Effects of accelerated human music on learning and memory performance of rats

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ABSTRACT

A total of forty, 7-week-old male rats were exposed to the 'rodentized' version (twice as fast as and one octave higher than the original) of Mozart's Sonata for Two Pianos in D major for ten minutes a day for 10 weeks. One group (10 rats) received the musical stimuli before ('B'), another during ('D') and the third before and during ('BD') the memory test, while the ten control ('C') animals were kept in silence. The animals' spatial learning and memory ability was tested in an 8-arm radial maze. Rats exposed to the music showed a significant (7.1%) improvement in task acquisition (Group BD), but it did not practically change in Group D and worsened by 10.5% in Group B. The 2-h working memory significantly improved by 12.1% (BD) while practically did not change in Groups B and D. The reference memory improved by 11.9% in Group BD, but did not change in Group B and D, compared to the Control. The performance of the groups during the 4-h working memory test did not differ significantly. During the long-term test period the spatial memory performance of the music-exposed rats did not show significant differences compared to the Control (Table 1). At the same time, most results obtained in the long-term period were better than the corresponding short-term data. In conclusion, this particular piece of music, falling within the rats' hearing range, was suitable for improving hippocampus-dependent spatial learning capacity, but only if the animals were exposed to it not only before but also during the task.

1. Introduction

The 'Mozart effect' (Rauscher et al., 1993) refers to a debatable (Pietschnig et al., 2010) scientific theory, according to which listening to classical music may improve the subsequent learning ability and IQ test results of spatial-temporal character. However, the above metaanalysis did not care of the new neurophysiological and gene expression studies. Mozart's Sonata for Two Pianos in D major, K 448 (hereinafter: the Sonata) and the Piano Concerto No. 23 (K 488) have been found to produce the most effective results. After listening to Mozart's music, the mental functions of Alzheimer's disease patients improved, and they were able to recall tunes (Fukui et al., 2012). The maze test performance of rats also improved under the influence of the Sonata (Rauscher et al., 1998). In contrast, silence, white noise or minimalist music have no similar effects. The question arises what type of music is generally able to produce the Mozart effect. Melodies similar in structure, harmony and predictability (recurrence) were also found to be efficient in producing similar effects. Hughes and Fino (2000) carried out computerized analysis of several pieces of music. In the case of Mozart and Bach, the long-term periodicity (the number of repetitions within 10–60 s, half a minute on average) is of decisive importance. The other common feature of Mozart's and Bach's music was their more frequent use of

G3 (196 Hz), C5 (523 Hz) and H5 (987 Hz) sounds. During and shortly after listening to the Sonata the firing patterns of the neurons in the left temporal and right frontal cortex were synchronized (Rideout and Laubach, 1996). The effective musical stimuli induced more beta waves in the brain (Sarnthein et al., 1997). Xu et al. (2009) kept newborn rats exposed to the Sonata eight hours a day for two months. According to their results, the ability to recognize sounds and the length of the sounds improved compared to the control group kept in silence. This ability was accompanied by increased expression of the NR2B subunit of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptors promote the maturation of glutamatergic synapses and ensure their plasticity during ontogenesis and learning.

Olton and Samuelson (1976) developed a maze of radial arms to measure the spatial learning and memory of rats. Since then the maze test has become a generally accepted method for studying the spatial memory of animals. According to Crusio and Schwegler (2013), the maze test results are in a strong correlation with the development of the hippocampal mossy fibre projections. In the field of spatial learning, there are two important types of memory, working memory and reference memory. While short-term memory means the storage of information for seconds, minutes, or a few hours, working memory refers to the short-time storage and manipulation of information for processing. In a maze test, working memory is responsible to remember the arms that have already been visited in search for food in the current trial. “Reference memory represents knowledge for aspects of a task that remain constant between trials.” (Nadel and Hardt, 2011). In a maze test, it means the knowledge about which arms of the maze always contain a food reward in each trial. In our recent work (Fekete et al., 2013), open-field (OF) activity and maze test performance were tested in rats after exposure to the Sonata. This particular music in the human hearing range was appropriate for improving hippocampus dependent spatial learning capacity: it resulted in a significant improvement of the reference memory and memory return after the resting period, as well as a 4-h-long working memory retention compared to the group without music exposure. The main critical argument

raised by Steele (2006) is that the hearing range of rodents is different from that of humans. The human audiogram extends from 0.02 up to 20 kHz, while that of rats from 0.5 up to approximately 90 kHz measured at 60–70 dB sound pressure level. He claims that rats cannot hear the lower 33–57% of the notes in this Sonata. Snowdon et al. (2015) emphasized, that music, used for animals, should be species-specific. In the experiments of Fekete and Bernitsa (2013) the ‘rodentized’ version of the Sonata (twice as fast as and one octave higher than the original) decreased kinetic activity and increased the time spent grooming and sitting more efficiently in an open-field trial than the original human version. The aim of the present work was to study the effect of ‘rodentized’ Mozart music as a typical classical piece on the spatial learning of rats, as well as on their memory retention performance in the 8-arm-maze. The present experimental question is the physiological-psychological effect on brain and not the possible subjective joy. Also, we wished to clarify how the timing of musical exposure (i.e. before, during, or before and during the memory test) might influence the results.

2. Material and methods

2.1. Animals, keeping, feeding

After a five-day acclimatization (week 0) forty naïve male 7 weeks old SPF Crl:WI BR clinically healthy Wistar rats (breed by ToxiCoop Ltd., four groups, 10 rats/group) have been used. Three groups were exposed twice during the habituation period (for 80 min, once at 8 a.m. and once at 2 p.m.) daily to a 10-min-long arrangement of the Sonata in the rodentized version (one octave higher and twice faster, looped). For the treatment, the animals have been carried into a different room in individual carrying cages. Sample size was determined by Power Analysis, assuming a significance level of 5%, a power of 90% (Festing and Altman, 2002). The control (C) group (10 rats/group) was not exposed to any music,

they spent the same time in a 3rd room, but in silence. From week 2, one group (10 rats) received the musical stimuli before the memory testing (before music=Group B), one group during the tasks (during music=Group D), and one group before and during the tests (before and during music=Group BD). The animals' spatial learning and memory ability was tested in an 8-arm radial maze (Columbus Instruments, Ohio, USA). During the acclimatization periods, the rats were fed a standard (CRLT/N, Charles River Ltd) rodent diet of 11 MJ ME/kg energy density ad libitum, and they had continuous access to tap water. During the spatial learning and memory ability test, the daily ration was restricted (20–40 g/animal/day), in order to assure a live weight of 80–85% of the standard (Beatty and Rush, 1983). Food restriction is necessary in order to motivate the animals in this type of maze test. The rats were kept in groups of four, in polypropylene boxes (24×36×18 cm, with wire lids, AnimaLab) placed in a conventional animal room. There were 10 boxes, one animal from each group in every box. The rats were individually marked with animal marking spray (RAIDEX). As bedding wood chips have been used (Aspen wood bedding CLASSIC, AimaLab). The light-dark period was set to 12:12 h (dark phase from 6 a.m. to 6 p.m.), the applied light intensity was 60 lx, the room temperature 22 ± 3 °C, and the relative humidity 60 ± 5%. The music was of standard CD quality (44.1 kHz 16-bit resolution, 'wav' extension) with an attenuation of −6 dB SPL (sound pressure level). The one octave higher and two times faster version was produced by the NCH WavePad Audio Editing 2012 software; the change of overtones was neglected. During making the higher and faster ("rodentized") version, the fine overtones remained the same.

2.2. Learning and behavioural studies

The testing (and the adaptation) was carried out in other separated rooms, with 40 dB background noise and 12–25 lx light intensity. Before the measurements, a 20-min adaptation time was applied in a resting room; the testing took place between 8:00 a.m. and 02:00 p.m in the dark phase. One box of rats has been carried at once into the resting room first. After the adaptation, one animal was carried into the test room, where the music-exposed group was exposed to the rodentized Sonata (10-min-long) before the test as a special acoustic stimulus of approximately 0.6–14 kHz pitch range. The SPL was 70 dB, using a Tamashi UX CD player and Genius speakers. Group D and BD were exposed to the same music during the test. The order of the animals from each box was the same: Group B, D, BD and C and the order of boxes was also constant during the different days and tests. The resting room and the testing room were far from each other, so animals in the resting room could not hear the music. After the test, the rat was put back into its homecage, so no animals were alone in the resting room, there was always at least three animals in a cage. Animals from group C also spent the same 10 min in the testing room before the maze test, but in silence. In the BD group, there was a pause in the music between the "before" and "during" phase while the animal was taken out from its cage and put into the maze. The rats' spatial learning and memory performance was studied using the maze test developed by Olton and Samuelson (1976) and modified by Vezer et al. (2000). The labyrinth stands in the middle of a dimly lighted room without windows, its position is constant. During the test, some or all the arms is supplied by a bait (sweet popcorn) and rats are expected to remember the place of the bait, or to the place, where they found and ate it. Rats orientate and memorize the situation of arms and the fact of a visit in it using the breaking points of the maze, by the sight of the objects around the maze and by other extramaze cues, but smell. The bait cannot be seen from the centre. Odour-controlled orientation was distracted by cleaning the maze with disinfectant after every run.

Week 1: In the 1 st week of treatment (Habituation), all animals had a 10-min training twice a day, adapting them to find food pellets in the maze arm ends. Perfect performance of this task required entering each arm only once. Week 2 (Acquisition of the task): During the 2nd week, the rats were first individually trained to learn the general cues of the task, that is, entering each one of the eight arms

only once in a given session, with no more than one error per session, which was done with one training per day in six consecutive days. Acquisition errors consisted of revisiting an arm previously visited in the same session. This way, the rats were forced to learn a win-shift food search strategy. The percent rate of correct responses was counted as: (correct responses – acquisition errors)×100, and was taken as performance indicator. All animals were expected to perform over 75%. Successful solution of the maze task required the rats to recall the arms already visited, mainly when the performance is interrupted (Beatty and Shavalia, 1982). On week 1 and 2, the animals received the music treatment in the testing room once daily, just before (and/or during) the training. Week 3 and 5 (Short-term working memory test): The rats (after the music treatment) were one by one put for ten min maximum in the centre of the maze, but were allowed to enter only 4 of the 8 open and baited arms (at their own random selection); this was the "event-to-be-remembered". After visiting the four arms, the animal was returned to its cage in the resting room and kept there for 2 (on week 3) or 4 h (on week 5). The rats (after a second music treatment) were then put again in the maze centre and allowed to complete arm choices 5–8 to obtain rewards in the 4 baited arms not visited before. In the working memory (WM) tests, WM errors meant re-entry into any of the arms visited in the first run. WM performance was thus counted as (correct responses – WM errors)×100. Week 4: Here, food reward was (Reference memory test) put only in the 4 arms previously preferred by the individual rats. Entering an unbaited (empty) arm constituted a reference memory error, from which performance was calculated as above. The music treatment was the same as before. Week 6 and 7 (Resting Period): the animals were kept in the housing room, were exposed to the music once a day (at 8 a.m.), but did not have any testing and were not exposed to new information. The long-term memory was tested in the 8 to 10th experimental weeks. In the 8th week (Return), memory return was observed in the same way as in week 2 (acquisition), and in the 9th and 10th treatment weeks, 2- and 4-h working memory (WM R) was studied in the same way as week 3 and 5.

2.3. Pathological and histopathological examinations. statistical analysis. Ethical issues

At the end of the trial all the rats (n=40) were euthanized by an intraperitoneal overdose of pentobarbital sodium (Euthasol® 40% Inj A.U.V., Virbac), and necropsied. The weights of the heart, liver, spleen, kidneys, suprarenal glands, thymus and testes were recorded. Samples for histopathological examination were fixed in 10% formaldehyde solution. The segments were frozen, stained with haematoxylin-eosin, periodic acid–Schiff (PAS) and Red-Oil (Fat Red), and embedded in paraffin. Sections were examined in a Nikon Microphot-5A microscope (Japan). During the study the OECD Directives of Good Laboratory Practice for Testing Chemicals (OECD, 1997) were followed. Validation of the histopathological examinations was assured according to Vos and Van Loveren (1996). Before the euthanasia blood samples have been collected by tail vein puncture under diethyl ether anaesthesia. Blood was collected using sterile Bekton-Dickinson Microtainer tubes. For the blood film K-EDTA was added into the tubes and Pappenheim stain was used. Samples have been stored in room temperature, and was processed in 8 h. The count of basophils, eosinophils, neutrophils, lymphocytes and monocytes have been measured from the film. The normal distribution of raw data was evaluated using the Kolmogorov-Smirnov test. Comparative statistical analysis of the memory tests was carried out by one-way ANOVA followed by post hoc Tukey HSD test on the basis of the theory described by Petrie and Watson (2013). The statistical software "R" has been used. Differences between the groups were considered significant in all cases at the level of $p < 0.05$.

The experiment was approved on the basis of SZTE I-74-04/2009, by the Ethical Committee of the Faculty of Veterinary Science Budapest (22.1/2877/3/2011).

3. Results

Rats exposed to the rodentized Mozart's music both before and during the test (Group BD) showed a significant difference in hippocampal-dependent spatial maze-learning capacity as compared to the group exposed to music only before the test (Group B) in the task acquisition period (Week 2: BD:83.92 ± 5.89 vs. B:70.87 ± 7.04%), in the short-term 2-h-interval working memory test (Week 3: BD:59.24 ± 3.97 vs. B:50.66 ± 2.46%), and in the reference memory (RM) test (Week 4: BD:56.31 ± 3.74 vs. B:48.09 ± 2.40%), and also compared to the controls (C) in the short-term 2-h WM (Week 3: BD:59.24 ± 3.97 vs. C:52.82 ± 3.59%) and in reference memory (Week 4: BD:56.31 ± 3.74 vs. C:50.26 ± 2.21%). The performance of the groups during the 4-h working memory test did not differ significantly.

During the long-term test period, no significant differences were found between any of the groups in spatial memory performance (Table 1). The pathological and histopathological examinations did not reveal alterations associated with the musical treatments or stress conditions in any organ except the adrenal glands. The relative weight of the adrenal glands (organ weight/body weight×100) in Groups B, D and BD was higher than in the control animals (One-way ANOVA, F3,39=3.93, P=0.016, C:0.026 ± 0.005%, B:0.035 ± 0.009%, D:0.028 ± 0.004%, BD:0.028 ± 0.006%), but only the B group differed significantly (Post-hoc C-B: p=0.0174) although they were free of histopathological changes. In the blood, the number of lymphocytes was slightly increased in all music-exposed groups, but only in group B significantly (2.02 ± 0.426, 2.35 ± 0.310, 2.11 ± 0.538, and 2.18 ± 0.509 million for the Group C, B, D and BD, one-way ANOVA, F3,39=3.209, p=0.0344, post hoc: C-B: p=0.0347). The number of neutrophils was decreased in the music exposed groups, but only in group B significantly (0.55 ± 0.222, 0.40 ± 0.216 0.40 ± 0.1050.53 ± 0.320 million for Group C, B, D and BD, one-way ANOVA, F3,39=3.341, p=0.0298, post hoc Tukey HSD: C-B: p=0.034). The neutrophil:lymphocyte ratio was elevated in the control group compared to the music treated groups, the difference is only significant between group C and B (One-way ANOVA, F3,39=3142, C: 0.27 ± 0.08; B: 0.18 ± 0.09; D: 0.19 ± 0.05; BD:0.23 ± 0.07; post hoc: C-B: p=0.037,).

Table 1
Spatial learning and memory performance of the animals during the whole course of the experiment (in experimental weeks 1–10).

Weeks		Spatial learning and memory task periods in the 8-arm radial maze		Weekly memory performance, %				Statistics (One-way ANOVA; post hoc: Tukey HSD test df = 3,39
				Control (C) group	Before (B) task group	During (D) task group	Before and during (BD) task group	
				Mean ± SD				
Weeks of the music exposure period	1st	Adaptation		–	–	–	–	
	2nd	Short-term retention tests (n = 10)	Acquisition	78.35 ± 6.02'ab'	70.87 ± 7.04 'a'	77.59 ± 5.14 'ab'	83.92 ± 5.89 'b'	F = 4.664, p = 0.013 post hoc: BD-B: p = 0.007
	3rd		Working memory 2h	52.82 ± 3.59'a'	50.66 ± 2.46'a'	54.56 ± 3.47 'ab'	59.24 ± 3.97 'b'	F = 5.694, p = 0.008 post hoc: BD-C: p = 0.041 BD-B: p = 0.005
	4th		Reference memory	50.26 ± 2.21'a'	48.09 ± 2.40'a'	52.02 ± 3.12'ab	56.31 ± 3.74 'b'	F = 7.079, p = 0.003 post hoc: BD-C: p = 0.023 BD-B: p = 0.002
	5th		Working memory 4h	61.32 ± 2.62'a'	54.81 ± 7.25'a'	54.76 ± 1.83'a'	55.88 ± 9.14'a'	F = 1.338, p = 0.297
	6–7th	Rest period		–	–	–	–	
	8th	Long term memory tests	Return	93.58 ± 2.82'a'	90.15 ± 4.08'a'	91.64 ± 1.94'a'	88.70 ± 2.67'a'	F = 2.457, p = 0.100
	9th	(n = 10)	Working memory return, 2 h	66.79 ± 7.10'a'	61.09 ± 6.06'a'	64.77 ± 5.66'a'	65.81 ± 5.30'a'	F = 0.840, p = 0.492
	10th		Working memory return, 4 h	58.66 ± 5.51'a'	56.50 ± 4.28'a'	57.01 ± 5.63'a'	61.40 ± 8.17'a'	F = 0.610, p = 0.619

Performance is given as weekly average of group averages. Mean ± SD, n = number of animals per group. Values marked with different letters are significantly different ($p < 0.05$).

4. Discussion

Musical stimuli may exert short-term effects (modifying the behaviour) and long-term influences (mitigating the clinical appearance of epilepsy, increasing weight gain or milk production) on mammals (Papoutsoglou et al., 2010; Lin et al., 2011; Leanna et al., 2013). The findings of the present study only partly confirm previous reports describing the improvement of temporal-spatial learning and memory performance (Rauscher et al., 1998; Ho et al., 2007). In the present study, the task acquisition, the 2 h working memory and the reference memory parameters of group BD were better than the parameters of group B and the 2 h working memory and the reference memory parameters of group BD were better than parameters of the control. Kanduri et al. (2015) performed genome-wide transcriptional profiling from the blood of human volunteers before and after listening to a Mozart violin concerto.

They found substantial differences in the gene expression of musically experienced and/or talented participants. It may refer to the importance of previous repetitive exposure to music ("training") and may explain the poor performance of the Acquisition period of Group B. Rauscher et al. (1998) exposed rats to Mozart's music, minimalist music or white noise before or during the test. When rats were exposed to it before the task, the Mozart sonata – similarly to the present data of the Group D – proved to be efficient in improving the maze test performance. When administered only during the task – similarly to the present data – the musical stimuli proved to be ineffective. In contrast to the present study, the experiment conducted by Rauscher et al. (1998) lasted longer, from the in utero stage until day 60 of the life. On the basis of our data, not only a long-term music exposure, but also a shorter but daily repeated musical stimulus is able to improve learning and memory performance, especially in short-term tests, if only the musical stimuli are administered not only before but both before and during the test (Group BD). Xing et al. (2016) exposed humans and rats to the Mozart K448 in original and in retrograde version. The original music improved and the retrograde worsened spatial performance. In the rats' hippocampus and auditory cortex, the concentration of BDNF (brain-derived nutritive factor) and its receptor, the tyrosine kinase receptor B (TrkB) increased in the Mozart group, and decreased in the retrograde Mozart group, compared to the control. The pathological and histopathological results obtained in the present study were negative, but the relative weight of the adrenal glands significantly increased in rats exposed to the musical stimuli before the task and slightly, but not significantly, in Group BD. It shows some habituation effect, that in the Group BD the novelty of the stimuli decreased. That stimulus may have elevated the glucose and cortisol concentrations of the blood and consequently, impairing the working memory 2 and 4 h in Group B. Histopathological examination of the adrenal glands did not show notable alterations associated with the musical exposure or the mild stress state, and the differences observed in learning and memory performance between the groups must have been of functional character, i.e. without histopathological impairments. This phenomenon of improved memory performance can be regarded as eustress or a higher arousal level, too (Kupriyanov and Zhdanov, 2014). By definition, eustress is a type of stress that increases the adaptive capacity of an animal. In the blood, the percentage of lymphocytes was slightly increased while that of neutrophil granulocytes was decreased, showing that the degree of stress was low and not pathological. Although the standard deviations were high, and the differences are not significant, the mean lymphocyte counts were higher in all three music-exposed groups, than in the control (2.02 ± 0.426, 2.35 ± 0.310, 2.11 ± 0.538, and 2.18 ± 0.509 million for the control, Group B, D and BD, respectively). The neutrophyl to lymphocyte ratio was the highest in the Control group, showing that no distress occurred in the music-treated groups (C: 0.27 ± 0.08; B: 0.18 ± 0.09; D: 0.19 ± 0.05; BD:0.23 ± 0.07; post hoc: C-B: $p=0.037$, $F_{3,36}=3142$) (Swan and Hickman, 2014). This may indicate a slight immunostimulated state. Verrusio et al. (2015) have found an increase in the alpha band and median frequency index of background alpha rhythm activity (arousal indicator). Good memory performers show significantly more upper alpha but less theta power. No similar event could be demonstrated in humans having listened to Beethoven's 'Für Elise'. Using a functional magnetic resonance device Bodner et al. (2001) observed during listening to Mozart a statistically significant, dramatic rise in cortical blood circulation, in particular in the dorsolateral prefrontal and occipital cortex, as well as in the cerebellum. These regions play an important role in the perception of space and time. Beethoven's 'Für Elise' or the jazz piano music of the 1930s were ineffective in this respect. Our hypothesis is that the responding animals' neurons show synchronized firings while listening to Mozart's or some similar music, and these firings continue for 10–15 min during and after the exposure. During and after listening to the music, the firing of the neurons in the right frontal and left temporal lobes of the brain get synchronized and the effective musical stimuli stimulate more beta waves (Rideout and Laubach, 1996; Sarnthein et al., 1997). By means of fNMR the hypothesis can be tested. The periodicity (basic frequency) is regarded as an important component of the Mozart effect

(Bizley et al., 2010; Hughes and Fino, 2000). Akiyama and Sutoo (2011) hypothised that music (like Mozart-K 205) containing high-pitch sounds stimulates dopamine production in the brain. The 4 h working memory tests were not affected by the musical stimuli, showing the known, 10–15-minlong, transient effect of the music listening on the spatial-temporal tests. The long-term memory did not differ between groups but interestingly enough, all the data of the long-term period were better than those of the short-term-phase. This later phenomenon may reflect the influence of continuous training and social contact with human. Anatomically, the hippocampus-dependent memory is organized into two major circles of memory, one of which has the hippocampus while the other the amygdala as its centre. The hippocampus is interconnected with the associative cortex fields of the frontal, temporal and parietal lobes, and it stores certain memory traces until the interconnections inside the brain cortex are established (Cowan, 2008). The hippocampus also helps in navigation and orientation in space. The amygdala and the stria terminalis with their nuclei store the emotional component of the memory. While the thalamus plays an important role in declarative memory, in the learning of motions the cerebellum is a major contributor. The question arises how the task acquisition, the 2 h working memory and the reference memory improved under the influence of musical stimuli. According to Tasset et al. (2012), exposure of rats to the Sonata for 2×2 h daily increased the activity of dopamine system. These data may explain the positive enforcement and association of music with getting the bait. Kirste et al. (2015) transposed the Sonata into the hearing range of C57BL/6J mice, elevating pitches by 5 octaves and thus putting 90% of the tones between 5 and 20 kHz. A 2-h exposure during three consecutive days resulted in the proliferation of precursor neurons in the hippocampus and the dentate gyrus, both being crucial in learning and memory performance. Total silence had a similar effect. In contrast, standard animal house noise, unstructured white noise (8–80 kHz) and the pups' voices (65 kHz) were inefficient. Escribano et al. (2014) found the exposure to the given Mozart music had an anxiolytic effect for rats. In the present study, the music exposure must have activated simultaneously, in a synchronized manner the otherwise consecutively joining regions (the anterior ventral and caudodorsal temporal lobe and the ventrolateral prefrontal cortex) (Rideout and Laubach, 1996; Sarnthein et al., 1997) and according to Crusio and Schwegler (2013), the maze test results are in a strong correlation with the development of the hippocampal mossy fibre projections. As a result, the alert rats executed their task in a more concentrated manner and visited the baited arms. At the end of the arm, eating up the treats, they actually performed self-rewarding which, in turn, could be fixed by the amygdala, facilitating future recall. The working memory is impaired by a powerful acute or chronic stress. This is due to catecholamine release in the prefrontal cortex which, in turn, decreases the firing in the surrounding neurons and impairs the performance of the working memory by modifying the intracellular signalling pathways (Arnsten, 2009). The final histopathological examinations performed in the present experiment show that the rats were not exposed to severe stress, because histologically the adrenal glands were normal. At the same time, the increased relative weight of the adrenal glands in the music-exposed groups indicates a certain level of stress, with a consequent elevation in blood corticosterone and glucose concentrations (Vezer et al., 2015). This later may explain the poor memory performance of Group B. The level of this stress is not pathological as most of the test results did not change or even improved. This phenomenon can be explained by a slight alertness and arousal (Steele, 2000). This state may result in a less distractibility and more focused concentration on the task and the mild stress may support the consolidating functions of the hippocampus and amygdala in Group BD (Cahill and McGaugh, 1996).

5. Conclusion

To sum up, in the present study the rodentized Mozart piece improved the learning and memory performance of rats if the animals were exposed to it before plus during the task. The results of Babb and Crystal (2006); Crystal et al. (2013) as well as those of Wright (2013) demonstrate the existence of

source and episodic memory in rats. This fact is promising as it allows the development of a rat model for studying human memory disorders. The rodentized version of the classical music, like Mozart, can be recommended both as background music and for improving the learning and memory performance, provided that the exposure is long enough.

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Table 1
Spatial learning and memory performance of the animals during the whole course of the experiment (in experimental weeks 1–10).

Weeks		Spatial learning and memory task periods in the 8-arm radial maze		Weekly memory performance, %				Statistics (One-way ANOVA; post hoc: Tukey HSD test df = 3,39
				Control (C) group	Before (B) task group	During (D) task group	Before and during (BD) task group	
				Mean ± SD				
Weeks of the music exposure period	1st	Adaptation		–	–	–	–	
	2nd	Short-term retention tests (n = 10)	Acquisition	78.35 ± 6.02'ab'	70.87 ± 7.04 'a'	77.59 ± 5.14 'ab'	83.92 ± 5.89 'b'	F = 4.664, p = 0.013 post hoc: BD-B: p = 0.007
	3rd		Working memory 2 h	52.82 ± 3.59'a'	50.66 ± 2.46'a'	54.56 ± 3.47 'ab'	59.24 ± 3.97 'b'	F = 5.694, p = 0.008 post hoc: BD-C: p = 0.041 BD-B: p = 0.005
	4th		Reference memory	50.26 ± 2.21'a'	48.09 ± 2.40'a'	52.02 ± 3.12'ab	56.31 ± 3.74 'b'	F = 7.079, p = 0.003 post hoc: BD-C: p = 0.023 BD-B: p = 0.002
	5th		Working memory 4 h	61.32 ± 2.62'a'	54.81 ± 7.25'a'	54.76 ± 1.83'a'	55.88 ± 9.14'a'	F = 1.338, p = 0.297
	6–7th	Rest period		–	–	–	–	
	8th	Long term memory tests	Return	93.58 ± 2.82'a'	90.15 ± 4.08'a'	91.64 ± 1.94'a'	88.70 ± 2.67'a'	F = 2.457, p = 0.100
	9th	(n = 10)	Working memory return, 2 h	66.79 ± 7.10'a'	61.09 ± 6.06'a'	64.77 ± 5.66'a'	65.81 ± 5.30'a'	F = 0.840, p = 0.492
	10th		Working memory return, 4 h	58.66 ± 5.51'a'	56.50 ± 4.28'a'	57.01 ± 5.63'a'	61.40 ± 8.17'a'	F = 0.610, p = 0.619

Performance is given as weekly average of group averages. Mean ± SD, n = number of animals per group. Values marked with different letters are significantly different ($p < 0.05$).