Studies on the ultrasonic vocalization (USV) during the copulatory behavior in the male rodent: Role of the USV after the ejaculation of the male rat

Summary of Doctoral Thesis

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Ultrasounds that are outer range of human audible one (20 Hz – 20 kHz) is applied for communication among some animals. Italian scientist, Spalanzani first suggested that animals possibly utilized ultrasounds we cannot perceive. His behavioral observation revealed that bats without sight could fly and catch foods successfully but that they could not fly without hearing (Spalanzani, 1799). However, the mechanisms had not been known until Pierce at Harvard University developed ultrasounds / audible converter in 1938. Next year, 1939, his colleague Griffin and him clarified bats emitted ultrasounds, using the instrument called "bat-detector". Furthermore, they also revealed that the animals used their own ultrasounds as sonar for echolocation which enable them to recognize surroundings. From this finding, research fields of ultrasounds in animals has proceeded.

The first research of ultrasounds in small rodents reported voles' one in 1948. After that, 23-28 kHz ultrasounds vocalizations (USVs) in socially isolated rats and USVs emitted by mice pups when they are isolated from their mother were identified. Following researches also suggested significance of USVs for rodents' communication mainly in rats and mice. Take rats for example, USVs can be classified into three groups based on the difference of duration and frequency: 40 kHz calls in pups, 50 kHz and 22 kHz calls in adults. The pup calls are named isolation calls as known in mice (as described above). The isolation calls seem to facilitate maternal behaviors such as retrieving. Adult 50 kHz calls is thought to be expression of positive emotion because this is observed when they play with cage mates including their brothers. Adult 22 kHz calls is regarded as negative emotion because it is emitted when the animals were subordinates in the context of aggression. In addition, the calls are also observed in footshocked ones.

In rodents of experimental models, USVs is also observed in sexual behaviors. In the case of rats, males emit 50 kHz USVs before ejaculation where 22 kHz after ejaculation. Barfield (1972) reported the hypothesis that post-ejaculatory calls could be a desist-contact signal. However physiological significance of this has been unclear.

Rats and mice are major model animals for every research field in the world. Therefore, understanding reproductive behaviors of them is one of the most important topics. Clarifying this courtship vocalization must contribute more understandings of this topic, which help improvement of

reproductive techniques. In the present study, first, I characterized and compared the acoustic properties of USV in rats, mice, and Syrian hamsters, which are major experimental rodents, during series of sexual behavior. Next, I investigated physiological function of specific USVs in 22 kHz band in rats after ejaculation because there are a lot of questions about this vocalization as described above.

1. Ultrasonic vocalization during copulatory behaviors in experimental rodents models

USVs in WisterImamichi rats, ICVS mice, and Syrian hamsters were observed during precopulatory and copulatory behavior, and after ejaculation.

My results revealed that rats' frequency band was 21 – 57 kHz with peak frequency at 22 and 55 kHz. In addition, the forms in spectrograms were discontinuous. Mice have wider continuous frequency band ranging from 42 to 84 kHz with peaks at 51, 66, and 75 kHz. As for hamsters, the forms were continuous with 24 – 42 kHz band and two peaks of 27 and 36 kHz. Average frequency were significantly different among these three animals ($P < 0.01$).

Comparing before and after ejaculation, vocalization in rats and hamsters were observed in both periods but mice vocalization was only observed before ejaculation. In hamsters, statistical significant changes in frequency and duration were not observed before and after ejaculation; only in rats, the significant changes were detected as reported in previous reports. In this manner, there were clear differences in frequency among these species and alteration of frequency after ejaculation was evaluated as rats specific phenomenon among these rodents.

2. Effects of females on ultrasounds vocalizations in male rats after ejaculation

In this section, I investigated whether or not presence of females during ejaculation or refractory period of male could be the trigger for 22 kHz USVs shortly after ejaculation. As described above, 22 kHz USVs in other contexts are possibly expression of negative emotion and this courtship 22 kHz sound have similar features to other 22 kHz sounds also in duration. If this is expression of negative emotion, too, is this USVs emitted toward females as communication? If so, it seems that this 22 kHz

vocalization can be diminished in the absence of females. Therefore, I removed females immediately after ejaculation and USVs were recorded. As a result, USVs was normally emitted, suggesting that not females during refractory period but the females before and/or during ejaculation could be trigger for the emission of USVs. White (1993) indicated that this 22 kHz vocalizations did not affect female behaviors. Taken together, this vocalization thought to be independent from female existence.

3. Effects of *p*-Chloroamphetamine (PCA) on rat USV after ejaculation

In this section, whether or not ejaculation itself is the trigger for USV shortly after ejaculation was investigated. Ejaculation was induced by intraperitoneally injected PCA. Thirty minutes after injection, ejaculation was successfully induced, but any USVs were not observed during 10-min recordings. It indicated that there were other factors triggering the USVs, such as sexual behaviors before ejaculation.

4. Comparison of *c*-fos protein expression in medial preoptic area (MPO) induced by PCA or copulation

In the previous section, it was demonstrated not only ejaculation itself but also presence of females before and/or during ejaculation is necessary for emission of USVs after ejaculation. So, next, I compared neuronal activity in MPOA, which is center for male sexual behavior, using c-fos immunohistochemistry as a maker for neural activity between rats ejaculated without female (PCA-induced) and with female. As a result, c-fos immunoreactivities were not observed in former while clearly observed in latter. This difference is well associated with emission of the USVs as demonstrated above. So, neuronal activity in MPOA thought to be important substrate for triggering USVs after ejaculation and the activities in MPOA were possibly induced by some female cues before and/or during ejaculation. It is reported that the 22 kHz USVs was emitted when temperature in the MPOA was raised. Taken these together, it is indicated that emission of the 22 kHz USVs is result from internal alteration of emotion or neuronal activity in males rather than male-female communication.

In conclusion, here, I demonstrated that there were interspecie acoustic differences of USVs in rodents, especially in frequency and duration among rats, mice, and Syrian hamsters during series of sexual behavior. Compared with mice and hamster, rats' USVs was uniquely characterized after ejaculation. Even though biological significance of this 22 kHz USVs remains unclear, I demonstrated the USVs was not utilized for communication, suggesting that the vocalization is spontaneously emitted as a result from internal alteration in males. This might have a roll of self-monitoring of males or, at least, there is a possibility that we can use this USVs as an index of male emotional states. If so, we could quantify the male emotional states by recording of vocalization. This would be a great proceeding in this field and such physiological studies should be needed further.