Locomotion Elicited by Electrical Stimulation in the Midbrain of the Lizard *Gekko gecko*

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Abstract. Locomotor behaviors evoked by stimulating the midbrain were studied in anesthetized and freely moving, awaking lizard *Gekko gecko*. Twenty *Gekko gecko* males were used in the anesthetized brain stimulation experiments; twenty were for microelectrode implantation. In the acute studies, four locomotor modes (lateral curvature of vertebral column, 's' shape curvature of vertebral column, limbs moving and walking or crawling manner, phonation reaction) were elicited successfully by normal electrical stimulation in anesthetized gekkos. The research show most effective points of stimulation for induced locomotion were located at the midbrain tegmentum. In the awake experiment, electrical stimulation was delivered through implanted electrode of certain regions (the regions were decided by the results of the acute experiments) of the midbrain in 20 gekkos. Locomotor modes, such as right and left turn , even the combined locomotion (going forward then turning around) was were successfully elicited. Results suggested that it is possible to carry out artificial induction on *Gekko gecko* through electrical stimulation on the related nuclus in their brain.

Keywords: *Gekko gecko,* electrical stimulation, midbrain, mesencephalic tegmentum, locomotion induce.

1 Introduction

Animals have wonderful locomotion abilities, especially those that can move on 3-dimensional, complex terrain. The remarkable motion capability of geckos has made them the hot spot for research, such as the investigations on the adhesion mechanism in gecko setae (Autumn, 2000; Gao, 2005; Rizzo, 2006; Sun, 2005; Sitti, 2002; Geim, 2003). The motion ability of a modern mobile robot system in an unstructured environment lags far behind animals in stability, flexibility, robust, environ- mental adaptation and efficiency of energy sources (Dickinson, 2000). Since the 1990s, bio-robotics has become one of the main directions for robotics research (Dai, 2007; Guo, 2005). The lizard *Gekko gecko*, located in Southwest China, North Vietnam, Northeast India and the Indo-Australian Archipelago (Holzer, 1979) was chosen as the target animal and was studied for developing a bio-robot, since it is big enough for investigating and for load-carrying. If we can modulate gekkos' locomotion just like the robo-rats (Talwar, 2002), it will be a revolution of Three Dimensional Obstacle-Free (TDOF) robots! As is commonly known among biological scientists, the

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midbrain in mammals contains circuits that modulate locomotion. Modern research on the brain mechanisms for the initiation of locomotion has discovered a site in the midbrain known as the mesencephalic locomotor region (MLR), and such a region has been detected in all species that have been examined (Adams, 1968 and 2006; Terry, 1978; McClellan, 1984; Jordan, 1998; Jean, 2003). However, there is no research on the *Gekko*'s midbrain for locomotion control. Most research on *gekko* has been on its forebrain, especially the neuroanatomical functions (Smeets, 1986; Hoogland, 1995; Bruce, 1995; Fokje, 1988; Gonzalez, 1990; Li, 2001), although there has been some research on gecko's vocalization (Kennedy, 1975; Lan, 1982) and locomotion mechanism of the toes controlled by peripheral nerve (Guo, 2006). In this study, we applied the electrical stimulation technique to locate the nerve corpuscle in *Gekko*'s middle brain that is responsible for *Gekko*'s locomotion.

2 Methods

2.1 Subjects

Adult *Gekko gecko* males (n=40), snout-to-vent length from 150 to 165 mm, body weight from 65 to 100 g, were bought from Nanning, Guangxi Province in China and were housed in a special room used for raising gekkos. In that facility, gekkos were raised for at least two weeks. Twenty were used in the anesthetized brain stimulation experiments; the remainders were for microelectrode implantation.

2.2 Surgery Procedures

2.2.1 Gekkos for Anesthetized Test

Gekkos in the acute experiment were lightly anesthetized with Nembutal (30 mg/kg). After the gekkos were anesthetized, they were placed in the stereotaxic apparatus and their heads were held firmly by the cranium holder (Model IBSS-Gecko-01, designed for *Gekko* used only and which was compatible with standard stereotaxic devices, Appl. 200610086008. X) (Wang, 2007). Opening the skull, cutting off the dura, and removing off the arachnoid, the craniotomy was carried out to expose the selected brain area for electrical stimulation.

2.2.2 Gekkos for Freely Moving, Awake Test

Gekkos for the freely moving, awake experiment did not perform the craniotomy. Instead, we used the micro dental motor handpiece which can be fixed on the stereotaxic apparatus to drill holes (diameter 600μm) on the skull of the gekkos' heads. And we used needle to penetrate the dura and arachnoid of the gekkos' brain. Then a number of bipolar stainless stimulating microelectrodes (Teflon coated for isolation) were chronically implanted into the gekkos' brain. Once the electrodes were lowered to the selected point they were fixed to the skull with dental cement. And a connector was used to connect the electrode for the next stimulation.

2.3 Stimulation Procedures and Behavioral Testing

In the acute experiments, a constant-current (negative pulses, 10-100 μA) was delivered through a electrical stimulator (Chengdu instrument factory, model YC-2-S). The

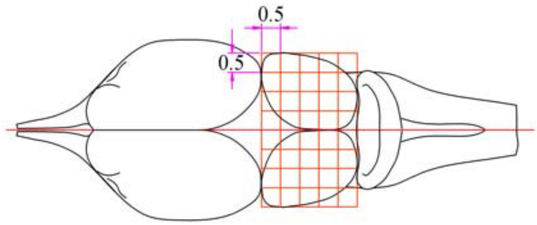


Fig. 1. Stimulation sites of the mesencephalon

brain was stimulated at various points on a grid of sites (Fig. 1) across the midbrain with a bipolar stainless electrode (diameter 100μm). And at each point, the electrode was lowered into the brain tissue by 50 μm steps. The stimuli were 2-5 sec trains of monophasic square waves, and the stimulus duration was shortened when the responses became too violent. The stimulus intensity was varied between 10-60 μA. At each stimulated site, the current was increased by steps of 10 μA. Response reproducibility was tested at three stimulus intensities. A rest period of 10 min was allowed between successive tests.

In the chronic experiment, the geckos were tested in an open field (on which the grid of 38 mm×38 mm was drawn) after recovery from surgery one weeks later. The stimuli consisted of biphasic pulses no more than 100 μA. Response reproducibility was tested at three times. A rest period of 5 min was allowed between two tests. We tested these stimulation points once a day after 5 o'clock P.M. and gave another two experiments in the following two days.

In the tunnel test, a cage with tunnel (Fig. 2) was made for the experimentation. In which, the geckos were made to crawling along the tunnel.

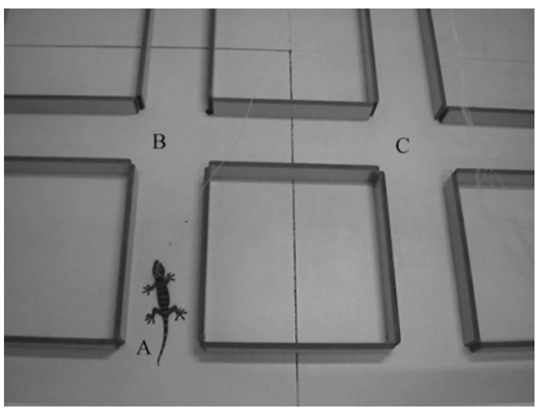


Fig. 2. The gecko was made to move from A to B then to C along the tunnel (The width of the tunnel is 20 cm, the distance form A to B is 50 cm—the same as that of B to C). In the test, Electrical stimulation was delivered to the gecko's brain through soft fine wire. gecko was made to start form A on the forward command; when the gecko got to B , the forward stimulation stop and the stimulation for right turn began; when the gecko turning the suitable angle, the turning command stopped and the forward crawling command was given.

2.4 Histology

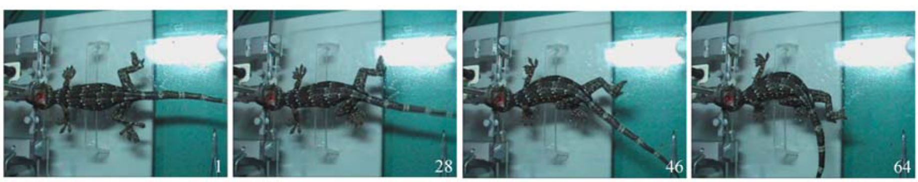
On completion of the studies for acute stimulation, a direct current (80 μA, 30 s) was passed between alternate electrode pairs to provide an anatomic referent for stimulation sites. The animals were sacrificed with Pentobarbital Sodium and perfused through the heart with 0.8% Nacl solution followed by 1% potassium ferrocyanide and a buffered 4% paraform solution. Their heads were detached and placed in a 4% paraform solution for at least 2 days. The fixed brains were removed and cut in 30 μm coronal frozen sections. The sections were stained with Neutral Red for light microscopy localization of the electrolytic lesions.

As to the awake studies, an electrolytic lesion (n=10) was also made at the stimulation site using DC current applied at the end of experiments for later histological identification.

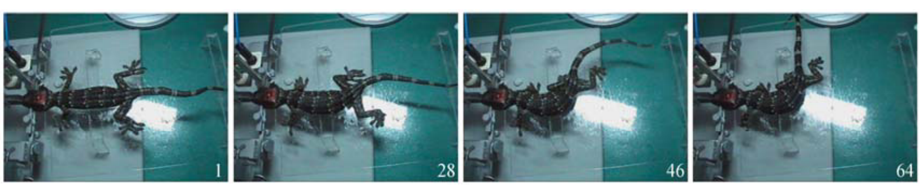
3 Results

3.1 Results of Acute Studies

We defined four locomotor modes: lateral curvature of vertebral column(LCVC), 's' shape curvature of vertebral column(SCVC), limbs moving and walking or crawling manner(LM&CM), phonation reaction(PR). These motion modes exhibited by gekkos were evoked by electrical stimulation (Fig. 3). The schematic distribution of the effective site to electrical stimulation was shown in Fig. 4. For the sake of modulation on *Gekko*'s locomotion, we pay more attention to the curvature of vertebral column. The research shows the curvature of vertebral column was often accompanied with limbs movement such as the limbs adduction and stretching. And there are two forms for the



(a)



(b)

Fig. 3. Locomotion of lateral curvature of vertebral column (bending toward the contralateral side of stimulation sites) induced by midbrain electrical stimulation of the lightly anesthetized geckos. From right to left, the recording pictures arranges on time successively (the number on which represents the serial number of the frame in the tape). (a) Vertebral column bent to the right and (b) to the left.

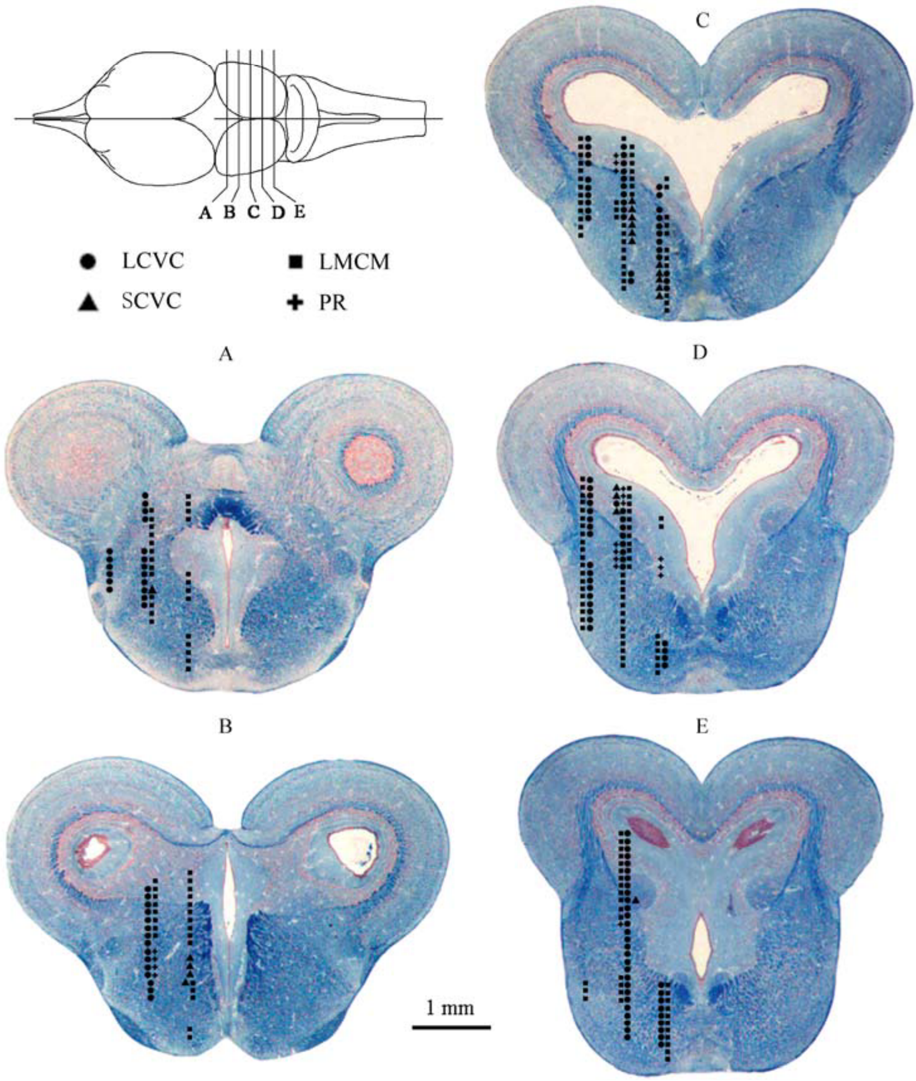


Fig. 4. Locomotion evoked by electrical stimulation in the midbrain of the gecko. Corresponded to the brain areas indicated by lines on the the dorsal view of the gecko's brain at the left-top, the right part of five brain maps were selected to show the positive sites to evoke locomotion.

lateral curvature of vertebral column: bending toward the ipsilateral or contralateral side of stimulation sites. According the *Gekko*'s crawl gait we presume that the 's' shape curvature of vertebral column is correlated with the movement of crawling forward.

And the anatomic study show most effective points of stimulation for induced locomotion were located at the mesencephalic tegmentum near the midline (Fig. 5).

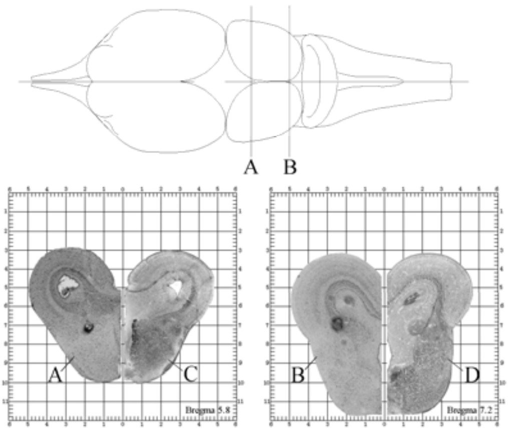
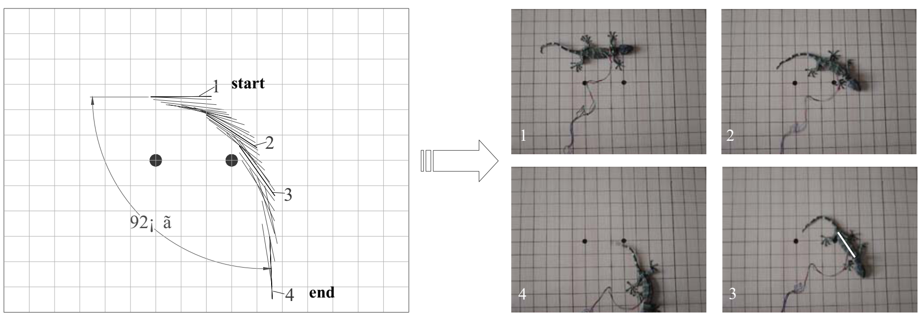


Fig. 5. Anatomic referent for stimulation sites in the midbrain of the gecko. A, B: Coronal section (frozen section, neutral red staining). C, D: Corresponding brain atlas (paraffin section, HE staining).



(a) (b)

Fig. 6. Right turning motion induced by midbrain electrical stimulation in the awake *Gekko gecko*. (a) Schematic of the *Gekko*'s motion route. (b) Recording pictures of the specified position in the left route schematic diagram. The white line (the midline of the body between the forelimbs and hindlimbs of the animal) in the picture 3 indicates the gekko's position.

3.2 Results of Awake Studies

According to the results of acute studies, electrical stimulation was delivered through implanted electrodes of certain regions of the midbrain in 20 *Gekko geckos*. Locomotor modes, such as right and left turn and forward crawling were successfully elicited. Fig. 6 shows the right turning of the gecko in the awaking test. The stimulus duration is about 3.72 s (93 frame total, 25 frames/s for the recording). We selected 28 frames of the recording to draw the route schematic diagram, and the midpoint of the gekko's forlimbs and hindlimbs were chosen as the two reference point. The line between the two reference points represents the position of the *Gekko* in each selected frame. From the Schematic of the gekko's motion route, we calculated that the turning angle was about 88°.Results show that the turning angle increases with the duration for electrical stimulation.

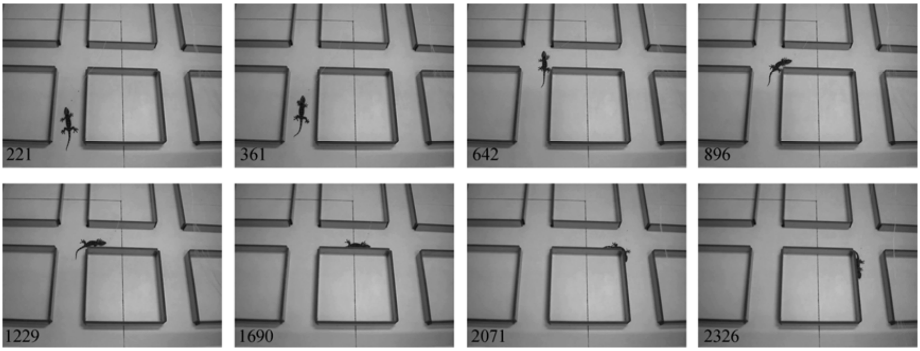


Fig. 7. Combined locomotion was induced successfully in a setting tunnel (forward going and right turning; the number in the picture indicate the serial number of the frame in the tape)

Furthermore, the experiment of combined locomotion which to combine the forward crawling and right turning was conducted successfully in a setting tunnel (Fig. 7).

4 Discussion

Our electrical stimulation study suggests that there are areas for locomotion control in gecko's midbrain. Anatomical studies revealed that the region might included the medial longitudinal fasciculus (FLM), medial longitudinal fasciculus□medial and lateral forebrain bundle (MFB), nucleus profundus mesencephali (PMI), stratum album periventriculare (SAP) and the red nucleus (Nrb).

The ongoing experiments of chemical microstimulation (L-glutamic acid microinjection) of gecko's midbrain have given some tentative confirmation to this conclusion. Locomotion of lateral curvature of vertebral column could be induced through chemical stimulation to the midbrain tegmentum. Whether there is MLR in the mesencephalic tegmentum is still to be identificated.

The response reproducibility in awake studies is not as good as that of anesthetized stimulation. This was caused by several difficulties.

Geckos lack the tightly adhering brain cases of mammals and birds and, instead they posses an enormous subarachniod space (Distel, 1978); the brain tissue is supported mostly by the cartilage and connective tissue. So the shape and position of the brain tissue is easy to change when it is pressed by electrodes. And most important, there was no midbrain atlas for *Gekko gecko*. For this reason, we carried out the preliminary preparation of the gekkos' brain atlas and engaged in the work now. However, it has a long road to go to set up a set of precise stereotaxic brain atlas for the *Gekko*.

In order to increase the accuracy markedly, we should perform the experiments on a larger sample of animals and improve the technique to implant the stimulated microelectrodes.

5 Conclusions

The present results imply a crucial role of *Gekko*'s midbrain neurons in the initiation of locomotion. And stimulation in freely moving, awake gekkos suggested that it is possible to carry out artificial induction on the *Gekko gecko.*

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References

[1] Autumn, K., Liang, Y.A., Flsieh, S.T., et al.: Adhesive force of a single gecko foot-flair. Nature 405, 681–685 (2000)
[2] Gao, H.J., Wang, X., Yao, H.M., et al.: Mechanics of hierarchical adhesion structures of geckos. Mech. Mater 37(2, 3), 275–285 (2005)
[3] Rizzo, N.W., Gardner, K.H., Walls, D.J., et al.: Characterization of the structure and composition of gecko adhesive setae. J. R. Soc. Interface 3, 441–451 (2006)
[4] Sun, J.R., Guo, C., Cheng, H., et al.: Comparison of the setae between the dung beetle Copris ochus and the gecko Gekko gecko and the effects of deformation on their functions. Acta Zoo Sin 51(4), 761–767 (2005) (in Chinese)
[5] Geim, A.K., Dubonos, S.V., Grigorieva, I.V., et al.: Microfabricated adhesive mimicking gecko foo-hair. Nat. Mat. 2(7), 461–463 (2003)
[6] Sitti, M., Fearing, R.S.: Nanomolding based fabrication of synthetic gecko foot-hairs. In: Proceedings of the IEEE Nanotechnology Conference, Washington DC, USA, pp. 137–140 (2002)
[7] Geim, A.K., Dubonos, S.V., Grigorieva, I.V., et al.: Microfabricated adhesive mimicking gecko foot-hair. Nat. Mat. 2(7), 461–463 (2003)
[8] Dickinson, M.H., Farley, C.T., Full, R.J., et al.: How animals move: an integrative view. Science 288(7), 100–106 (2000)
[9] Dai, Z.D., Sun, J.R.: Research progress in gecko locomotion and biomimetic gecko-robots. Prog. Nat. Sci. 17(1), 1–5 (2007) (in Chinese)
[10] Guo, C., Dai, Z.D., Sun, J.R.: Current status and prospect of research on bio-robots. Robot 27(2), 188–192 (2005) (in Chinese)
[11] Talwar, S.K., Xu, S.H., Hawley, E.S., et al.: Rat navigation guided by remote control. Nature 417(2), 37–38 (2002)
[12] Adams, D.B.: Cells related to fighting behavior recorded from midbrain central gray neuropil of cat. Science 159, 894–896 (1968)
[13] Adams, D.B.: Brain mechanisms of aggressive behavior: An updated review. Neurosci. Biobehavi. Revi. 30, 304–318 (2006)
[14] Robinson, T.E.: Electrical stimulation of the brain stem in freely moving rats: I. Effects on behavior. Physiol. Behav. 21(2), 223–231 (1978)

[15] Robinson, T.E., Vanderwolf, C.H.: Electrical stimulation of the brain stem in freely moving rats: II. Effects on hippocampal and neocortical electrical activity, and relations to behavior. Exp. Neurol. 61(3), 485–515 (1978)

[16] McClellan, A.D., Grillner, S.: Activation of 'fictive swimming' by electrical microstimulation of brainstem locomotor regions in an in vitro preparation of the lamprey, central nervous system. Brain Res. 300, 357–361 (1984)

[17] Jordan, L.M.: Initiation of Locomotion in Mammals. Annals of the New York Academy of Sciences 860, 83–93 (1998)

[18] Jean, M.C., Céline, B.L., Réjean, D.: Bimodal locomotion elicited by electrical stimulation of the midbrain in the salamander Notophthalmus viridescens. J. Neurosci. 23(6), 2434–2439 (2003)

[19] Smeets, W.J.: A forebrain atlas of the lizard Gekko gecko. J. Comp. Neurol. 254, 1–19 (1986)

[20] Hoogland, P.V., Vermeulen-Vanderzee, E.: Efferent connections of the lateral cortex of the lizard Gekko gecko: evidence for separate origins of medial and lateral pathways from the lateral cortex to the hypothalamus. J. Comp. Neurol. 352(3), 469–480 (1995)

[21] Bruce, L.L., Neary, T.J.: Afferent projections to the lateral and dorsomedial hypothalamus in a lizard, Gekko gecko. Brain Behav. Evol. 46(1), 30–42 (1995)

[22] Russchen, F.T., Jonker, A.J.: Efferent connections of the striatum and the nucleus accumbens in the lizard Gekko gecko. J. Comp. Neurol. 276(1), 61–80 (1988)

[23] Gonzalez, A., Russchen, F.T., Lohman, A.H.: Afferent connections of the striatum and the nucleus accumbens in the lizard Gekko gecko. Brain Behav. Evol. 36(1), 39–58 (1990)

[24] Li, G.F., Meng, S.Q., Jiang, S.Y.: Connections of rostrollateral area of the anterior dorsal ventricular ridge in lizards Gecko gecko. Zoological Res. 22(1), 74–77 (2001) (in Chinese)

[25] Kennedy, M.C.: Vocalization elicited in a lizard by electrical stimulation of the midbrain. Brain Res. 97, 321–325 (1975)

[26] Lan, S.C., Zhang, G.Q.: A study of the production of phonation reaction in Gekko gecko by stimulation of the midbrain. Acta Zoo Sin 28(1), 15–21 (1982) (in Chinese)

[27] Guo, C., Dai, Z.D., Ji, A.H., et al.: Study on the regulation and control mechanism of the gecko's toes. Chin. J. Biomedi. Engin. 25(1), 100–104 (2006) (in Chinese)

[28] Wenbo, W., Zhendong, D., Hua, T., Ce, G., Jiurong, S.: A stereotaxic method and apparatus for the Gekko gecko. Chinese Science Bulletin 53(7), 1107–1112 (2008)

[29] Distel, H.: Behavior and electrical brain stimulation in the green iguana, Iguana iguana L. 11. Stimulation effects. Exp. Brain Res. 31, 353–360 (1978)