·Original Article·

Motor assessment of developing common marmosets

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ABSTRACT

Motor development has been extensively studied in human infants and children, with several established scales for the evaluation of motor functions. However, the study of the neuronal mechanisms underlying human motor development is hampered by the lack of good animal models. The common marmoset (*Callithrix jacchus*), a small New World monkey, has recently attracted much attention as a potential non-human primate model for understanding human physiology and diseases. However, little is known about its gross motor development. In the present study, we found that marmosets have a critical period for motor development in postnatal weeks 2 to 5, and acquire most of their motor skills by 8 weeks of age. We also developed methods to assess their motor functions, which will be useful for the evaluation of motor performance in marmoset models of human diseases. In addition, we found that marmosets exhibit a "head-to-tail" sequence of motor development similar to that found in humans, further supporting the notion that they provide a good animal model for studying the neuronal mechanisms underlying human motor development.

Keywords: marmoset; animal model; motor development; motor behavior

INTRODUCTION

Motor development is one of the most important areas in developmental biology, and has been extensively studied over the past five decades. In human infants and children, the development of motor behaviors has been thoroughly characterized, resulting in the formulation of several scales for its assessment. For example, the Albert Infant Motor Scale has been widely used to measure the maturation of gross motor functions from birth up to the age of independent walking[1]. These methods have important clinical applications in the identification and treatment of premature newborn infants with motor dysfunction. Efforts have also been made to study the underlying neuronal mechanisms of human motor development. Two major theories of motor development, neuronal maturation *versus* dynamic systems theory, have been proposed. They reflect the traditional "nature-nurture" controversy in understanding human development[2, 3], and the exact neuronal circuit mechanisms remain largely unknown. One important reason is the lack of useful animal models that are amenable for experimentation.

Non-human primates, with close physiological and genetic similarities to humans, have been used as animal models for human diseases, such as Huntington's disease[4] and Parkinson's disease (PD)[5]. Monkey models of PD show motor dysfunctions typical of human patients. However, the Old World primates, such as rhesus monkeys, have significant limitations as models, such as the large body size, slow sexual maturation (>3 years), and long gestation period. Thus, the common marmoset (*Callithrix jacchus*), a small New World monkey, has recently attracted attention as a non-human primate model in neuroscience, immunology, drug toxicology, and stem-cell research[6]. Marmosets have a significantly smaller body size (300–500 g), faster sexual maturation (>1 year), relatively short gestation period (~144 days), and more offspring during their lifetime (40–80). Recently, transgenic marmosets with germline transmission have been successfully generated[7], further promoting the potential their use as models to study human physiology and diseases.

Several studies have described the development of specific behaviors in marmosets, such as hand preference[8] and head-cocking[9]. However, their gross motor development is still unknown. Furthermore, to model the motor dysfunctions in human diseases, it is essential to set up a scale to evaluate the motor abilities of marmosets. Therefore, in the present study, we mainly examined the gross motor development of marmosets from postnatal week 1 to week 8, by which time they have acquired most of the motor skills of the adult.

MATERIALS AND METHODS

Animals

Eleven newborn common marmosets (*C. jacchus*) were used in this study (Table 1). The animals were maintained and tested in the Jiuting Non-human Primate Facility of the Institute of Neuroscience, Shanghai, China. They were housed in cages as families with male-female pairs. Food and water were available *ad libitum*, except during the experimental sessions. Animal care and the experimental procedures were approved by the Animal Care Committee of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

Experimental Protocol

The tests were performed in a single sound-isolated room. Animals were taken from their home cages, handled by experienced breeders, and then tested in different tasks. In brief, as shown in the supplementary movies, each animal was (1) handled to test whether it could grasp small sticks, (2) observed in an open field for 10 min to test the righting reflex, postural control, and locomotion, (3) placed on a smooth inclined plane (45°) to test negative geotaxis, (4) placed on a vertical plastic rod (2.5 cm diameter) to test climbing ability, (5) observed in a small cage for 10 min to test hanging and jumping, and (6) placed behind two 10-cm high barriers to test barrier-crossing. No reward was given during the tests. To ensure the health of the young animals, the duration of tests was limited to 30 min.

Data Recording and Analysis

The marmoset behaviors in all experiments were recorded by digital video cameras. All data were scored offline by two independent experimenters in a blinded manner. Data are shown as mean ± SEM. All statistical analysis was performed using Origin 7.0 (OriginLab, Northampton, MA).

RESULTS

Grasping, Hanging, and Righting Reflex

We chose 11 newborn marmosets for this study (Table 1), consisting of five pairs of twins and one singleton. At the age of 1 week, they were all able to grasp a small stick (Fig. 1A; Supplemental Movie 1) and hang from the cage (Fig. 1B; Supplemental Movie 1), and showed a righting reflex when placed on their back (Fig. 1C; Supplemental Movie 1).

Postural Control

Postural control is the ability to achieve a stable vertical posture of the head and trunk against the force of gravity. We scored postural control as follows: 1, raising the head and looking up; 2, standing with forelimb support; and 3, sitting with hindlimb support (Fig. 2A; Supplemental Movie 2). We found that marmosets could only raise the head during the first two weeks, and then stood with forelimb support from the third week (Fig. 2A; Tables S1–11). In the

Table 1. Animal information

Animal No.		Birth date (yyyy.mm.dd)	Gender
2-1	Twins	2012.03.22	M
2-2		2012.03.22	M
8-1	Twins	2012.03.23	M
8-2		2012.03.23	M
23	Singleton	2012.04.07	F
14-1	Twins	2012.04.22	F
14-2		2012.04.22	F
25-1	Twins	2012.04.27	M
25-2		2012.04.27	M
13-1	Twins	2012.05.03	M
13-2		2012.05.03	F

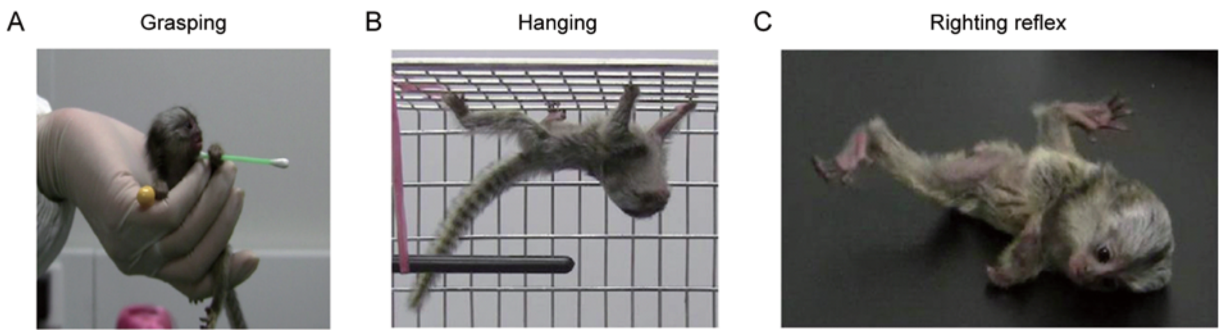

Fig. 1. Representative images showing the behaviors of grasping (A), hanging (B), and the righting reflex (C).

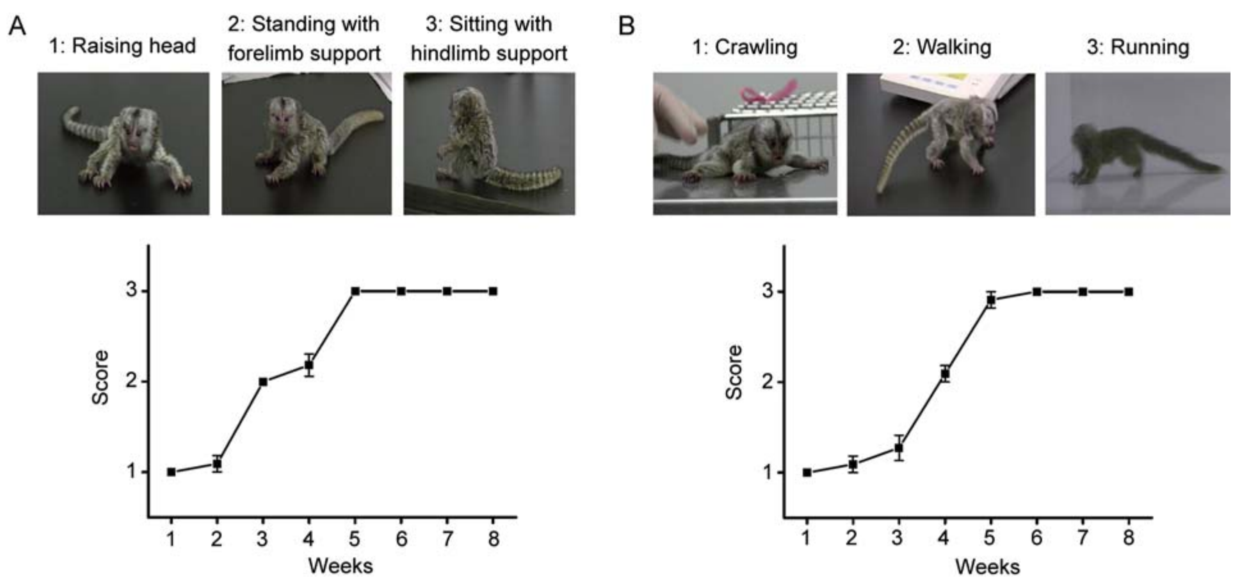

Fig. 2. Representative images and summary of results for postural control (A) and locomotion (B). ***n*** **= 11.**

fifth week after birth, they were all able to sit with hindlimb support, similar to adults (Fig. 2A; Tables S1–11).

Locomotion

Locomotor behavior was scored as follows: 1, crawling; 2, walking; and 3, running (Fig. 2B; Supplemental Movie 3). Most marmosets could only crawl during the first three weeks, and then walked from the fourth week. In the sixth week, they could all move by running (Fig. 2B; Tables S1–11).

Negative Geotaxis

Negative geotaxis, the movement response against gravity, was tested by placing the animals facing down on a smooth inclined plane. This behavior was scored as: 1, sliding down; 2, holding onto the inclined plane; and 3, orienting upward and moving up the plane (Fig. 3A; Supplemental Movie 4). Most animals could hold onto the inclined plane in the second or third week, and showed negative geotaxis in the fourth week, as they successfully oriented themselves upward (Fig. 3A; Tables S1–11).

Climbing

Climbing is an important motor skill for monkeys, and is also complicated as it involves the coordination of the whole body. Climbing behavior was assessed using a vertical plastic rod and scored as: 1, sliding down the rod; 2, holding onto the rod; and 3, climbing up the rod (Fig. 3B; Supplemental Movie 5). Marmosets could hold onto the rod

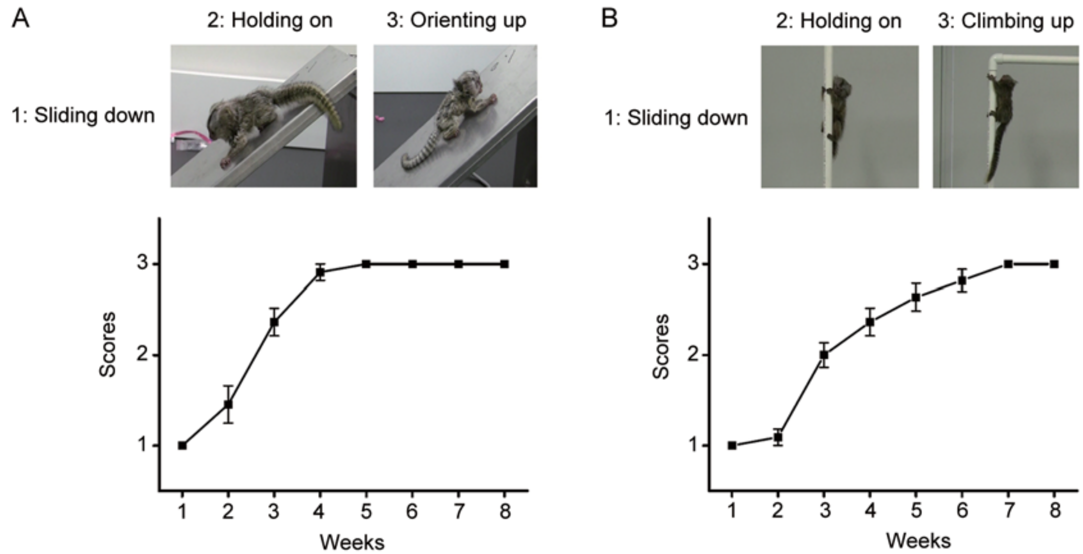

Fig. 3. Representative images and summary of results for negative geotaxis (A) and climbing (B). *n* = 11.

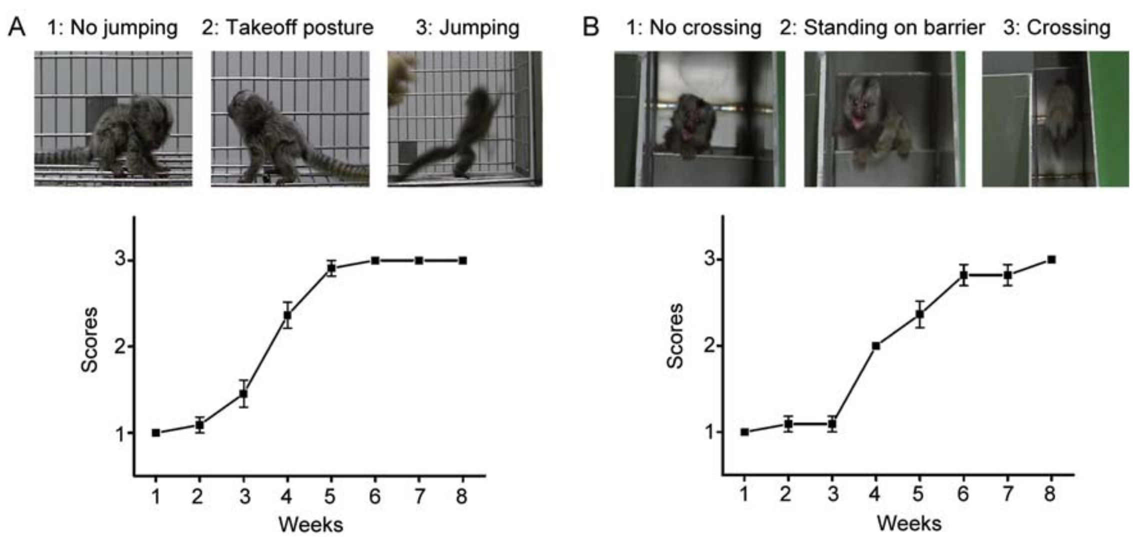

Fig. 4. Representative images and summary of results for jumping (A) and barrier-crossing (B). *n* = 11.

from the third week, and all animals could climb up it in the eighth week (Fig. 3B; Tables S1–11).

Jumping

Jumping is also especially important for monkeys, and depends especially on the power of the hindlimbs. The jumping behavior was observed in a cage and scored as: 1, no jumping; 2, takeoff posture; and 3, jumping (Fig. 4A; Supplemental Movie 6). Similar to the results above, they showed the takeoff posture from the third week after birth, while most marmosets were able to jump in the fifth week (Fig. 4A; Tables S1–11).

Barrier-crossing

Finally, we designed a barrier-crossing task since the ability to cross a barrier is necessary for marmosets, and requires a variety of basic motor skills. We placed two 10-cm high barriers in front of the marmosets. The barrier-crossing behavior was scored as: 1, no crossing; 2, standing on the barrier; and 3, barrier-crossing (Fig. 4B; Supplemental

Movie 7). We found that from the fourth week, they could stand on the barrier, and in the eighth week, all successfully crossed it (Fig. 4B; Tables S1–11).

DISCUSSION

In this study, we examined the gross motor development of common marmosets. In the first week after birth, they exhibited grasping, hanging, the righting reflex, raising the head, and crawling, all of which are important for newborn marmosets to tightly cling to their parents. In the third and fourth weeks, they could stand using the forelimbs, walk, and hold onto the inclined plane and vertical rod. By 6 to 8 weeks, when marmosets start to obtain food by themselves, they had acquired essentially all the common motor skills of the adult, including sitting with hindlimb support, running,

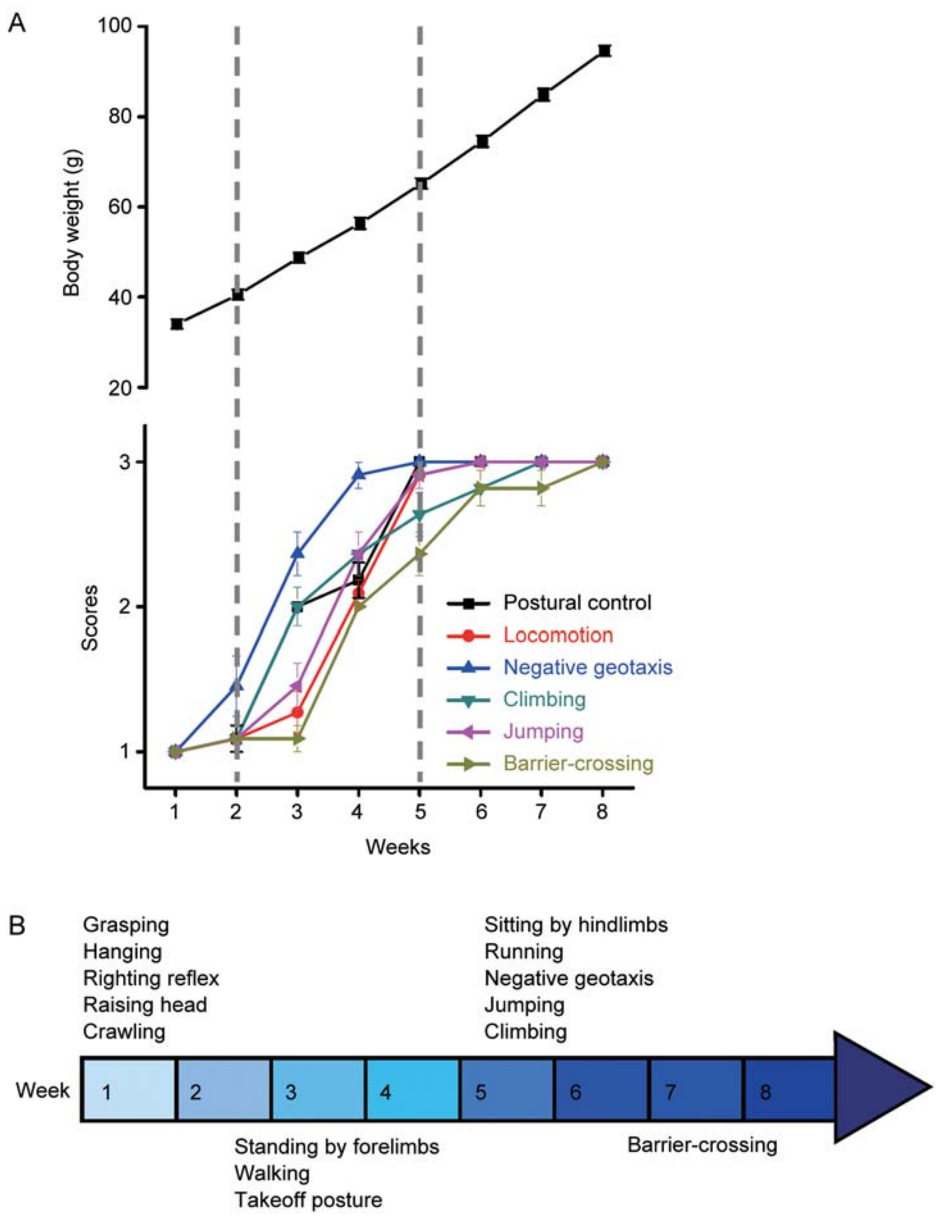

Fig. 5. Summary of the motor development of marmosets. A: The increase of body weight (upper panel) and the development of motor abilities (lower panel) during the first 8 postnatal weeks. *n* = 11. B: Flow-chart depicting the motor development of marmosets.

negative geotaxis, jumping, climbing, and barrier-crossing. These results showed a "head-to-tail" sequence of motor development and indicate that weeks 2 to 5 is a critical period for the motor development of marmosets (Fig. 5). Furthermore, by analyzing the developmental change of body weight (Fig. 5A), we found a linear increase with age, with no critical period like that of motor development, suggesting that the motor development is not simply due to growth but reflects the maturation of the nervous system.

Motor development has been extensively studied in humans and some animal models, but little is known in marmosets. Motor behaviors of newborn marmosets are difficult to observe in the home cage, because they are usually carried on the back of the mother. In this study, we removed the infants from their home cage once a week, and were the first to describe the gross motor development of marmosets. The various tests and parameters used in the present study were designed based on both the motor assessment methods for human infants (such as grasping, postural control, and locomotion) and the natural characteristics of marmosets (such as climbing, jumping, and barrier-crossing). Interestingly, in a previous study, a specific marmoset behavior (head-cocking) was reported to occur on day 13 after birth and reach a stable level by days 24–29[9], over the same developmental period as our study. Motor performance has also been studied in several marmoset disease models, such as PD[10], spinal cord injury[11], stroke[12], and aging models[13]. In the future, marmosets could be further used for developing models of other motor-related human diseases, such as transgenic models for motor-related genes. Our study establishes a basis for the further study of motor development in marmoset disease models, and provides a method for the assessment of their gross motor functions.

A controversial aspect of human motor development is the underlying mechanisms. The traditional neuronal maturational theory proposed by McGraw and Gesell[14, 15] posits that changes in motor development are due to the maturation of the central nervous system. On the contrary, the "dynamic systems theory" proposes that movement patterns do not arise from the maturation of neuronal centers but from the cooperation of multiple subsystems[16, 17]. Given that the motor development of marmosets resembles the "head-to-tail" sequence in humans, marmosets could be used for neuronal circuit analysis to resolve this "nature-nurture" issue in motor development. Interestingly, as shown in Table S5, the only singleton (23#), supposed to obtain more nutrition from its mother than twins, showed earlier motor development with a critical period from 2 to 3 weeks after birth, while the other twinned animals showed a critical period from 3 to 5 weeks (Tables S1–11). In the future, genetic manipulation in marmosets can be used to study the intrinsic determinants of motor development. Also, postnatal training or interference in marmoset models can be used to study the nurture mechanism of motor development.

SUPPLEMENTAL DATA

Supplemental data include 7 movies and 11 Tables, and can be found online at http://www.neurosci.cn/epData.asp?id=140.

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REFERENCES

[1] Piper MC, Pinnell LE, Darrah J, Maguire T, Byrne PJ. Construction and validation of the Alberta Infant Motor Scale (AIMS). Can J Public Health 1992, 83 (Suppl 2): S46–50.

[2] Thelen E. Motor development. A new synthesis. Am Psychol 1995, 50: 79–95.

[3] Heriza C. Motor development: traditional and contemporary theories. Contemporary Management of Motor Control Problems: Proceedings of the II STEP Conference. Alexandria, Va: Foundation for Physical Therapy 1991: 99–126.

[4] Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC*, et al.* Towards a transgenic model of Huntington's disease in a non-human primate. Nature 2008, 453: 921–924.

[5] Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L*, et al.* Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. Science 2000, 290: 767–773.

[6] Mansfield K. Marmoset models commonly used in biomedical

research. Comp Med 2003, 53: 383–392.
[7] Sasaki E, Suemizu H, Shimada A, Hanazawa K, Oiwa R, Kamioka M*, et al.* Generation of transgenic non-human primates with germline transmission. Nature 2009, 459: 523–527.
[8] Hook MA, Rogers LJ. Development of hand preferences in marmosets (*Callithrix jacchus*) and effects of aging. J Comp Psychol 2000, 114: 263–271.
[9] Kaplan G, Rogers LJ. Head-cocking as a form of exploration in the common marmoset and its development. Dev Psychobiol 2006, 48: 551–560.
[10] Verhave PS, Vanwersch RA, van Helden HP, Smit AB, Philippens IH. Two new test methods to quantify motor deficits in a marmoset model for Parkinson's disease. Behav Brain Res 2009, 200: 214–219.
[11] Iwanami A, Yamane J, Katoh H, Nakamura M, Momoshima S, Ishii H*, et al.* Establishment of graded spinal cord injury model in a nonhuman primate: the common marmoset. J Neurosci Res 2005, 80: 172–181.
[12] Marshall JW, Ridley RM. Assessment of cognitive and motor deficits in a marmoset model of stroke. ILAR J 2003, 44: 153–160.
[13] Tardif SD, Mansfield KG, Ratnam R, Ross CN, Ziegler TE. The marmoset as a model of aging and age-related diseases. ILAR J 2011, 52: 54–65.
[14] McGraw MB. The Neuromuscular Maturation of the Human Infant. London: Mac Keith Press, 1943. Reprinted 1989: Classics in Developmental Medicine No.4.
[15] Gesell A. The ontogenesis of infant behavior. In: Carmichael L (Ed.). Manual of Child Psychology. Hoboken, NJ: John Wiley & Sons Inc, 1946.
[16] Bernstein NA. The co-ordination and regulation of movements. Oxford, UK: Pergamon Press Ltd., 1967.
[17] Schoner G, Kelso JA. Dynamic pattern generation in behavioral and neural systems. Science 1988, 239: 1513–1520.