



Attachment of Turner's thick-toed geckos (*Chondrodactylus turneri* GRAY 1864) during weightlessness and their responses to flotation

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ABSTRACT

We investigated the behavior of 15 female Turner's thick-toed geckos (*Chondrodactylus turneri* GRAY 1864) during a 30-day orbital experiment on the unmanned spacecraft “BION-M” No. 1. During weightlessness, the geckos maintained their ability to attach to the surfaces using the subdigital pads on their toes. On average, the geckos spent 99.9% of the time adhering to surfaces during the flight and only 0.1% floating freely. The active geckos, when starting to float, immediately restored attachment by a number of behavioral responses. The floating quiescent geckos, when resuming their active condition, responded in the same manner. The responses during flotation are similar to the behavioral reflexes triggered by a fall under normal gravity; i.e.: 1) the ventral extension of the limbs, 2) a skydiving posture, and 3) postural righting reflexes. Ventral extension of limbs was described for the first time in weightlessness. Individual variability in the frequency of flotations was found for both active and quiescent geckos during the flight. The findings show that the ability to attach to surfaces is an important factor in the geckos' adaptation to weightlessness. The behavioral responses that originated during freefall in conditions on Earth (one-G) appear as adaptations to weightlessness and remain partially effective.

1. Introduction

Weightlessness causes complex reactions in organisms (Clément and Reschke, 2008; De la Torre, 2014), including behavioral changes (Mori, 1995). In conditions which occur on Earth, vertebrates experience weightlessness when falling from a height. Some species of wingless vertebrates have developed reflex responses to free-fall, allowing them to soften the impact of touchdown (Jusufović et al., 2011; Wang et al., 2013). One such reaction is rotation of the body, or a righting reflex. This occurs when an inverted animal that is falling turns in the air to land on its feet. The righting reflex, which was first observed in cats (Marey, 1894; Magnus, 1924, quoted in Gerathewohl and Stallings, 1957), has also been observed in rabbits (Schonfelder, 1984), rats (Laouris et al., 1990), guinea-pigs (Warkentin and Carmichael, 1939; Schonfelder, 1984), tree frogs (Wang et al., 2013), the aquatic frog, *Xenopus* (Wassersug, 2001) and, among reptiles, in turtles, *Maur-emys japonica* (Wassersug and Izumi-Kurotani, 1993), and in geckos, *Cosymbotus platyurus* (Jusufović et al., 2008), which are often classified in the genus *Hemidactylus*.

Another important adaptation is the ability for controlled gliding, or “directed aerial descent”, in which animals convert gravitational

potential energy to useful aerodynamic work and reduce the impact forces of landing (Dudley et al., 2007; Socha et al., 2015). For geckos, some of which are characterized by a unique ability to attach and move on a variety of surfaces, this is a skydiving posture (Wassersug et al., 2005).

The behavior of amphibians and reptiles, including six gecko species, was studied during parabolic flights with short-term (up to 24 s) weightlessness (Wassersug et al., 2005). Reptile behavior varied from calm, slow movements in amphisbaenians to violent and massive movements in snakes, and body undulation and tail beating in terrestrial lizards. Arboreal geckos, as well as terrestrial ones, extend their limbs laterally, slightly inflecting the body dorsally and lift their tails (i.e. take up the skydiving posture), which increases their frontal surface area as if to increase drag and slow their free-fall speed in one-G. In addition, Wassersug and co-authors observed the gecko *Uroplatus* during weightlessness to be in contact with container surfaces, but whether it was actively adhering to or passively in contact with the surfaces was not reported.

We supposed that the adhesive toes of the geckos would allow them to attach to surface under conditions of weightlessness. Indeed, in a 12-days Foton-M3 orbital experiment, video recordings confirmed that

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Turner's thick-toed geckos preserved the ability to attach to surfaces and move more or less normally during a prolonged space experiment (Nikitin et al., 2008). We also documented a motionless gecko, which was floating free for more than 13 min. Histological study of brain tissue from flight geckos revealed structural cytological changes in the neurons of rhombencephalon vestibular nuclei and brain reticular formation motoneurons, testifying the enhancement of their metabolic activity (Proshchina et al., 2008).

This lead us to hypothesize, that in weightlessness the nervous system of thick-toed geckos controls their attachment to the surfaces by means of inhibition of vestibular signals about “free fall”, inadequate in these conditions, but that in some cases (for example, in sleep) this control could fail. Nevertheless, due to the features of life-support in the 12-day experiment onboard Foton-M3 (small container, absence of food and water) there were still some doubts as to whether the revealed changes in geckos' brain morphology and peculiarities of behavior during flotations were caused by the factors of spaceflight (Nikitin et al., 2008), or by poor housing conditions (Almeida et al., 2006). That motivated us to study the adaptations of geckos during prolonged weightlessness and in more accommodating housing conditions. Our focus was on the possible reasons for flotation and behavioral responses to weightlessness in Turner's thick-toed geckos over a 30-day orbital flight of the Bion-M1 biosatellite.

2. Materials and methods

2.1. Animals and life-support

The orbital experiment was conducted with 15 adult virgin female Turner's thick-toed geckos (*Chondrodactylus turneri* GRAY 1864) aged 1.5–2 y. The average weight of the animals was 19.8 ± 1.7 g, the average snout-vent length was 79.2 ± 3.3 mm and the average total length was 148.4 ± 9.1 mm. The animals stayed on board the Bion-M1 biosatellite for 30 day (April 19–May 19, 2013; near-circular orbit with an average height of 575 km and inclination 64.9°). The geckos were placed in three flight containers, called research and support blocks (RSB-1, –2 and –3), with five females in each RSB. The RSB volume was 5.9 L. The walls were lined with hardboard (a type of fiberboard) and the floor was textile laminate (a fabric reinforced laminate). Five tubular shelters for geckos, made of American oak, were fixed on the walls.

In the center of the floor of each RSB there was a hole for a revolving-type feedbox, sealed with a plug when not in operation. The feedbox had ten cavities for the geckos' food. The food consisted of mealworms (*Tenebrio molitor*) mixed with bran particles, dried carrots, crushed eggshell and particles of potable gel. During the flight, the feedbox was opened for four hours every third day, starting from the day of the launch. Two heating zones, each of 5 cm in diameter, were positioned on the floor. These zones functioned during the day, creating a local surface temperature of 31–32 °C. Daytime was 08.00–20.00, and night-time was 20.00–08.00. The average temperature on board the satellite during the flight was 21–22 °C. LEDs, a video camera and a fan were placed on the RSB lid. Lighting at the bottom of the RSB was approximately 485 lx during the daytime and 8 lx at night. The ventilation fan operated continuously, with an air flow of approximately 3.0 L/min.

The geckos in all RSBs were individually marked with colored polyurethane collars. White or black marks were applied to each collar, allowing the geckos to be identified under both daytime and night-time lighting.

2.2. Ethical statement

The study was approved by the Biomedical Ethics Commission of Russian Federation State Research Center —Institute of Biomedical Problems, Russian Academy of Sciences /Physiology Section of the

Russian Bioethics Committee of Russian Federation National Commission for UNESCO (minute №319 from 4 April 2013), and was conducted in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

2.3. Video registration

Video recordings were made with a KPC-VBN190HDV digital camera (KT&C, S. Korea) and Digital Video Recorder TRAL-31-500 (SMP-Service). The frame rate was 25 frames per second. Recordings were carried out continuously from day 1 to day 4 of the flight. Starting from day 5, the camera was switched on for 2 h periods, 6 times a day with 2 h intervals. The whole duration of video recording during weightlessness was 396 h 39 min (or 55.3% of the total flight time in weightlessness of 711 h 56 min) for each RSB. There was no video recording during the launch and landing periods.

2.4. Analysis of gecko behavior

The behavior of geckos when partially detached from surfaces and during flotations was described. The flotations demonstrated by quiescent geckos were classified as either short or long, based on the distance traveled by the quiescent and inactive geckos as they passively drifted from the point where they detached from the surface to where they shifted from passive floating to active movements. Whenever the distance was comparable with the length of the gecko's straightened limbs (approximately 2.5 cm), flotation was labeled as a “short flotation.” Whenever a gecko became active at a longer distance from the point of detachment, this was classified as a “long flotation.” Three specified reactions were recorded: 1) ventral extension of the limbs, 2) the skydiving posture, and 3) righting reflex. We also estimated the angles and calculated the frequency of turns in different directions during righting reflex. The duration of turns was counted based on the number of frames with a recorded turn, and the frame rate.

In addition, we distinguished different conditions for floating geckos related to the geckos losing contact with a surface. When active lizards lost contact with the surface, they immediately performed very vigorous attempts to reattach to a surface. No geckos died during the experiment. Therefore, when we saw living geckos floating motionlessly and retaining the posture of initial attachment, we considered them to be sleeping. This distinction was relevant because the geckos' behavior during the active (awake) and passive flotations was very different.

The frequency of flotations in active and passive geckos, as well as flotation dynamics, was studied. Experimental variables were analyzed using the Wilcoxon matched-pairs test. The values were presented as means \pm SEM. A probability of $p < 0.05$ was considered significant.

3. Results

Video recording was interrupted during the launch period of the biosatellite Bion-M1 and resumed 5 min after the onset of weightlessness. At that time, all 15 geckos were attached to the surfaces of containers (walls, shelters and floor). No geckos were observed floating. Therefore, during approximately the first 5 min of weightlessness, the geckos successfully attached to the surfaces or managed to stay attached from rocket ignition to Bion orbit. Being attached to surfaces was the typical location for the geckos throughout the flight.

The ability of geckos to adhere to surfaces during weightlessness varied between the surfaces available to the animals. The lizards held on best to oak and hardboard. They were less commonly attached to the plexiglass and showed the least tendency to adhere to the textile laminate. The geckos displayed a variety of behaviors while on orbit. They preferred surfaces with the best adhesion properties and demonstrated sociability, and explorative, foraging and even play behaviors. They also were immobile for long periods, suggestive of resting. Surface

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