Key signature differences in music and their effects on cortisol and behavior in Rattus norvegicus

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Abstract

Sound is a powerful stimulus that affects organisms in a variety of ways. Music, for example, can change how animals act in certain situations. It can be used in farming to increase animal production or it can be used in a doctor's office to create a more calming atmosphere. The type of music present can elicit more or less active behavior; e.g. a person dancing to a fast-paced song, or falling asleep to a ballad. The path sound takes through the brain directly meets the body's stress response system in two places: the amygdala and the hippocampus. Thus, it is likely that cortisol (stress hormone) levels in the body change when music is perceived. This study attempted to discover if different types of music (major, minor, and atonal violin sonatas, and a control of white noise) could change the hypothalamic-pituitary-adrenal axis response of music to the stress of being forced to hang from a pull-up bar. Blood serum was analyzed with an ELISA for cortisol. The cortisol concentrations were above those of the standards; therefore cortisol levels in all samples were unexpectedly high. Also in this study, animal activity was analyzed in the presence of music. The behaviors of groups of rats differed in pre-treatment assessments; differences in normalized data were insignificant. Rats exposed to atonal music did not bleed from tail cuts; this observation was attributed to a tail shunt, which occurs in stressed animals.

Introduction

Sound is one of the most powerful stimuli on the planet. It can signal to animals that predators are near, or it can be an avenue for communication. What makes some sounds affect organisms more than others? Are there sounds that are

not consciously monitored, but affect body function or response? Music can be one of these sounds. There are numerous examples of music changing behavior and hormones in the body (Papoutsoglou et al., 2007).

Music's Effects on Animals

Carp (*Cyprinus carpio*) are strongly affected by the presence of music waves in their water. These fish have slower growth rates and higher cortisol levels with music present (Papoutsoglou et al., 2007). In contrast, broiler chicks grow more rapidly in the presence of music (Gvaryahu et al., 1989). These differences are likely due to the fact that sounds traveling through water are normally from an impending threat, like a predator or a boat propeller, whereas chickens experience more neutral and communicative sounds (Papoutsoglou et al., 2007). Domesticated mammals are affected by music as well. Cows, for example, are more willing to go to the milking station on dairy farms if music is played (Uetake et al., 1997).

Humans have lower perceptions of pain when music is played during a minor operation (Zengin et al. 2013), and music positively affects people with debilitating neurological diseases (Takahashi & Matsushita, 2006). Sounds that induce fear create a smaller stimulus in the brain than joyful sounds in humans. Fear stimulates a specific area of the brain, but joyful sounds have a radiating effect and enhance activity in the brain (Koelsch et al., 2013).

Music affects humans through (at least in part) effects on cortisol. This hormone can be lowered in stressful situations in the presence of music (Miluk-Kolasa et al., 1994). The amount of cortisol in the body depends on how the music is

played. When the music is played normally cortisol levels drop, but when the music is played in reverse, cortisol levels spike (Jezova et al., 2013).

Cortisol

Cortisol is a glucocorticoid and corticosteroid, which acts as a hormone in the body during stressful situations (Vélez-Marín et al. 2012). At its most basic level, stress is the response to an aversive stimulus, which activates the hypothalamic-pituitary-adrenal axis. Corticotropin-releasing hormone is discharged from the hypothalamus and sent to the anterior pituitary. From there, adrenocorticotropic hormone is released into the blood stream, and then stimulates the adrenal cortex to send cortisol through the body. The main function of cortisol is to increase the amount of sugars in the blood stream, and ultimately provide fuel for skeletal muscles when needed (Engelmann et al., 2004). Animals also have a circadian rhythm of cortisol release. Typically cortisol levels are highest when the animal starts its day, e.g. in the morning for diurnal species and in the afternoon for nocturnal animals, then gradually decrease (Vélez-Marín et al. 2012).

Music in The Brain

The connection between music and stress can be found by tracing the path of sound through the brain. When sound enters the brain, the auditory cortex processes it in both of the temporal lobes. Then, signals move medially to the amygdala. Finally, the signals pass through the amygdala to the hippocampus in the medial temporal lobe of the brain (Koelsch et al., 2013). The hippocampus provides the negative feedback for the hypothalamic-pituitary-adrenal axis during a stress response. The hippocampus has abundant glucocorticoid receptors. The binding of

cortisol to these receptors ultimately lowers corticotropin-releasing hormone production by the hypothalamus. The amygdala stimulates the hypothalamic-pituitary-adrenal axis when a stressor is perceived (Moser et al., 2007). Thus, sound can trigger two different effects on the stress response from the body based on the path that it takes through the brain.

Properties of Music

According to Webster's Dictionary, music is the art of combining sounds to produce beauty of form, harmony, and expression of emotion. There is great diversity within music stemming from the formation of scales. A scale is a combination of seven tones within an octave that flow together. This 'flow' comes from the wavelengths of the sounds produced. They line up well mathematically, and the sound waves blend together. The specific pattern of whole and half steps within a scale makes it unique. Of the seven scale types, two are most commonly used: major and minor. A major scale has a more warm and happy tone, whereas minor scales are more gloomy or sad. These scales are written into key signatures of music to keep the musician in the correct key (Wood, 1950).

There is another way to write music, and that is without the use of a scale. An atonal piece does not follow any scale. This creates an 'uneasy' tone because the sound waves cannot blend together. This phenomenon is called dissonance. Two dissonant pitches compete for dominance rather than blending together. This tension between the tones produces a sound that is bothersome to humans. Atonal music is rarely written because it is not pleasant to hear (Wood, 1950).

Hypotheses

This study examined whether differences in types of music have varying effects on mammals. *Rattus norvegicus* was used as the model system because it is a widely accepted mammalian model system. They cannot detect the lowest frequencies that humans can hear. Their hearing threshold is about 200 Hz and climbs to 75,000Hz compared to the human range of 20Hz to 20,000Hz (Fey, 1988). This study focused on two main questions:

(1) Do different types of music have varying effects on cortisol levels in the body? It was hypothesized that major and minor pieces would reduce cortisol levels compared to a control of white noise, and a dissonant piece, which would increase cortisol levels.

(2) Do different types of music elicit different types of behaviors? Music can increase human activity levels via dancing or foot tapping; it was a curious question if an animal's activity would change too. It was hypothesized that there would be more activity with all types of music compared to white noise before the stress.

Methods

Animal Collection and Rearing

R. norvegicus was taken from a lab colony, n=16 all male, at Allegheny College. They were held at 23°C with an 18:6 photoperiod. Animals were fed normally as mandated by the Animal Review Committee. The animals were caged in groups of 4 and the tails were marked in order to identify individuals. Animals were tested at 18 weeks old.

Music

There were 4 groups with 4 animals in each condition. First was a control in which white noise (sound with many frequencies at equal intensities) was played for twenty minutes. Three groups were exposed to music. The first music group heard J.S. Bach's Violin Sonata no. 3 in C major (part 2). The second group listened to J.S. Bach's Violin Sonata no. 2 in A minor (part 2). The last dissonant piece was Bartok's Solo Violin Sonata: Fuga, which is atonal. To eliminate possible other variables, these pieces are all similar in tempo. All sound was played at 65dB (about normal human conversation volume) for twenty minutes.

Behavioral Analysis

R. norvegicus were individually placed into a wooden box roughly 2'x 2' with 1.5' high walls, and given two minutes to acclimate to the new environment. The box was partitioned into a 5x5 grid. The number of times the rat crossed into a different quadrant was counted for twenty minutes before music was turned on as a control assessment of activity. I recorded how many times the rats stood on their hind paws and tried to reach the top of the box; this was defined as an escape attempt. Also, the amount of time the rat stayed in one place longer than two minutes was recorded as another behavioral measurement. The rat was removed from the box for one minute and the box was cleaned of waste. Music was turned on and the rat was reintroduced to the box at the end of the minute. It was allowed one minute to acclimate again, then activity in the presence of music was assessed using the same three parameters as before. A stressor was applied after both activity tests were completed. During the stressor, behavior was analyzed by timing the interval the animal is able to hang on a horizontal bar. The longest duration was recorded.

Stressor

The stressor was applied immediately following the twenty minutes of music. Rats were forced to hang from a pull-up bar four times until they could not hold themselves any longer. Blood was drawn immediately after the four hangs had been completed. Music was played for the duration of the stressor as described in Escribano et al. (2014). The tests were all conducted between noon and 1:30 pm to make sure no difference is caused by the circadian rhythm of cortisol.

Blood Drawing and Analysis

Blood was drawn from the caudal vein as described by Fluttert et al. (2000). A small incision was made crossing a vein roughly in the middle of the tail. The tail was stroked gently to encourage bleeding until sufficient blood was collected for the ELISA (about 0.5 mL). Three of the four groups had no problem with the blood collecting method through the tail. The animals exposed to atonal music did not bleed from their tail and had to be sacrificed to collect sufficient blood. Those four rats were sacrificed by decapitation and thoracic blood was collected for analysis.

The serum was separated directly after drawing the blood. At least 100 μL of serum was needed for the ELISA. Serum samples were frozen until all samples were collected, and then all samples were run at the same time. Blood was tested using an ELISA for cortisol. The ELISA was run as described by the manufacturer (Enzo Life Sciences).

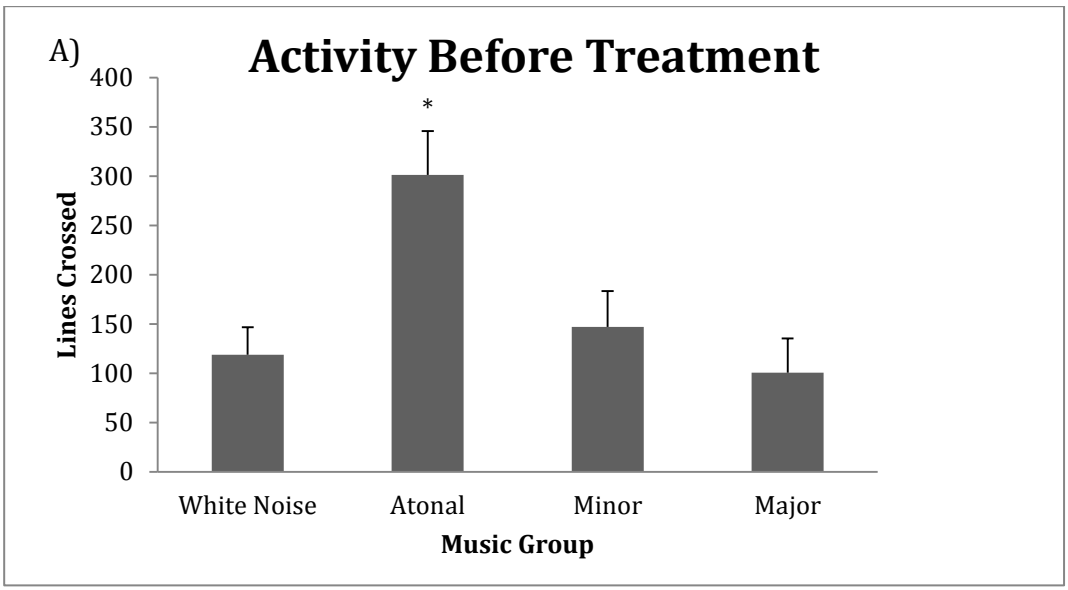
Statistical Analysis

Results from the ELISA and the behavioral analysis were analyzed using a one-way between analysis of variance (significance, $p < .05$).

Results

Activity Before Treatment

During the pre-treatment assessment of activity in the absence of sound the animals exposed to white noise, minor, and major conditions crossed a similar number of lines in the box ($\bar{X}$= 118.75, 147.25, and 100.75 line crosses respectively). Those three groups also were similar in escape attempts as well ($\bar{X}$= 18, 23.5, and 16.5 escape attempts respectively). However, the group assigned to atonal music had significantly higher activity ($\bar{X}$= 301.5 line crosses, P=.008) and escape attempts ($\bar{X}$= 54.75 escape attempts, P=.007) than the rest of the groups (figure 1).



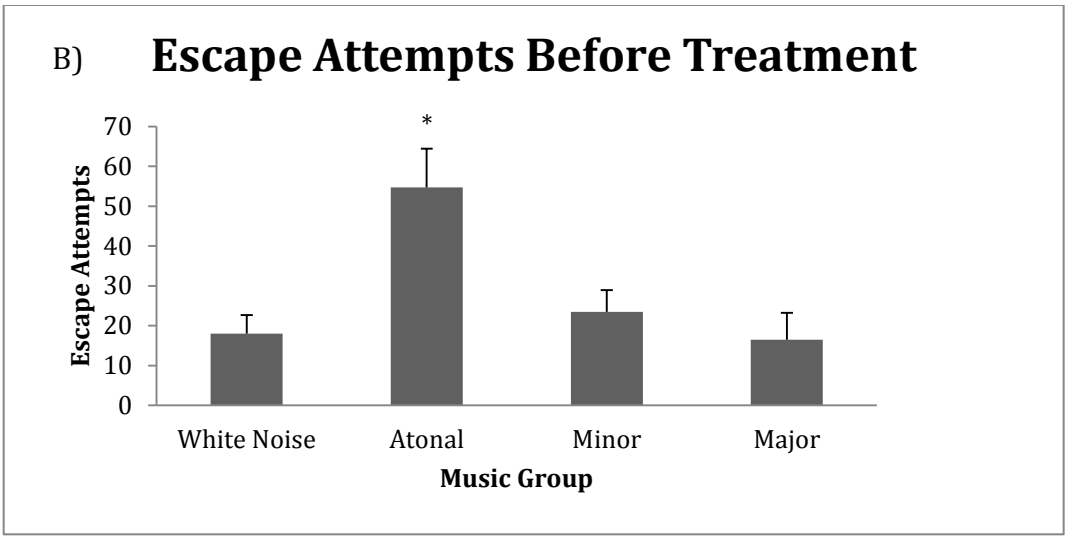
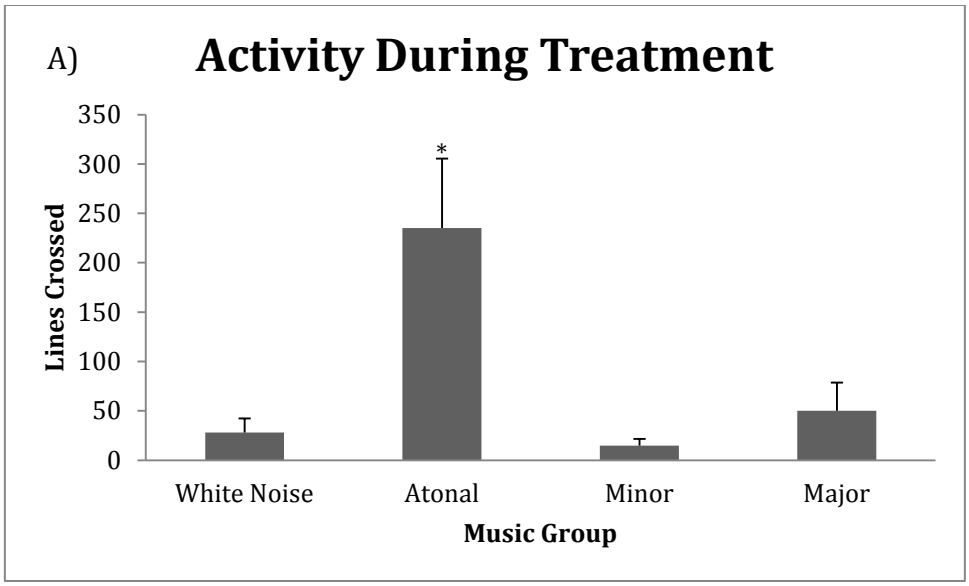


Figure 1: (A) The number of times a line in the grid was crossed by those exposed to 20 minutes of silence. The activity of the atonal group was significantly higher than that of the other groups (P= .008). (B) The number of escape attempts was compared between the four groups. The number of escape attempts by animals assigned to the atonal group was significantly higher than the other groups (P=.007). Results are expressed as means ± SEM. N=16, four groups of four. Significance (*) was found with the Tukey Post–Hoc test.

Activity During Treatment

Activity during the music conditioning mirrored that of the preceding activity period. The rats exposed to white noise, minor, and major pieces were similar ($\bar{X}$=28.25, 14.75, and 50.25 line crosses respectively). The group exposed to atonal music had significantly higher activity levels ($\bar{X}$=235 line crosses, P= .005) (figure 2A). Escape attempts did not differ between any of the four groups (P= .117) (figure 2B).



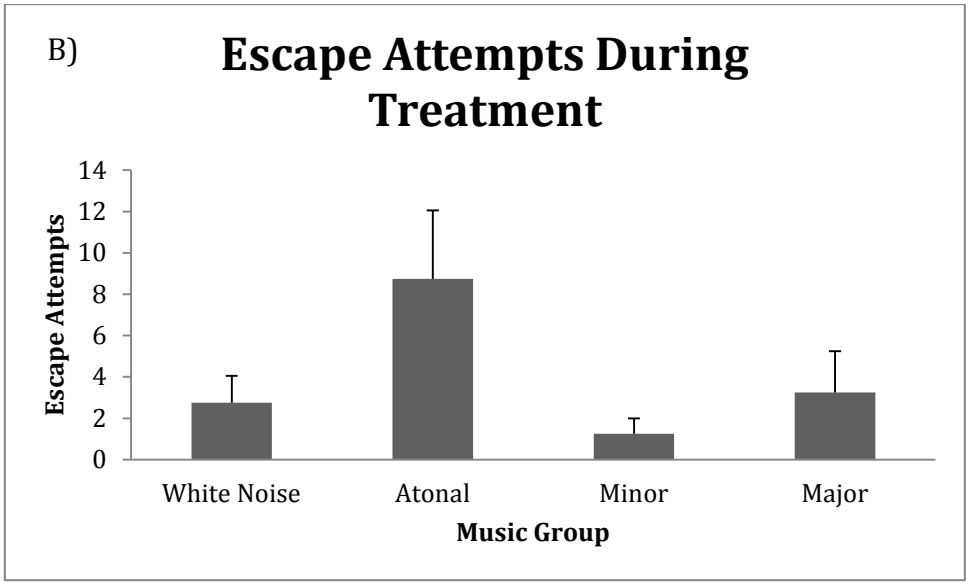


Figure 2: (A) The number of times a line in the grid was crossed by those exposed to 20 minutes of music. The number of line crosses by those exposed to atonal music was significantly higher than the rest (P= .005). (B) The number of escape attempts did not differ significantly (P= .117). Results are expressed as means ± SEM. N=16, four groups of four. Differences between groups were assessed with the Fisher's PLSD Post–Hoc test.

Stress Test: Hang Duration

The duration rats were able to hang on the pull-up bar was not affected by treatment (P= .119). Means ranged from 12±2.67 seconds for those exposed to white noise to 5.5±0.65 seconds in the rats exposed to atonal music (figure 3).

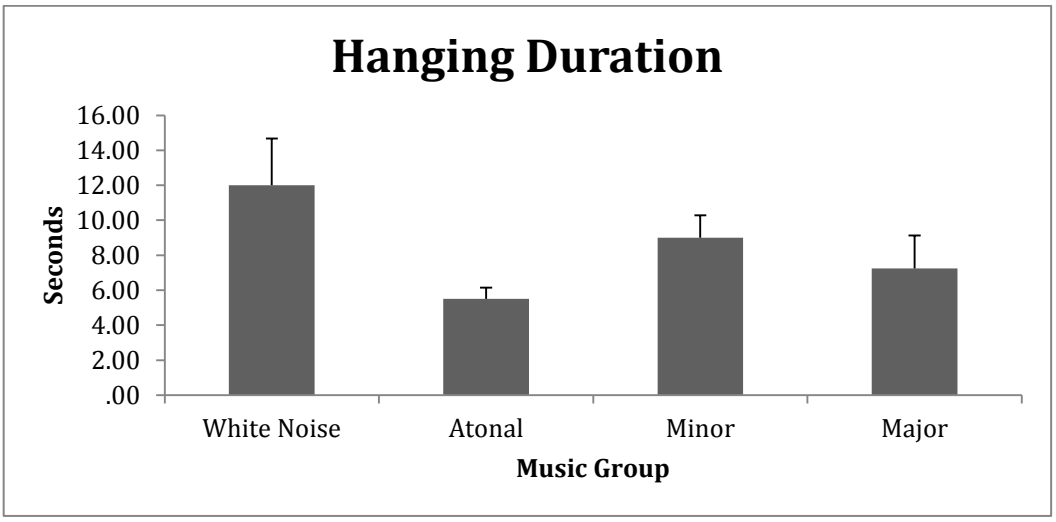
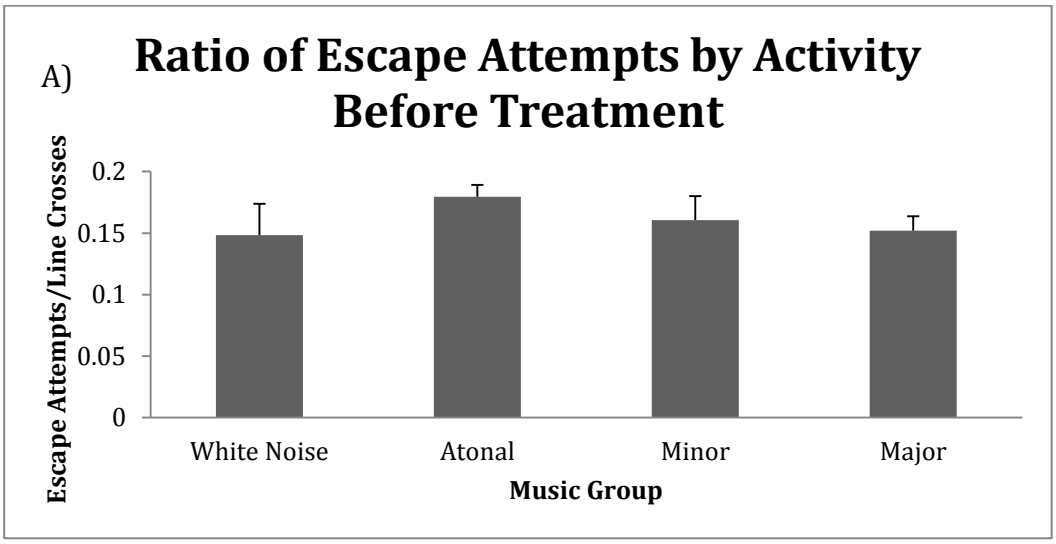


Figure 3: The duration the rats were able to hang on the pull up bar. There was no significance between the groups (P= .119). Results are expressed as means ± SEM. N=16, four groups of four.

Normalizing Data

The unexpected pretreatment behavior of rats exposed to atonal music necessitated further analysis. In an attempt to normalize activity levels, I examined the ratio of escape attempts to line crosses. This eliminated significant differences in activity levels during the pre-treatment period (P= .619)(figure 4A). Similarly, behavior during the treatment period showed no significant difference (P=.433)

(figure 4). The ratio of activity before treatment to activity during treatment was also analyzed. There was a significant difference (P= .028) between the behavior of the rats exposed to atonal music ($\bar{X}$= 0.735) and those exposed to white noise and minor groups ($\bar{X}$= 0.236, and 0.130 respectively). The group exposed to the major key was not different from the other three groups ($\bar{X}$= 0.468)(figure 5).



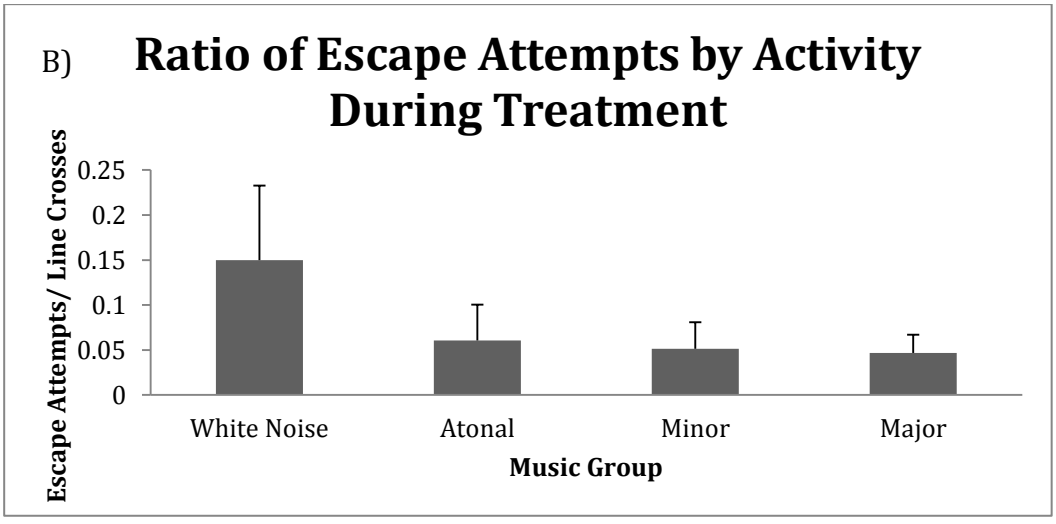


Figure 4: The ratio of number of escape attempts by the number of lines crossed during the 20-minute period. (A) The pre-test data shows no significance and even distribution across the groups (P= .619). (B) The during treatment data also shows no significance but seems to show a lower ratio within the music groups (P=.433). Results are expressed as means ± SEM. N=16, four groups of four.

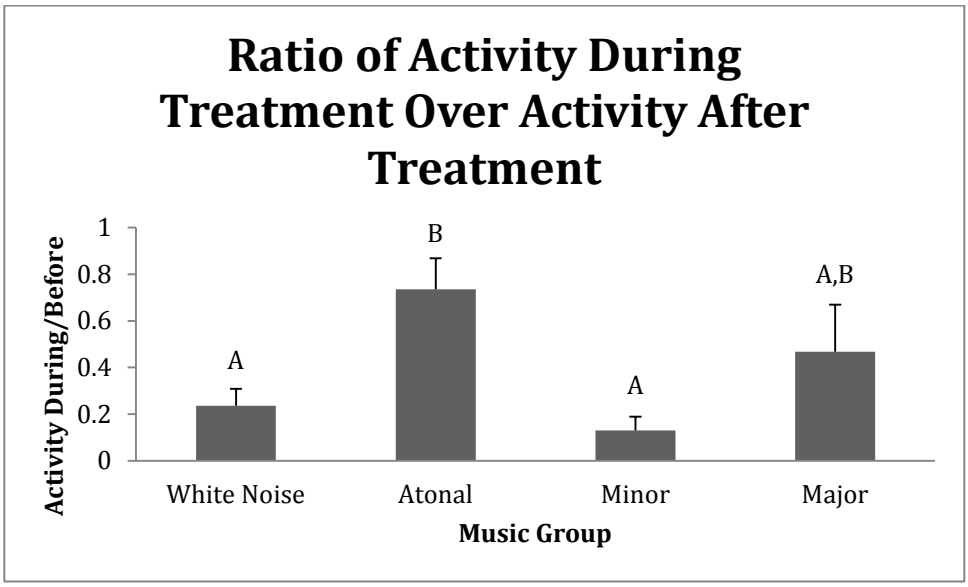


Figure 5: The ratio of number of times the rat crossed a line in the grid before treatment by the number of lines crossed during the treatment. There was no difference between the white noise and minor groups' ratios. The atonal group ratio was significantly higher than the white noise and minor groups' ratios, and the major group ratio was not significantly different from any of the other three (P= .028). Results are expressed as means ± SEM. N=16, four groups of four. Differences between groups were assessed with the Tukey Post-Hoc test.

Cortisol and Blood Drawing

The cortisol concentrations from the serum were above the range of the ELISA standards, so all animals had large amounts of cortisol in their blood after the tests had concluded.

Discussion

Activity

Due to the unfortunate reality that the rat groups differed significantly during the pre-treatment period it is difficult to say whether the music affected subsequent behaviors. If one is willing to assume that music was effective, then the following explanation is plausible. People have a harder time emotionally relating to atonal music than with tonal music, especially if they lack a music background (Daynes, 2011). If rats are similar in that regard, then exposure to atonal music may have increased their anxiety levels. Rats are more active in terms of distance traveled when they are under acute stress, possibly because they are trying to run away from danger (Shoji, 2010). Thus, if the atonal music created the acute anxiety it would have induced more line crossings on the grid.

In an attempt to offset the discrepancy between groups present before testing, the ratio of escape attempts by the number of lines crossed was analyzed. This would show which type of activity was more frequent. A lower ratio would indicate that roaming was more frequent, whereas a higher ratio indicated more escape attempts relative to total behavior. The ratios of behavior before or after stress did

not differ significantly; however, music seemed to correlate more with a roaming behavior when compared to the behavior of those exposed to white noise. This trend alludes to the idea that music as a whole changes behavior, but the scale chosen does not have an effect.

In contrast, the second attempt to normalize data indicated the opposite affect. It was assumed that looking at the percentage of activity during the treatment compared to the activity before treatment would nullify the original differences. This points to the scale used making a difference in the rat's level of stress because the rats exposed to minor music showed a greater reduction in activity than those exposed to atonal music. The fact that these attempts to normalize data allude to different ideas brings the validity of each ratio into question.

In comparison to literature it is hard to say if the rats in this study were showing a 'normal' amount of activity in the pre-treatment tests for a few reasons. First, not all lines are the same distance apart in all studies. For example Roth & Katz& (1979) used a grid with 30.5 cm boxes and Gregus et al. (2005) used 9.5 cm boxes. Also, the rats are not kept in the open field for the same amounts of time. Rat activity decreases the longer they stay in the open field (Roth & Katz, 1979), meaning that averages become more skewed the longer the rat is being tested. Studies also chose different times to test the rats activity, for example Roth & Katz (1979) tested for 9 minutes taking averages every 3 minutes, and Gregus et al. (2005) tested for 5 minutes and only took one average. Females are more active

than males (Frye & Wolf, 2002), which also will skew data in comparison to this study because most studies use both sexes, and this study only used males.

The rats exposed to atonal music were more in the range of activity compared to other studies in terms of distance traveled per minute, but as stated above the duration rats were tested skews the results since rats continually become less active. The other three groups moved a lot in the first 5-10 minutes then sat still the rest of the time. If the test was finished after 5 minutes the rats not exposed to atonal music might have been closer to the activity levels in other studies than those exposed to atonal music.

Tail Blood Flow and Cortisol

The average amount of cortisol in a rat's blood is about 3,800pg/mL (Dugovic et al., 1999); however the amount changes depending on the time of day and between rats (Vélez-Marín et al. 2012). The Elisa kit standards highest readable concentration was 10,000 pg/mL, so all rats had elevated stress levels at the conclusion of the tests.

The problem with blood drawing was fascinating because all rats from the major, minor, and white noise groups easily bled enough to test the serum. The atonal group might have bled one drop (~10μL) from the incision before the bleeding stopped. Rats have a defense mechanism that allows them to reduce their tail blood flow from about 30cm/s to about 6cm/s if a threat is detected. This is specifically the case when the raphe palladius of the medulla is electrically

stimulated, which is part of the sympathetic nervous system (Blessing & Nalivaiko, 2001).

The sympathetic nervous system mediates the body's fight or flight response, which is connected tightly to stress. The raphe palladius is stimulated when a body experiences pain (Senbo et al., 1993). That itself is not the connection to the reduced tail blood flow since these rats did not experience pain until the blood was being drawn, and this tail shunt is maximized 5 seconds after stimulation (Blessing & Nalivaiko, 2001). The pain stimulus can also be triggered in the presence of fear or anxiety (Senbo et al., 1993). The hanging would surely elicit either fear or anxiety, which could have activated the tail shunt in the rats. The fact that only the atonal group showed this result and that they were more active during treatments suggests that they were significantly more stressed than all other groups.

The rats in the atonal music treatment group were the only ones affected to this extent; so, it is probably due to chronic stress. Rats that are chronically stressed have an increased physiological response to acute stress (Adell et al., 1988); therefore, the rats exposed to atonal music would have been more affected by the fear of hanging from the pull-up bar. Their response was amplified so much that their tail shunt was activated, whereas the other groups had a normal response to the acute stress and blood flow to their tails was not affected.

Limitations

Obviously, the unusual behavior of the rats exposed to atonal music was problematic in this study. In addition, a better control for activity would have been advantageous. Had the music activity tests not immediately followed the control activity test there would not have been a testing acclimation effect. It is possible the reason the music tests showed less activity than the pre-treatment control is because the rats were just accustomed to that environment when they entered it for a second time. The tests could have been a day apart from each other and that would have reduced this effect.

Behavioral analyses were vague. More behaviors should have been defined and recorded; this would have cleared up the ambiguity in what type of activity the rats were performing and what emotion was being portrayed. Things like time grooming and time spent in the middle versus on the edges of the box should have been recorded as well. The definition of 'still time' was too vague and it should have been a shorter duration of time. Two minutes is a long time for a rat not to move: a duration of one minute might have been more appropriate. It also would have helped to videotape the tests because it is hard to get accurate data by taking notes as the test is in progress.

Future

If this study were to be recreated a few adjustments should be made. The first problem was doing behavioral analyses consecutively. This nullified the differences between pre-treatment and treatment results. The pre-treatment analysis should be done a day before the treatment analysis. The intervals that the rats were analyzed

should have been shorter, and broken into intervals. This would more specifically define rat activity at various times during the testing.

An interesting finding of this study was the tail shunt in relation to stress. Something to consider in the future would be how cortisol affects blood flow in the tail. A possible test could be how much blood could be collected from the tail in a specific amount of time, and test the blood for cortisol concentration. Another possibility could be stress the rat and take a blood pressure measurement from the tail before and after the stress to see if blood flow has changed.

Another direction could be to look at behavior in different environments. It would be interesting to do a behavioral analysis with the three music types in this study. It did appear to me that the rats were acting differently but I was not quantifying that. A similar test could be to analyze behavior in a stressful environment. One could do a pre-test behavioral analysis, stress the animal, and do a post-test behavioral analysis and analyze the differences. Last it would be interesting to see if the pain response of rats is different in the presence of different types of music. A microphone could be used during the blood letting protocol to capture the rat's vocalizations, and that could be used to measure pain perceived. While I drew blood sometimes the rat would make an audible sound and jump, but others would not budge. Perhaps the music they were listening to played role in that.

Conclusion

In the end there are some tantalizing trends to be seen in the data, but no definitive conclusions can be drawn due to initial differences in the rat groups. There is still a lot to be learned from this study even though the original hypotheses were not answered; more research could be done looking at the connection to stress and blood flow in the tail of a rat, better behavioral analyses should be done in the presence of music. The connection between music and stress is a complex system and deserves to be researched further.

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