

Original Research Article

Hearing Range Analysis of *Rattus Argentiventer* the Paddy Field Pest

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Abstract: Rice is the second most-grown cereal crop and one of the important staple foods in Malaysia. Seasonal rodent attacks from a family of *Rattus argentiventer* have shortened rice production by up to 80%. Yet, the acoustical method in controlling rodent populations is one of the topics that have not been properly explored. Under the field study radius, this study aims to analyse the hearing range of *R. argentiventer* under a controlled environment. Knowing a rodent's ability to hear is essential for evaluating whether human activities, especially in terms of noise pollution, have an impact on hearing and consequently on rodent behaviour. Thirteen subjects were randomly cage-caught in the paddy field and were tested in a reward-based go/no-go procedure. Fully trained subjects were observed after acoustically exposing the subjects to a 10 kHz of pure tone less than 60 dB SPL for 25 sessions. Hearing range analysis commenced by revealing a pure tone to the fully trained subjects at low (1 kHz–5 kHz), median (10 kHz–40 kHz) and high (45 kHz–80 kHz) frequencies. The results showed the subjects have sensitive frequencies at 5 kHz for low frequency, the median frequency at 25 kHz and high frequency at 45 kHz. From the sensitivity hearing results, it can be indicated that the hearing range of *R. argentiventer* is between 1 kHz to 55 kHz at sound levels of 60 dB. The results responded to the lack of timely response which is within an average of 5 sec and the minimum licking period at an average of 13 sec as the inaudible cut-off hearing range.

Keywords: Hearing Range; Pest; Rodents; Pesticides; Acoustical Method.

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1. Introduction

Rattus argentiventer has the widest native range of any omnivore, spreading from the Indochina region, Thailand, Indonesia, Philippines, New Guinea and as well as in Malaysia

(Sekarweni *et al.*, 2019). *R. argentiventer* prefers to live in cultivated regions like rice fields and meadows. It is heavily reliant on human rice crops and fields (Sudarmaji & Herawati, 2018). Long year follow-up study discovered that the rice yield losses by the rodents at a bare minimum of 5% to a maximum of 10% in Malaysia (Brown *et al.*, 2017). These surprising percentages categorized the sub-seasonal rodent attacks as a significant pre-harvest pest disrupting yield yearly (Omar *et al.*, 2019). A study clarified in seasonal details that the rodent attacks during the booting stage period to the very harvesting stage of rice paddy (Rachmawati & Herawati, 2021).

A number of methods have been introduced in order to control the rodent population: chemical approach (Hazra *et al.*, 2017), mechanical approach (Ratnadass & Deguine, 2021) and schedule management approach. These approaches, however, showed ineffective improvement in the rice yield output (Babendreier *et al.*, 2019). Nonetheless, there is one acoustical approach superficially explored by mimicking an exact frequency wave pressure of mosquitoes to control them in a particular area (Pantoja-Sánchez *et al.*, 2019). In parallel to hominid application, the acoustical approach or to be exact the use of a long-range acoustic device (LRAD) was proven to disperse uncontrolled mop, sea pirates and others (Basuki & Palupi, 2020). The study hinted that the important keys to this acoustical approach are an exact frequency level of continual emission (Lubner *et al.*, 2020). Typically, compared to other animals, rodents have wider frequency filters. Therefore, the hearing range of *R. argentiventer* needs to be explored at either low frequencies or high frequencies to know at what certain frequencies rodents can respond.

Analysis of hearing ranges determining the ability of rodents to hear pure tones and intervals. This testing is done by training the rodents to respond to a frequency tone until the rodents fail to respond. In order to ascertain the spectrum of frequencies and how effectively the rodents can hear across those frequencies, behavioural hearing studies are carried out. Its hearing threshold for that frequency is the softest or lowest volume at which the rodents can detect a sound. Behavioural studies are performed with trained rodents. The rodents are trained to lick the water portable drinking when a sound at 10 kHz is played. In this sense, if the rodents hear the sound, it responds in a particular way. The rodents do not react if the sound is not heard by it (nor if there is no sound played). The frequencies and sound levels the rodents can hear were found by varying the volume of the test signal. A hearing threshold curve represents these measurements.

There are notable parallels and differences between rodents and humans in terms of hearing. Rodents' hearing doesn't fully develop until after birth, although humans can hear before birth (Escabi *et al.*, 2019). The ability to research hearing in more ways than with humans is made possible by this crucial distinction. In other words, because the human cochlea develops before birth, scientists are unable to study the cochlea's development concerning characteristics like hearing sensitivity. This creates the possibility for creative developmental investigations into the maturation and development of the mammalian cochlea's unique capacities and traits. Additionally, when compared to humans, this

particular trait of rodents offers researchers a clinically applicable insight into developmental, anatomical, and functional malformations and how these eventually affect hearing outcomes.

The hearing ranges of *R. argentiventer* are crucial especially for creating an effective ultrasonic-repellent device to combat rodents. Previous studies have shown that ultrasonic devices are ineffective due to the general hearing range of each rodent. Perhaps the hearing studies of *R. argentiventer* may contribute to the researchers to create an efficient ultrasonic device to control the population of specific rodents without killing them. Hearing ability studies have been performed on several species of rodents. For instance, the hearing range of gerbils (*Meriones unguiculatus*) is between 125 Hz to 60 kHz with thresholds comparable to human hearing, which operates over a shorter frequency range, and the most sensitive frequency at such levels (Pyott *et al.*, 2020). Masterton and Heffner, 1980 stated that the hearing range of feral house mice is between 2.3 kHz to 92 kHz at an intensity of 60 dB SPL. Meanwhile, the hearing range of albino rats is between 250 Hz to 80 kHz at 70 dB SPL with sensitivity at 8 kHz and 38 kHz (Kelly & Masterton, 1977).

The LRAD application properties highlighted the weak side of the human eardrum capable of tearing at a frequency above 20 kHz. Meanwhile, for *R. argentiventer* at the current repository study archive, the hearing range of the omnivore still has not been properly explored. The closest *Rattus* family studies showed that the hearing range differs from one to the other because different *Rattus* have different physical attributes (Chen *et al.*, 2017). In order to further study the controlling of the *R. argentiventer* population in the paddy field, this research aims to analyse the hearing range of *R. argentiventer* using low frequency to high frequency of acoustical pure tone from 1 Hz to 80 kHz.

2. Materials and Methods

2.1 Rodents

A total of thirteen subjects consisting of nine males and four females of *R. argentiventer* were labelled as R1 to R13. The subjects were weighed between 35.52–204.35 g at the beginning of testing. The subjects were first normalised in a larger cage for 4 weeks. The subjects then were apprehended in an individual test cage with a dimension size of 18 cm × 12 cm × 28 cm kept shaded outdoors along with unlimited access to the food prepared.

2.2 Behavioural Testing Chamber

Testing was conducted in an acoustic chamber with a dimension size of 110 cm × 190 cm × 110 cm. The floor, walls and ceiling were covered with egg crate foam to reduce sound reflection. The subjects were viewed by closed circuit video and all acoustic and behavioural equipment were situated outside the acoustic chamber. A portable water drinking protruded through the floor at a comfortable drinking height inside the test cage. The portable water bottle was manually refilled with exactly 20 mL for each session. A shock generator was also

installed on the test cage floor to provide feedback and a modest penalty for not responding to the emitted tone.

2.3 Acoustical Apparatus

A pure tone of sine wave was harnessed and aired for 5 min at a 10 kHz frequency level. The tone was generated, amplified and attenuated using a KMOON FY6800 DDS signal generator and directly connected to the two loudspeakers of a horn type. Audax Horn Tweeters with dimensions $130 \times 130 \times 100$ mm and feature of speakers are 8 Ohm of nominal impedance, have a power capacity for 28 Watts RMS, a sensitivity of 109 dB, and a frequency range between 1.5 kHz to 20 kHz were put inside of the test cage, facing the subjects' head while they drink from the water portable drinking. The loudspeakers were placed precisely 45 cm apart from the test cage.

2.4 Behavioural Training Procedure

The subjects were trained to establish constant contact between its mouth and the portable water drinking. This training is also to obtain a slow, steady drip of water using a conditioned avoidance procedure (Heffner & Heffner, 2014). A pure tone at a frequency of 10 kHz and 60 dB SPL was presented in each session for 5 min long. In these sessions, mild electric shock was introduced to the subjects if only no contact was established between the subjects' mouths and the portable water drinking during the airing of the tone. Following the mild shock, the test session was promptly stopped and replaced with the next subject. A total of 25 sessions were accomplished in the subject training procedure. Additionally, a series of assessments were observed during the training sessions majorly on the average of licking time and notably on the average of response time. The licking time is rendered as a steady licking time acquired by the subject during the tone aired. The response time, on the other hand, is translated as time acquired by the subject to react from the start of the tone aired till the subject initiated a first lick. The evaluations of the fully trained subjects were elucidated by attaining more than 2 min of the average licking time and less than 2 min of the average response time (Heffner *et al.*, 2006).

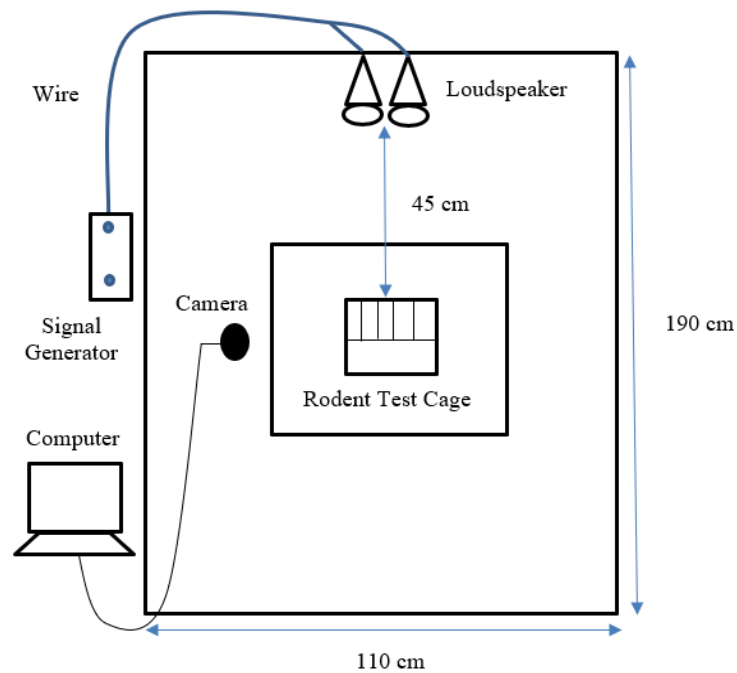


Figure 1. Behavioural testing was conducted in an acoustic chamber with a size of 110 cm × 190 cm × 110 cm. The tone was generated using a KMOON FY6800 DDS signal generator and directly connected to the two loudspeakers of a horn type. The loudspeakers were placed precisely 45 cm apart from the test cage.

2.5 Hearing Range Test

For the hearing range test, three groups of frequency levels were classified. A low-frequency sound was categorised as 1–5 kHz, a medium frequency sound as 10–40 kHz and a high-frequency sound as 45–80 kHz (Beltrame *et al.*, 2021). Each of the frequency sounds was presented for 5 min at 60 dB SPL during hearing range test analysis. The average licking time and the average response time of each subject were observed in the behavioural training procedure via a camera. The out-of-hearing range ability of the subjects was translated as 41 sec lower average of licking time and 5 sec lower for average response time.

3. Results

3.1 Behavioural Training Sessions

In the early stages of training, the subjects were observed climbing and standing in undesirable positions and normally shocked by the sound of emitted tones. Figure 2 showed the average licking period among the subjects. Subject 7 had the highest average licking period for 3 min 16 sec for 25 training sessions while the lowest was subject 4 for 1 min 36 sec. One of the reasons that subject 7 had the highest average licking period was because of the size of subject 7 being bigger compared to subject 4. As a consequence, subject 7 required more water after fasting for 24 h to maintain its body weight than subject 4. Detailed observation showed that the highest average licking period of the subjects was at session 8

for 3 min 6 sec and the lowest average licking period found at 10 kHz was at session 9 for 1 min 14 sec.

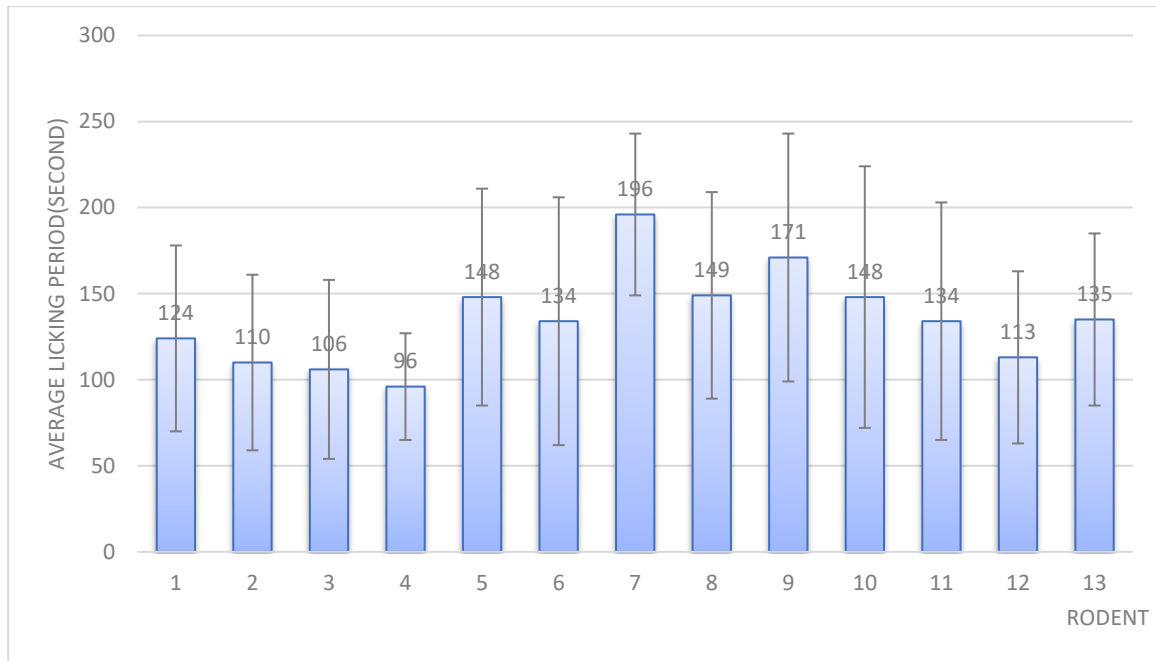


Figure 2. The Average Licking Period result from the behavioural training sessions showed that the subjects were fully trained after 25 completed sessions. Wide error bars in the initial sessions represented that the subjects were newly adapting to the new environment. At the end of session 25 the error bar showed significant reduction.

The average time of response is referred to the time taken for the rodents to start hearing the tone and lick the portable water drinking for the first time for each session. After several training sessions, the rodents became more familiar and understood the task that was given to them. Based on Figure 3, the bar graph showed that subject 3 and 5 responded earlier compared to others with response times of 48 sec when they heard the emitted tones, while the second fastest response was performed by subject 9 with 49 sec. Meanwhile, subject 7 was among the slowest in response with a time record of 1 min 36 sec. Hence, it was expected that all the subjects were well trained even though other subjects had a later response for 5 min emitted tones. This is due to environmental factors that have affected the subjects during training sessions such as temperature and undesired noises. Gaskill and Garner (2017) also indicated that environmental factors such as temperature that could affect the data variability for as much as 42% during animal training. They also stated that some animals prefer different temperatures for different behaviours, times of day and genders. The body weight of subjects has also been affected by the stress during the fasting session. This result is similar to Jeong *et al.* (2013). They claimed that stressed mice's body weights remained noticeably lower than the control mice.

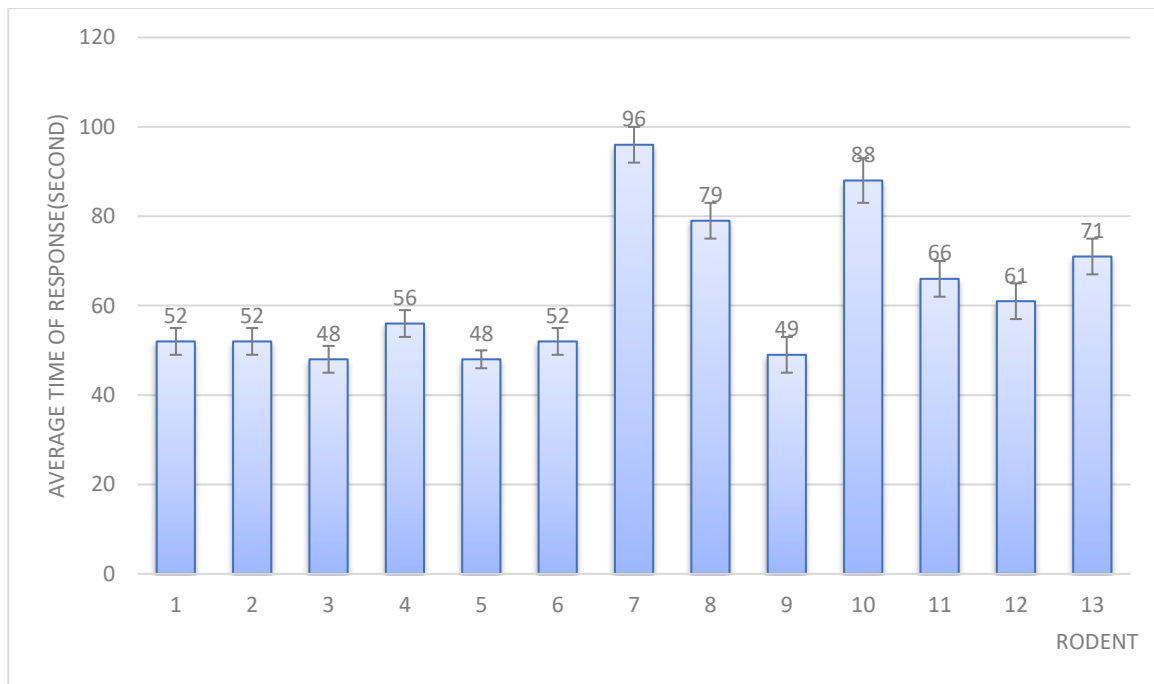


Figure 3. Average Time of Response results showed the subject's time taken lick the portable water drinking for the first time.

3.2 Audible Hearing Ranges

The audible hearing range refers to the range of sound frequencies that can be heard by subjects. Figure 4 shows the low between 1 kHz to 5 kHz, the medium between 10 kHz to 40 kHz and the high between 45 kHz to 80 kHz of sound frequencies. The bar graph illustrates that the subjects have the most sensitivity to 4 kHz because the subjects responded early with a time of 1 min 46 sec. At medium frequency sound, the rodents were sensitive to frequencies between 25 kHz and 30 kHz with a response time of 2 min 18 sec while at the high frequency sound, the rodents were sensitive to a frequency of 45 kHz with a time of 2 min 14 sec. The rodents showed good agreement, with the difference between individuals ranging between 1 kHz to 80 kHz at 60 dB SPL. Beginning at 1 kHz, there was rapid improvement in sensitivity as frequency increased to a distinct best frequency at 4 kHz. At higher frequencies, the rodents remained sensitive up to 45 kHz, beyond which there was a sharp decline in sensitivity at 60 kHz and a slightly rose of sensitivity until 80 kHz. Taken together, the rodents showed a relatively broad range of good audible hearing from 1 kHz to 55 kHz.

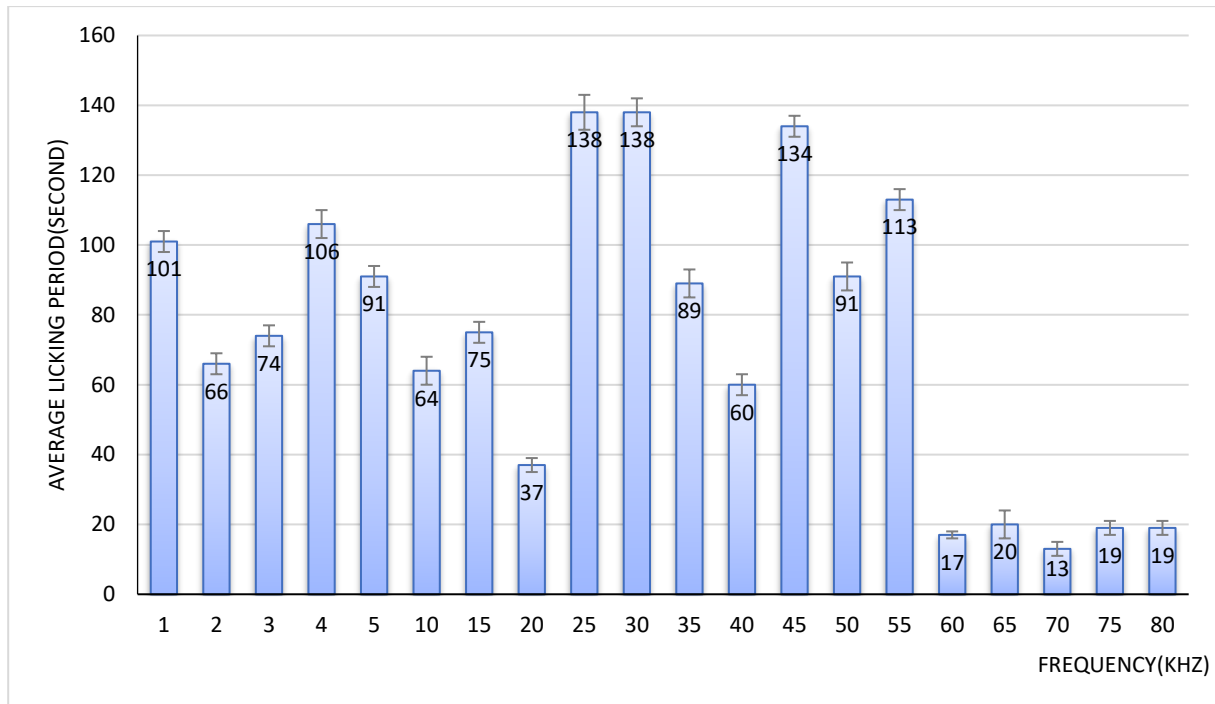


Figure 4. Average Licking Period results showing the audible hearing range of subjects for low (1–5 kHz), median (10–40 kHz) and high (45–80 kHz) frequency sound.

By comparison, the *R. argentiventer* can hear up to 55 kHz at 60 dB SPL compared to the Norway rats which can hear a higher frequency of about 80 kHz (Modlinska & Pisula, 2020). In addition, according to Gerhardt *et al.* (2017), mole-rats are able to hear up to 18.5 kHz at 60 dB SPL which had a lower hearing range compared to the *R. argentiventer*. The main reason for this difference is due to the small head size of the rodents which generally have good high frequency hearing than rodents with larger head size (Old *et al.*, 2020). Meanwhile, the external ear, or pinna, too can amplify or attenuate sound, thereby limiting the spectrum of excellent hearing (Yoshitomi & Cole, 2018). This is because the behavioural test was conducted with a loudspeaker located in front of subjects that causes the subjects with moveable pinnae to position their pinnae ideally for sensing sound (Miller-Klein, 2020). Apart from that, Dent *et al.* (2018) also claimed that high-frequency hearing is better in rodents that dwell entirely or partially above ground.

3.3 Sensitivity Hearing

The sensitivity hearing of low-frequency sound is shown in Figure 5. The bar graph shows a gradual increase in sensitivity as frequency is increased to a well-defined best frequency near 5 kHz. Indeed, the rodents appear to be quite sensitive at this frequency at an intensity of 60 dB SPL. All rodents responded to the lowest frequency presented from 1 kHz to 5 kHz. Based on low-frequency sound, it can be seen that the rodents probably could not hear much below 4 kHz. This result is quite similar to Dent *et al.* (2018). They claimed that mice have poor hearing sensitivity below 4 kHz and the availability of interaural time difference (ITD) cues for horizontal sound localisation is limited due to mice's small heads.

Besides that, Gerhardt *et al.* (2017) stated that naked mole-rat *Heterocephalus glaber* has the most sensitive hearing to 4 kHz with an intensity of 35 dB SPL. Meanwhile, the observation of the sensitivity hearing at medium frequency sound from 10 kHz to 40 kHz indicated that all the rodents are most sensitive to 25 kHz, while for higher frequencies from 45 kHz to 80 kHz, the rodents are most sensitive to 45 kHz. Thus, it can be expected that the subjects are most sensitive to 5 kHz for low frequency, 25 kHz at medium frequency and 45 kHz at high frequency ranges.

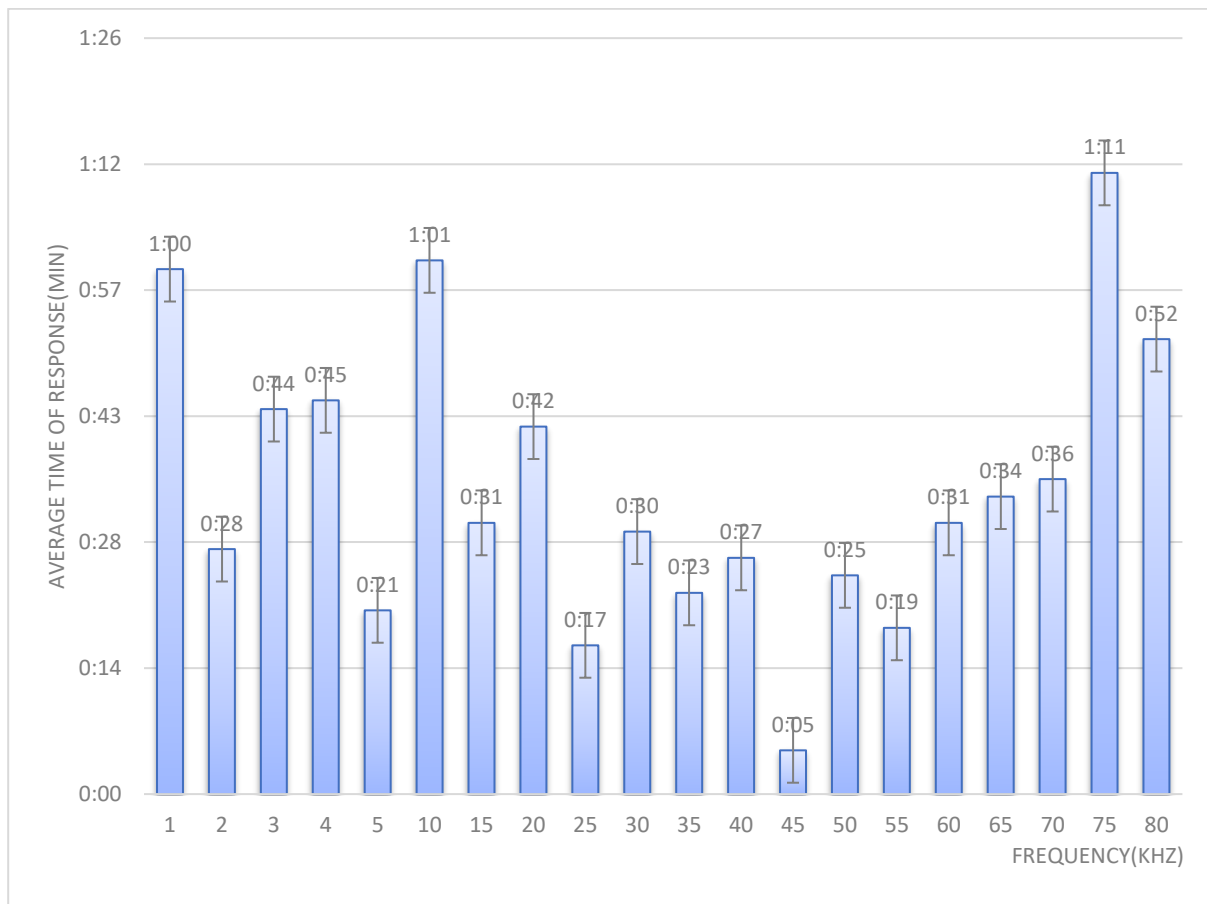


Figure 5. Average Time of Response results showed the sensitivity hearing of subjects for low (1–5 kHz), median (10–40 kHz) and high (45–80 kHz) frequency sound.

Overall, it may be said that the *R. argentiventer* has relatively good hearing sensitivity with a significantly wider range than that found in humans. Escabi *et al.* (2019) mentioned that the greatest sensitivity of rats' hearing occurs between 8 and 38 kHz while Holt *et al.* (2019) indicated that the hearing sensitivity of rats between 0.20 to 85 kHz revealed an upper range of hearing that was more than four times of humans. The main reason for this sensitivity hearing variation among rodents is because of ear morphology. Based on previous studies, one of the key parameters determining sensitivity in the low-frequency range in small mammals is the volume of the middle ear cavity (Gerhardt *et al.*, 2017). In addition, the

cochlear also play role in charge of encoding and transmitting auditory impulses to the brain (Escabi *et al.*, 2019).

5. Conclusions

Thirteen rodents responded steadily whenever an audible tone was presented. They were quick in learning the task. Given that punishment was imposed when they do not respond for each session, their incentive was to avoid a shock circuit. Subsequently, the hearing range of rodents at low, medium and high frequencies were obtained. The results showed that the rodents were able to hear low to high frequencies. From those above, it was concluded that the hearing range of *R. argentiventer* was between 1 kHz to 55 kHz at 60 dB. Above 60 kHz until 80 kHz, the rodents were not sensitive to the emitted sounds but still able to respond. The best hearing sensitivity of the *R. argentiventer* at 5 kHz, 25 kHz and 45 kHz is different from other rodent species with respect to the frequency range and absolute sensitivity. This was discovered to be related to the frequencies that most frequently cause audiogenic seizures. It was also thought to be significant for the selection of acoustic stimuli in future rat hearing experiments. Hence, it proved that though *R. argentiventer* can hear low-frequency sound, it is more sensitive towards high-frequency sound above 20 kHz at 60 dB SPL. Since humans are inaudible to high frequencies above 20 kHz, we need to use a special instrument to detect sounds that are easily audible to rodents. Finally, the ability of animals to adapt to noisy situations must also be acknowledged, even while the mere knowledge of auditory sensitivity is insufficient to answer the question of whether rodents would find a particular sound psychologically upsetting.

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Conflicts of Interest: The authors declare no conflict of interest.

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