

the 22nd
International
Congress
of Zoology

Joint
Events

the 87th
meeting of
Zoological
Society
of Japan

in Okinawa
Japan
14-19 November, 2016

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事業内容

- 基礎研究用試薬・体外診断用医薬品・動物用医薬品・化学工業製品の販売
- 理化学機器・医療用機器・分析用機器・その他機器、器具の販売・修理
- 家電製品・コンピュータおよび医療関連ソフトウェアの開発・販売

swimming speed and direction of the individual cells with respect to the gravitational vector was analyzed.

Gravikinesis, defined as the active control of the propulsive thrust depending on the swimming direction, was observed when the magnitude of gravity was more than 0.2xg. The extent of gravikinesis increased with increase in the magnitude of the gravitational force. On the other hand, gravitactic characteristics assessed by the vertical distribution of the cells did not change significantly during partial gravity.

Downward plume of convective motion disappeared when the magnitude of gravity was less than 0.6xg, although we suspect that the plume might have disappeared even in the greater magnitude of partial gravity, if the partial gravity had been prolonged beyond the limit of flight maneuver. These results may imply that gravity plays different roles in collective motion and in individual swimming.

497 [Physiology] [Protozoa]

Re-exploring gravitactic mutant strains of the unicellular green alga *Chlamydomonas reinhardtii*

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How negative gravitaxis, biased swimming against gravity, emerges in unicellular protists has been controversial in the fields of protozoology, fluid mechanics and gravitational biology for more than a century. The "passive" mechanisms are pure mechanics due to the fore-aft asymmetry of the body, and emerged even in the non-living, self-propelled colloid particles (ten Hagen et al., 2014), while the "active" physiological mechanism has also been proposed. For relatively large protists such as *Paramecium* and *Euglena*, the "active" mechanism seems to take place (Häder et al., 2005). The problem now I am focusing on is how about *Chlamydomonas reinhardtii*: a unicellular green alga with the cell body of just 10 µm in diameter and with rigid cell wall. Previously we showed that two kinds of "passive" mechanisms actually worked in *C. reinhardtii*: combining the experimental and the computational approaches, we dissected which passive mechanism worked more in the actual *C. reinhardtii* (Kage et al., submitted; ZSJ2014). How about the "active" physiological mechanism? We do have a clue.

Yoshimura et al. (2003) isolated two gravitactic mutant strains of *C. reinhardtii*, which they named *gtx1* and *gtx2*. Except weaker negative gravitaxis than that of the wild type, they reported that both of the strains do not have anomalies in morphology or most of the major swimming phenotypes. Unexpected from the original description, the strains having been kept in the Mogami Lab, Ochanomizu University (Tokyo, Japan), have "obtained" negative phototaxis or "agging" phenotype, and the *gtx2* strain sometimes could not be distinguished from the wild type in gravitaxis when reassessed in 2015. From the PCR analysis, it was turned out that the "agging" mutation in the *gtx* strains was different from the *aggl* mutation in the CC124 strain, which is sometimes treated as the "wild type" of *C. reinhardtii* in motility. The unidentified "agging" mutation was segregated from *gtx1*. In addition, our data so far suggested that the *gtx2* strain has a tendency of temperature-sensitivity, which might have confused our initial reassessment. The relationship between *gtx1* and *gtx2* is being investigated.

498 [Physiology] [Protozoa]

Arginine kinase (AK) and arginine phosphate shuttle in *Paramecium tetraurelia*

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Arginine kinase (AK) is the enzyme that catalyzes reversible transfer of gamma phosphoranyl group of ATP to arginine yielding ADP and a phosphorylated arginine.

The ciliate *Paramecium tetraurelia* contains four AK genes (AK1, AK2, AK3 and AK4). In this study, we confirmed that three of the four genes (AK1, AK2 and AK3) are expressed in the cell by detecting their mRNAs. The recombinant enzymes of the four AKs were expressed in *Escherichia coli* and their kinetics parameters were determined.

The AK3 of *P. tetraurelia* showed a typical substrate inhibition toward arginine, namely that the enzyme activity was markedly decreased when the concentration of the substrate arginine was increased. This is the first finding of substrate inhibition in AKs. To explore the mechanism of substrate inhibition, the site-directed mutagenesis was introduced in the amino acids sequence D-D-S-Q-V at position 77-81 in AK3, and the kinetics parameters of the mutants were determined. The three mutants, D78A, S79A and V81A showed a strong substrate inhibition, like the wild-type AK3, but the substrate affinity for arginine was increased about 10-fold, while the substrate inhibition of the S79A mutant was almost disappeared. These results indicate that the residue S79 is responsible for the substrate inhibition mechanism in AK3.

AK3 and AK4 show 91% identity in the amino acid sequences. However, the catalytic constant, k_{cat} (or V_{max}) of AK4 was extremely low when compared with that of AK3 (only 3%). From the sequence alignment of AKs, the unusual amino acid replacement of the conservative residue Gly at position 298 by Arg was observed in the AK4. We constructed two mutants,

G298R in AK3 and R298G in AK4. The k_{cat} of the R298G in AK4 was remarkably increased and the value was almost the same as that of wild-type AK3, while the k_{cat} of the G298R in AK3 was considerably reduced. Thus we concluded that the low k_{cat} value in AK4 is due to the R298.

Interestingly, the amino acids sequences of AK1, AK3 and AK4 were found to have typical prenylation signal sequences in C-terminal regions. In order to determine whether the enzymes are prenylated in the native form or not, the AK1 and AK3 were synthesized by cell-free protein synthesis system in the presence of farnesyl diphosphate, and their tryptic peptides were analyzed by peptide mass fingerprinting (PMF). Although the prenylated peptide of AK1 was not observed, the target peptide of AK3 was obtained by PMF analyses. Thus, *P. tetraurelia* AK3 may be farnesylated also in vivo and would be anchored to membrane. Western blot analyses indicated that the cilia of *P. tetraurelia* contain AK3.

Noguchi et al. (2001) demonstrated that phosphoarginine supplies energy for ciliary beating in the ciliate *P. caudatum*, suggesting that it functions not only reservoir of energy but also as a transport of energy in conditions that continuously consume energy (phosphoarginine shuttle). They prepared an intact ciliated cortical sheet from live *P. caudatum*. The AK activity was clearly observed on this sheet, indicating that some of the AK enzymes are attached to the membrane matrix fraction. Our work suggests that the attached AK is the AK3 in *P. tetraurelia* and the AK enzyme is anchored to the membrane by farnesylated group.

499 [Behavior] [Mammalia]

Biphonic vocalizations in canids: considering anatomical sources of frustration calls

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Biphonation, i.e. two independent fundamental frequencies in a call spectrum, is a prominent feature of vocal activity in dog-like canids: African wild dogs *Lycaon pictus*, Asiatic wild dogs or dholes *Cuon alpinus*, timber wolves *Canis lupus*, domestic dogs *C. lupus familiaris*, dingos *C. lupus dingo* and red wolves *C. rufus*. Dog-like canids can produce a low (f0) and a high (g0) fundamental frequencies simultaneously. In our study of domestic dog frustration whines, the biphonic calls comprised 451 (17.0%) of 2643 whines recorded from nine individual dogs, from 0 to 39% of calls depending on individual. In our study of dhole vocalizations, the biphonic calls comprised 583 (44.3%) of 1317 contact calls recorded from 14 individual dholes, from 20 to 92% of calls depending on individual. In domestic dogs, the range of the low fundamental frequency is 0.4-1.4 kHz and the range of the high fundamental frequency is 3.1-11 kHz. In the dhole, the range of the low fundamental frequency is 0.5-1.4 kHz and the range of the high fundamental frequency is 5.5-10.7 kHz. In contrast to dog-like canids, biphonic calls are lacking in all studied fox-like canids: red fox *Vulpes vulpes*, swift fox *V. velox* and Arctic fox *V. lagopus*. The fox-like canids are only capable of producing the low fundamental frequency (f0). In our study of red fox, a detailed analysis of 12,964 whines recorded from 75 individuals did not reveal one single biphonation. In red fox, the range of the low fundamental frequency is 0.32-1.21 kHz, while the high fundamental frequency is missing. To reveal macroscopic structures potentially responsible for canid biphonation, we used a comparative anatomical approach. We investigated the vocal anatomy for 4 (1 male, 3 female) captive dholes and for 2 (1 male, 1 female) wild red fox. In addition, we analyzed the acoustic structure of vocalizations in the same dholes that served postmortem as specimens for the anatomical investigation. All study dholes produced both high-frequency and biphonic calls. The anatomical reconstructions revealed that the vocal morphologies of the dhole are very similar to those of red fox. These results suggest that the high-frequency and biphonic calls in dog-like canids can be produced without specific anatomical adaptations of the sound-producing structures. We discuss possible production modes for the high-frequency and biphonic calls involving laryngeal and nasal structures. This study was supported by the Russian Science Foundation, grant 14-14-00237 (to IAV, EVV and SSG).

500 [Behavior] [Mammalia]

Anatomical and vocal divergence between sexes in a ungulate species with prominent and descended larynx in males: parallels with "Adams apple" of humans

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Male goitred gazelles *Gazella subgutturosa* bear a handicap of an en-

larged and descended larynx, whereas female larynx is much smaller and less descended in the neck. This sexual dimorphism is reminiscent of the situation in adult humans, with pronouncedly enlarged and descended 'Adam's apple' of human males and only slightly descended larynx in human females. Goitred gazelles, as humans, are not born with a descended larynx, therefore the sexual dimorphism of larynx size and position develops during ontogeny. We studied the vocal ontogeny of male and female goitred gazelles across five age classes from neonates to adults. The acoustic variables of nasal contact calls were measured in 53 (24 male, 29 female) individuals, body mass and neck dimensions in 63 (31 male, 32 female) live individuals and nasal vocal tract and vocal fold lengths in 26 (16 male, 10 female) anatomical specimens. Call fundamental frequency, the acoustic correlate of the ontogenetically enlarging larynx, decreased significantly in either sex, being lower in males, although non-significantly. Call formants (second, third and fourth), the acoustic correlates of the ontogenetically elongating vocal tract, did not differ significantly between sexes up to early adulthood, but clearly diverged in adults. Significant differences between sexes in neck circumference at the level of the larynx emerged already at 2-3 months of age, whereas body mass, neck circumference at the neck-body transition and the degree of larynx descent significantly differed in adults only. We discuss that in contrast to humans, the accelerated enlargement of the larynx in male goitred gazelles starts early in ontogeny. A moderate descent of the larynx develops equally in both sexes before early adulthood, whereas the additional prominent descent of the larynx in males is shifted to late ontogeny. This might avoid selective disadvantages of this sexually dimorphic trait on males during their period of growth. Vocal changes are gradual in females but undergo drastic changes in premature males. The emergence of the strong male-specific descent of the larynx goes along with the increasing social status and the males' increasing chances of siring offspring. Similar to the Adam's apple of human males and pronouncedly enlarged and descended larynx Mongolian gazelles *Procapra gutturosa* and fallow deer *Dama dama*, this may indicate the important role of the enlarged and descended larynx for signaling male status via a deep masculine voice with low fundamental and formant frequencies. This study was supported by the Russian Foundation for Basic Research, grant 15-04-06241 (to EVV, IAV and KOE).

501 [Behavior] [Mammalia]

Neonatal fluoxetine treatment restored prenatal hypoxia-induced behavioral changes in male and female mice offspring.

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Decrease in the fetal heart rates and the subsequent hypoxia during prenatal period need an emergent treatment to save neonatal lives in clinical practice. It, however, still remains unknown how such hypoxic event during the prenatal period affects behaviors in adulthood. Therefore, we developed a rodent model to mimic the prenatal hypoxia in human by uterine artery occlusion (UAO). The UAO was performed by clipping the uterine vessels near the lower and upper ends of the right uterine horn for 30 min at gestational day (GD) 16. On GD 19 (full term), the fetuses were delivered by Caesarian section. The fetuses in the right and the left uterine horns were designated as UAO and control mice, respectively. After the delivery, pups were fostered out to ICR mice dams. By using these model mice, we examined whether the UAO induces longitudinal changes in behaviors and neurochemistry in grown-up offspring. Significant decrease in weight was observed only in male UAO mice at birth, but not at 8-week-old when we started behavioral tests. Then we performed a battery of behavioral tests, consisting of open field test, light/dark transition test, elevated plus maze, prepulse inhibition test, forced swim test, and fear conditioning test. In male UAO mice, longer immobility was observed in the forced swim test. On the other hand, female UAO mice showed decreased inhibition in the prepulse inhibition test. Neurochemical analyses revealed that dopamine was increased in the hippocampus of female UAO whereas such kind of change was not detected in the prefrontal cortex and hippocampus of male UAO mice. Next, we tried to intervene these behavioral changes in adulthood with neonatal fluoxetine (FLX), a selective serotonin reuptake inhibitor, treatment. The FLX treatment from postnatal day 3 to 21 ameliorated the behavioral changes in both male and female UAO mice and also improved the DA level in the hippocampus of female UAO mice. The present study suggests that the prenatal hypoxia is an environmental factor for differential behavioral abnormalities in male and female offspring in adulthood, associating with distinct changes in monoamines in their brains. Further, the effect of the FLX treatment implicates that restoration of serotonergic system has a potential to prevent behavioral disabilities caused by the hypoxia.

502 [Behavior] [Mammalia]

First observation of the unique breeding behavior of the Amami rabbit *Pentalagus furnessi*, an endemic species distributed only on two islands in southwestern Japan

奄美大島と徳之島に分布する固有種アマミノクロウサギのユニークな繁殖行動に関する初めての観察

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The Amami rabbit *Pentalagus furnessi* is an endemic mammal distributed on only two islands, Amami-oshima and Tokunosima, in southwestern Japan. The IUCN Red List has categorized this animal as Endangered, because the population has declined owing to invasive predators and habitat loss caused by deforestation. The Amami rabbit inhabits subtropical, evergreen, broad-leaved forests, preferring dense forest as its breeding habitat. The rabbit is active mainly at night, and bears offspring in an underground breeding burrow. Its nocturnal activity and elusive behavior make it difficult to observe in the wild; therefore, its breeding ecology has been poorly understood until now. In the present study, we observed a breeding burrow over an entire breeding period, using camera traps and remote-controlled video-cameras. On September 15, 2015, a new burrow was found in the forest. Cameras were placed approximately 5 m from the burrow during the daytime when the rabbit was not in/around the burrow. The presence of cameras did not disturb the rabbit's activity from that time on. On September 20, 2015, a rabbit visited the burrow at night, carrying many fallen leaves and live fern leaves into the burrow using its mouth. Subsequently, the rabbit visited the burrow every two to eight days, but rarely entered it. On October 30, 2015, the rabbit brought leaves into the burrow and stayed there for approximately two hours. A delivery is inferred to have occurred during this period. From that day on, the rabbit visited the burrow every two days, probably for lactation. Ten days after birth, the offspring was observed with its face out of the burrow when the mother visited the burrow for lactation. Lactation lasted for approximately two minutes, after which the mother covered the entrance of the burrow with soil for approximately 20 minutes. On December 7, 2015, the offspring exited the burrow for the first time. The mother tried to push the offspring back into the burrow, but failed. Both offspring and its mother left the burrow and did not return. The breeding period (from the day of delivery to the day of offspring emigration) was 39 days. The unique method of breeding (burying the offspring in the ground) is inferred to be an adaptation to avoid predation by the Habu (*Protobothrops flavoviridis*), a poisonous snake and presumably the only predator of the rabbit. Recently, introduced predatory mammals (especially mongooses and feral cats) have had a serious impact on the native animals of Amami-oshima and Tokunosima. The Amami rabbit has evolved on the islands with no predatory mammals, so it lacks the ability to avoid attack by these mammals. Although the mongoose eradication project has progressed steadily with the efforts of the Ministry of the Environment, the feral cat population is not presently under control. For the conservation of this unique, endangered rabbit, there is an urgent need to control these introduced animals.

503 [Behavior] [Mammalia]

Behavioral and gene expression analyses on CRMP4-KO mice revealed sexual different disorders in CRMP4-KO mice

CRMP4 ノックアウトマウスの行動解析および遺伝子発現解析から雌雄で異なる異常が発見された

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Our proteomics study identified collapsin response mediator protein (CRMP) 4 as a protein exhibiting sex-different expression during sexual differentiation of the sexually dimorphic nucleus of the hypothalamus (Iwakura et al. 2013). Recently, we found a *de novo* mutation of CRMP4 in a male patient with autism spectrum disorders (ASD) from whole exome sequencing (Nakano et al., the 39th Annual Meeting of the Japan Neuroscience Meeting, 2016). Statistics show that more men and boys than women and girls have a diagnosis of autism and the most widely reported male-female ratio for autism prevalence is 4.5:1. Although many genes have been reported to be involved in symptoms of ASD, only a few genetic variants have been discovered to be related to the sexual difference in ASD. Combined our results with the above information, the possibility is arose that the impairment of CRMP4 molecule may cause signs and symptoms of ASD, especially in terms with sex differences. For getting more information, the aim of the present study is to investigate whether CRMP 4 is related to the sex difference in behaviors and gene expressions of neurotransmitter receptors by using wild-type (WT) and CRMP4-knockout