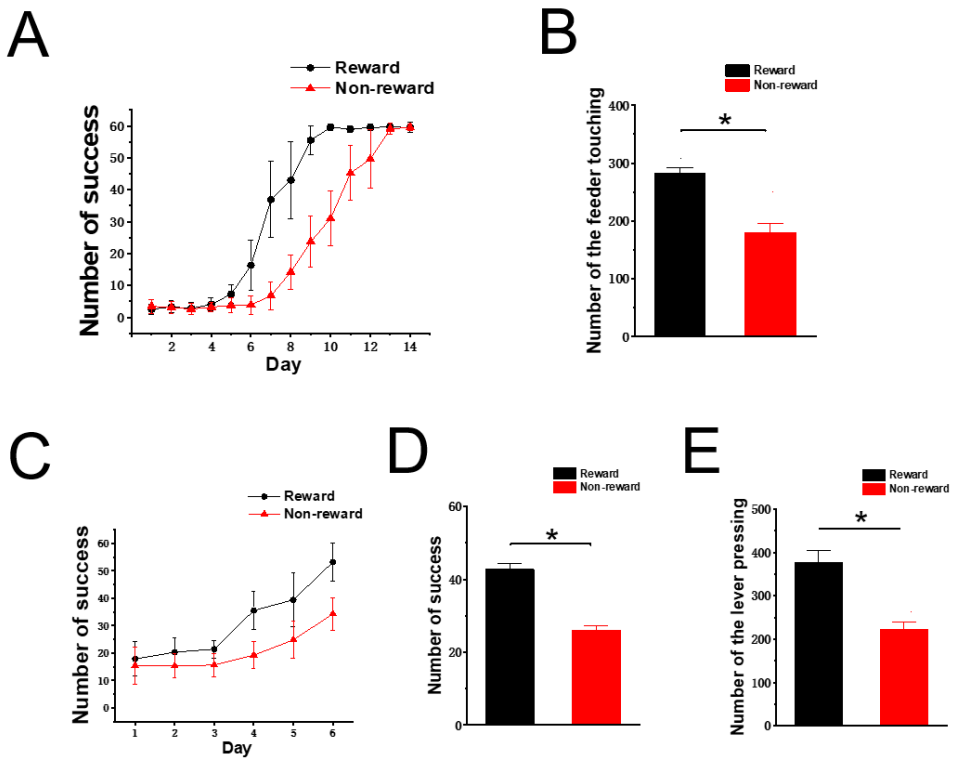

Figure 2. Experimental result. A, results of each day's training in Experiment 1 and show the learning trend of mice. B, the average times of mice touching the feeder in Experiment 1were compared. C, results of each day's training in experiment 2. D, the average number of successes in recording phase of experiment 2. E, the average number of levers pressing recorded in recording phase of experiment 2 and show the difference of enthusiasm of mice.

What should lead to the improvement of training efficiency? We think it may be the learning motivation of mice. Because in Experiment 1, the mice pressed down the lever, and the reward would be provided, so the number of levers pressing and the number of successes were basically equal. But according to the analysis in Experiment 1, the mice with post training reward had more feeder touching than those without reward (**Figure 2B**). This means that the rewarded mice have higher reward enthusiasm, which may be the reason for their higher learning efficiency. We think that the post training reward can stimulate the mice to expect the reward in training, and make the mice have a more positive attitude towards training, which is of great benefit to their learning.

3.2. Post training reward can improve the efficiency of behavioral training

The experiment 2 lasted for 6 days, which is on the basis of the experiment 1 and sound was added as a new condition. Only the mice press the lever when the sound played can get a syrup reward. This is actually a test of the sound judgment for mice. Through the analysis of the experimental results of six days, it can be seen that the learning trend of the mice with and without post training reward is roughly the same, which are constantly improving (**Figure 2C**). Since Experiment 2 was carried out after the mice completed the requirements of Experiment 1, the performance of the two groups of mice was almost the same at the beginning of Experiment 2. But everything was different from the second day of Experiment 2. The training results of mice with post training reward were better than those without reward, and the gap increasing with time. This confirmed again that post training reward can improve the learning efficiency of mice during training. By the last three days of data recording, the number of success of mice with post training reward was far more than that without reward. Although from the trend, the mice without reward can achieve the same number of successes as the mice with post training reward, but more training is needed. So, we consider that the training efficiency of mice with post training reward was higher than that without reward.

The number of successes between mice with and without post training reward show that success times of mice with post training reward is significantly more than that without reward (**Figure 2D**). It is in the whole training that the final training result is influenced by post training reward. The mice with post training reward have better training results. This time, we analyzed the number of pressure lever, and found that the number of pressure lever of mice with post training reward was significantly more than that without reward (**Figure 2E**). The number of levers pressing directly indicated the attitude of mice towards training, and is a direct indicator of training motivation and enthusiasm of mice. The higher number of levers pressing showed that the mice with post training reward were more active in training than the mice without reward. We believe that this is one of the important reasons for the higher training efficiency of mice with post training reward.

4. Discussion

Mice are very common experimental animals, such as monkeys, rabbits, rats and so on. Due to the price and docility of mice, mice have always been excellent experimental animals in biological research. As mentioned in introduction, one of the important methods in biological research is to establish animal behavior paradigm. The establishment of behavior paradigm is generally divided into two stages: conditional training and goal behavior training. The corresponding stage are experiment 1 and experiment 2. Experiment 1 is a typical reward conditional training, which is widely used in the establishment of many experimental behavior paradigms. In Experiment 2, sound was added as a new condition, and the goal behavior was to let mice learn to listen the sound. Experiment 2 was designed to imitate the goal behavior training stage in the establishment of behavior paradigm. The combination of Experiment 1 and Experiment 2 is a complete behavioral paradigm.

A total of 20 mice were divided into two groups. One group received a certain reward after training, while the other group did not. The final experimental data show that post training reward can effectively improve the learning efficiency of mice in Experiment 1 (conditional training stage) which may be caused by stimulating the expectation of the reward offer in training. Similarly, post training reward also improved the learning efficiency of mice in Experiment 2 (goal behavior training stage) and achieved better training results. This may be due to the mice with post training reward had more pressing lever times which means the activity of the training enthusiasm.

All in all, whether it is the conditional training stage or the goal behavior training stage, post training reward plays an obvious positive role. This means that post training reward can help the establishment of behavior paradigm. We strongly suggest that researchers can design appropriate post training reward when they need to establish behavior paradigm, which is very beneficial to the establishment of paradigm in experiments. However, it should be noted that when the reward behavior paradigm needs to be established, reward will be used to lure experimental animals to learn and train in the training process, which is likely to conflict with post training reward. Therefore, the selection and dosage of post training reward should be decided by pre-experiment.

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