EMOTION TRANSFER THROUGH TRANSMISSION OF VOCAL MESSAGES IN MICE

MA Vassiliadis Department of Music Technology and Acoustics, Technological Educational Institute of Crete, Rethymnon, Crete, Greece
C Theochari Department of Biology, University of Crete, Heraklion, Crete, Greece
P Georgiakakis Museum of Natural History, University of Crete, Heraklion, Crete, Greece
V Boura Department of Music Technology and Acoustics, Technological Educational Institute of Crete, Rethymnon, Crete, Greece
I Athanassakis Department of Biology, University of Crete, Heraklion, Crete, Greece
S Kouzoupis Department of Music Technology and Acoustics, Technological Educational Institute of Crete, Rethymnon, Crete, Greece

1 INTRODUCTION

Psychological welfare and relaxation ensured by music hearing offers beneficial effects to the pathophysiology of an organism, whereas undesirable noise negatively affects the defensive mechanisms of the organism.[1,2,3] In mice, music has been shown to reduce the stress-induced inhibition of protective immunity as well as the development of metastatic foci provoked by carcinosarcoma cells.[4]

Hearing is an important sense for mice and differs from humans as to the range of frequency sounds are perceived. Although the hearing range in humans varies from 20 Hz to 20kHz, mice generally hear sounds in the range of 10-70kHz but may also hear sounds in excess of 100kHz. Except from hearing, vocalization in mice includes important sounds in ultra-sound frequencies. Neonate rodents use ultra-sounds to call their mothers when they become isolated from them by emitting messages recorded at ~40kHz.[5,6] During the reproductive copulation, when males meet female mice or their pheromones emit ultrasound frequencies ranging from 30-110kHz.[7,8,9] These sounds present a periodicity and have been characterized as “syllables” that constitute the so-called “song of male mice”.[10] In contrast to ultra-sounds, sounds at lower frequencies can be transferred at longer distances and therefore are used as warning messages to the community.

Hearing is one of the most important senses for communication and transfer of messages. Sound waves reach hair cells in the inner ear where they are translated into electro-chemical energy, trigger the central nervous system (CNS) and are transferred to the whole organism *via* hormonal regulation. Using the mouse as an experimental model in the present report we attempted to transfer the sensation of stress from one organism to another by reproducing voluntary calls previously recorded, over loudspeakers. The transduction of stress to chemical messages was visualized through the study of urine. The responsiveness of CNS to stress is spontaneous and is expected to result in an increase or decrease of production of various components in blood, urine and all body fluids. Serum would be the best body fluid to measure hormonal variations, but blood collection in mice is by itself a stressful manipulation which could thereafter mislead data analysis. Although there is no supportive evidence in the literature as to whether stress markers are present in the urine, the choice of this body fluid in the present study was based on the fact that its collection is naturally performed and does not require any further manipulation of the animals. In humans proteinuria represents a pathological condition, whereas in mice it is a physiological procedure where the major urinary proteins (MUPs) that represent low molecular weight components (~18,000 daltons) can cross the kidney membranes and be excreted in the urine.[11]

Experimental animals were recorded under two stress conditions named “fear/pain” and “quarrelling” in the auditory and ultra-sound spectrum and the urine protein profile was determined. Within the scope of emotion transfer, the recorded sounds were analyzed, monitored and emitted to other animals. Comparing the protein profile in the urine of these animals with the animals that had received the direct stimulus, a successful emotion transfer was demonstrated, showing that sound perception directly affected the physiology of the organism.

2 RESULTS

2.1 Recording and Analysis of Sound Messages in the Audible Spectrum

The primary stimulus of BALB/c and CeH/HeN mice, referred as “fear/pain emotion” consisted of direct pinching mouse foot by hand, while holding it. Among the approximately 100 mice tested, only 60% reacted by emitting sounds in the audible spectrum. The sound messages emitted during manipulation were recorded using two Shure SM57 microphones, placed at a constant distance from the animal. Spectrum analysis was performed using various software programs (Batsound, Matlab and WaveLab). Typical sound signatures for both male and female mice appear in Figure 1. Two types of “spectral signatures” were detected, both in male and female mice, termed as ”curved” and “non-curved” spectra (Figure 1). “Curved” signatures in male mice showed either symmetrical (Fig. 1E) or not symmetrical shape (Fig. 1F) and their forms were different than those for the females (Fig. 1A, B, C). Male mice formant traces also spanned a wider range of frequencies than female formant traces. On the average both male and female calls showed most of their acoustic energy in 4 ± 1 distinctive ‘formants’, (extreme values being 2 and 8, number of samples n=85). Frequency range in males varied from 2 - 22 kHz and in females from 2.5 -18 kHz, whereas the duration of sound messages varied in both cases from 100 - 200 ms. The sound pressure level (SPL) of the reproduced vocal activity at the mice location was adjusted to 50 dB (re 20 μPa) for both male and female mice. During the above analysis, no differences were detected between BALB/c and CeH/HeN mice and similarly no statistically significant changes were detected between the different ages of mice tested.

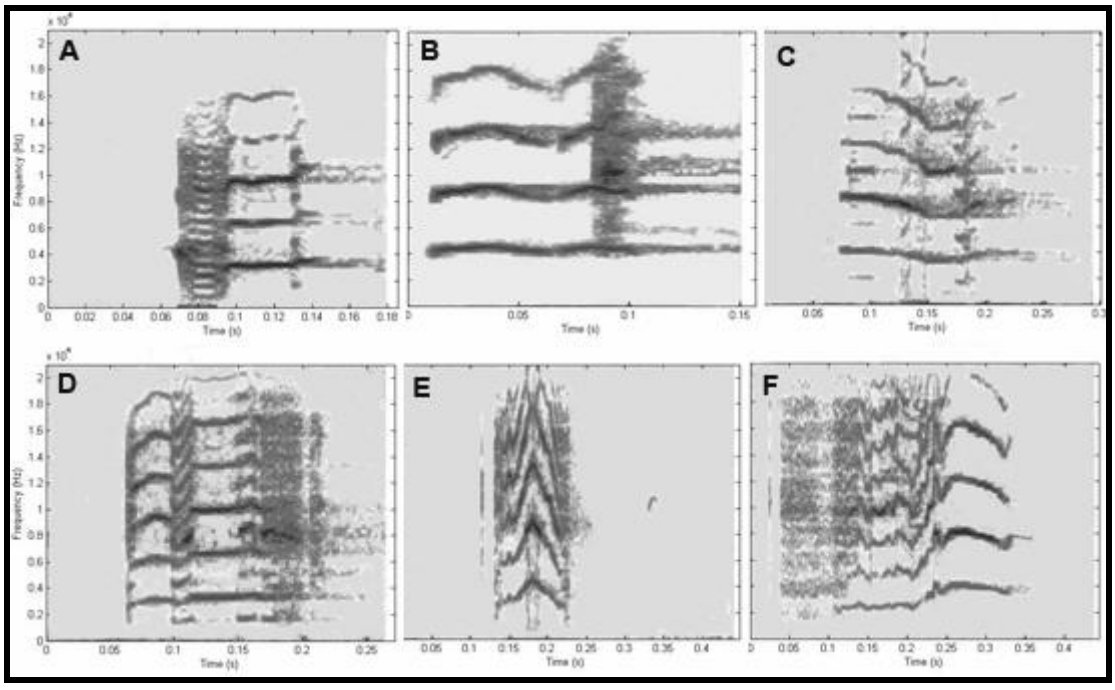

Figure 1: Spectrograms of sound messages emitted from female (A, B, C) and male (D, E, F) mice during the “fear/pain” emotion. The frequency axis in all graphs spans linearly the range 0-20 kHz.

Another manipulation included “quarrelling” between male mice. A female mouse in estrus was placed in a cage with a male and when the reproductive copulation was about to start (~10 min later) a second male was placed in the cage. The quarrelling sounds between males were recorded and the spectrograms for three typical calls are presented in Figure 2.

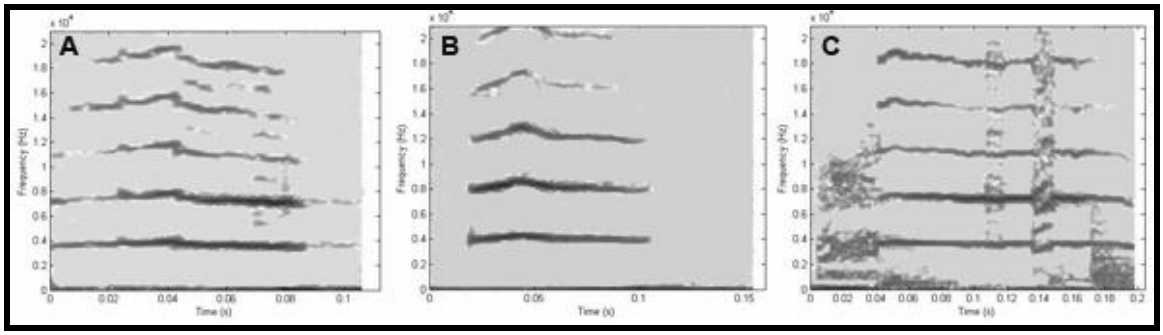

Figure 2: Spectrograms of sound messages emitted from male mice during “quarrelling”. The frequency axis in all graphs spans linearly the range 0-20 kHz.

Most of the acoustic energy for these calls is concentrated in 5 ± 1 distinctive ‘formants’, (extreme values being 4 and 6, number of samples n=35), whereas the frequency spanned the range of 2-25 kHz (SPL being approximately 50-dB). It has to be noted that during this manipulation, two male mice were recorded at the audible spectrum, whereas during the whole experiment the female remained silent.

Finally, the last experiment included the induction of anxiety to neonates. A few minutes after removing the mother from her nest, neonates (8 to 13 days old) expressed an anxiety which was recorded as described previously. Example oscillograms for a few cases appear in Figure 3. The number of distinctive formants was 3 ± 1 for neonates and 4 ± 1 for their mothers. Most of the energy was in the range 4-15 kHz in both neonates and their mothers.

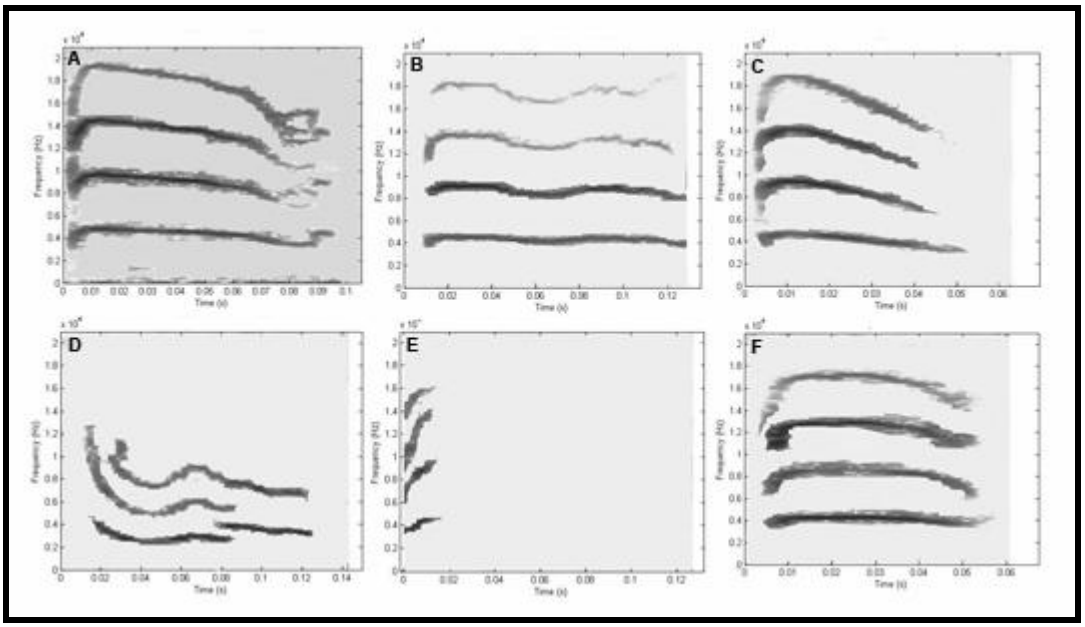

Figure 3: Spectrograms of various vocalizations from female mice (A, B, C) and neonates (D, E, F) after removing the mother from the nest.

2.2 Recording and Analysis of Sound Messages in the Ultrasonic Spectrum

Mice use the emission of messages in the ultrasonic spectrum in order to communicate among them, usually when they are calm and they don’t want to be noticed by the hunter. In the present study, ultrasonic vocalizations were recorded using an ultrasonic receiver (Pettersson Elektronik

A.B. D980, Uppsala, Sweden) in both “fear/pain“and “quarrelling between males” manipulations. During the “fear/pain“manipulation the number of formants of the messages recorded were 11±5 (n=56), spanning a frequency range of 10-250 kHz (150 kHz being the most common frequency detected). During the “quarreling” manipulation 12±6 formants were identified (n=45), spanning a frequency range of 10-150 kHz. Spectrograms and oscillograms for two characteristic vocalizations during quarrelling and fear/pain emotional state, are shown in Figure 4. It has to be noted that because of the recording method used, the ultrasonic signal was expanded in time so that frequency appears 10 times lower.

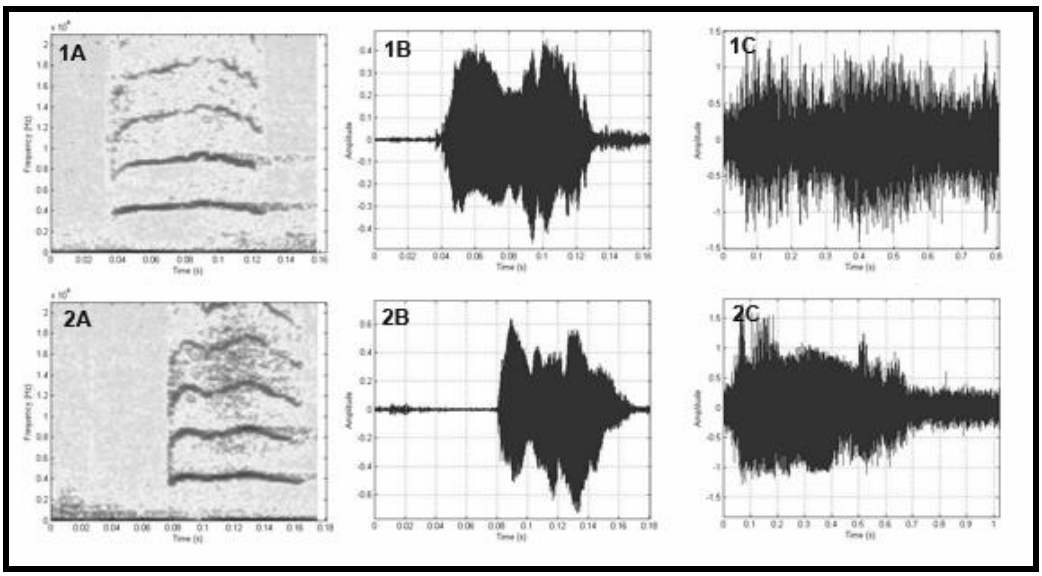

Figure 4: Spectrograms and oscillograms for two vocalizations during the fear/pain emotional state in the audible spectrum (1A, 1B, 2A, 2B) and ultrasonic spectrum (1C, 2C).

2.3 Protein Profile in the Urine of Experimental Mice

Urination is one of the direct biological excretions in response to an external stimulus in mice. Urine samples were collected from individual BALB/c or C3H/HeN mice during the fear/pain manipulation in sterile eppendorf tubes. The protein profiles of 2 or 5 μl of urine samples from individual male or female mice were examined by SDS-page gel electropheresis[12] and visualized upon silver staining.[13] Ten mice or more were tested in each case and characteristic examples are shown in Figure 5. This type of manipulation showed a significant quantitative difference in all protein products between male or female negative controls (Figure 5A, lanes 1, 2, 7, 8) and groups that had received the stimulus (Figure 5A, lanes 4, 5, 6, 9). Except from the 5-fold quantitative increase of proteins, in the urine of mice that were submitted to the fear/pain manipulation, an additional band at ~16 kD was detected (Figure 5B) which was absent from the negative controls, even in the case where a greater volume (5μl) of urine was used (Figure 5B, lanes 7, 8).

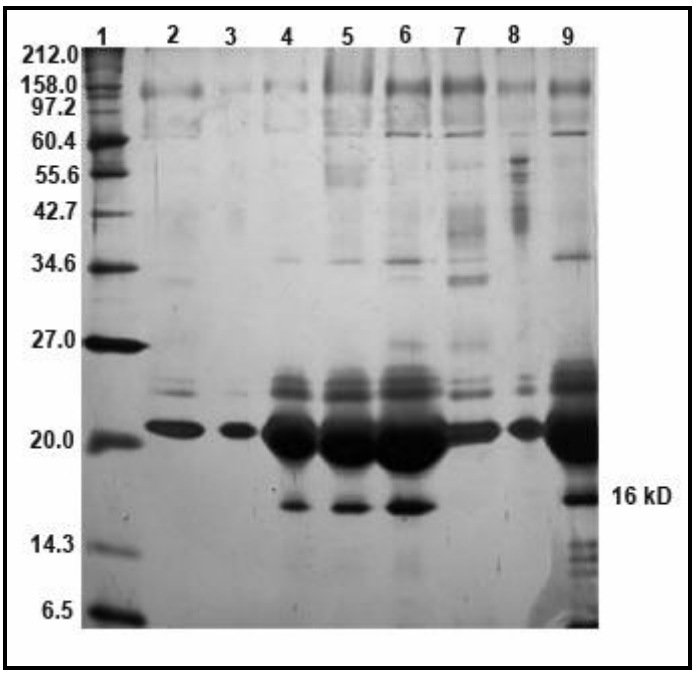

Figure 5: Electrophoretic analysis of urine samples from individual mice in SDS-polyacrilimide gel. 1: Molecular weight marker (kD), 2: negative control female BALB/c 1 (2μl of urine), 3: negative control female C3H/HeN 1 (2μl of urine), 4: BALB/c male during the fear/pain manipulation (2μl of urine), 5: BALB /c female during the fear/pain manipulation (2μl of urine), 6: C3H/HeN female during the fear/pain manipulation (2μl of urine), 7: negative control BALB/c female (5μl of urine), 8: negative control C3H/HeN female (5μl of urine), 9: BALB/c male during the fear/pain manipulation (5μl of urine).

2.4 Emotion Transfer through Sound Messages

That sounds that were recorded in the audible spectrum during the direct manipulations of experimental mice were isolated and concentrated into two files, one containing the fear/pain manipulation and the other the quarrelling manipulation. Some simple signal processing of recorded sounds involved normalization of sounds, denoising and deletion of any unrelated external noise. Sound emission was performed using a desktop computer, Hi-Fi amplifier and speakers (TEAC POWERMAX 80.2 system, frequency response 60-20.000 Hz). Experimental mice were individually caged and placed at equal distances and angles from the speakers. Male and female mice which were not submitted to any previous manipulation were emitted with the sound messages previously described, while collecting urine from each individual animal. SDS-page gel electrophoretic analysis of each separate urine sample clearly demonstrated the development of a secondary reaction in response to the indirect emotion of stress through the perception of sound messages (Figure 6). Both groups of mice (those submitted to the fear/pain and quarrelling emotion) showed a protein profile similar to that of animals that had received the direct stimulus. It is interesting to note that males and females showed a different perception of the indirect stimuli, while no such difference was detected during the primary stimulus.

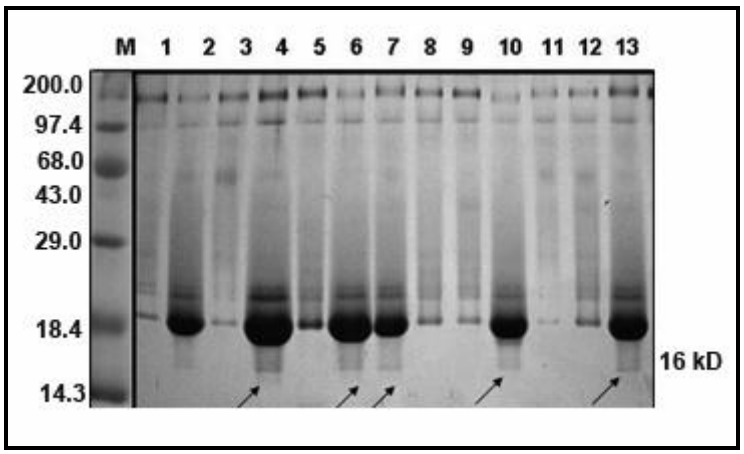

Figure 6: Electrophoretic analysis of 2 µl urine samples in SDS-polyacrilimide gel collected upon emission of fear/pain (lanes 1-8) and quarrelling (lanes 9-13) sound messages. M: Molecular weight marker (kD),1: negative female control BALB/c, 2: female BALB/c_1 3: male BALB/c_1, 4: female BALB/c_2, 5: male BALB/c_2, 6: female C3H/HeN, 7: male C3H/HeN, 8: negative female control C3H/HeN, 9: male BALB/c, 10: female BALB/c, 11: male C3H/HeN, 12: male C3H/HeN, 13: female C3H/HeN. Arrows indicate the 16 kD band.

Transfer of the fear/pain emotion in BALB/c or C3H/HeN females provided a response similar to the animals that had received the primary (direct) stimulus (Figure 6, lanes 2, 4, 6). In contrast, BALB/c males (Figure 6, lanes 3, 5) were not affected by the secondary (indirect) sound messages. C3H/HeN males showed a protein profile similar to females (Figure 6, lane 7).Female mice were also shown to be more sensitive to the transfer of "quarrelling" emotion sound messages as well, since they showed the quantitative increase of proteins in urine and developed the 16 kD protein product (Figure 6, lanes 10, 13). Male mice (Figure 6, lanes 9, 11, 12) were not affected by this secondary sound message, since they showed a protein profile similar to negative controls. Unfortunately, the nature of the experimental procedures did not allow the collection of urine from individual mice during the direct "quarrelling" manipulation between the two males in the presence of the female and consequently the results can not be compared to the primary stimulus.

One kind of proteins detected in the mouse urine are soluble major histocompatibility complex class I proteins (sMHCI), which are also known as odortypes[14] and act as pheromones for the choice of the male or female partner for reproduction. To test whether the experimental manipulations applied in the present study also affect sMHCI production, enzyme linked immunoassays (ELISA) were performed[15] in the various urine samples isolated from mice which had received the indirect emotion stimuli. As shown in Figure 7 only the emotion fear/pain induced an increase of sMHCI production in the urine of male as well as female mice by ~2- and 65-fold respectively. The transfer of the quarrelling emotion did not affect the levels of sMHCI proteins.

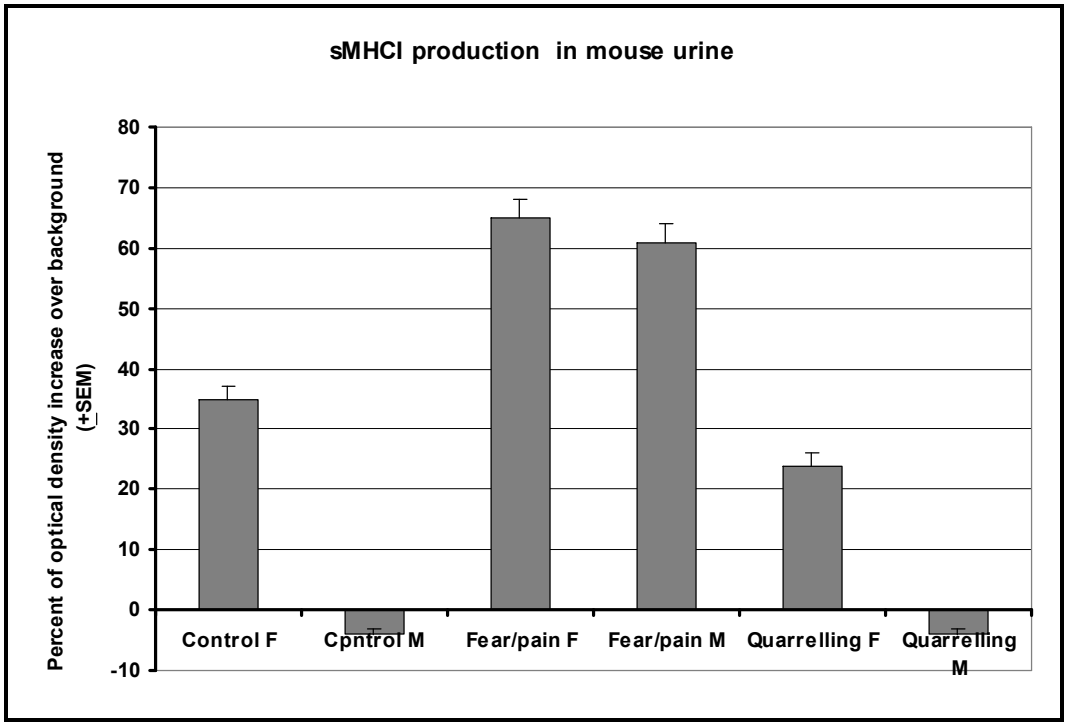

Figure 7: Detection of sMHCI molecules in the urine of male (M) or female (F) BALB/c mice collected upon emission of fear/pain and quarrelling sound messages. Urine was used at 1/50 dilution and sMHCI detection was performed using a monoclonal anti-H-2Kd antibody. The results are expressed percent of optical density increase over background ±SEM (n=5). The experiment was repeated three times and similar results were obtained.

3. CONCLUSIONS

The biology of hearing directly links acoustic stimuli to the nervous system of the organism which in turn triggers various biologic phenomena. The correlation of sound hearing to specific regulatory mechanisms of the organism is a very interesting scientific field to study, since it may reveal simple combinations of sound frequencies which could stimulate or suppress the production of hormones, growth factors, cytokines, etc. These factors, in turn, could affect various cellular systems and modify the whole physiology of the organism. Using as experimental model the mouse, in the present study sound messages emitted during the externally induced fear/pain emotion were recorded in the audible spectrum, while at the same time the direct changes in the urine protein profile were estimated. Sound messages were analysed, monitored, emitted to virgin mice and their urine protein profile was evaluated as to whether it resembles the urine protein profile obtained by the primary manipulation of mice. The results showed that sound messages may indeed transfer an emotional state which is translated into chemical signals, an approach that may be very useful in the therapeutic biomedical research.

Spectrum analysis showed that males emitted sounds at higher frequencies and intensities as compared to females. In all cases, the emission in the audible spectrum was accompanied by ultrasound messages, which reached frequencies up to 250 kHz, with most commonly emitted frequencies in the region of 150 kHz.

The recorded messages were monitored and given back to virgin mice. The anxiety of animals to the hearing of these messages was obvious and could successfully be detected in the urine protein profile. Upon electrophoretic analysis of the urine samples, it was shown that the animals submitted to the exogenous stress were showing a quantitative increase of MUPs, while a new protein product

was obtained at 16 kD, which was not present in the urine samples of negative controls. A similar protein profile was obtained with urine samples that had received the indirect stimulus through the emission of sound messages. The different reaction of the sound messages of females from male mice, as this was observed during the sound emission process, was also obvious in the electrophoretic profile of MUPs. Furthermore, sMHCI proteins which act as partner selection pheromones were only detected in the urine of mice during the fear/pain emotion transfer.
In conclusion, this study presents for the first time a possibility of emotion transfer through sound messages, which are directly translated into chemical messages. Using initially simple frequency compositions, one could attempt to stimulate or suppress protective or harmful mechanisms to the organism respectively, so that sound compositions could support or even replace various pharmacologic therapeutic procedures.

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