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# Rock influences spatial memory in adult rats, while classical music do not

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Music can be used as a therapeutic tool and has several effects in cognitive and physiological functions. The aim of this work was to evaluate the effect of different musical pieces in spatial memory in adult male rats. A T maze was used to investigate spatial memory, with 2h and 6h inter trial interval between training and testing phases. Before training, animals were exposed to an active or relaxing musical stimulus corresponding to rock and classical pieces. It was found that in the experiment with 2h interval, animals explore more the novel arm in comparison with the other arm, which indicated that animals still had a good spatial memory. This was not observed in rats that were exposed to the relaxing rock piece, which could indicate that this stimulus diminished memory. With the 6h interval the rats explored both arms equally, except animals that were exposed to the activating rock piece of music which indicated a slightly enhance in memory. Thus, there were found different effects of music corresponding to the time of interval and the stimulus characteristics. This data provides information to use music as a possible treatment to modulate memory.

A growing body of literature has started to document the effect of music in several cognitive and physiological functions, both humans and animal models. Music is used as a therapeutic tool in several disorders, such as Parkinson disease (de Dreu, van der Wilk, Poppe, Kwakkel, & van Wegen, 2012), Alzheimer disease (Simmons-Stern, Budson, & Ally, 2010;

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Simmons-Stern et al., 2012), depression (Maratos, Gold, Wang, & Crawford, 2008), schizophrenia (Mössler, Chen, Heldal, & Gold, 2011), autistic spectrum disorder (Gold, Wigram, & Elefant, 2010), aphasia (Norton, Zipse, Marchina, & Schlaug, 2009; van der Meulen, Samdt-Koenderman, & Ribbers, 2012), among others. Also has been demonstrated its effect in regular population, for instance music presented at 20 min, but not immediately or 45 min post-learning, significantly enhanced long-term word-list retention (Judde & Rickard, 2010).

According to the physiological effects that music had on organisms the parameters that help to determine if a musical piece has arousing or relaxing effects were determined (Wigram, Pedersen, & Bonde, 2002). The musical elements that have an activating effect are unpredictable tempo changes, unpredictable or sudden shifts in intensity, rhythm, timbre, harmony, unexpected dissonances, unexpected accents, hard timbres, lack of structure or musical form, *accelerandos*, *ritardandos*, *crescendos* and *diminuendos* unexpected, and surprising cut on the music. On the other hand, the musical elements that induced a relaxing effect are: steady tempo, stability or gradual shifts in volume rhythm, timbre, harmony; consistent texture, predictable harmonic modulation, appropriate cadence, predictable melodic lines, repetition of material, soft timbre, few accents, among others. Activating musical pieces improve visual memory in young (Justel & Rubinstein, 2013) and older adults (Justel, O'Conor & Rubinstein, 2015), while relaxing music can deteriorate this cognitive function (Rickard, Wing Wong, & Velik, 2012).

In animal models, classical music reduces anxiety in an elevated plus maze (Chikahisa et al., 2006; Chikahisa, Sano, Kitaoka, Miyamoto, & Sei, 2007; Escribano et al., 2014), it was an effective method to reduce blood pressure (Akiyama & Sutoo, 2011; Nakamura et al., 2007), also it had a positive short term effect attenuating the spontaneous high-voltage spike discharge in rats (Lin et al., 2013). Furthermore, the exposure to music during perinatal or postnatal period enhanced neurogenesis in the hippocampus (Angelucci, Fiore, et al., 2007, Angelucci, Ricci, Padua, Sabino, & Tonali, 2007; Chikahisa et al., 2006, 2007; Kim et al., 2006; Marzban et al., 2011; Rauscher, Robinson, & Jens, 1998; Xu, Yu, Cai, Zhang, & Sun, 2009); it is effective antagonizing the adverse effect of stress on immune system and cancer development (Nuñez et al., 2002) and music protects memory against callosal lesions (Amagdei, Baltes, Avram, & Miu, 2010). Besides, music increased learning and memory in rodents since music exposure could enhance significantly learning performance of rats in the water maze test (Xing et al., 2016) and music-exposed mice completed a maze learning task with fewer errors than the white noise-exposed mice

(Chikahisa et al., 2006). It is relevant to highlight that music effects had been study in many animal species, such as cows, horses, piglets, chicks, monkeys, among others, (Campo, Gil, & Davila, 2005; de Jonge, Boleij, Dudink, Baars, & Spruijt, 2008; Stachurska, Janczarek, Wilk, & Kedzierski, 2015; Uetake, Humik, & Johnson, 1997; for a review see Rickard, Toukhsati, & Field, 2005).

An experimental way to study spatial memory in rodents is by a T maze. This paradigm is based on the natural tendency of these animals to prefer novel environments compared to known ones (Zhang, He, Chen, Wang, & Ma, 2008). The ability to detect novel spatial contexts is a feature that acquires a great ecological and adaptive importance. Since T maze does not utilize punishment or reward, which are commonly used in memory paradigms, non-specific effects are minimized. Also, does not require the use of a rule, therefore in addition it is useful for studying memory in rats (Zhang et al., 2008). Memory can be tested by evaluating the influence of various intertrial intervals (ITIs) on recognition performance (Dellu, Contarino, Simon, Koob, & Gold, 1997). It was previously found that maze recognition memory in rodents may be retained for up to 2 h post-training, and does not usually last for more than a few hours, such as 4 h. Since relaxing music deteriorates memory, we choose a 2h ITI (in which recognition memory is retained) to test the deleterious effect of music. Also, because is reported that activating pieces enhances memory we choose a 6h ITI (in which recognition memory is no longer retained) to test if music could improve memory.

Considering the great potential that music could offer there is a growing need to develop animal models to study the mechanism through which music exert its effects. To our knowledge there are no researches that study the acute effect of different music styles in memory, therefore that was the aim of this work. Based on previous literature it was hypothesized that rats exposed to the classical music piece will exhibit better memory (Chikahisa et al., 2006; 2007; Xing et al., 2016) in comparison to control animals, based on human studies the rock music will deteriorate memory (Burns et al., 2002).

# METHOD

**Subjects**. One-hundred and sixteen adult male Wistar rats, born and reared at the vivarium of Instituto de Investigaciones Médicas Alfredo Lanari (IDIM-CONICET, Buenos Aires, Argentina) were used. The animals were approximately 150 days olds at the start of the experiment. They had ad-libitum access to food and water. They were weighed daily and the average weight was 373 g (range: 280-480 g). Animals were kept in a daily light-dark cycle of 12 h (lights on at 07:00 h). The housing and testing rooms were maintained at a constant temperature (around 22 °C) and humidity (around 60-70%). They have previous experience in sucrose consumption.

**Apparatus**. Four T mazes were used to measure spatial memory, they were made of black and white acrylic, and they had four parts: the start white alley (36x13.5x17 cm), the black main corridor (39x13.5x17 cm) and two black arms (novel and other) which were perpendicular to the corridor (39x13.5x17 cm). The start alley and main corridor were separated by a guillotine door (Figure 1). The apparatuses were located in the floor of the room and the animals were exposed to ambient noise. A light bulb (100 W) was suspended on top of the apparatuses to provide illumination. The light and the ambient noises remain constant in all the experimental phases.

**Auditory stimuli exposure.** The effect of classical and rock music was tested in the T maze. Musical pieces with high frequencies, marked rhythms and high intensity induced tension, excitation, alert, which in turn generates energy and activity. In addition, low frequencies pieces, with high chronometric density and low intensities induce calm and relaxation (Burns et al., 2002; Grocke & Wigram, 2007). Based on these parameters and previous research the musical pieces were selected (Fancourt, Ockelford, & Belai, 2014; Judde & Rickard, 2010; Justel & Rubinstein, 2013; Knight & Rickard, 2001; Kreutz, Ott, Teichmann, Osawa, & Vaitl, 2007; Rickard et al., 2012). The relaxing rock stimuli was What a difference a day makes (Dinah Washington); the relaxing classical stimuli was the cannon, D major (Pachelbel); the activating rock stimuli was I just can’t get enough (Depeche Mode); the activating classical stimuli was the Symphony No. 70, D major (Haydn).

The sound level was between 50 and 70 dB, the sounds were reproduced by a laptop which was situated at approximately 80cm from the animals (Escribano et al., 2014). The control group was in the same training room as the music and white noise conditions, where there was no sound except ambient noise.

The rats were transported in squad of 4 animals to the room where they were exposed to the assigned auditory stimulus for 5 minutes. Six groups were run in each experiment. The control group (with no music exposure; CTRL), the animals exposed to white noise (WN group), the group exposed to the classical activating piece of music (Clas/Act), animals exposed to the relaxing classical musical piece (Clas/Rel), the group exposed to the rock activating piece of music (Rock/Act) and the rats that were exposed to the rock piece with relaxing properties (Rock/Rel). Immediately after, they were transported to the room where the behavioral procedure took place, where two trials (acquisition and test) were administered. The behavior in the apparatuses was videotaped for later scoring by 2 experimenters who were blind to the conditions of the subjects.

**Figure 1. T maze apparatus. Four T mazes were used to measure spatial memory, they were made of black and white acrylic, and they had four parts: the start white alley (36x13.5x17 cm), the black main corridor (39x13.5x17 cm) and two black arms (novel and other) which were perpendicular to the corridor (39x13.5x17 cm). The start alley and main corridor were separated by a guillotine door.**

**Training phase**. In the first trial (training or acquisition trial) animals were placed in the start alley and the guillotine door was lifted up. Once animals step through the main corridor the guillotine door was lifted down. If rats did not step through after 60 seconds, they were gently push into the main corridor. They could freely explored for 10 minutes the main corridor and one of the arms (other arm), while the remained arm was closed (novel arm). After training trial animals were relocated in their homecages, where they stayed until the test trial. The other and novel arm were counterbalanced to right and left positions so no bias could be present in this regard.

**Test phase.** In Experiment 1 after a 2h interval animals were submitted to the test trial. In the Experiment 2 after a 6h interval rats had the test trial. Animals were placed again in the start alley, the guillotine door was lifted up, they step through the main corridor, the guillotine door was lifted down and they could explored for 5 minutes the entire apparatus, i.e. the main corridor and both arms (the novel and other one). Two video cameras were located in the top of the T mazes to record performance in both trials.

**Data analysis.** The datasets were tested for normality and homogeneity of variance. These assumptions were tested through the Shapiro-Wilk and Levenes´s tests, respectively. The results indicated that the assumptions of homogeneity and normality were violated. Therefore, Kruskal Wallis (K-W) and Mann-Whitney U test were used to analyze differences between groups, and the comparison intragroup were made with Wilcoxon T test, with an alpha value set at the 0.05 level, for a 2-tailed distribution. SPSS software package was used to compute all statistics.

During the acquisition trial, duration and total number of visits of the corridor and other arm of the T maze were taken as an index of locomotor activity (Contarino et al., 1999). During test trial two dependent measures were recorded: entries in novel and other arms and duration of time in each arm. Spatial recognition memory can be measured by the absolute and percentage duration spent in the novel arm. Since analyses of absolute time spent in each arm was similar to that observed with percentage duration, the absolute time is not shown for simplicity. Thus, all data are expressed as percentages of total time spent in each arm during the 5 min retention period (Zhang et al., 2008). The videos were analyzed by J-Watcher-Video V1.O software.

# RESULTS

**Experiment 1. Music effect on T maze after a 2h inter trial interval**

In the first trial (training or acquisition trial) there were no differences in percentage of time or entries to main corridor or the other arm between groups (Table 1; $p > 0.05$).

**Table 1. Percentage of time and entries to central area and the other arm.**

| Exp | Groups | Percentage in other arm | Percentage in main corridor | Entries to other arm | Entries to main corridor |
|---|---|---|---|---|---|
| 2h | Ctrl | 24.22+-2.23 | 75.73+-2.23 | 9.43+-0.25 | 14.14 +-0.57 |
| | WN | 31.87+- 1.27 | 68.01+-1.25 | 12.28+-0.61 | 17+-0.39 |
| | Clas/Act | 22.73+-2.78 | 77.25+-2.78 | 7+-0.66 | 13.6+-0.29 |
| | Clas/Rel | 27.91+-2.57 | 72.07+-2.56 | 9.67+-1.18 | 15.11+-0.45 |
| | Rock/Act | 30.73+-2.27 | 69.22+-2.27 | 12+-0.89 | 15.57+-0.33 |
| | Rock/Rel | 22.79+-1.97 | 77.14+-1.96 | 9.43+-0.86 | 14.43+-0.47 |
| 6h | Ctrl | 24.31+-1.56 | 75.66+-0.37 | 17.00+-0.18 | 9.50+-1.56 |
| | WN | 37.09+-1.48 | 62.77+-0.53 | 12.75+-0.80 | 11.50+-1.51 |
| | Clas/Act | 32.84+-7.87 | 67.04+-1.09 | 11.50+-1.08 | 7.83+-7.9 |
| | Clas/Rel | 27.73+-4.24 | 72.23+-0.76 | 14.00+-0.57 | 8.25+-4.24 |
| | Rock/Act | 18.60+-3.35 | 81.32+-0.38 | 12.80+-1.07 | 6.60+-3.34 |
| | Rock/Rel | 31.92+-4.13 | 67.86+-0.62 | 14.88+-0.76 | 9.13+-4.18 |

Data are expressed as mean ± S.E.M. CTRL: Group not exposed to any stimuli (n=7). WN: Group exposed to white noise (n=7). Clas/Act: Group exposed to Haydn (Symphony No. 70, D major, n=6). Clas/Rel: Group exposed to Pachelbel (canon D major, n=9). Rock/Act: Animals exposed to Depeche Mode (I just can't get enough, n=7). Rock/Rel: Rats exposed to Dinah Washington (What a difference a day makes, n=7).

In the T maze test after a 2h interval between training and test trials, it is expected that control animals explore more the novel arm in comparison with the other (known) arm (Zhang et al., 2008). Besides there are authors that indicated that white noise conditions behave similar than control or silence conditions (Chikahisa et al., 2006, 2007) but other authors indicated that white noise deteriorates learning (Kim et al., 2006). It was expected that activating pieces enhance learning and memory and it was expected that relaxing pieces deteriorate it (Chikahisa et al., 2006; Escribano et al., 2014; Rickard et al., 2012).

As observed in Fig. 2A, rats explore more the novel arm in comparison with the other arm, and this effect was observed in all groups except for animals that were exposed to the relaxing rock piece of music. These observations were corroborated by the analysis.

Related to the variable "*percentage of arm duration*" (Fig. 2A) the Kruskal Wallis analyses indicated no differences between groups ($p$>0.05). Subsequent Mann-Whitney´s U tests indicated that Rock/Act group explored more the other arm in comparison to Clas/Act group [$U$(7,6)=7, $p$<0.047]. No other between-groups differences achieve significance ($p$>0.05). Wilcoxon T test indicated that in each of the groups there were significant differences between exploration of novel and other arm [CTRL: Z=-2.36, $p$<0.019. WN: Z=-2.36, $p$<0.02. Clas/Act: Z=-2.04, $p$<0.042. Clas/Rel: Z=-2.1, $p$<0.036. Rock/Act: Z=-2.19, $p$<0.029] except for the Rock/Rel group (Z=-0.845, $p$=0.398) who explored similarly both arms.

In the variable "*number of arm visits*" (Fig. 2B) the Kruskal Wallis analyses indicated no differences between groups ($p$>0.05). The subsequent Mann-Whitney´s U tests indicated that the Rock/Act group enter more to the novel arm than Clas/Act [$U$(7,6)=5.5, $p$<0.022] and Rock/Rel groups [$U$(7,7)=8.5, $p$<0.039]. Also, the WN group had more entries to the novel arm than Clas/Act group [$U$(7,6)=6.5, $p$<0.033]. Related to the other arm the WN group made more entries than CTRL [$U$(7,9)=9, $p$<0.038] and Clas/Act groups [$U$(7,6)=2.5, $p$<0.007]. No other between-groups differences achieve significance ($p$>0.05).

Wilcoxon T test indicated that there were significant differences between the access to novel and other arm in the following groups: CTRL ($Z$= -2.21, $p$<0.027), Clas/Act ($Z$= -2.041, $p$<0.042), Clas/Rel ($Z$= -2.1, $p$<0.036) and Rock/Act ($Z$= -2.05, $p$<0.04). In the WN and Rock/Rel groups there were no significant differences between entries to both arms ($p$>0.05).

Inter observer reliability was substantial and significant in every of the measures ($r$> 0.98, $p$ < 0.0001). These results indicated that the white-noise and silence (control) conditions are not similar, besides animals exposed to the relaxing piece of music had a decrement in their spatial memory but only the rock not the classical piece, which indicated that arousal is not the only variable implicated but also the valence or interaction between arousal and valence is responsible for the results.

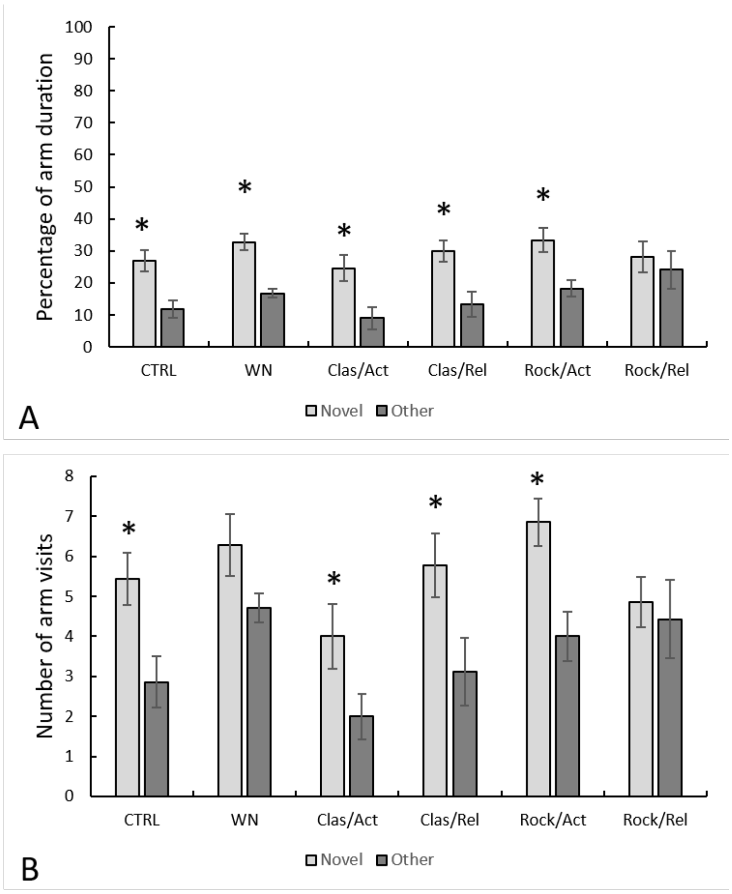



**Figure 2. Effect of classical and rock music on consolidation of spatial memory in the T maze after a 2h inter trial interval. A. Percentage of arm duration. B. Entries to the arms of the maze. Data are expressed as mean ± S.E.M. CTRL: Group not exposed to any stimuli (n=7). WN: Group exposed to white noise (n=7). Clas/Act: Group exposed to Haydn (Symphony No. 70, D major, n=6). Clas/Rel: Group exposed to Pachelbel (canon D major, n=9). Rock/Act: Animals exposed to Depeche Mode (I just can't get enough, n=7). Rock/Rel: Rats exposed to Dinah Washington (What a difference a day makes, n=7) *Significant differences between novel and other arm.**

**Experiment 2. Music effect on T maze after a 6h inter trial interval**

In the first trial (training or acquisition trial) there were no differences in percentage of time or entries to the main corridor or the arm between groups (Table 1; $p>0.05$).

After a 6 hour interval between training and test trials there is evidence that points out that animals explored both arms as new ones because there is no spatial recognition of the novel versus known arm (Dellu, Mayo, Cherkaoui, Le Moal, & Simon, 1992; Zhang et al., 2008). The results of the 2nd experiment indicated that our results are in accordance with this, with this window time between training and testing the rats explored both arms equally.

In the "*percentage of arm duration*" dependent measure (Fig. 3A) the Kruskal Wallis analyses indicated no differences between groups ($p>0.05$). The Mann-Whitney test indicated that the only significant difference was between CTRL and Rock/Act groups, the former explore more the other arm than the last group [$U(14,13)=48$, $p<0.038$]. No other statistical analysis achieve significance in any of the measures ($p>0.05$).

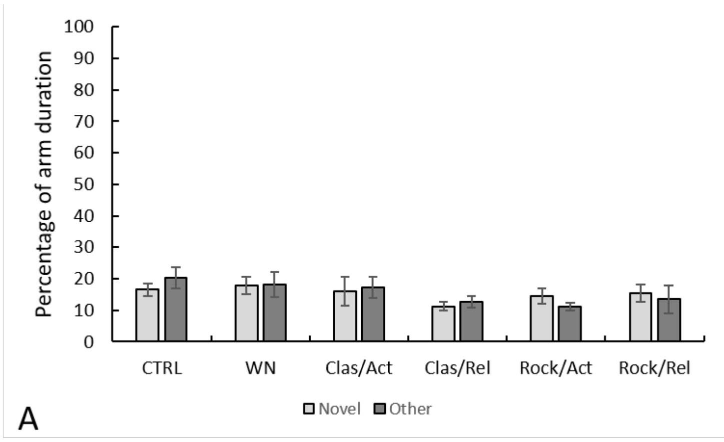
In the other dependent variable "*number of arm visits*" (Fig. 3B) the Kruskal Wallis and Mann-Whitney analyses indicated no differences between groups ($p>0.05$). The Wilcoxon test indicated that all groups enter to both arms equally except the Rock/Act group, which enter more to the novel arm than to the other one ($Z= -2.40$, $p<0.017$).

Inter observer reliability was substantial and significant ($r> 0.99$, $p < 0.0001$). These results indicated that there was a slightly enhance in memory in animals that were exposed to the rock activating piece of music.

## DISCUSSION

Mechanisms involved in music perception generated a great amount of questions about cognitive neuroscience since music makes some unique demands on the nervous system. Understanding this phenomenon could reveal specific aspects of the neuronal function (Zatorre, Chen, & Penhune, 2007). For that reason in the last year's music has been used as a tool in cognition research.

There are several researches that indicate that an elevated arousal modulates memory (McGaugh & Roozendaal, 2002; 2009). It is well documented that music elevated arousal, for instance music affects heart rate, blood pressure among others parameters (Akiyama & Sutoo, 2011;



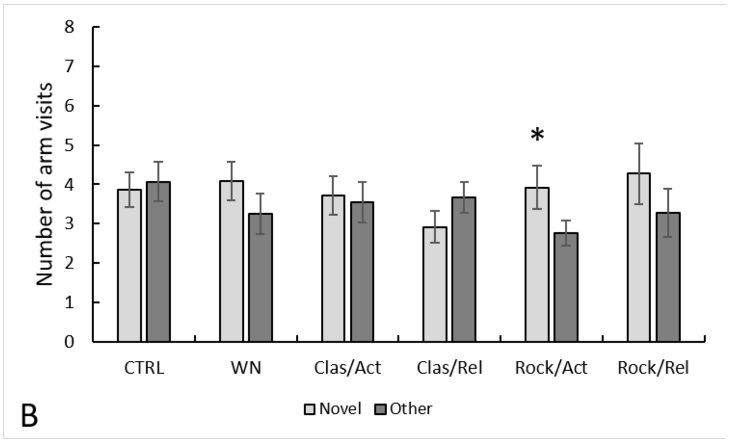




**Figure 3. Effect of classical and rock music on consolidation of spatial memory in the T maze after a 6h inter trial interval. A. Percentage of arm duration. B. Entries to the arms of the maze. Data are expressed as mean ± S.E.M. CTRL: Group not exposed to any stimuli (n=14). WN: Group exposed to white noise (n=12). Clas/Act: Group exposed to Haydn (Symphony No. 70, D major, n=11). Clas/Rel: Group exposed to Pachelbel (canon D major, n=12). Rock/Act: Animals exposed to Depeche Mode (I just can't get enough, n=13). Rock/Rel: Rats exposed to Dinah Washington (What a difference a day makes, n=11) *Significant differences between novel and other arm.**

Nakamura et al., 2007). According to this background the aim of this study was to evaluate the effect of music that modulates arousal on memory in adult male rats. For that reason the animals were tested in a T maze to study spatial memory. Since the vast majority of the papers investigated the effect of classical pieces we test this style but also rock music to add a new variable of research. Our hypothesis was that the activating pieces will enhance memory while relaxing pieces will deteriorate it. The main results indicated that animals exposed to the rock relaxing musical piece had a decrement in their memory, also the results indicated that the activating rock musical piece slightly enhanced memory.

These results support only in part our hypothesis, because if arousal was the only variable involved, then in each of the experiments the relaxing or activating pieces would have had the same effect. But this was not the case. In the 1st experiment the relaxing rock piece of music deteriorated memory, not the classical one. In the 2nd experiment only the activating rock piece of music enhanced memory not the classical piece. Apparently the interaction between the variables is the responsible for the results obtained. Therefore is necessary to unravel the elements that are effective in each of the pieces.

A possible explanation is that music acts as a proactive interference (Justel, Pautassi, & Mustaca, 2014; Justel, Psyrdellis, Pautassi, & Mustaca, 2014), this phenomenon occurs when previously acquired information (music) modifies the storage or retrieval of new information (spatial learning).

In the second experiment the rock activating piece of music only showed an effect in one of the two dependent variables under study. Our work employed a 5 minutes exposition to musical pieces, while in other researches the music exposition has a longer duration, for hours or weeks (Chikahisa et al., 2006, 2007; Kim et al., 2006; Nakamura et al., 2007). Is possible that if same stimuli are employed but with longer duration a different result would appear. Future research could address this issue. Also, another interesting topic for future research is to study the specific contribution of the different musical elements such as pitch, rhythm, tempo, or timbre.

Because music was provided before training it could not be dissociated if the experimental manipulation was affecting the acquisition and/or consolidation of the spatial learning (Justel & Psyrdellis, 2014). In future experiments would be interesting to discriminate which memory phase is the affected one, to achieve this purpose is necessary to perform the treatment after the information is acquired.

It is worth noting that white noise and silence conditions were not completely comparable in experiment 1. A possible explanation is that white noise could have anxiogenic properties (Chikahisa et al., 2007; Escribano et al., 2014; Naqvi, Haider, Batool, Perveen, & Haleem, 2012).

The findings of this work provide evidence about the effects of music without cultural bias because animal's models are not constrained to these issues. It can be considered that humans acquire culture-specific knowledge about music because they are exposed in everyday experiences, such as listening to the radio, singing and dancing. This is known as a musical enculturation process (Hannon & Trainor, 2007). Just as there are different languages and cultural styles with particular scales, categories and grammatical rules governing pitch and rhythmic structures, differential effects of music can be expected according to cultural affiliation of subjects. Thereby, the data in this article can be understanding as a universal effect of music and cannot be attributed to any previous experience with the stimulus, which can be a common critic if a human model was used (Koelsch, 2014).

To sum up, the present study indicated that relaxing music deteriorated memory and activating music enhanced it. This would be useful to modulate cognitive functions and to be used in several disorders where the memory need to be improved it (as Alzheimer disease) or diminished (like in Post-Traumatic Stress Disorder). The use of this simple and non-invasive technique that modulates memory could become a practical tool in different memories issues, and it is need more basic research to underpin this result.

## RESUMEN

**El rock afecta la memoria espacial en ratas adultas, mientras que la música clásica no.** La música puede ser usada como una herramienta terapéutica y ha demostrado tener múltiples efectos en las funciones cognitivas y fisiológicas. El objetivo de este trabajo fue evaluar el efecto de diferentes piezas musicales en la memoria espacial de ratas adultas macho. Se utilizó un laberinto en forma de T para evaluar la memoria espacial, con 2 y 6 horas de intervalo entre ensayos entre el entrenamiento y fase de testeo. Antes del entrenamiento los animales fueron expuestos a estímulos sonoros activantes o relajantes correspondientes a piezas de rock y música clásica. Se encontró que en el experimento con 2 horas de intervalo los animales exploraron más el brazo novedoso en comparación con el ya conocido, lo cual indica que los sujetos tenían un buen índice de memoria espacial. Esto no se observó en las ratas que fueron expuestas a la pieza de rock relajante, lo que indicaría que este estímulo disminuyó la memoria. Con el intervalo de 6 horas los animales exploraron ambos brazos por igual, excepto los que fueron expuestos a la pieza activante de rock, lo cual indicaría una mejora en la memoria. Con lo cual, se hallaron diferentes efectos de la música en relación al intervalo de tiempo aplicado entre entrenamiento y test, así como al tipo de estímulo empleado. Estos datos

proveen información acerca del uso de la música como un posible tratamiento de modulación de la memoria.

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