# Changes in Food Intake and Abnormal Behavior Using a Puzzle Feeder in Newly Acquired Sub-Adult Rhesus Monkeys (Macaca mulatta): A Short Term Study

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***Abstract:*** *The majority of newly acquired nonhuman primates encounter serious problems adapting themselves to new environments or facilities. In particular, loss of appetite and abnormal behavior can occur in response to environmental stresses. These adaptation abnormalities can ultimately have an affect on the animal's growth and well-being. In this study, we evaluated the affects of a puzzle feeder on the food intake and abnormal behavior of newly acquired rhesus monkeys for a short period. The puzzle feeder was applied to 47- to 58-month-old animals that had never previously encountered one. We found that there was no difference in the change of food intake between the bucket condition and the puzzle feeder condition. In contrast, the time spent for consumption of food was three times longer in the puzzle feeder condition than in the bucket condition. Two monkeys initially exhibited stereotypic behavior. One showed a decreasing, and the other an increasing pattern of abnormal behavior after introduction of the puzzle feeder. In conclusion, this result suggests that over a short period, the puzzle feeder can only affect the time for food consumption since it failed to affect the food intake and did not consistently influence stereotypic behaviors in newly acquired rhesus monkeys.*
***Key words:*** *intake, puzzle feeder, rhesus monkey, stereotypic behavior*

## Introduction

The preparation of domestically bred animals for research usually involves some combination of social separation, relocation and re-socialization, as well as alterations to their physical space, photoperiod, and diet [2]. Investigators who use animals in biomedical and behavioral research have an obligation not only to conduct high-quality research, but also to promote the health and well-being of their animal subjects to the greatest extent possible given the research objective [9]. Most single-housed nonhuman primates in laboratory facilities, however, are not provided with environments that are sufficient to maintain their psychological well-being.

Foraging for food is part of a primate's natural behav-

ior and is very important for its psychological well-being [16]. It disperses animals, reduces tension and aggressive interactions, occupies time and reduces stereotypies [16]. The puzzle feeder has been used to assess the cognitive ability of young and aged monkeys [7, 14, 15], as a feeding enrichment tool for common marmosets [5], and the effects on self-inflicted rhesus monkey [11]. When laboratory primates are relocated to a new environment, they must adapt to a variety of stresses including temperature, humidity, water, food and cage size. Sauzeat *et al.* have mentioned that acute gastric dilatation in nonhuman primates can be induced by predisposing factors such as environmental disturbances, food in pellets, feeding schedules and behavior [13]. Similary, various stresses may affect the food intake and temperament of the animals. This study only addresses the short-term effect of the puzzle feeder on the food intake and abnormal behavior which was observed in newly acquired rhesus monkeys.

## Materials and Methods

*Animals*

Five female rhesus monkeys (*Macaca mulatta*) weighing 3.6 to 4.6 kg, 47–58 months of age, were used in this study. The monkeys were imported from China and were placed in quarantine for 30 days at the Korea Research Institute of Bioscience and Biotechnology. After quarantine, they were moved to our laboratory facility and housed in individual cages (WHD; $760 \times 760 \times 800$ mm), made of stainless steel plate. The room was maintained at $24 \pm 4°C$ with a relative humidity of $50 \pm 10\%$, artificial lighting on a 12:12 light-dark cycle (7:00 AM onset) and 13–18 air changes per hour. The animals had daily access to food (PS DITE®, Oriental Yeast Co., Ltd., Japan; plus fresh fruits) and unlimited access to water. All animals used in this study were cared for in strict accordance with the National Institutes of Health "*Guide for the Care and Use of Laboratory Animals.*"

*Apparatus*

The test apparatus was a Puzzle Feeder® (PP Inc., USA) commonly used as a feeding enrichment device. The puzzle feeder consisted of a thin Plexiglas box (WHD; $241.3 \times 222.3 \times 44.5$ mm) encasing removable



**Fig. 1.** The 5 to 6 levels of the puzzle feeder are made from individual horizontal or vertical pieces.

flat pieces which can be arranged both horizontally and vertically such that a variety of maze configurations can be formed (Fig. 1). The side of the box accessible to the monkey contained several small holes through which a monkey can insert its fingers and manipulate the food treat. However, the treat can only be removed from the large openings at the bottom of the box. The puzzle feeder was fixed in front of the cage with a plastic tie which prevented the monkey from removing the box.

*Procedure*

The subjects were five monkeys that were acclimating themselves to our laboratory facility after quarantine. Two of them showed stereotypic behavior, that is rubbing the cage's floor by hand, and pacing and bouncing. The monkeys experienced two experimental conditions: one condition was provision of food using a bucket and the other condition was provision of food using the puzzle feeder. Initially, the monkeys were fed with the bucket feeder after four weeks, they were fed with the puzzle feeder. All animals experienced each condition twice during this study. The maze configuration of the puzzle feeder was moderate (level 5 to 6) as described by Watson *et al.* [15]. The study was performed for 16 weeks. Food was supplied to the monkeys three times a day: apple (1/2, 50 kcal), banana (1/2, 40 kcal) and pellet (10 ea, 38 kcal) in the morning; seasonal fruits (20 kcal) and pellet (20 ea, 76 kcal) in the afternoon; only pellet (20 ea, 76 kcal) in the evening. The meal times were 9 h, 14 h, and 17 h on week days, and 10 h and 16 h on

weekends with the daily volume remaining the same. In the puzzle feeder condition, we gave the fruits to the animals inside the cage to prevent clogging of the passage of the maze. We measured the body weight of the animals every week, and before and after the study. The food intake was expressed as the number of pellets consumed divided by the number of supplied in 24 h as a percentage. The food consumption times of both feeder types were assessed from video records. It was the time spent retrieving pellets in the two feeding conditions, and did not include the time spent eating, such as filling or emptying of cheek pouches, masticating and swallowing. Abnormal behavior assessment was conducted by daily observation record in which an animal handler documented the incidence of the behaviors mentioned above. We counted the days showing abnormal behavior during observation period (about 16 weeks).

*Statistical analysis*

The results of all of the experiments are expressed as the mean ± SD. The paired *t*-test was performed on the mean intake rate, intake time and body weight using Microsoft Excel. The level of significance was set to 1% ($P<0.01$).

## Results

*Changes in food intake*

The data for change of intake rate, consumption time and body weight are presented in Table 1. The average food intake rate of the bucket condition was 88.6 ± 10.9% and that of the puzzle feeder condition was 87.4 ± 11.9%. One monkey showed an increased intake rate while the other four monkeys showed decreased or same intake rates between the experimental conditions. There was no significant difference between the two conditions. For food consumption time, the monkeys in the puzzle feeder condition (812.4 ± 429.4 sec) spent a longer time retrieving food than in the bucket condition (241.9 ± 108.3 sec). There was a significant difference between the two conditions ($P=0.0037$). After the quarantine, the body weight of subjects significantly decreased from 3.7 ± 0.3 to 3.5 ± 0.4 kg ($P=0.022$) during the acclimation period. Although, we recorded a slight increase (0.02 kg) of body weight compared with before the study in the puzzle feeder condition, it was not statistically significant. In conclusion, we found that there were no differences in the change in body weight between the two conditions.

*Change in abnormal behavior*

One monkey that showed the behavior of rubbing the cage's floor with both hands showed a decrease in behavior frequency from 9 to 1, the day after receiving the puzzle feeder. This monkey was interested in retrieving pellets from the maze of the feeder. The monkey that showed pacing and bouncing behavior, however, showed an increase in behavior frequency from 20 to 27 through-

**Table 1.** The change of intake rate, consumption time and body weight by feeder type

| No | Condition | Intake rate (%) | Consumption time (sec)** | Body weight (kg) |
|---|---|---|---|---|
| R001[†] | Bucket | 77.7 ± 14.3 | 344.7 ± 175.6 | 4.6 ± 0.3 |
| | Puzzle Feeder | 79.1 ± 15.6 | 1154.1 ± 974.8 | 4.8 ± 0.3 |
| R002[†] | Bucket | 79.1 ± 15.2 | 136.0 ± 94.7 | 4.5 ± 0.0 |
| | Puzzle Feeder | 72.9 ± 14.9 | 824.8 ± 285.0 | 3.9 ± 0.0 |
| R003 | Bucket | 86.3 ± 11.7 | 650.7 ± 238.7 | 4.3 ± 0.0 |
| | Puzzle Feeder | 86.1 ± 18.0 | 922.1 ± 376.6 | 4.3 ± 0.1 |
| R004 | Bucket | 100.0 ± 0.0 | 48.7 ± 13.9 | 3.7 ± 0.0 |
| | Puzzle Feeder | 98.7 ± 5.6 | 674.3 ± 305.3 | 3.6 ± 0.1 |
| R005 | Bucket | 100.0 ± 0.0 | 29.3 ± 18.4 | 4.5 ± 0.1 |
| | Puzzle Feeder | 100.0 ± 0.0 | 486.8 ± 205.1 | 5.0 ± 0.0 |

The changes of food intake rate and body weight were not significant between using the puzzle feeder and a bucket. The time taken to consume food was longer in the monkeys fed with the puzzle feeder than those fed with a bucket. **$P<0.01$; statistical significance between the experimental conditions. [†]Monkeys that showed stereotypic behavior.

out the 16-week period. This monkey was neither faster nor slower at retrieving the pellets from the puzzle and bucket feeders than the other monkeys. Consequently, we assume the puzzle feeder was not attractive to this monkey. This, the changes in abnormal behavior differed with the individual.

## Discussion

In this study, we evaluated the puzzle feeder and bucket as methods of delivering food to newly acquired rhesus monkeys. We recorded the effects of the different feeding devices in terms of both food intake and behavior. The relocation of animals from a standard-sized individual cage to an identical cage in a new room is moderately distressing for long-tailed monkeys, as indexed by their behavior and food intake [4]. The relocation of a monkey from social housing to individual housing requires it to adapt to the new environment. However, this adaptation process can often affect the food intake of the animals and result in abnormal behavior. Therefore, newly acquired animals may exhibit a decrease in their interest for food and an increase in abnormal behavior. Although we did not observe a serious change in body weight in any of the animals in this study, we detected an initial reduction of 0.2 kg after relocation. However, this reduction of body weight was a temporary phenomenon induced by environmental change, and it improved irrespective of the feeding condition.

Over the course of the study there was no significant difference in the amount of food consumed between the puzzle feeder condition and the bucket condition. However, we found an increase in the consumption time in the puzzle feeder condition that was probably due to the novelty of the puzzle feeder. It has previously been reported that some monkeys are in danger of serious problems, such as acute gastric dilatation (AGD), due to food restriction or accidental over feeding [12], as well as altered feeding schedules [3, 10]. To the author's knowledge, many monkeys that are housed individually at laboratory facilities die because of AGD regardless of the experiment. Therefore, the puzzle feeder could be used in this situation as a tool to regulate food intake and hence prevent this type of disorder.

A variety of studies have reported the behaviors of nonhuman primates that are associated with the use of different foraging devices [5, 6, 8, 11]. Monkeys that are individually-housed appear to be more vulnerable to the development of abnormal behavior than monkeys housed in social groups [1]. Two of the monkeys used in this study displayed stereotypical stress behaviors which included rubbing on the floor with the hands, and pacing and bouncing in the cage in an unchanging pattern. Although we had expected the puzzle feeder to reduce stereotypes, there was no remarkable change in this study. Also, there were no differences in food intake and consumption time between normal and abnormal behaviors. Novak *et al.* reported that the puzzle feeder was more effective than treats alone in alleviating whole body stereotypes in the short term [11]. Further study with more subjects on the relationship of the puzzle feeder with stereotypes in newly acquired rhesus monkeys will be need.

In this study, we evaluated the effects of the puzzle feeder on the food intake and abnormal behavior of individually housed rhesus monkeys over a short period of time. Use of the puzzle feeder caused the feeding time to increase compared to monkeys fed with a bucket; this mimics foraging for food that is a natural behavior for these monkeys. This study evaluated food intake and behavior over a short period of time. It would be very interesting to investigate the use of the puzzle feeder in a long-term study in the future.

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