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Abstracts













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# Okinawa Medix

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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・化学工業薬品の販売
- 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

(KO) mice.

Eight behavioral tests were performed in young adult (4-8 weeks old) WT and CRMP4-KO mice: open field test, novel object recognition test, social interaction test, tube test, food exploring test, elevated plus maze, automated three-chambered social approach task and hot plate test. Among those tests, we found some sex different disorders in CRMP4-KO mice, compared to WTs. For example, in the social interaction test (10 min), the total duration of active interactions was significantly shorter in CRMP4-KO males than WT males, though its difference was not significant between CRMP4-KO females and WT females. We next compared gene expressions including 5-hydroxytryptamine (5HT) receptors, alpha-amino-3hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-selective glutamate receptor subunits (GluR1 and GluR2) and dopamine D1-D2 receptors (D1R and D2R) in the hippocampus between WT and CRMP4-KO mice of both sexes. We found some significant changes in expressions of GluR1. GluR2, and D1R mRNAs in CRMP4-KO mice, compared to those of WTs. In addition, sex different changes were detected in two of them. The expression of GluR2 mRNA was significantly higher in the hippocampus of CRMP4-KO males than that in WT males, though the difference was not found between CRMP4-KO and WT females. On the other hand, the expression of D1R mRNA was significantly higher in CRMP4-KO females than that in WT females, though the difference was not found between CRMP4-KO and WT males. These studies showed effects of CRMP4-deficiency on some of behaviors and gene expressions in sex-dependent manner. It means that sex differences appeared in some behaviors and gene expressions when CRMP4 was deleted. In our previous studies, we revealed that CRMP4 contributes to the regulation of dendritic elongation and inhibitory excitatory balance in male prenatal mice (Tsutiya et al., 2015, 2016). The present study provides new questions why those sex-dependent effects come out in CRMP4-KO mice and how CRMP4 naturally contributes to making these sex-differences inconspicuous.

### (504) [Behavior] [Mammalia]

Follower and hider neonate ungulates: Whether anti-predatory strategy in first days of life influences mother-offspring acoustic communication? Olga Sibiryakova¹, Ilya Volodin¹², Frey Roland³, Elena Volodina² (¹ Lomonosov Moscow State University, Moscou, Russian Federation, ²Scientific Research Department, Moscow Zoo, Moscow, Russia, ²Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany)

Neonate ungulates have two main strategies for avoiding predators: hiding in grass and reunion with a mother only for nursing or following the mother in the herd soon after birth. In both cases, mothers and young use individualized contact calls for mutual recognition. However, the differences in the anti-predatory strategies can affect individuality of contact calls and their acoustic structure. We collected data from three species with different anti-predatory strategies in neonates: the "follower" saiga antelope Saiga tatarica, the "hider" red deer Cervus elaphus and the "hider" goitred gazelle Gazella subguttorosa. We expected more individualistic calls in the "follower" species, because in the "hider" species mothers may additionally rely on spatial landmarks for recognition their young. Calls of 39 mothers and 22 neonate saigas were collected on their natural breeding grounds in Northern Kazakhstan in May 2014. Calls were collected in the absence of humans using automated recording systems SongMeter SM2+ placed near the neonates; call sequences of distinctive animals were selected from 235 hours of the automated recordings. Calls of red deer were collected at the experimental farm of University of Castilla-La Mancha (Albacete, Spain) in June 2011-2012. We analysed a sample of 1094 individually identified contact calls, recorded from 28 mothers and 31 neonate red deer. Calls of goitred gazelle were collected at the Ecocenter "Djeiran", (Bukhara, Uzbekistan) in May-June 2008-2010. We analysed a sample of 453 individually identified contact calls, recorded from 7 mothers and 21 neonate goitred gazelles. Mothers and neonates of all three species produced two types of contact calls: oral (emitted with an opened mouth) and nasal (emitted through the nose with a closed mouth). The individual identity was well expressed in all three species and exceeded two-to-three times the random value in either nasal or oral calls. However, inter-species differences were observed. In saigas, discriminant analysis based on 10 acoustic variables accurately classified individual identity in 97.9% oral calls of 21 mothers, 93.5% nasal calls of 18 mothers and 93.4% oral calls of 22 neonates. Variables mostly contributing to vocal individuality (the fundamental frequency, the 2nd and 3rd formants) were the same in both mother and young and in both the nasal and oral calls. In red deer, discriminant analysis based on 11 acoustic variables, accurately classified to individuals 77.0% oral and 61.8% nasal calls of 22 mothers and 61.1% oral and 64.2% nasal calls of 17 neonates. Variables mostly contributing to vocal individuality were a few variables of the fundamental frequency and the duration. In goitred gazelles, discriminant analysis based on 6 acoustic variables, accurately classified to individuals 69.2% nasal calls of 7 mothers and 83.6% oral and 69.7% nasal calls of 12 neonates. Variables mostly contributing to vocal individuality (the fundamental frequency. 3rd and 4th formants) were the same in mother and young and in

nasal and oral calls. As was expected, the "follower" saigas had the most individualized calls among the three studied species. This study was supported by the Russian Foundation for Basic Research, grant 16-34-01230 (to OVS).

### 505 [Behavior] [Mammalia]

Copulation behavior by males observed during hibernation period in Japanese little horseshoe bat.

Takahiro Sato<sup>10</sup>, Nobuhiko Hoshi<sup>2</sup>, Tsuneo Sekijima<sup>1</sup> (<sup>1</sup>Niigata Univ, Niigata, Japan, <sup>2</sup>Kobe Univ, Kobe, Japan)

In bats having delayed fertilization, it has been recognized that gametogenesis and mating occur during autumn but the timing of actual fertilization is delayed until the termination of hibernation. The inseminated spermatozoa by autumn copulation are stored within female reproductive
tracts throughout wintering. And males also retain motile and fertilizable
spermatozoa in cauda epididymis. The copulating behavior during hibernation period has been reported in some bat species, however, it is not
known about the adaptive significance of winter copulation. Recently, we
found out that the Japanese little horseshoe bat showed the distension of
gonads in males and the vaginal opening in females during wintering. And
we confirmed the flight activity of bats within and outside the hibernacula
during periodic arousal. In this study, to reveal whether the winter copulation is occurred in the Japanese little horseshoe bat, and to infer the
roles of winter copulation in terms of increasing fertilization success, we
investigated the behavior of bats during hibernation period.

Field study was conducted at Osawa lime stone cave that was located on Gosen city. Niigata prefecture from April 2013 to March 2016. Some bats within the cave were captured once per month using mist net or hand net. The external sizes of testis and cauda epididymis in captured males were measured and whether females show the vaginal opening was also recorded. During hibernation period, the behavior of bats during periodic arousal was observed once a week using video camera. Furthermore, periodically aroused bats were captured, the cauda epididymis size was measured in males and the vaginal smears were collected from females to confirm the evidence of insemination.

Males retained active spermatozoa within cauda epididymis throughout wintering despite testis atrophied. During hibernation period, periodically aroused bats aggregated on the cave entrance and formed dense colony at dusk. The cauda epididymis size of males after leaving the aggregated colony was significantly smaller than that of soon after bats aggregated at the cave entrance. Additionally, we found out the spermatozoa from vaginal smear of the aggregated female. These results show that the ejaculation was occurred during aggregation at the cave entrance. In addition to copulating at the aggregated colony, periodically aroused males forcibly copulated with torpid females on the hibernating colony that was formed at interior part of the cave. By estimating the ratio of copulated females that was occupied in the number of wintering females, we found out that 52% of wintering females were copulated by males. The roles of winter copulation for increasing fertilization success in the Japanese little horseshoe bat will be discussed in our poster presentation.

### 506 [Behavior] [Mammalia]

FFemale mate choice in Campbell's hamster (Phodopus campbelli Thomas, 1905): Whether the sexual traits, stress, testosterone, aggression, and immunocompetence are indeed real predictors?

Nina Yu. Vasilieva<sup>o</sup>, Konstantin A. Rogovin, Anastasia M. Khrushchova, Olga N. Shekarova, Nina A. Vasilieva (A.N. Severtsov Institute of Ecology and Evolution, Moscow, Russian Federation)

Androgen-dependent male sexual traits as well as immunocompetence are theoretically assumed to be key indicators of a male's quality for the mate-choosing female. The goal of this study was to estimate a relative role of sexually dimorphic traits, stress and sex hormone levels, immunocompetence and behavior of males in female choice of potential sexual partner. Campbell's hamster (Phodopus campbelli Thomas, 1905) is a sexually dimorphic species. Males of this species possess a testosterone dependant midventral skin gland producing smelly secretion used in territorial marking and involved in sex and individual recognition. In the choice experiment sexually motivated (SM) females (transitional state from vaginal proestrous to estrous) were able to interact with two tethered male siblings that differed in expression of sexual traits. Males were unrelated to the female and able to contact and copulate with her. In both males, we measured body mass, size of midventral gland, ano-genital distance and external testicular diameter. We also estimated levels of blood testosterone and cortisol, specific T- and B-cell immune responses to antigens, as well as aggressive and sexual dominance through additional encounter experiments with another SM female (male sibs could freely compete for the female). We found that SM females chose a partner among two male sibs and spent over 80% of their time on average with the preferred male compared with the non-preferred one. Their choice was not associated neither with the first visit of the chosen male, nor with the higher expression of sex-related morphological traits, higher levels of