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HOW THE DIFFERENT NOISE TYPES  
MAY INFLUENCE  
THE OPEN-FIELD BEHAVIOUR OF RATS ?

by

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- 2013 -

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## Introduction

It is said that the different tools of environmental enrichment influence the animal behaviour and/or physiological status. In the frame of the 3rd „R” (Refinement), the laboratory animal science focuses on the feeding and on the physical environment of the animals, which are related to sensory stimuli, like visual, olfactory, auditory and tactile (BAUMAN et al. 2011).

Noise is also one of the elements of the environment. It has been thought as a powerful stressor. Generally, the acoustic environment does have an influence on the behaviour and physiological state of the humans and animals. Amongst those the different noises have an important role. According to the general definition the noise is the ratio of the meaningful signals and those, of carrying no information. In the bioacoustics, noise is equal to the unpleasant sounds.

The noise music is an avant-garde music and sound art, which is akin to the futurism and dadaism. It is employing the elements of cacophony, dissonance, atonality, noise, irregularity and repetition. The manifesto of Luigi Russolo: *The art of noise* (1913) is considered as the first step of this movement. Its features are the distortion, acoustic or electric noises, a combination of randomly generated electric signal and non-traditional music (TORBEN, 2002). The noise musician uses modified music, hissing-humming, feedback, machine sound, software of common noises, non-musical vocal, in order to organise the whole noise towards a particular feeling. As characteristic examples the compositions of Takechisa Kosugi, the record „The Two Virgins” of John Lennon and Yoko Ono, the „Machine Music” works of Lou Reed (*Metal Machine Music*) can be mentioned. Maybe the best known contemporary representative of the noise music is undoubtedly is Takechisa Kosugi, the composer-performer, who was born in Tokyo in 1938. Together with Yaunao Tone and Mieko Shiomi, he is a co-founder member of the Ongaku Musical group (Tokio, 1961). This movement was searching for the mechanism of action, while the non-musical, sensual stimuli may cause a musical feeling in humans. They considered, that each object sonore is able to be part of a – predominantly chaotic – music (DORIS, 1998). The sounds of his music works used everyday materials (e.g. rattling of the bunch of keys) and electronic technology. The “Violin Improvisation” is one of his compositions and it was recorded in New York, September in 1989.

Thus, the 3 „R” principles (RUSSEL and BIRCH, 1959) declare, that to make animal experimentation humane, the number of animals used should be reduced, if possible, replaced

by lower organisms or computer simulation and finally, the keeping and handling, as well as the experimental techniques should be refined. This third „R” includes also the species-specific, comfortable environment. Sounds and noises are important components of the acoustic environment in animal facilities. There studied dealing with the auditory, the non-auditory effects and there are also noise-stress models (SZOMBATH, 2011). The Guide for the Care and Use of Laboratory Animals (INST. LAB. ANIM. RES. 2011) stated that over 85 dB SPL the noise may alter the physiological state of the animals and may influence the outcome of the experiments. If background music is used, the recommended loudness is 60 dB. In their classical paper (GEBER et al. 1966) the influence of duration and level of noise in albino rat model. They have found that the number of circulating eosinophils, an elevation of the serum cholesterol and a depression in adrenal ascorbic acid are good indicators of the noise stress.

TURNER et al. (2007) classify the non-auditory effects of the noise into six classes: cardiovascular, hormonal-biochemical, reproductive, sleep, behaviour and the others. The latter included the increase of the microvascular permeability, the disruption of the intestinal lining, irritability, depressed mood, impairments in the cognitive functions, elongation of the tail flick latency, slower wound healing and an increase in leukocyte number, liver and adrenal gland relative weight. The question of environmental noise and background masking acoustic stimuli is important both for the scientific community and for the practical laboratory animal science, because it is known (KROHN et al. 2011) that for longer time interval, adult rats prefer silence above all type of sound stimuli, but they prefer speech, pop music or the mix of the two latter (talkshow in the radio) over white noise. The present work will focus on the behaviour changes, caused by different types of noises (natural and technical, as well as of noise music (in original, human version and in rodentized version, i.e. two times faster and two octave higher in pitch).

## Review of the Literature

The noise is inevitable in the animal facilities: cleaning, changing cages, feed distribution, running tap water, ventilation, the vocalisation and movements of the animals and humans. The first studies, dealing with the noise loading of laboratory rodents have been published in the early fifties. The basic question was, how the noise may be an important source of stress and the physiological-immunological consequences of it (KELLEY, 1980). Although the animals may have some adaptation to the noise-induced stress, there is an continuous immunosuppression, which in turn, may alter the results of the experiments (MONJAN, 1977; HOLT, 1978). The overview of WESTMAN (1981) introduced the notion of „sound environment” and consider the sound and noise effects as a complex system. PATTERSON-KANE and FARNWORTH (2006) classify the noxious noises into two groups: the non-controlable acute (e.g. siren, street clamour etc.) and the non-controlled, chronic noises like the ventilator, air conditioning, noises from the other animals and care takers. Both influence the physiological parameters of the animals, therefore the biological samplings should be scheduled according to them. VOIPIO (1997) propose seven parameters for measuring the change of rats' behaviour after noise exposure: no response, watching (listening), moving, be frightened, be frightened with freezing and fear with flight/escape. In addition, the vocalisation of the animals (even in the ultrasound range) may be used. In the study of KREBS et al. (1996) an abrupt noise stimulus (white noise of 95 dB) decreased the previously conditioned eating time of rats, the incidence of spontaneous defecations increased and they spent more time with exploratory behaviour.

In their paper REYNOLDS et al. (2010) compared the noise perception of human and mice in an animal facilities, using sounds of reconstruction. They have taken into consideration that the hearing range of human and animal is different: sensitive frequency range for humans is from 1 to 4 kHz, and 1-16 kHz for mice. Age, disease, noise exposure and strain can also affect the hearing in individual humans or mice. The audible sound for human might not be or less perceived in the animal facilities due to the frequency content of the sound. Although loud noises can cause teratogenic and reproductive effects. and may also affect to growth rate in mice or rats, the everyday life is different. In this experiments, jackhammer, small jackhammer, vacuum, ridged, organe, grinder and vacuum, terrazzo grinder and shot blaster were used for producing sound. The distance of the noise source s important factor, too. The pitch of the sound that rodents would hear declined quickly than

that for humans, because part of the animal communication is performed at ultrasound altitude. *The conclusion of the present work is, that inside the cage the rodent heard less of the reconstruction noises, than human did.* BJÖRK et al. (2000) propose a transformation of the dB-values according to the hearing range of the rats: sound pressure level weighted for rats (R-weighted dB), instead of the unweighted linear SPL.

PRIOR (2002) studied the effects of predictable and unpredictable intermittent noise (68 dB) on spatial learning in rats. They delivered noises through speakers, in form of background noise predictable (rhythmic) and unpredictable (continuously changeable in pitch) PC-sounds. According to the number of errors, the time required to perform the multiple T-maze test, *the effects of predictable and unpredictable background noise were similar, compared to the no-noise ( $\leq 35$  dB) groups, namely both significantly improved learning performance and spatial memory.* Not only differences in acute noise exposure, but also individual difference of history of acoustic experience can affect learning experiments. The effects of noise-induced arousal in humans and animals have similar basis. The facilitating noise effects might be caused due to increased cholinergic activity, but the intensity and the duration of noise exposure might be essential. Acoustic stimulation increases the activity of central noradrenergic system, included the locus coeruleus immediately, which in turn, enhance attention. The emotional factors and long-term memory formation are in close relationship.

According to GALENO et al. (1984) the central amygdaloid nucleus lesion attenuates exaggerated hemodynamic responses to noise stress in the spontaneously hypertensive rat (SHR). The central amygdaloid nucleus plays a role in the development of spontaneous hypertension by mediating cardiovascular responses to environmental stimuli. The lesion SHR and lesion normotensive Wistar-Kyoto rats (WKY) responded with almost identical arterial pressure, heart rate and regional vascular resistance changes under the circumstances of acute noise stress. The hemodynamic shortages produced by amygdaloid lesions are site specific. Vasoconstrictor effects are more prominent in SHR. Enhanced heart rate and renal and mesenteric vascular resistance changes observed in SHR are considered due to greater activation of sympathetic nervous system. *The central amygdaloid nucleus and its output pathway mediate the cardiovascular responses of both SHR and WKY to an acute unconditioned environmental stimulus like the noise stress.* After the extirpation of the central amygdaloid nuclei, rats showed decreased renal and mesenteric blood flow and increased hindquarter hemodynamic during the acute noise stress both in SHR and WKY animals. The major changes of neural cardiovascular regulation in SHR may belong to in abnormal neurogenically induced vasoconstrictor and pressor responses to acute

environmental stress. The central amygdaloid nucleus is an element of the central system that change acute environmental stress into increased sympathetic activity. HIRANO et al. (2006) using fMRI (blood oxygenation level-dependent signal), studied the effect of unpleasant loud noise on hippocampal activities during picture encoding. The amygdala and prefrontal area has been considered to play a role in unpleasant emotion processing network. Effect of short-term stress caused by unpleasant noise was examined using blood oxygenation level-dependent signal in the amygdala, hippocampus and auditory area. The picture encoding significantly activated the posterior part of the hippocampus and *loud noise significantly activated the auditory association area, amygdala and the auditory area*. The fMRI showed that loud noise activated the auditory association part and amygdala. It means the path from auditory association area to amygdala was not only direct but also indirect via the auditory area under loud noise. Loud noise not only stimulated amygdala but also decreased hippocampus activity during picture encoding. The unpleasant emotion stimulated both the prefrontal area and the amygdala, and high activity of amygdala may suppress hippocampus activity leading to decreased memory formation.

Noise can induce escape behaviour. The acquisition of avoidance was more rapid for 87.5 dB than for 90 dB SPL noise in the study of MOLLENAUER et al. (1992) using C57BL/6J mice. The gender of the animals did not affect the activity with time. The continuous noise is more effective than pulsed noise, moreover, the continuous noise is more aversive than pulsed. Mice were able to learn to avoid continuous noise more rapidly. No interaction has been shown between noise condition and time. Pulsed noise is appropriate for experiments involving long-term exposure. Noise of moderate intensity is a mildly aversive stimulus for the C57BL/6G mouse.

The noise is a powerful stressor. Even human feels uncomfortable in loud environment. CABRERA and LEE (2000), as consequences of exaggerated noise effect described not only the damage of the sensory cells of the *Corti* organ, but also a decrease in the learning capacity and hypertension. On the contrary, the environmental enrichment using background music is advantageous both for the laboratory and production animals (VAN HORNE and ACHTERBOSCH, 2008; FEKETE, 2012a). Besides the sound pressure level, decibel (SPL, dB) the colour of the noise counts, too, but the related data are scarce and sporadic. According to VOIPIO (1997) the monotonous white noise is deteriorating even at a low SPL.

NÚ(NES) et al. (2002) have demonstrated, that the noise decreases both the cellular and the humoral immune answer and makes rodents prone to cancer. Noise-induced stress was not only immunosuppressive, but slowed down wound healing by decreasing the IL-1 (interleukin-

1) production of the macrophages and that of the free-radicals by the neutrophils. The stress could have been attenuated by classical music. Loud noise exposure decreased the neurogenesis in the hippocampus of rat embryos and pups (KIM et al. 2006). KOUTSOS and KLANSING (2008) exposed four-week-old chickens to a noise stress of two hours, which caused an increase of the thymus and bursa *Fabricii* and an elevation of the lutein and zeaxanthin concentration of the liver. The phytohemagglutinin- and concanavallin-stimulated lymphocyte proliferation decreased. The duration of the noise exposure has a great influence, too: in rats, expose only to a 24-hour-long, 80 dB rock music, there was a significant decrease in the superoxide anion and IL-1 production of the neutrophil granulocytes and macrophages, but the lymphocyte proliferation did not change (MCCARTHY et al. 1992).

Noise has not only auditory, but also non-auditory effects. These impact on the endocrine and cardiovascular function, sleep-awake cycle disturbances, seizure susceptibility and behavioural modifications. The extent of the above changes are determined not only by the noise intensity, duration and predictability, but also from the related animal species and even strain (TURNER et al. 2005). Beside the acoustic component, the noise includes vibration, too. „Vibration is motion that is not constant but assumes an oscillatory or wave-like form. This motion is characterized as cycles, in which the structure oscillates around an equilibrium position, that is, the position of the object when vibration is applied.” (NORTON et al. 2011). Contrary to the noise itself, towards the humans are more sensitive, than the rodents do (VOIPIO et al. 2006), the vibration would be of greater detrimental influence for the animals. The legs and other body components (abdomen, thorax, head) of the rodents may absorb some vibration, causing gastrointestinal alteration, impaired nervous functioning and increased respiratory rate.

There is a massive body of evidences, that some of the animal specieses are able to discriminate complex musical stimuli and generally they prefer the classical music (FEKETE, 2012b). On the contrary, it is less known, that do animals differentiate among the noises of different types and colour, and is there any differences in the physiological and behavioural effect of the them. The aim of the present work was to study the ethological effect of noises on the growing rats. The animals were exposed to noises of different character (noise music in original and in rodentized, higher and faster version, a selection of natural and a selection of technical noises, registering the changes of their OF-behaviour.



# Own Investigations

## Material and methods

### *Animals*

Twelve, experimentally naïve, 29-day-old weaning male rats [Charles River (W1) Barrier Raised, Specified Pathogen Free] (live weight  $112 \pm 10.07$  g) were used. The animals were kept in pairs, in plastic cages (37 cm × 22 cm × 18 cm), on aspen bedding (2 × 2 × 1 cm, TAPVEI®) helyezettük el. Tap water and feed pellets (producer: SSNIF) continuously were offered ad libitum. According to the approximate analysis (AOAC, 1990) the feed contained 88.0% dry matter, 8.1% ash, 20.5% crude protein (N × 6.25), 3.6% ether extract, 4.7% crude fibre and 50.7% N-free extracts. For easier handling and socialisation, rats received name (the 12 months) and they were signed with stain of different colours. The open-field (OF) tests were carried out in a glass vivarium (60 × 27.5 × 30 cm), in which each occasion a feeder was place, filled with the above pellets. To adjust the noise music to the rat's hearing threshold (Borg, 1982), the WavePad software (2012) has been used. The noise music and the noises were were transmitted at 70 dB SPL, using the speaker of an Apple Macbook Pro laptop.

### *Treatments*

The animals were exposed to four different, approximately 7-min-long acoustic stimuli. 1. noise music (NM), in original version (Takehisa Kosugi: Violin Improvisation, 1989 New York); 2. twice faster and two oktaves higher, “rodentised” version (NM2X), 3. a selection of natural noises (NatN) and 4. a selection of technical-industrial noises (TechN). The Table 1 summarizes the used, from the sound collection of the Hungarian Radio received noises.

The real experiment was preceded by a week of adjustment-acclimatisation period. We socialised the animals every day, placed them one-by-one into the observation vivarium to spend there 10 minutes quiet, undisturbed environment. At the beginning the experiment the basic behaviour (without music, noise or noise music) was registered, using eight minutes observation time for each individual rat. First the animals received musical treatments on every second days (for details see Fekete and Bernitsa, 2013), afterwards the acoustic stimuli of the NM, the NM2X, the NatN and finally the TechN continued.

The OF-behaviour of the rats during listening to the acoustic exposures was registered by two independent observers on mm-paper (one remark/10 sec) and by video recorder. The following ethological elements were considered: eating, moving by the wall, crossing the

centre, sitting, sitting in the corner, freezing, sleeping, grooming, watching-sniffing, sniffing-raising (prancing, rearing, in the following: raising). The OF-tests were taken one by one, one at a time, during that the other rats were placed in a remote room, where there were not able to hear the acoustic stimuli of the experiment. The registered values of the two independent observers were averaged out and in case of uncertainty, correction have been made on the basis of the video records.

**Table 1.** List of the applied noises (Collection of TM Century Effect)

Natural noises	Technical noises
Mosquito buzz	Air compressor
Piglets' grunting	Car engine starting-roaring-driving off
Cat miaowing I	Bowling
Horse neighing	Dentist drill
Owl ululating	Hair dryer
Crickets chirping	Hammer
Screaming woman	Welding
Cat miaowing I	Circular saw
Dog snarling	Big lorry working
Fly buzz	Closing a squeaking iron gate
Thunder-lightning-rain	Vacuum cleaner in work
Lion's howling, growling	Old train: tinkles-hoots-draws away
Seagull screeching	Fireworks with crowd noise
Dog barks and howls	
Cock crows	

### *Statistical Evaluation*

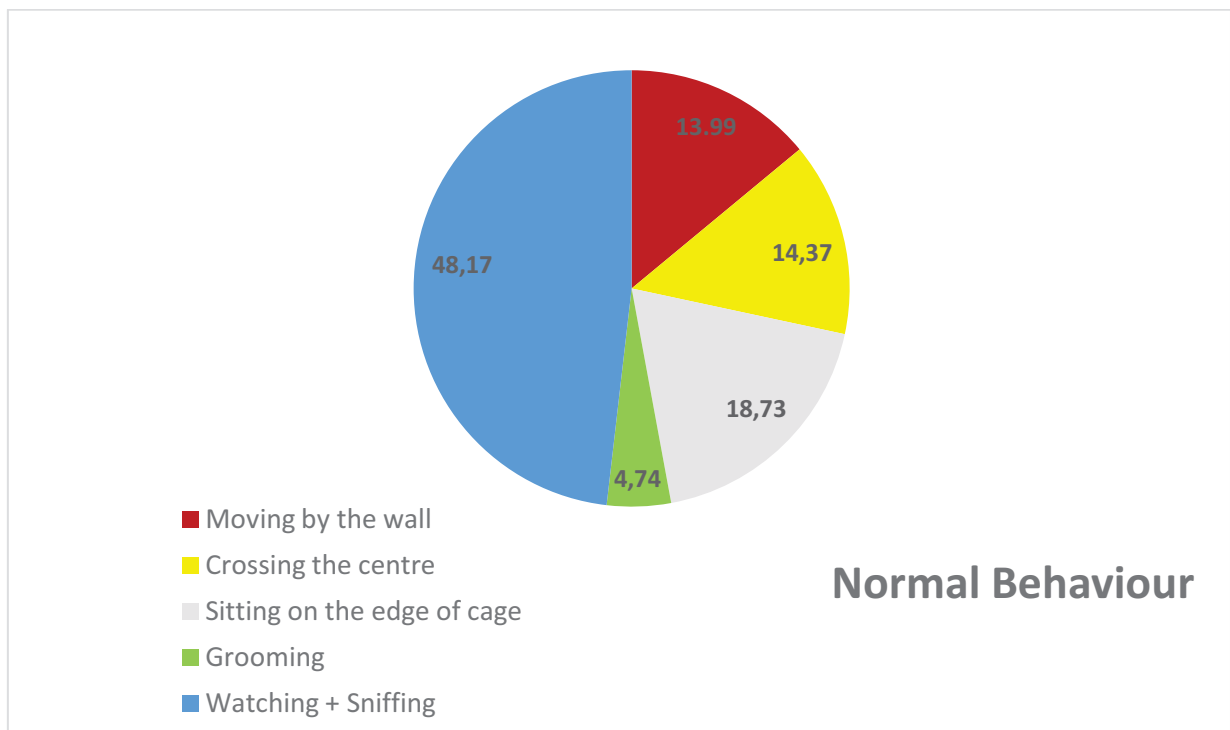
The statistical calculations (mean, standard variation, ANOVA, level of significance) were made using the Microsoft Office 2011 Excel software and the Statistica 10 (2012) program, on the theoretical basis of the textbook of Petrie és Sabin (2005). The differences were taken as significant if the p-value reached the 5%.

### *Ethical Issue*

This experiment was allowed by the Ethical Committee of the SzIU Faculty of Veterinary Science, under the number 22.1/2877/3/2011.

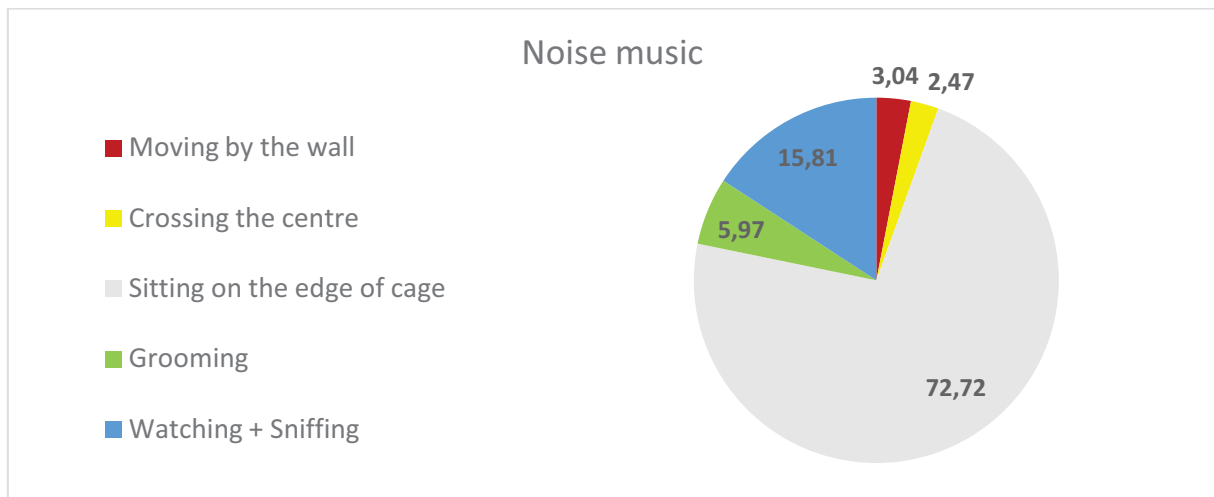
## Results

The final live weight of the rats were  $227 \pm 22,9$  gram. Considering the mean of the noise treatments ( $167 \pm 15.1$  grammes), the average daily gain is 6.8 gram. The method of analysis, compared to the musical treatments, has been improved, so for example the time of watching and sniffing was contracted. It worth mentioning, that this did not change the main tendencies. First the detailed data and the level of significane between treatments will be given, the Table 10 will summarize the percentual averages of the elements of action catalogue during the noiseless (normal behaviour) and the four sound treatments session.



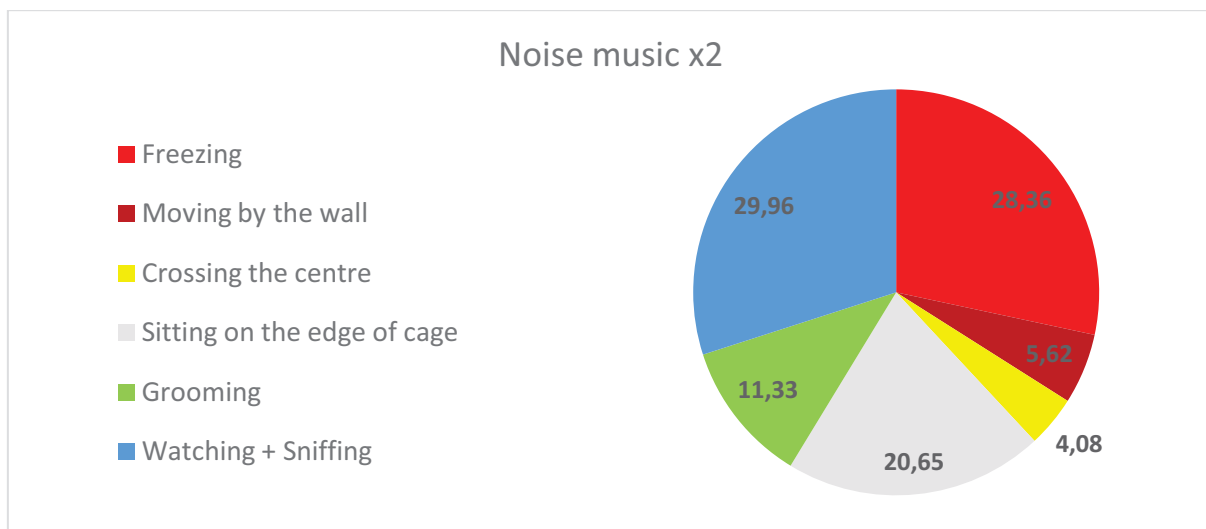
**Figure 1.** Percentage distribution of elements of open-field behaviour without sound treatment (and without „eating”)

Under normal conditions (absence of sound stimulus), the rats spent the highest proportion of time with watching and sniffing (48.17%) followed by sitting in the corner (18.73%), crossing the centre (14.37%), moving by the wall (13.99%), and grooming (4.74%). The number of eating individuals was four, therefore this behaviour element was not visualized.



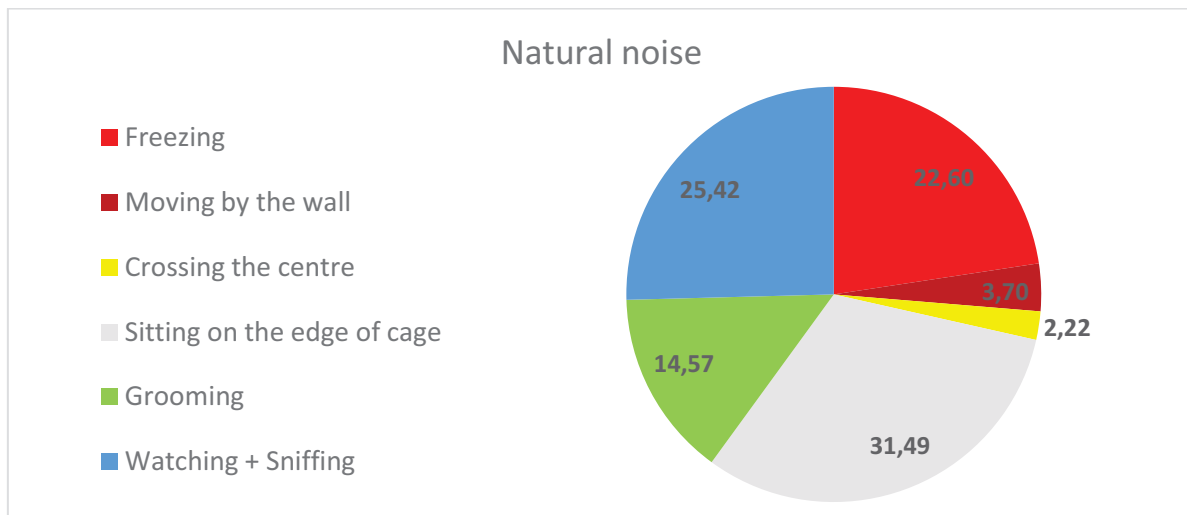
**Figure 2.** Percentages distribution of elements of open-field behaviour when listening to noise music (without eating)

When the rats were exposed to noise music, they spent the highest proportion of time sitting in the corner (72.72%), followed by watching and sniffing (15.81%), grooming (5.97%), moving by the wall (3.04%) and crossing the center (2.47%). The number of eating individuals was two, therefore this behaviour element was not visualized.



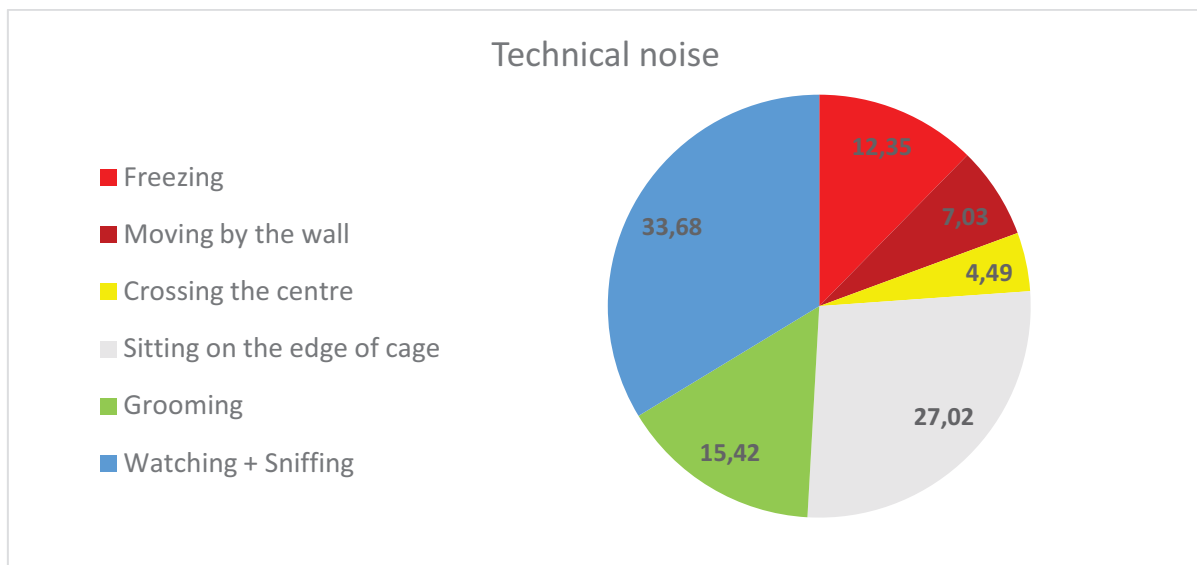
**Figure 3.** Percentages distribution of elements of open-field behaviour when listening to rodentized noise music (without eating)

When the rats were exposed to rodentized noismusic they spent the highest proportion of time with watching and sniffing (29.96%), followed by freezing (28.36%), sitting in the corner (20.65%), grooming (11.33%) and moving by the wall (5.62%). The lowest amount of time was spent with crossing of the centre (4.08%). The number of eating individuals was only one, therefore this behaviour element was not visualized.



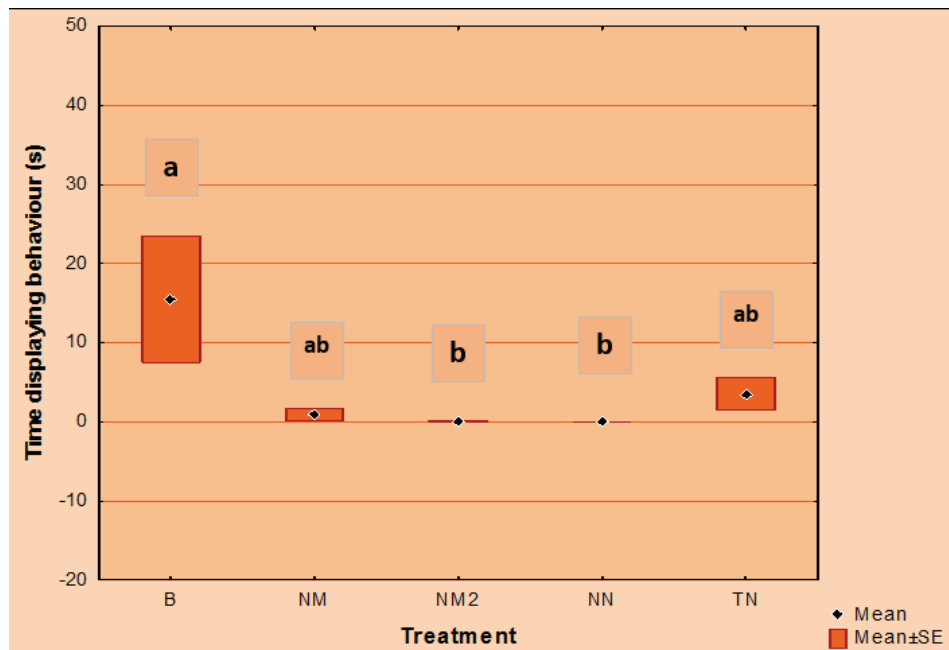
**Figure 4.** Percentages distribution of elements of open-field behaviour when listening to natural noise (without eating)

When the rats were exposed to natural noise treatment they spent the highest proportion of time with sitting in the corner (31.9%), watching and sniffing (25.42%), freezing (22.60%), grooming (14.57%), moving by the wall (3.70%) and crossing the centre (2.22%). There was not any eating behaviour during this treatment.



**Figure 5.** Percentages distribution of elements of open-field behaviour when listening to technical noise (without eating)

On the last session the rats were exposed to technical noise. The highest proportion of time was spent watching and sniffing (33.68%) followed by sitting on the corner (27.02%), grooming (15.42%), freezing (12.35%), moving by the wall (7.03%) and crossing the centre (4.49%). The number of eating individuals was three, therefore this behaviour element was not visualized. Figure 6 and Table 2 show the data of the eating behaviour.

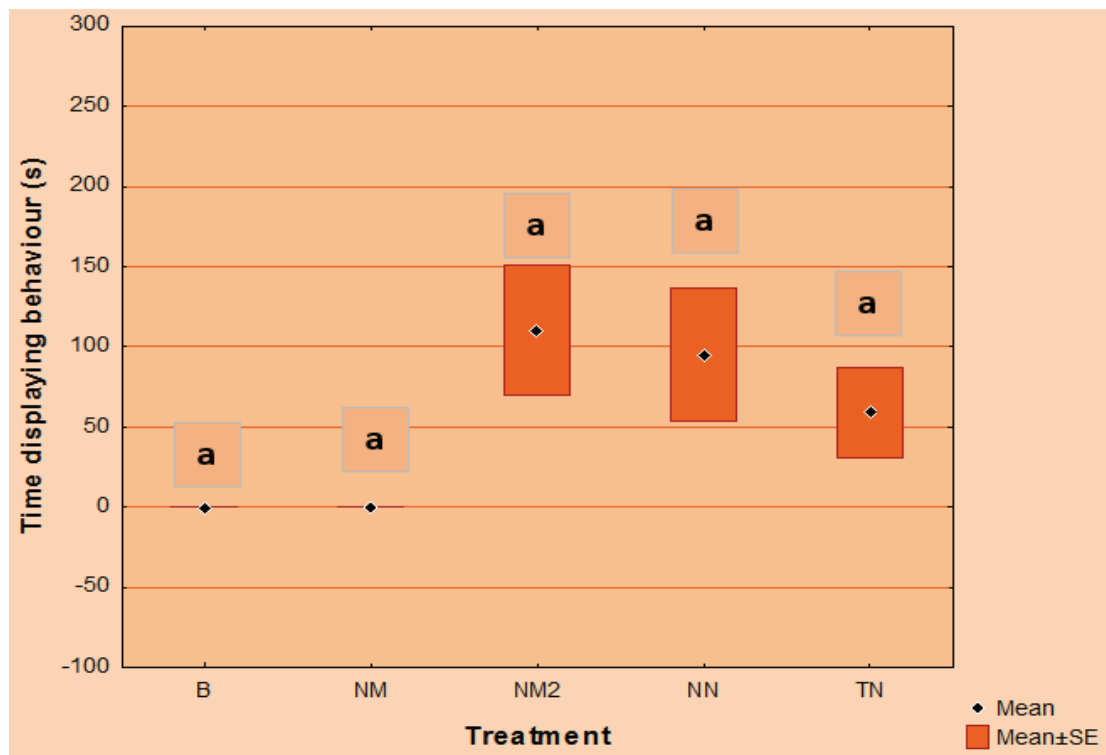


**Figure 6.** Means and Standard Errors of eating behaviour in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

**Table 2.** Means and Standard Errors of eating behaviour in seconds. The significances among the treatments, red colour means a level of significance of  $p < 0.05$ . Numbers in parentheses are the numbers of individuals concerned.

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	15.5±13.8 (4)	B		0.054	0.036	0.035	0.162
Noise music	0.92±1.85 (2)	NM	0.054		1.000	1.000	0.988
Noise music 2x	0.08±0.29 (1)	NM2	0.036	1.000		1.000	0.965
NatNoise	0±0 (0)	NN	0.035	1.000	1.000		0.962
TechNoise	3.5±4.15 (3)	TN	0.162	0.988	0.965	0.962	

There was a significant difference in the proportion of time the rats spent eating during the different treatments. During to the small sample size, one cannot make a general conclusion. The highest amount of time spent eating was detected during the basic (no treatment) followed by the technical noises, noise music and rodentized noise music. During natural noise treatment there was not any eating behaviours.

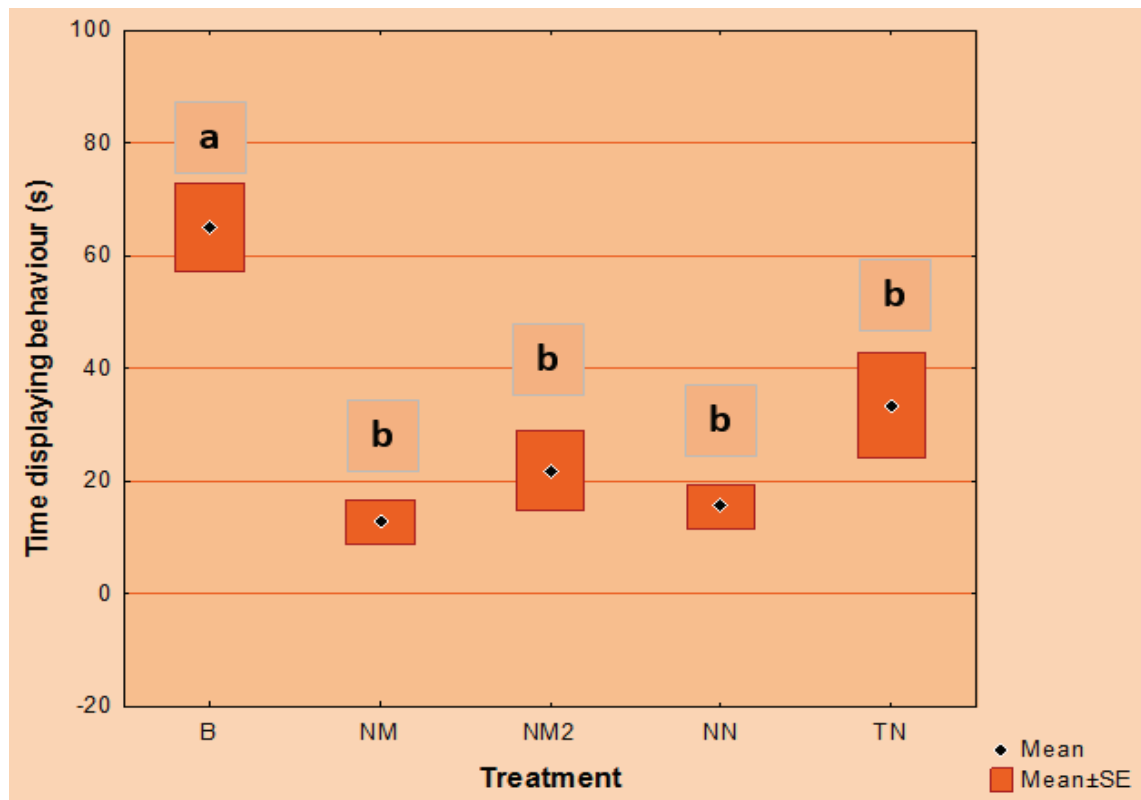


**Figure 7.** Means and Standard Errors of freezing behaviour in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

**Table 3.** Means and Standard Errors of freezing behaviour in seconds. The significances among the treatments (Red colour means a level of significance of  $p < 0.05$ )

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	0±0 (0)	B	1.000	0.066	0.151	0.605	
Noise music	0±0 (0)	NM	1.000	0.066	0.151	0.605	
Noise music 2x	110.58±44.23 (10)	NM2	0.066	0.066	0.996	0.713	
NatNoise	95.17±48.34 (9)	NN	0.151	0.151	0.996	0.900	
TechNoise	58.83±36.82 (7)	TN	0.605	0.605	0.713	0.900	

There was no significant difference in the proportion of time the rats spent frozen during the different treatments, but the noise music versus the basic has a strong tendency. Although there was no significant difference the animals spent the maximum amount of time freezing while listening to noise music followed then by natural noise and technical noise. During the basic and noise music treatment the animals did not almost display any freezing behaviour.



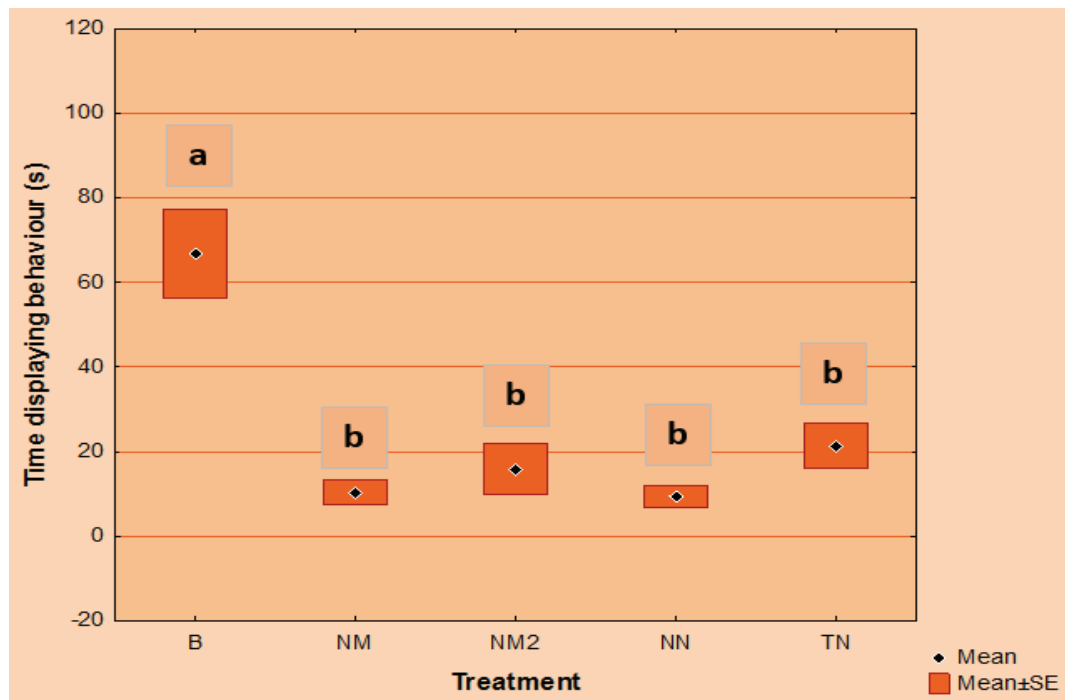
**Figure 8.** Means and Standard Errors of moving by the wall in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

**Table 4.** Means and Standard Errors of moving by the wall in seconds. The significances among the treatments (Red colour means a level of significance of  $p < 0.05$ ).

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	65±7.92 (12)	B	0.000	0.000	0.000	0.015	
Noise music	12.75±4.48 (9)	NM	0.000	0.875	0.998	0.212	
Noise music 2x	21.9±8.27 (9)	NM2	0.000	0.875	0.964	0.750	
NatNoise	15.58±4.26 (10)	NN	0.000	0.998	0.964	0.350	
TechNoise	33.5±9.77 (11)	TN	0.015	0.212	0.750	0.350	

There was a significant difference in the portion of time the rats spent moving by the wall during the different treatments. During basic the rats spent significantly more time moving by the wall than they did during the rest of the treatments. Although there was no significant differences between the other treatments, rats were moving by the wall for a longer period during technical noise followed by rodentized noise music, natural noise and noise music.



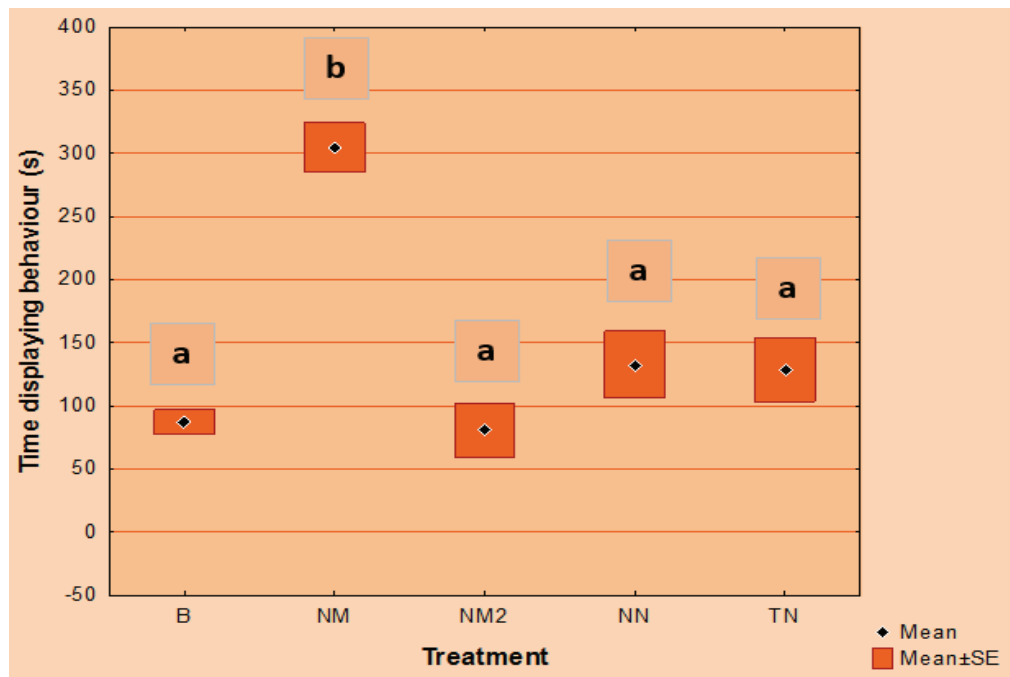


**Figure 9.** Means and Standard Errors of crossing the centre in seconds (values having different upper case letter are significantly different, p 0.05)

**Table 5.** Means and Standard Errors of crossing the centre in seconds. The significances among the treatments (Red colour means a level of significance of p < 0.05)

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	66.75±10.46 (12)	B		0.000	0.000	0.000	0.000
Noise music	10.33±3.47 (9)	NM	0.000		0.969	1.000	0.715
Noise music 2x	15.92±8.65 (6)	NM2	0.000	0.969		0.944	0.970
NatNoise	9.33±3.06 (9)	NN	0.000	1.000	0.944		0.645
TechNoise	21.42±5.7 (11)	TN	0.000	0.715	0.970	0.645	

There was a significant difference in the portion of time the rats spent in the centre if the cage during the different treatments. During basic the rats spent significantly more time crossing the centre than they did during the rest of the treatments. Though there was no significant differences between the other treatments, the animals were crossing the centre for a longer period during technical noise followed by rodentized noise music, noise music and natural noise.

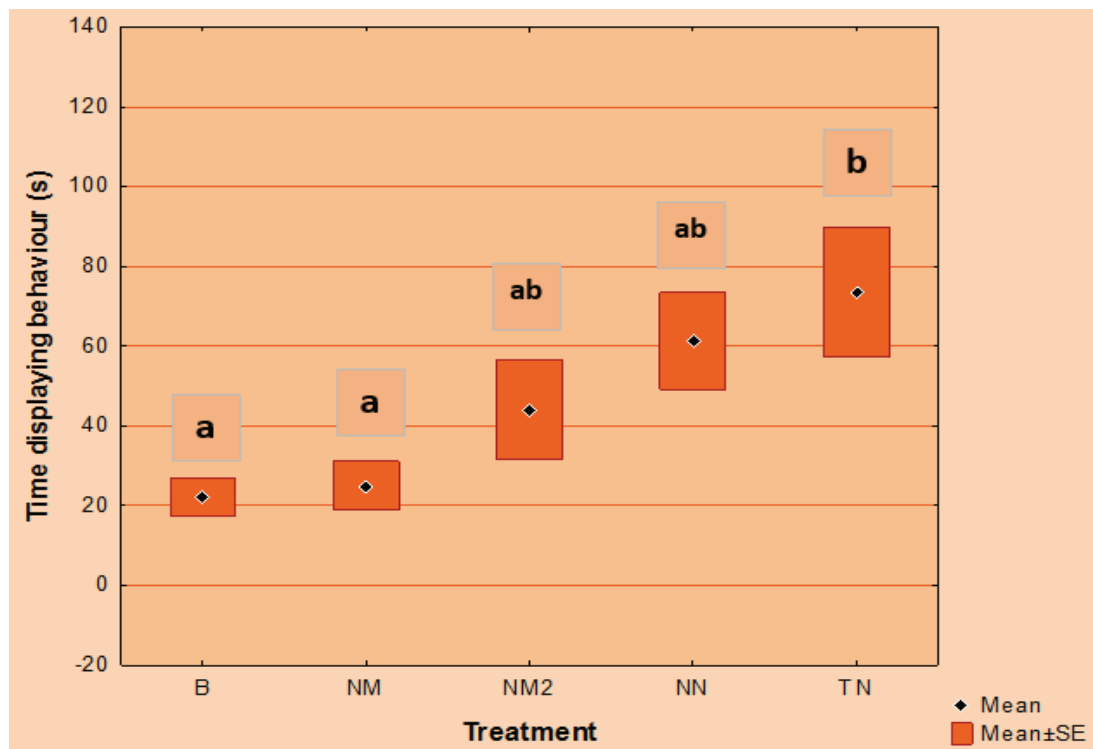


**Figure 10.** Means and Standard Errors of sitting in the corner of the cage in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

**Table 6.** Means and Standard Errors of sitting in the corner of the cage in seconds. The significances among the treatments (Red colour means a level of significance of  $p < 0.05$ )

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	65±7.92 (12)	B		0.000	1.000	0.566	0.646
Noise music	12.75±4.48 (12)	NM	0.000		0.000	0.000	0.000
Noise music 2x	21.9±8.27 (10)	NM2	1.000	0.000		0.434	0.511
NatNoise	15.58±4.26 (11)	NN	0.566	0.000	0.434		1.000
TechNoise	33.5±9.77 (11)	TN	0.646	0.000	0.511	1.000	

There was a significant difference in the portion of time the rats spent sitting in the of the cage during the different treatments. During noise music the rats spent significantly more time sitting in the corner of te cage than they did during the rest of the treatments. Though there was no significant differences between the other treatments, the animals were sitting in the corner for a longer period during technical noise followed by rodentized noise music, natural noise and noise music.



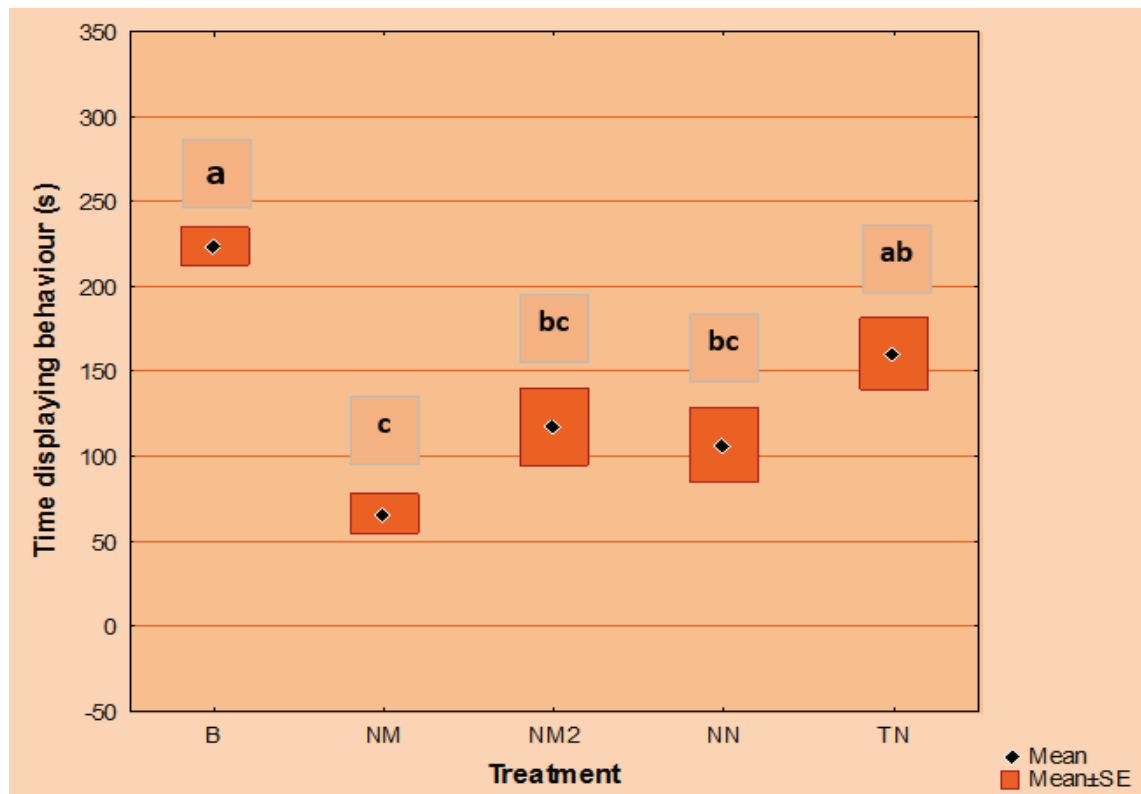
**Figure 11.** Means and Standard Errors of grooming in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

Table 7: Means and Standard Errors of grooming in seconds. The significances among the treatments (Red colour means a level of significances of  $p < 0.05$ )

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	22±5.22 (10)	B		1.000	0.635	0.112	0.017
Noise music	25±6.69 (10)	NM	1.000		0.749	0.166	0.028
Noise music 2x	44.17±13.78 (10)	NM2	0.635	0.749		0.817	0.360
NatNoise	61.33±12.16 (12)	NN	0.112	0.166	0.817		0.940
TechNoise	73.5±17.06 (11)	TN	0.017	0.028	0.360	0.940	

There was a significant difference in the portion of time the rats spent grooming during the different treatments.

The highest amount of time spent grooming was detected during the technical noise followed by natural noise, rodentized noise music, noise music and the basic behaviour.



**Figure 12.** Means and Standard Errors of watching and sniffing behaviour in seconds (values having different upper case letter are significantly different,  $p < 0.05$ )

**Table 8.** Means and Standard Errors of watching and sniffing behaviour in seconds. The significances among the treatments (Red colour means a level of significance of  $p < 0.05$ )

Treatment	Mean±S.E.	P=	B	NM	NM2	NN	TN
Basic	223.75±11.36 (12)	B		0.000	0.002	0.000	0.125
Noise music	66.25±12.13 (11)	NM	0.000		0.295	0.529	0.006
Noise music 2x	116.83±23.71 (11)	NM2	0.002	0.295		0.994	0.480
NatNoise	107±21.9 (12)	NN	0.000	0.529	0.994		0.258
TechNoise	160.5±21.03 (12)	TN	0.125	0.006	0.480	0.258	

There was a significant difference in the portion of time the rats spent with watching and sniffing during the different treatments.

The animals spent significantly more time watching and sniffing during basic than when they were exposed to noise music, rodentized noise music and natural noise. And they spent

significantly less time with these behaviours during noise music than during basic and technical noise.

**Table 9.** Incidence of spontaneous defecation (#D) and urination (#U) during observation period (D=faeces pellet, U=urination stain)

NAME	BASIC		NOISE MUSIC		NOISE MUSIC2X		NATNOISE		TECHNOISE	
	#D	#U	#D	#U	#D	#U	#D	#U	#D	#U
Jan	2	0	0	0	0	0	0	1	2	1
Feb	0	0	0	1	0	0	0	0	0	0
Mar	0	1	0	1	0	0	0	0	0	0
Apr	5	0	1	0	0	0	4	1	2	0
May	3	0	4	1	0	0	0	0	0	0
June	2	0	4	0	0	0	0	0	1	2
July	2	0	0	1	0	0	0	0	0	0
Aug	0	1	0	0	3	0	0	0	0	0
Sept	3	1	0	0	0	1	0	0	0	0
Oct	4	0	0	0	0	0	0	0	0	0
Nov	0	0	0	0	0	0	0	0	0	0
Dec	2	1	0	0	0	0	0	0	0	1
Σ	23	4	9	4	3	1	4	2	5	4

The incidence number of spontaneous urination and defecation did not show a significant difference ( $p>0.05$ ), which is a sign that they are not a really good sign to evaluate the emotional state of the rats.

Summarizing, during the soundless basic OF-session the rats spent the majority of their time with watching and sniffing (53.5%), which was followed by the moving at the wall (19.6%) and the crossing the centre (11.5%). They spent few time for the grooming (4.3%), for the sitting in the corner (11.1%) and the very least for eating (5.1%). The latter is the average of only seven animals, because five did not eat at all (**Table 10**). This *action catalogue* has been drastically change under the influence of the four different noise stimuli (in the order of NM, NM2X, NatN and TechN), moreover the phenomena of stereotyp movement and freezing emerged. The time of running by the wall (3.0, 5.6, 6.5 and 7.0%), the crossing of the centre (2.5, 4.1, 2.2 and 4.5%) and the watching-sniffing (15.8, 30.0, 24.2 and

33.7%) drastically dropped, the sitting in the corner (72.7, 20.6, 30.5 and 27.0%) significantly ( $p < 0.001$ ) increased; the nervous grooming also got longer (6.0, 11.3, 13.8 and 15.4%) (NM vs. basic:  $p < 0.001$ , the others  $p < 0.05$ ). During the noise music and rodentized noise music session stereotyp behaviour occurred: every single rat, in sitting position, continuously moved its head from one side to another, as if had nodded 'NO'. During the rodentized noise music, the natural noises, and although for less time during listening the technical noises, the freezing phenomenon emerged, too.

## Discussion

GARNER (2005) defines the stereotypies as an abnormal, repetitive behaviour. They basically derive from the disturbance of the homeostasis and can be classified into two groups: troubles of the adaptation and the neuropsychological troubles. According him, the real stereotypes are aimless series of movements or the repetition of a posture. The stereotypic behaviour of the present experiment fit into both categories, because rats were not able to solve the situation (they could not stop the noise or escape, at the same time they would not deliberate themselves from it). This may be the model of some human stereotypies, like some acts of compensation like the hair tearing (barbering), allowing even some extrapolation. The neurophysiological background is in relationship with the basal ganglions of the motoric system (TURNER et al. 2002). The basal ganglions play an important role in the learning and memorizing process, too (ROBINSON, 2008).

Some of the selected natural noises (meowing, dog snarling etc.) were similar to those, which could signalise for the free, wild living wandering rat a real danger. Generally the higher (2-5 kHz pitches) sounds are considered – both for human and rat – as unpleasant, uncomfortable (KUMAR et al. 2012). The very question is, that it is an acquired, learned, or inherited/congenital discriminating capacity of the rats. The rats of the present study did not meet any of the noises before, nevertheless they reacted violently to the natural, potentially dangerous noises. It can be assumed that the similar and strong reaction on the rodentized noise music can be explained by the similarity to the sound of one of a natural danger sources. The phenomenon of freezing or startling means that the animals, sitting or bending, become immobile and they are showing only respiratory movements. The abrupt freezing on an acoustic signal develops through three synapses: the cochlear root neurons, the nucleus reticularis pontis caudalis and the motoric neurons of the spinal chord (Lee et al. 1996). The behaviour and neurophysiological reaction of rats, given on the unpleasant noises is similar in many respects to those of the human (GARNER, 2005; KUMAR et al. 2012). The cortex is the reservoir of the objective, and the amygdala, in its turn, that of the subjective, emotional memory (MAREN and FANSELOW, 1996). Today the finest mechanisms of this topic is studied by the most developed methods. KAWAHARA et al. (1993) using *in vivo* microdialysis in rats, have shown that the psychological stress (the sight of their suffering companions) enhances the serotonin release of the amygdala and prefrontal cortex. HIRANO et al. (2006) by means of functional magnetic resonance imaging (fMRI), have demonstrated that the unpleasant, too

loud noise (sound of the fire alarm signal at 95 dB SPL) in human increases the activity of the amygdala and decreases that of the hippocampus, deteriorating by this way the memorizing ability, too.

It is not totally clear, what does mean as unpleasant stimulus, furthermore it is a congenital or learned fear or aversion. A series of data are suggesting that both in case of the human and rat, there is a congenital aversion to smells and sounds (*LeDoux*, 2000, *Williams et al.* 2005). In the study of ZALD and PARDO (2002) the dental drill and the scraping sound of the violin, which is also part of the repertoire of the present experiment, proved to be disgusting and repugnant for human, too. The parallel fMRI measurement has shown an increase of the activity in the tissues of the lateral amygdala-claustrum, the dorsal brain stem and the medial temporal field, especially in the nucleus accumbens, the auditory cortex, the thalamus and the cerebellum.

In their study KREBS et al. (1996) have chosen the eating, grooming, resting and exploratory behaviour as the most characteristic parameters. The latter included the movement, the sniffing and the watching in raising position, too. Under the influence of repeated, short noise stressor (white noise of 95 dB) the incidence of defecation and the time of nervous grooming increased.

The average daily gain during o the noise treatments (6.8 grammes) is physiological in this period of life. It indicates that the noise treatments meant only mild and transient discomfort to the animals. The incidence of urination did not difer between the treatments, but the basic defecation incidence is higher during the basic and human noise music session (**Table 9 and 10**). It may be explained by the sitting and freezing during the accelerated noise music, natural noises and technical noises, which may prevent defecation activity.

The ethological data of the exeriment can be organised into three categories according to their significances: if  $p > 0.05$  is, when the means are not significantly different; if  $p < 0.05$ , when the results are significantly different and if  $p < 0.001$  when the data are highly significantly different from each other.

### **Non-significant ( $p > 0.05$ )**

#### *Freezing*

The results were not significantly different during the treatments (**Table 2**). But one cant state that the presence of some stimuli have effect on the rats' freezing behaviour because the animals did not almost display any freezing behaviour during the basic and human noise



music treatments, while it achieve a fairly high percentage of the total time of the session: 28.36%, 22.6% and 12.35%, NM2X, NatN and TechN, respectively.

### Significant ( $p < 0.01$ )

#### *Eating*

There was a significant difference in the proportion of time, that the rats spent eating during the different treatments, namely the noise decrease the eating time (**Table 3 and 9**). The highest amount of time spent eating was detected during the basic (no treatment) followed by technical noise, noise music and rodentized noise music. During natural noise treatment there was no eating behaviour. However, because of the few number is animals, this is rather a tendency than a real statistical difference.

**Table 1:** Time spent eating during the different treatments (seconds)

Treatment	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Okt	Nov	Dec
Basic	48	0	12	0	0	84	0	0	0	0	42	0
NM	0	0	0	0	0	0	0	0	2	0	0	9
NM2	0	0	0	0	0	0	0	0	0	0	0	1
NN	0	0	0	0	0	0	0	0	0	0	0	0
TN	0	5	0	0	0	20	0	0	0	17	0	0

Grooming

Overall, the treatments increased the amount of time rats spent grooming (**Table 7**). The technical noise produced a significantly higher frequency of this particular type of behaviour than the other treatments, which are not significantly different from the basic.

### Highly significant ( $p < 0.001$ )

#### *Moving by the wall*

The treatments decreased significantly the amount of time rats spent moving by the wall (**Table 4**), compared to the basic. Although there was no significant difference between the noise treatments, the animals were moving by the wall for a longer period during technical noise, followed by rodentized noise music, natural noise and the shortest time during human noise music.

### *Crossing the centre*

This behaviour is affected by certain noise stimuli, especially if compared to the basic. The treatments decreased the amount of time that the rats spent crossing the centre of the cage (**Table 5**). Though there was no significant differences between the other treatments, the animals were crossing the centre for a longer period during technical noise followed by rodentized noise music, noise music and natural noise.

### *Sitting in the corner*

The noise music treatment significantly increased the amount of time rats spent sitting in the corner of the cage (**Table 6**). Though there was no significant differences between the other treatments and basic, the animals were sitting in the corner for a longer period during technical noise followed by rodentized noise music, natural noise and noise music.

### *Watching and sniffing*

The treatments, except the technical noise, significantly decreased the amount of time rats spent with watching and sniffing (**Table 8**). The technical noise also decreased the time spent with this behaviour, but not significantly.

## Conclusions

To help the overview of the behaviour changes, the **Table 10** below shows the percentage distribution of the different ethological elements.

**Table 10.** The percentage distribution of the different ethological elements (eating not included) and the mean of the defecation incidence and urination number

Element, %	B	NM	NM2X	NatN	TechN
Eating <sup>□</sup>	3.2 (4)	0.2 (2)	0.02 (1)	-	0.7 (3)
Freezing	-	-	28.4	22.6	12,3
Moving by the wall	13.5	3.0	5.6	6.5	7.0
Crossing the centre	13.9	2.5	4.1	2.2	4.5
Sitting in the corner	19.2	72.7	20.6	30.5	27.0
Grooming	5.7	6.0	11.3	13.8	15.4
Watching/Sniffing	47.7	15.8	30.0	24.4	33.7
Urination, N <sup>□□</sup>	0.3	0.3	0.1	0.2	0.3
Defecation, N <sup>□□</sup>	1.9	0.75	0.25	0.25	0.4

Legend: A=basic open-field behaviour, NM=noise music (original version), NM2X=rodentized noise music (two times faster and higher noise music), NatN=natural noises, TechN=technical noises <sup>□</sup>number of touched individuals (in all the other cases: 12); it is not included into the percentage, <sup>□□</sup>number of event; not included into the percentage

Owing to each of the noise treatments, the eating, moving by the wall, the crossing the centre, watching and sniffing and time decreased. The efficacy of the influence of different noises is diverse: moving by the wall is the lowest in case of the noise music, then natural noise, rodentized noise music and the technical noises follow. The eating behaviour is very few in all cases, compared to the basic. Watching sniffing decreasing order is B>TechN>NM2X>NatN>NM. The shortening of the crossing the centre time is similar in each treatment. The human noise music caused the highest increase in the length of sitting in

the corner, but no freezing occurred, in contrast to the other treatments. The freezing is present in the case of rodentized noise music (the longest), natural noises and technical noises.

Since the moving by the wall, crossing the centre and the watching and sniffing definitively decreased under the influence of each noise treatment, in turn the sitting in the corner and the grooming time increased, and with the exception of the human noise music the freezing appeared (NM2X, NatN, TechN), one can confirm that the treatments meant real stress to the rats. In case of human musical stimuli (FEKETE and BERNITSA, 2013) the decrease of the movement was interpreted as a sign of relaxation, and in case of this study as a stress situation. The reason is, that in the first case, instead of the moving, the resting and here the nervous grooming (KREBS et al., 1996) increased and even the freezing appeared. Already in the early work of ANTHONY et al. (1959) was demonstrated, that noise exposure increased washing-grooming activity of rats. The eating behaviour seems to be mostly as an individual character. Such as the numbers of the urination and the defecation incidence.

The means of the different noise treatments did not differ significantly, with the exception of human noise music, when no freezing occurred, but the sitting in the corner was definitively longer and the watching and sniffing time shorter. The grooming time gave also the lowest number, but the difference was significant versus the technical noises. As a whole, the rodentized noise music and the natural noises seem to be the most disturbing for the rats. If we compare the moving by the wall and the crossing the centre, and compare the basic versus the means of the four noise treatments (movement: 27.5 vs. 8.8%, sitting in the corner: 18.1 vs. 37.7%, grooming: 4.6 vs. 11.6% and watching+sniffing: 46.6 vs. 26.1%), it can be stated that the noises and noise music are stressful for the rats. Consequently, the weakening or covering noises by appropriate music should be part of the animal welfare.

As final, *general conclusions*, one can state that under the influence of unexpected, unpleasant sound stimuli, rats

-increase the length of sitting in the corner, answer with freezing (startling), or/and stereotypic behaviour;

- with the exception of sitting in the corner, the rodentized version of the noise music is more efficient on the OF-behaviour;

- natural noises have more influence than the technical ones, for example the freezing time is two times longer;

- some of the sound stimuli, unpleasant for the rats, act similarly in case of the human and it seems to exist a possible congenital aversion to certain sounds for both species.

Concerning the physiological answer on the noise, like wound healing, cytokin production, the rat may be an appropriate model for the production animals and even for human. The horrifying reaction (freezing, stereotypic behaviour) shows the noise music, aesthetic for some reason for the man, may be unpleasant and irritating for the animals. Consequently, the background music in an animal facility must carefully be chosen.

**Abstract. Sukikara, Chihiro: “HOW THE DIFFERENT NOISE TYPES MAY INFLUENCE THE OPEN-FIELD BEHAVIOUR OF RATS ?”**

The acoustic environment, including noises, is an utmost important component of the animals' welfare. Environmental noises are harmful to the laboratory animals, therefore the author tested the effect of different noise types. Before starting the noise exposures, a week preliminary period was inserted to socialize the animals. After that 12 young rats were exposed to musical stimuli (MAGY. ÁO. LAPJA, 2013. 135. 246-253.), thereafter a particular noise type only once, every second day, in the afternoon. Each rat spent approximately 7 minutes inside the open-field (OF) box and with simultaneous manual and video registration of behaviour was carried out by two independent observers. The following activities were evaluated: eating, moving by the walls, crossing the centre, sitting, grooming, watching and sniffing, freezing, stereotypical movement, urination and defecation rate. First the basic OF-behaviour, without any sound effect was registered and then the noise stimuli were presented in the following order: noise music (Takehisa Kosugi's Violin Improvisation: 1989) in original and in two-times accelerated rhythm and two octaves higher in pitch (“rodentized” version”), selection of natural and technical noises. Predominantly the basic ethogram consisted of watching and sniffing (47.7%), moving by the wall (13.5%) (exploratory behaviour), sitting in the corner (19.2%), animals crossed the centre (13.9% of the time (showing the lack of fear) and no freezing or sleeping occurred. Noise music, especially the “rodentized” noise music, as well as the natural noises drastically decreased time spent with moving, watching and sniffing, the duration of sitting in the corner and the nervous grooming increased. Freezing and stereotypical behaviour (moving the head from one side to another in sitting position) appeared, too. The noise music, especially the higher and accelerated noise music, as well as the natural noises drastically decreased the time of ambulation, watching and sniffing. In turn, the duration of sitting in the corner and the nervous grooming increased. Freezing, and stereotypical behaviour (moving the head from one side to another) appeared, too. Listening to the natural noises (containing also cat and dog voices) basically had similar effect on the OF-behaviour as the “rodentized” noise music and rats crossed the centre the few times (sign of anxiety). The technical noises had less influence on the OF-behaviour: the freezing time reduced by 50%. The time for eating, the urination and defecation rate were reduced by all the four noise stimuli. From the point of view of animal welfare, the background music (e.g. radio) of the animal facilities should carefully be chosen to avoid the possible negative side-effects.

## **Összefoglalás.**

### **Chihiro Sukikara: HOGYAN BEFOLYÁSOLJÁK A KÜLÖNBÖZŐ ZAJTÍPUSOK A PATKÁNYOK PORONDTEST-VISELKEDÉSÉT ?**

Az akusztikus környezet – a zajokat is beleértve – jelentős befolyást gyakorol az állati jóllétre, de az ide vonatkozó adatok száma kevés. A szerzők célja ezért a különböző típusú zajoknak a porondtest (PT) viselkedésre gyakorolt hatásának összehasonlítása volt. A zajingerek előtt az állatok szocializálására egy hét előszakaszt iktattak be. Ezt követően két naponta délután 12 fiatal hím patkányt egy-egy zenei (MAGYAR ÁLLATORVOSOK LAPJA, 2013. 135. 246-253.), majd zajtípusnak tettük ki. Minden patkány kb. 7 percet töltött a PT-viváriumban, mialatt két független bíráló figyelte az állatokat, továbbá folyamatos videofelvétel készült. Az alábbi viselkedéselemek kerültek rögzítésre: evés, mozgás a fal mellett, a középpont átszelése, ülés, önápolás, figyelés és szimatolás, “lefagyás”, sztereotíp mozgás, vizelet- és bélsárürítés. Először a zaj nélküli alapviselkedést rögzítettük, majd a zajhatásokat a következő sorrendben vizsgáltuk: zajzene (Takehisa Kosugi Hegedű Improvizáció: New York, 1989 Szeptember) eredeti, valamint kétszer gyorsabb és két oktávval magasabb (“rágcsálósított”) változatban, majd természetes és technikai zajok válogatását. Az alapetogram főként figyelésből és szimatolásból (47,7%), valamint a fal melletti mozgásból (13,5%) (fölfedező magatartás) és a sarokban való ülésből (19,2%) állt, az idő 13,9%-ában keresztülfutottak a központi területen (félelem hiánya), sem lefagyás, sem alvás pedig nem fordult elő. A zajzene, különösen “rágcsálósított” változatban, valamint a természeti zajok drasztikusan csökkentették a mozgásokra, figyelésre és szimatolásra fordított időt, a sarokban ülés és az ideges önápolás tartama megnőtt. Lefagyás, valamint sztereotíp viselkedés (ülő testhelyzetben a fej mozgatása egyik oldalról a másikra) is megjelent. A PT-viselkedés a természetben előforduló (macskanyávogást és kutyaugatást is tartalmazó) zajkeverék hallgatása alatt alapvetően a “rágcsálósított” zajzenéhez hasonlóan változott meg, a központot ezalatt szeltek át a legkevesebbszer (félelem jele). A technikai eredetű zajok kevésbé módosították a PT-viselkedést: 50%-kal rövidebb volt a lefagyás ideje. Az evés tartama és a vizelet- bélsárürítés gyakorisága minden zajtípus hatására csökkent. Következtetésként megállapítható, hogy mind a négy hanginger hatott a patkányok viselkedésére. Az állati jóllét szempontjából fontos, hogy egy állatházban a háttérzenét (pl. rádió) gondosan kell megválasztani, hogy az esetleges káros mellékhatásokat elkerüljük.

## Acknowledgement

The author owes a debt of gratitude to *Emese Andrásófszky*, head of the laboratory of the Institute of Animal Breeding, Nutrition and Laboratory Animal Science, *Gabriella Korsós dr.*, assistant research fellow of the Unit for Laboratory Animal Science, *Krisztina Sényi* secretary, *Bálint Szalai* voice technician, *Theodora Bernitsa* and *Emily Barret* veterinary student and *Kiss Józsefné Edit Oláh* librarian for their multiple help in laboratory analyses, data registration, sound technique questions, biomathematical evaluation and providing with literature. This work was supported by a grant from the NKB Grant of the Faculty of Veterinary Science, Szent István University, Budapest.



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