·Basic Research·

Experimental Tibetan monkey domestication and its application for intraocular pressure measurement

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Abstract

• AIM: To train Tibetan monkey (Macaca thibetana) for intraocular pressure (IOP) measurement in conscious state and obtain normal IOP in conscious Tibetan Macaque.

• METHODS: The training was based on award-conditioned behavior. Food stimulation and human-animal interaction were used in this training.

• RESULTS: Trained Tibetan monkeys calmly accepted IOP measurement by the TonoVet® rebound tonometer without sedation or anesthesia and their IOP values were similar to other primates.

• CONCLUSION: Human-cultivated Thibetan monkeys are tamable, and can be used for biomedical research such as ophthalmic research without anesthesia.

• KEYWORDS: Macaca thibetana; domestication; conscious intraocular pressure measurement
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INTRODUCTION

Macaca thibetana (Macaca genera), a unique nonhuman primate in China, which widely distributed in western and northern Sichuan province [1,2], is a class Ⅱ national protected wildlife. Compared to rhesus and cynomolgus monkey, tibetan macaque have larger body, longer life span (over 20 years), calmer and gentler temperament, and it is suitable for domestication [3]. They are legally approved to be used for scientific research by the State Forestry Administration in the People's Republic of China. In 2005, the research project of artificial breeding of Tibetan Macaque was included in the science and technology infrastructure platform project (No.140107) which was regulated by the Science and Technology Department of Sichuan Province. Parts of the basic research works have already been done [4-7]. Non-human primates are very valuable because of their anatomic and functional similarities to human in ocular structures relevant to the disease, such as glaucoma, *etc* [8,9]. Most commonly used monkeys in intraocular pressure related studies are Macaca fascicularis and Macaca rhesus [10-12]. All approved IOP-lowering medications lower IOP in the monkey [13]. The intraocular pressure (IOP) measurement in monkey are all under anesthesia previously [14-16], while the accuracy and authenticity of the results would be affected by a variety of stress response triggered by the anesthesia or mandatory operation [10,17-19]. To eliminate anesthesia and other factors affecting the monkey intraocular pressure, we tried to use directed domestication of artificial-bred Macaca thibetana to measure intraocular pressure under conscious condition.

MATERIALS AND METHODS

Animals Twenty artificially-bred Macaca thibetana monkey in equal numbers of males and females were used in this study. All monkeys are aged 2-3 years old, weighted 2-3kg. National key protected wild animal domestication and breeding license is 2004, Sichuan 21-01. The experimental animal license number is SYXK [chuan]2008-058. Animal management including environment, cage specifications,

Figure 1 Basic domestication A: Getting food from the trainer; B:Close contact with the trainer; C:Crowding in the trainer's arm.

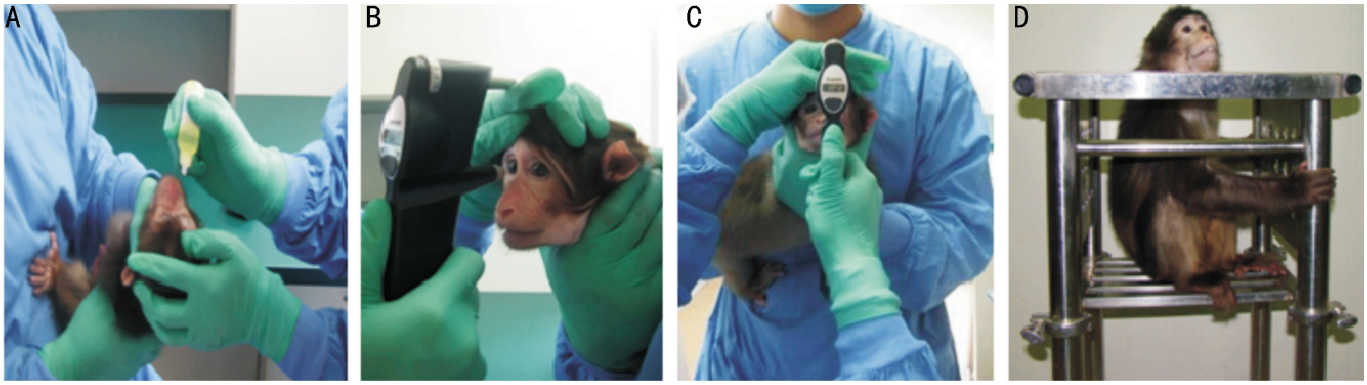

Figure 2 Train monkey for IOP measurement A: Accepting eyedrops; B, C: Trained monkey calmly accepting IOP measurement under conscious condition; D: Monkey in monkey chair.

feeding, nutrition, water, control of microorganism and parasite are all conformed to the forestry industry standard of the People's Republic of China LY/T 1784-2008. All animal experiments were conducted in compliance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research, the Guide for the Care and Use of Laboratory Animals (National Research Council, Chengdu Sichuan), and under the supervision of the Institutional Animal Care and Use Committee in Sichuan University. The instruments and drugs included TonoVet® rebound tonometer (Tiolat Oy, Helsinki, Finland), Ketamine injection, *etc.*

Methods

Basic domestication Experimental monkey was housed separately in cages. Behavioral training was conducted based on the principle of award-conditioned behavior. Newborn monkeys were raised in the laboratory, with daily close and friendly interactions with caretakers and trainers. Training for IOP measurement started at the age of two. Two trainers participated in the training, one holding the monkey gently in his arms, and the other one using the tonovet to measure IOP. The animal was gently restrained but not stressed. The trainer placed food on his palm and let monkey gradually adapted to get food from the trainer (Figure 1A). After monkey got used to it, the trainer could have more physical contact with monkeys such as shaking hands etc. If the monkey was willing to have physical contact with trainer, a peanut was given as a reward, which was one of the monkey's favorite foods but not provided in its daily food ration. If a monkey refused to do so, peanuts would not be given (Figure 1B, C). We tried to domesticate one monkey at first, it would give example to others, and more monkeys were trained together in the same way and the training was done one or two times each day.

Training method1: Training for IOP measurement by carrying the monkey in the arms One trainer in sitting position carried the monkey in his arms with one hand holding the monkey's forelimb, another hand lightly holding the monkey's lower jaw. The animal was gently restrained but not stressed. Supine position was used during training for drug delivery (Figure 2A). When giving medication, upper and lower eye lid was gently opened, liquid medication was dripped into the conjunctival sac (0.9% sodium chloride injection was given during the training). When training monkey calmly accepting IOP measurement under conscious condition, the monkey was carried by one trainer in upright position, another trainer's left hand gently hold monkey's forehead, the right hand hold Tonovet to measure IOP by using electromagnetic induction probe to contact the center of cornea in vertical Angle (Figure 2B,C) letting the monkey gradually accept IOP measurement under conscious condition. During early stage of training, trainer used mandatory way to give drug to those monkeys who were not cooperative. Food reward was given to those monkeys who were cooperative. To monkeys who were too excited,

Table 1 IOP values of conscious Macaca thibetana (mmHg)(n=12, 6 monkeys, binoculus) mean±SD

Detection time	Day time					Night time (start after three days of daytime test)				
	09:00	12:00	15:00	18:00	21:00	21:00	24:00	03:00	06:00	09:00
	23.0±1.5	29.3±1.5	24.2±1.5	24.2±1.5	22.3±1.5	20.4±1.5	20.5±1.5	19.7±1.5	22.6±1.5	22.7±1.5

struggling and not cooperative, the rewards would be canceled during training process and the training would be repeated. IOP measurement and drug delivery were performed on both eyes for two to three times per day.

Method 2: Training monkey for IOP measurement by monkey chair Self designed monkey chair was used for training monkey (Figure 2D). Trainers caressed monkey to help it overcome the fear and food was given as a reward. Training was given twice a day, each time lasted for more than one hour. Drug delivery and IOP measurement were undertaken when the monkey adapted to monkey chair.

IOP measurement All IOP measurements were conducted in the same room at room temperature, with humidity approximately 70%. Illumination intensity was set at 200 lx during the day (6 AM to 6 PM) and 10 lx at night (6 PM to 6 AM). IOP was measured using the TonoVet ® rebound tonometer (Tiolat Oy, Helsinki, Finland) according to the manufacturer's recommended procedures. The tonometer was programmed to calculate and display the mean IOP value of six consecutive, acceptable measurements. In this study, six mean values were obtained from each eye, at each time point, under each condition, and the mean of means treated as a single datum.

Statistical Analysis The highest and lowest time points during 24 hour time period were selected for measuring intraocular pressure of Tibetan Macaque under anesthesia. Ketamine (8mg/kg body weight) was used intramuscular for general anesthesia. SPSS 16.0 statistical software were used for single factor analysis of variance and paired T-test, A P<0.05 was considered to be statistically significant.

RESULTS

After three months of basic domestication, it was easy for monkeys to get along with trainers. Once the door was opened, monkeys would jump to the trainer and got food from the trainer"s pocket and played well with the trainer. When monkey chair was used for training, monkeys appeared to have fear and resistance. It's hard to eliminate this feeling in a short time. When training by carrying the monkey in the arms, monkeys seemed to be pleased and cooperative, and IOP measurement and drug delivery could be performed in the calm, conscious condition and the IOP measurement would be much easy and results would be more stable. It took us five months to tame 20 Macaca thibetanas for IOP measurement, visual evoked potential measurement and laser dazzling experiment and so on. The domesticated monkeys have been used for two years, and could still cooperate well with the trainer for various experiments.

Table 2 Effects of anesthesia on IOP of Thibetan monkeys (mmHg)(n=12, 6 monheys, binoculus)(mean±SE)

Detection state	Detection time	
	12:00	03:00
conscious	29.3±3.1	19.7±2.8
anesthesia	22.1±2.4[a]	19.7±1.4

[a]P<0.05 *vs* Conscious.

We got normal basic IOP values (Table 1) of six Macaca thibetana (half male and female) at different time points in a 24-hour period in the non-anesthetized state by carrying them in the arms.

We use different kinds of IOP-lowing mediations on domesticated Macaca thibetana to verify IOP lowing effect and compared it with the IOP values under anesthesia conditions. The results showed that the normal IOP of Macaca thibetana is similar to other non-human primates and the IOP values are significantly different between conscious and anesthetized state (Table 2). The domesticated Macaca thibetana has been used on IOP-lowing research for many new drugs [20].

DISCUSSION

It has been proved that the animal model of domesticated Macaca thibetana can last for a long time after training, and can be used repeatedly for long-term research. It may reduce the amount of monkeys used and therefore lower the cost of animal research. The IOP of domesticated Macaca thibetana is similar to that of Macaca rhesus and Machin [12]. The IOP of Macaca rhesus and Machinin is much higher in the morning and afternoon than that in other time points [10,11]. However, the IOP of Macaca thibetana increased significantly at noon, which may be due to that the measuring time is too close to feeding time (within 30 minutes) [20]. Anesthesia by Ketamine could lower the IOP, and the effect is prominent at noon, but not in 3 a.m. The possible reason may be that the IOP is already low at 3 a.m[20]. The success of using domesticated Macaca thibetana for IOP measurement indicated that it is feasible for domesticated monkey to be cooperative with researcher for blood collection, medicine injection and non-invasive blood pressure measurement by stretching out arms or legs.

Macaca thibetana in this study is M.mulatta zimmermam [1], which belongs to the old world monkey. The sequences of interferon and interleukin-6 genes in Macaca thibetana ar e

highly consistent with that of Macaca rhesus's [4]. In the evolutionary of primates, the relationship between old world monkeys and human is only second to the relationship between anthropoid and human, and it is likely that the old world monkeys are the direct ancestor of humans and anthropoid [21]. It implies that Macaca thibetana could be potential non-human primate resources with great biomedical research value. Artificially-bred Macaca thibetana used in biomedical application has not been reported in China.

The successful application of domesticated Macaca thibetana for IOP measurement indicated that it is feasible to domesticate Macaca thibetana for using in the ophthalmic research. Experiments with Macaca thibetana may play an important role on laboratory animal research and biomedical application, and promote the protection and application of Macaca thibetana.

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