The Mozart Effect in Rats: Response to Steele

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Abstract

Steele (2003) raised several concerns regarding Rauscher, Robinson, & Jens' (1998) study that found improved maze running following early music exposure in rats. Steele's primary criticisms were that the rats in the Rauscher et al. study were only able to hear 31% of the notes, and that a selection bias resulting in pre-existing differences between groups could account for the disparity in their performance. Here we provide evidence that the rats heard a substantially higher percentage of notes than Steele reported and that there were no pre-existing differences between groups. A recent replication is discussed that shows a neurophysiological basis for a Mozart effect in rats.

Rauscher, Robinson, & Jens (1998) reported that Long Evans rats exposed *in-utero* and 60 days after birth to the first movement of Mozart's Sonata for Two Pianos (K. 448) completed a spatial maze faster and with fewer errors than rats exposed to white noise, a composition by Philip Glass, or silence. In his review of this work, Steele (2003) raised several concerns regarding the interpretation of these data. Steele's primary criticism was that rats are able to hear only 31% of the notes of the sonata. He speculated that a selection bias and pre-existing differences between groups could account for the performance differences, rather than exposure to the music.

Auditory Threshold and Note Count

Steele (2003) presents auditory threshold data to support his argument that rats are insensitive to sounds below 500 Hz at 65 to 70 dB (the sound level used in the Rauscher et al. (1998) study). In Steele's nomenclature, adapted from Reblitz (1976), octaves are numbered starting with A, rather than the traditional C. Solely in order to maintain consistency with Steele, the same nomenclature is used throughout this paper. According to this system, 500 Hz on the piano corresponds to a frequency between B5 (493.883 Hz) and C5 (523.251 Hz). C5 corresponds to an octave above middle C, and B5 corresponds to one half-step below that note. Steele assumed that the presence of a ventilation fan would reduce sensitivity such that the rats would hear notes above C5 only, and

he calculated that rats could only hear 37 (42%) of the 88 notes of the piano.[1]

However, other researchers report rat hearing thresholds starting at 250 Hz at 70

db (for a review, see Kelly & Masterton, 1977). Because 250 Hz corresponds to

a frequency between B4 (246.942 Hz) and C4 (261.626 Hz, middle C), this

research implies that the rats in Rauscher et al.'s study heard the fundamental

frequencies of notes a full octave lower than Steele presumed—49 of the 88

piano notes (56%). Moreover, the thresholds reported by Steele were averages

recorded from two 3-month-old and two 9-month-old rats over a period of 20 – 80

days (Heffner, Heffner, Contos, & Ott, 1994). Heffner et al. found that the 9-

month-old rats had thresholds of 290 Hz at 70 dB (H.E. Heffner, personal

communication, January 7, 2004). Due to technical difficulties, auditory

thresholds were not recorded for the 3-month-old rats below 500 Hz (H.E.

Heffner, personal communication, January 7, 2004), but their true thresholds

were presumably similar to or lower than those of the 9-month-old rats. Rauscher

et al.'s rats were neonates during the exposure. In addition, it is well known that

young rats have greater auditory sensitivity than older rats (Cowles &

Pennington, 1943). Furthermore, early auditory exposure has been found to

influence functional development of the rat primary auditory cortex (Zhang, Bao,

& Merzenich, 2001), which in turn affects auditory threshold (Sakai, Kudoh, &

Shibuki, 1999). Therefore, the rats in Rauscher et al.'s study may have had lower

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[1] The level of sound produced by the ventilation system in the rooms where the animals were exposed did not register on a decibel meter. The rooms were virtually silent. It is therefore unlikely that the rats' auditory thresholds were reduced accordingly.

frequency thresholds than typical rats due to their early exposure to the Mozart sonata.

Steele reports "a count of the amount of each note" in the first movement of K.448, which leads him to assert that rats would not have heard 1,913 of the 2,790 (69%) notes in the first movement. Our own analysis disagrees considerably with Steele's estimate of the total number of notes in the Mozart sonata.

To perform the note-count analysis, we acquired a Musical Instrument Digital Interface (MIDI) version of the first movement of K.448 (Classical Music Midi Page, n.d.) and compared it and a written score (Sheet Music Archive, n.d.) against the recorded version played to the rats (Mozart, 1781) to assure they were equivalent. We then used MIDINote (Nagler, n.d.) to produce a list of the notes in the MIDI performance. Our count produced 9,363 notes, not 2,790 notes as Steele (2003) reported. The discrepancy between Steele's note count and our own is inconsequential using Steele's threshold of 500 Hz, because it did not substantially alter the proportion of notes that the rats may have potentially heard. However, the proportion of the 9,363 notes with a fundamental frequency above 250 Hz was 57% (5,329 notes). Considering the data showing that rats can hear down to 250 Hz at 70 dB SPL (Kelly & Masterton, 1977), this analysis suggests that the rats in Rauscher et al.'s (1998) study could hear the fundamental frequency of 57% of the notes played in the first movement recording. This conclusion contradicts Steele's statement that "adult rats are deaf to most notes in the sonata" (p. 251). One partial explanation for the discrepancy

between our numbers and those claimed by Steele (2003) may be found in the way the repeats in the score are counted. The first movement of the Mozart sonata contains two repeats, denoted in the score by repeat signs (:ıı:). In the recorded and MIDI performances, the first repeat in the score is performed. Therefore, there are substantially more notes in the actual performance than are literally represented on the pages of the score. (The repeated section consisted of 2,642 notes.) A second source of variance in any performance is the exact number of notes played within each trill or other musical ornament not explicitly represented on the pages of the score. Nevertheless, the large difference (6,573) between the number of notes Steele counted and those revealed by our analysis remains largely unexplained. In addition, Steele's Figure 4, which shows the distribution of notes of the Mozart movement by each octave on the piano, indicates that no notes of the movement fell within the first octave. In fact, both the musical score and the actual performance have 146 notes in that octave.

　　Just as in research with human infants, one cannot really "know" what rats hear when exposed to the first movement of the Mozart piano sonata K.448. The critical point, however, is that Rauscher, Robinson, and Jens (1998) clearly demonstrate that some aspect or aspects of that sensory stimulus influenced the rats' maze-running performance in a distinctly different manner than did white noise, a Phillip Glass composition, or silence. This finding must be explained. Although rats certainly hear a higher range of frequencies than do humans, studies designed to test auditory sensitivity generally employ sine wave stimuli. Applying those sine-wave thresholds will lead to invalid conclusions with respect

to musical stimuli, which are much more complex auditory events that typically exhibit substantial energy peaks at higher frequencies, called overtones. These can either be harmonic partials, integer multiples of the fundamental, or non-integer multiples of the fundamental. Indeed, it is the higher order partials of the fundamental frequencies played on different instruments that impart to those instruments their characteristic timbre, a musical component to which rats are sensitive (Poli & Previde, 1991).

We performed a spectral analysis of piano tones to emphasize why this point is critical. As mentioned previously, published studies of rat hearing show that they are capable of discriminating between silence and a 250 Hz sine-wave tone played at 70 dB sound pressure level (SPL) (Kelly & Masterton, 1977). One might then infer that rats would not be able to detect a B4 (246.942 Hz) piano tone played at 70 dB. However, as discussed above, a note played on a piano also produces substantial energy at partials above its fundamental frequency. Furthermore, partials of a note played on the piano induce resonance in higher pitched strings (when those strings are not damped), thus adding to the total energy at these higher frequencies. Figure 1 illustrates this principle with a spectral analysis of a B4 played on a grand piano.

Insert Figure 1 here

The spectrogram of this note shows a prominent peak at the fundamental frequency of 247 Hz that has an amplitude of 74 dB. The first partial of B4 (494 Hz—B5) is an octave above the frequency of the fundamental, yet its amplitude is 4 dB higher. B4's first partial at 494 Hz causes the B5 string to vibrate at its

fundamental frequency, which adds to the total energy at this peak. One can observe a similar phenomenon with B4's third partial (988—B6), which is two octaves above the fundamental, and again with the fourth, fifth, and sixth partials. Thus, it is probable that rats would be able to hear a substantial number of the partials of quite low notes on the piano, even if they were unable to hear the actual fundamental frequencies of those notes.

Another important issue to consider is that K. 448 is scored for four hands, and its lowest notes are typically played as chords, often with the same notes played at successively higher octaves (for example, the G1s in the second and third measures, in which the four lowest Gs are played at the same time, two on each piano). According to Helmholtz (as cited by http://www.vibrationdata.com/piano.htm), "A note accompanied by its Octave consequently becomes brighter in quality, because the higher upper partial tones on which brightness of quality depends, are partially reinforced by the additional Octave." This phenomenon probably accounts for the subjective experience of brightness at many points in K. 448. Thus, not only would rats likely have heard partials of the lowest notes in the music, many of those partials would have been amplified further by simultaneously played octaves.

A further problem in Steele's (2003) approach to determining what the rats actually heard during exposure to the Mozart sonata lies in his assumption that pitch was the primary musical feature the rats perceived. The Mozart effect in rats may not be due to the percentage of different fundamental frequencies the animals heard. Other relevant musical values may include melodic contour, the

rhythmic or temporal pattern of the notes, the ratio of filled to unfilled durations, timbre, harmonic interplay, intervallic relationships, etc. All these musical components can be clearly discerned merely by hearing partials of the fundamentals. Although very little is known regarding how animals perceive complex auditory stimuli, it seems likely that not all aspects of a musical composition may affect frequency perception equally (D'Amato & Salmon, 1984; Poli & Previde, 1991; Schulkind, Posner, & Rubin, 2003). Therefore, fundamental frequency discrimination may not be at all relevant to the discovery of a Mozart effect in rats.

In-utero Exposure

Steele (2003) claimed that "Rauscher et al. (1998) treated rats as if they were humans by exposing the rats to the music *in-utero*" (p. 255), and that *in-utero* exposure would be ineffective because "rats are born deaf" (p. 251). The decision to begin exposure *in-utero* was made for practical rather than theoretical reasons. In an earlier pilot study, Rauscher et al. exposed the animals to the auditory conditions after birth. The result was that the mothers in all auditory exposure groups cannibalized the pups, an event that has been found to occur in approximately 33% of litters of mothers exposed to peri-natal stress (DeSantis & Schmaltz, 1984). Although Rauscher et al. expected post-birth exposure to affect maze performance, the rats were exposed to the music *in-utero* as well to avoid future occurrences of cannibalism. Steele's contention that "they thought they

were studying the effect of music on the developing fetal brain" (p. 261) is incorrect. A further study, discussed below, has replicated the effect using only post-natal exposure.

The Mozart Effect in Humans

Steele (2003) focused his review of the literature on failures to replicate Rauscher, Shaw, & Ky's (1993) report that college students who listened to 10 min of the Mozart sonata scored higher on a spatial-temporal task than after they listened to relaxation instructions or silence. He refers to two meta-analyses, one that did not find a significant Mozart effect (Chabris, 1999) and one that did (Hetland, 2000). Chabris' analyses included 16 studies with 714 subjects combining spatial measures, and 12 studies with 522 subjects using spatial-temporal measures. Hetland analyzed 36 studies with 2,465 subjects combining spatial measures, and 31 studies with 2,089 subjects using spatial-temporal measures. Steele criticized Hetland's analysis on the basis of her having included unpublished as well as published studies. Statisticians agree that published studies in the social sciences are a biased sample of the studies that are actually conducted (Bakan, 1967; McNemar, 1960; Smart, 1966; Sterling, 1959), since journals are filled with the 5% of studies that show type I errors, while approximately 95% of the unpublished studies show nonsignificant results (Rosenthal, 1979; Wachter, 1988). Hetland's analysis may have produced a higher effect size than Chabris' due to the unpublished successful replications

she included. Nevertheless, it is important to acknowledge that there are many reasons other than significance that determine whether or not a study gets published, including study quality. In an attempt to address this, Hetland coded the studies in her analysis according to three indices relating to study quality: random assignment/counterbalancing of conditions, efforts to reduce expectancy effects, and whether subjects were naïve as to the purpose of the study. She found larger effect sizes for studies indexed as higher quality, and concluded that "…there really is an effect of music on spatial tasks, since the better designed [studies] actually demonstrate a stronger, not a weaker, relationship between music and spatial-temporal reasoning" (p. 134). In addition, she found no significant difference in effect size between the published and unpublished studies included in her analysis.

In Steele's (2003) review of the literature, he cited numerous failures to replicate Rauscher et al.'s (1993) research but did not consider that most of the studies used different tasks, subject populations, and/or musical compositions than those employed in the original research (Bridgett & Cuevas, 2000; Carstens, Huskins, & Hounshell, 1995, Kenealy & Monsef, 1994; McCutcheon, 2000; McKelvie & Low, 2002; Newman, Rosenbach, Burns, Latimer, Matocha, & Vogt, 1995; Steele, Ball, & Runk, 1997; Stephenson, 2002; Stough, Kerkin, Banks, & Mangan, 1994; Weeks, 1996). In fact, the only laboratory that consistently failed to replicate the Mozart effect using the same spatial-temporal task, musical composition, and subject population as Rauscher et al. is Steele's (Steele, Bass, & Crook, 1999; Steele, Brown, & Stoecker, 1999; Steele et al., 1999). Many other

laboratories have successfully replicated the effect (Husain, Thompson, & Schellenberg, 2002; Ivanov & Geake, 2003; Nantais & Schellenberg, 1999; Rauscher, Shaw, & Ky, 1995; Rideout, 1999; Rideout, Dougherty, & Wernert, 1998; Rideout & Taylor, 1997; Rideout & Laubach, 1996; Thompson, Schellenberg, & Hussain, 2002; Wilson & Brown, 1997), sometimes offering compelling explanations other than those proposed by Rauscher et al. (1993). One possible explanation as to why Steele's studies did not replicate the effect may be that he did not control for experimenter expectancy effects. It is well known that expectations held by investigators can influence how their subjects perform (Rosenthal & Rubin, 1978). Rauscher (2000) found that experimenter expectancies can indeed contribute to the outcome of Mozart Effect experiments, even when experimenters are provided with identical instructions and scripts.

Pre-Existing Group Differences?

To explain Rauscher et al.'s (1998) findings, Steele (2003) proposed that there were pre-existing differences between the groups of rats on the first day of testing. He suggested that the Mozart group was "behaving differently from other groups on the first day of training" (p. 260), and supported this statement by claiming that "Mozart-reared groups showed significantly less errors on the first day of training" (p. 260). In fact, the number of errors produced by the Mozart group ($M = 3.46$) did not differ from that of the Glass group ($M = 3.79$) on the first day of testing. (There were five days of testing, three trials per day.) However,

the noise group (M = 4.67) did produce more errors than the Mozart group averaged across all three trials on Day 1. To determine if Steele's contention of pre-existing group differences is correct, we re-analyzed our data for the first day of testing according to trial. If pre-existing differences between the groups were present, one would expect to find differences between the groups at baseline, i.e., on Trial 1 of Day 1. A one-factor between-subjects analysis of variance performed on errors made during the first trial of Day 1 was not significant (F(2,87) = 1.62, p = .20). Subsequent t-tests found no differences between any of the three groups. It is therefore unlikely that the groups differed behaviorally at the start of the study.

Steele also contended that group differences might have occurred because "all offspring from a particular mother were assigned to the same music condition" (p. 260). While this was in fact true, it is important to bear in mind that 30 pups from 6-8 mothers were assigned to each group. It is not true that each group consisted only of rat pups from the same mother. It would, of course, have been better to cross-foster the pups, and future studies requiring neo-natal exposure will follow this procedure. Nevertheless, the likelihood of pre-existing group differences due to a litter effect in this study is low due to the large number of litters in each group.

Selection Bias?

Steele (2003) contends that Rauscher et al. (1998) introduced a selection bias when they culled rat pups for testing. He suggests that the undergraduate laboratory assistant who selected animals for testing paid inadvertent attention to AGD (anogenital distance—the distance between anus and genitalia), and assigned animals likely to perform better in the maze to the Mozart group. (Increased AGD indicates increased androgen exposure, which is related to spatial behavior in rats (Isgor & Sengelaub, 1998)). Even if the assistant were aware of AGD and its relationship to spatial behavior in rats, it is unlikely that an untrained observer could reliably select 3-week old pups with increased AGD, especially given that their AGD would be on the order of millimeters. Moreover, the sampling plan used by Rauscher et al. (1998) involved a random digit generator. All rat numbers were entered into a computer and randomized. The first 15 males and females generated in each group were then chosen for testing. This procedure eliminated any possible selection bias on the part of the research assistant.

Replication

Since the publication of Rauscher et al. (1998), an additional study has shown a Mozart effect in rats. The goal of the study was to determine the neurophysiological basis of the effect (Li, Rauscher, Cai, Cooper, Ying, & Gomez-Pinilla, 2005). Sprague Dawley rats were randomly assigned to two auditory conditions at weaning: the same Mozart sonata used by Rauscher et al. (1998) or white noise (WN) for 12 hours/day during the dark cycle. Animals were tested at 21 days in the same manner as Rauscher et al. (1998). Analyses of variance found that music-exposed rats completed the maze faster and with fewer errors than WN-exposed rats. Significant differences were found starting on Day 2 (p<.03 all). To determine the molecular mechanism underlying these effects, the researchers isolated mRNA and protein in the hippocampi and spinal cords of rats exposed to music or WN. They found a 150% increase in BDNF, 140% increase in synapsin I, and 176% increase in CREB mRNAs, compared to WN controls. In addition, levels of phosphorylated proteins were 107% and 190% higher in music-exposed rats for synapsin I and CREB, respectively. Microarray study showed that music exposure resulted in up-regulation of several genes involved with synaptic function/plasticity and intracellular signaling. Effects were found in the hippocampus but not in the spinal cord. These results indicate that music exposure both improves maze performance and affects molecular systems associated with the action of BDNF on synaptic plasticity in the brain. Because BDNF is critically involved with molecular mechanisms underlying cognitive

function, music exposure may play an important role in supporting neuronal function and cognition through modulation of the BDNF system.

Conclusion

The research cited above provides evidence for a Mozart effect in rats. The histological study, although not yet published, replicated the behavioral findings of Rauscher et al. (1998) and suggests a neurophysiological mechanism. It should be noted, however, that this study has not yet undergone peer review, and should therefore be interpreted with caution. Steele's (2003) concerns regarding auditory thresholds, pre-existing group differences, and selection biases have been addressed as well. These data, however, should not be confused with findings of a Mozart effect in humans. My colleagues and I do not claim that listening to classical music will improve children's mathematical or spatial scores—a common myth regarding the Mozart effect. Furthermore, the mechanism responsible for the Mozart effect in rats may be quite different from that which is responsible for the effect in humans, as suggested by other researchers (e.g., Thompson, Schellenberg, & Husain, 2001). In addition, it would be unethical to expose humans to 12 hours per day of the same composition. We therefore caution against generalizing the rat data to human populations. However, the research described herein addresses one of the basic inquiries of cognitive neuroscience: Are there general principles in all animals' neural codes that underlie the organization of their behaviors, despite the diverse

complexity of the brains of lower and higher animals? Although we do not yet

know how an account of auditory processes can explain complex cognitive

functions, bridges between animal and human study are useful because they

help guide research across levels of analysis.[2]

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Figure Caption

Figure 1. Spectral analysis of a B4 played on a Steinway model M grand piano,

performed using SoundForge (v. 5.0, Sonic Foundry, Inc.). The leftmost peak

represents the fundamental frequency of 247 Hz at an amplitude of 74 dB. The

amplitude of the first six partials of B4 are all within 15 dB of that of the

fundamental—and well above the hearing threshold of the Long-Evans rat.