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Induction of Fear but No Effects on Cognitive Fluency by Theta Frequency Auditory Binaural Beat Stimulation

Graham Pluck
Universidad San Francisco de Quito

Marco Antonio López-Águila
Escuela Superior Politécnica de Chimborazo

When pure tones of different frequencies are presented dichotically, participants report a subjective beat at a frequency of the difference between the 2 tones. These *binaural beats* are illusory in that they do not exist in the physical stimuli. Electro- and magnetoencephalographic evidence suggests that these psychological binaural beats can induce physiological synchronous electric activity in the brain. Therefore, they have potential experimental and therapeutic applications. The present study reports on a fully controlled, double-blind experiment of acute exposure to binaural beats at 6 Hz (theta) on concurrent mood and cognitive function as measured by a range of fluency tasks on healthy adults. The present study could not find any detectable influence of binaural beat exposure on four different measures of cognitive fluency (phonological, semantic, ideational and design). However, regarding moods measured with the Visual Analogue Mood Scales, a significant induction of fear in the binaural beat condition compared to control was detected. A suggested mechanism of action for this fear induction is the entrainment of theta activity in the amygdala or primary auditory cortex. Furthermore, as the beat was embedded within music, participants were unaware of its presence. The present data suggest that no cognitive enhancement, nor for that matter cognitive suppression, at least as measured by fluency tasks, is induced by theta frequency binaural beat stimulation. However, the fact that theta frequency stimulation can induce fear without participants being aware of the stimulation could be of practical experimental use, for example in human anxiety or conditioned fear research.

Keywords: fear, auditory stimulation, cognitive fluency, dichotic stimulation, anxiety

When two pure tones with slightly different frequencies are presented dichotically, for example, 306 Hz to the left ear and 300 Hz to the right ear, this often produces a subjectively heard beat phenomenon of the difference between the two frequencies, that is, at 6 Hz (Oster, 1973). These illusory *binaural beats* do not exist in the physical stimuli and must, therefore, have an intracerebral origin. Single cell recording in the cat inferior colliculus shows that this region is the probable generator of the binaural beat signal, as cells here selectively respond to the frequency of the beat (Kuwada, Yin, & Wickesberg, 1979). The normal inferior colliculus function of this interaural frequency difference detection is probably sound localization. For example, it is known that individual neurons in the guinea pig inferior colliculus respond to intraaural time delays as low as 10 to 20 microseconds (Skottun, Shackleton, Arnott, & Palmer, 2001). In natural circumstances, intraaural sound delays are caused by the different locations of the two ears, and their detection within the inferior colliculus represents one of the three principal ways that sounds can be

Graham Pluck, Quito Brain and Behavior Laboratory, Universidad San Francisco de Quito; Marco Antonio López-Águila, Escuela de Física y Matemática, Escuela Superior Politécnica de Chimborazo.

Correspondence concerning this article should be addressed to Graham Pluck, Colegio de Ciencias Sociales y Humanidades, Universidad San Francisco de Quito, D316, Diego de Robles y Vía Interoceánica, Cumbayá, Ecuador. E-mail: g.c.pluck@gmail.com

localized (the other two are sound level differences and spectral cues; Palmer & Kuwada, 2005), Furthermore, those inferior colliculus neurons that respond to static intraural delays of tonal stimuli also respond to binaural beat stimuli (Yin & Kuwada, 1983). It is only the artificial context of pure-tone dichotic stimulation that produces the phenomena of illusionary beats (Oster, 1973).

This binaural illusory beat also seems to entrain the normal brain wave activity as measured by electroencephalography (EEG) and produces patterns of wave synchronization across the entire cerebrum at the same frequency as the binaural beat stimulation (Jirakittayakorn & Wongsawat, 2017). The effects on brain electrophysiology have also been demonstrated directly from human brain tissue with intracerebral EEG recordings of epilepsy patients with implanted deep electrodes (Becher et al., 2015). Additionally, this neural response to an illusory binaural beat has been confirmed with magnetoencephalography (Karino et al., 2006; Ross, Miyazaki, Thompson, Jamali, & Fujioka, 2014). This spread of activation is likely to be an incidental feature of normal auditory processing, as the binaural beat phenomena would rarely occur naturally.

The interaural frequency difference determines the frequency of the binaural beat, and this tends to physically entrain neural responses at the same frequency (Jirakittayakorn & Wongsawat, 2017). The most accepted mechanism behind this entrainment is that cochlear neurons discharge according to the volley principle, preserving the phase information to each ear, and these discharges converge on the ascending auditory system, activating binaurally receptive neurons in the brain stem. This activity then generates neurophysiological beat phenomenon that can be recorded with EEG at the cortex, particularly on the left temporal lobe (Pratt et al., 2009). Thus, in theory, it should be possible to reproduce some of the psychological correlates of neuroelectrical synchronous firing with binaural beat stimulation. For example, EEG recordings show that alpha range synchrony (i.e., 8 Hz to 12 Hz) is associated with active task-relevant processing, inhibition of task-irrelevant processing and fronto-executive control of behavioral responses (Palva & Palva, 2011). Whereas in contrast, EEG recording shows that theta range synchrony (i.e., 4 Hz to 7 Hz) likely has a role in learning by coupling the activation between more local and distant brain regions including the hippocampus and amygdala, via synchronized theta oscillations (Colgin, 2013).

Binaural beat phenomena are already widely used for their supposed therapeutic effects. This is partly because of their simplicity and tolerance. They can be produced with freely available sound mixing software, or they can be downloaded as prerecorded sound files from various alternative health sources. They can be listened to with any music player and a pair of stereo headphones. Although the binaural beat research published from the alternative health perspective has generally been supportive of therapeutic effects, it has also tended to be methodologically weak. Nevertheless, several well-controlled studies have demonstrated behavioral or physiological effects of binaural beat stimulation (Chaieb, Wilpert, Reber, & Fell, 2015; Padmanabhan, Hildreth, & Laws, 2005; Wiwatwongwana et al., 2016), as well as several neurophysiological studies that have demonstrated effects within the brain (Becher et al., 2015; Jirakittayakorn & Wongsawat, 2017; Karino et al., 2006; Ross et al., 2014). For example, Jirakittayakorn and Wongsawat (2017) used a 6 Hz binaural beat stimulus (therefore in the theta range), and this produced significant increases in theta power as measured by quantitative EEG. Similarly, Pratt et al. (2009) have shown that binaural beats of either 3 Hz or 6 Hz (theta range) provoke event related potentials above the temporal lobes at the same frequencies (3 Hz or 6 Hz).

That binaural beats are easily produced, effective at stimulating the brain and well tolerated, suggesting that they could offer an alternative to therapeutic brain stimulation techniques such as electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS). For example, typical protocols for treatment of depression with ECT involve AC stimulation to the brain with frequencies of 10 Hz to 70 Hz, two to three times each week over several weeks; typical TMS treatment involves multiple stimulations at 10 Hz every day for three weeks (Eranti et al., 2007). Both ECT and TMS have to be administered by clinicians and have relatively high operating costs (Knapp et al., 2008). Binaural beat stimulation is considerably simpler to apply. However, the effectiveness of the various stimulation parameters on different psychological states is not well understood and observed effects tend to be relatively weak and short-lived (Chaieb et al., 2015). Nevertheless, the research that exists suggests acute effects, with several reports demonstrating short-term changes to cognitive processing (Reedijk, Bolders, Colzato, & Hommel, 2015; Reedijk, Bolders, & Hommel, 2013) or mood (Padmanabhan et al., 2005; Wahbeh, Calabrese, Zwickey, & Zajdel, 2007) associated with listening to binaural beats.

However, the current evidence for measurable effects of binaural beat stimulation on mood or behavior is mixed and therefore requires further elucidation. There is evidence that listening to gamma frequency binaural beats (i.e., 40 Hz) may enhance some aspects of feature binding in episodic memory (Colzato, Steenbergen, & Sellaro, 2017). Although evidence suggests that low-frequency binaural beats, ranging from 4 Hz to 7 Hz, appear to produce detectable synchronization across much of the cortex (Karino et al., 2006; Ross et al., 2014), the neural synchronization associated with higher frequencies, such as within the gamma range (i.e., 25 Hz to 100 Hz), seems to be mainly driven by activity in the primary auditory cortex (Pastor et al., 2002). One group have suggested that gamma frequency binaural beats could be used therapeutically for cognitive enhancement (Reedijk et al., 2015).

Another study suggested that performance in a fluency task was enhanced by either alpha (i.e., 10 Hz) or gamma (i.e., 40 Hz) frequency binaural beat stimulation in some people, but this depended on individual variation in an indirect measure of striatal dopaminergic tone, spontaneous eyeblink rate (Reedijk et al., 2013). This is an example of a biological individual difference moderating the effect of binaural beats on cognitive functioning. However, in the same research study, the authors measured mood on several scales and could not find any change in affective state related to binaural beat stimulation. Furthermore, a study of five different binaural beat frequencies (i.e., theta, 4.5 Hz; alpha, 9.0 Hz; beta, 17.9 Hz; gamma, 34.5 Hz; and upper gamma, 57.3 Hz), which included objective measures of emotional arousal, heart rate, and skin conductance, failed to show any physiological effects of binaural beat stimulation, including in the EEG signal (Lopez-Caballero & Escera, 2017). Alternatively, a randomized controlled trial of alpha frequency binaural beats (i.e., 10 Hz) to reduce anxiety during cataract surgery under local anesthetic reported a significant reduction in heart rate with binaural beat stimulation (Wiwatwongwana et al., 2016). In addition, a recent review has summarized a range of behavioral studies that also reported acute alterations to clinical anxiety levels, mood, and cognitive functions caused by binaural beat listening (Chaieb et al., 2015).

Therefore, the current evidence regarding the potential for binaural beat stimulation to influence mood or neurocognitive functioning remains unclear. In order to address this issue, the present study reports a fully controlled experiment directed toward assessing the effects of binaural beat stimulation on cognition and mood in healthy adults. The present study employed a dichotic task that was hypothesized to produce a theta frequency binaural beat, as this has been shown to entrain brain phasic activity across the cerebrum with some evidence of a bias toward the left hemisphere and medial-dorsal brain regions (Jirakittayakorn & Wongsawat, 2017). However, as the beat follows the ascending auditory pathway through the ipsilateral midbrain to the contralateral auditory cortices (Oster, 1973), binaural beats also invoke activity in temporal lobe structures, which are probably the source of the cortical binaural beat (Pratt et al., 2009). This latter point of information is relevant, as the present study used a neurocognitive assessment battery predicted to be sensitive to alterations in frontotemporal functioning (i.e., fluency tasks), as well as measurements of current subjective mood states. It was hypothesized that 15 min to 25 min of exposure to theta frequency (i.e., 6 Hz) binaural beat stimulation carried on a 400-Hz tone would alter cognitive fluency or mood.

Method

Participants

A sample of 24 participants was recruited. Twenty-two were psychology undergraduate students of Universidad San Francisco de Quito (Ecuador), and two were master's students. Sixteen participated in return for course credits; the remainder received no compensation. All participants were screened for psychiatric or neurological illness prior to recruitment, as well as hearing problems and the use of any illicit substances during the previous 48 hr. The mean age of the recruited sample was 23.2 (SD = 2.89) and nine (37.5%) were male. On verbal report 21 (87.5%) were right-handed and the other three reported being left-handed.

Research Design

A double-blind repeated-measures experimental design was employed. Each participant took part in two counterbalanced test conditions, an experimental condition with binaural beat stimulation embedded within a piece of music, and a control condition involving the same piece of music, but with a similar embedded tone that would not produce a binaural beat. In both conditions, cognitive function and emotional responses were assessed during the auditory stimulation.

Binaural Beat Stimulation

In both the experimental and control conditions the participants listened to the same piece of music, which was a slow-paced instrumental track called "En Attendant Cousteau" by the artist Jean-Michel Jarre. This track was 47 min long and was played continuously from the beginning of each condition, and each condition was completed in approximately 30 min.

For the experimental condition, a binaural beat stimulation at 6 Hz (i.e., theta range) was inserted into the music track using the Audacity digital audio editing software package (www.audacityteam.org). This was achieved by adding a 400-Hz sinusoidal pure tone to the left channel and a 406-Hz sinusoidal pure tone to the right channel. When listened to dichotically, this would be expected to produce an intracerebral binaural beat of 6 Hz. For the control condition, Audacity was used to add sinusoidal pure tones of 403 Hz to both the left and right channels, and it is important to note that such a stimulation would not produce an intracerebral binaural beat. The stimulation paradigm is shown graphically in Figure 1. The added tones were all set to a gain of 13 dB. The compound music tracks were played via Audacity on a personal computer through a pair of stereo headphones. The music track was included in an attempt to mask the conscious perception of the binaural beat and so not to reveal or inadvertently bias the participant as to which condition contained the experimental auditory stimulus. The overall volume level in both conditions was set to

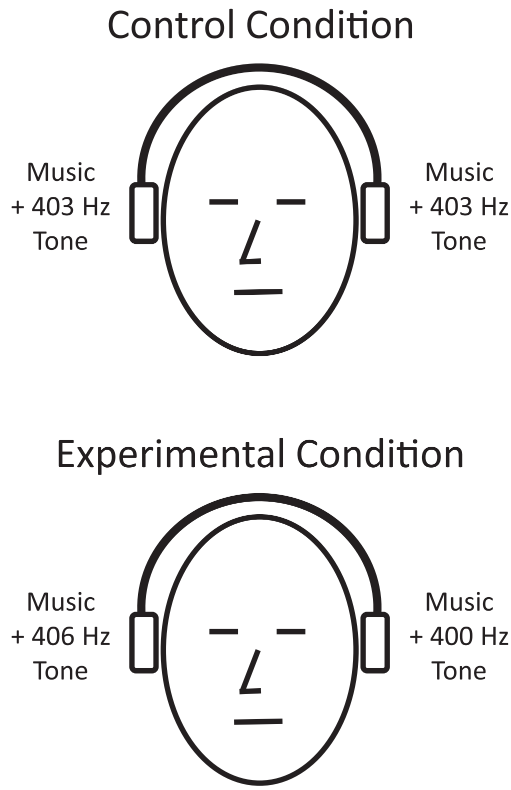

Figure 1. Details of the auditory stimulations in the control and experimental conditions.

50%, which was considered a loud, yet tolerable, intensity.

Assessment of Cognition and Mood

The assessment of cognitive function was focused particularly on fluency tasks, as such tasks are known to be among the most sensitive cognitive measures associated with factors that transiently alter one's neurological status. For example, statistically large effects on fluency task performance are associated with induced hypoglycemia (Graveling, Deary, & Frier, 2013), alcohol intoxication (Peterson, Rothfleisch, Zelazo, & Pihl, 1990), induced positive mood (Phillips, Bull, Adams, & Fraser, 2002), state anxiety (Buckelew & Hannay, 1986), and physical exercise (Chang, Labban, Gapin, & Etnier, 2012) among other factors. In addition, fluency tasks are well-known assessments of fronto-executive functions (Robinson, Shallice, Bozzali, & Cipolotti, 2012), and significantly involve language processing areas in the left temporal cortex (Baldo, Schwartz, Wilkins, & Dronkers, 2006; Kleibeuker, Koolschijn, Jolles, De Dreu, & Crone, 2013). The left temporal cortex was identified via EEG recordings as the probable source of the cortical binaural beat (Pratt et al., 2009). Finally, of the tests used, ideational fluency has already been shown to be affected by binaural beat stimulation (Reedijk et al., 2013). The present study assessed four different forms of fluency: phonemic, category, design, and ideational.

Phonemic fluency. Phonemic fluency involves participants saying words beginning with prespecified target letters within time-limits (Borkowski, Benton, & Spreen, 1967). The target is to produce as many different words as possible within one minute. To allow repeated testing, the experimental procedures were comprised of two parallel versions. This was done by piloting the letters *O*, *G*, *F*, *M*, *D*, *S*, and *E* on 11 participants. Analysis of this data revealed that a set comprised of the letters *O*, *D*, and *S* produced an equal number of words as the set comprised of the letters *E, G*, and *F* ($M_{O\text{-}D\text{-}S}$ = 12.21 and $M_{E\text{-}G\text{-}F}$ = 12.36 words per set, respectively). These two sets were therefore used for the repeat testing.

Category fluency. Category fluency is similar to phonemic fluency. However, participants are asked to produce unique words within specific semantic categories (Reitan & Wolfson, 1993). The present study used the same pilot sample as described above to define parallel sets of categories (i.e., from eight piloted categories). These were the categories of musical instruments, marine animals, and colors in one set, and of forms of transport, emotions, and land animals in the other set. These produced equivalent numbers of exemplars in the two sets ($M_{MI\text{-}MA\text{-}C}$ = 14.91 and $M_{FOT\text{-}E\text{-}LA}$ = 14.39, respectively) and so were used for the repeat testing in the main experiment.

Design fluency. Design fluency involves participants drawing as many different meaningless designs as possible within a time constraint (Jones-Gotman & Milner, 1977). The present study used the basic task (i.e., Trial 1) from the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplan, & Kramer, 2006). This version of the task is controlled because participants must join dots together. However, in the present study the rules were slightly altered, specifying that all designs must compose five lines (i.e., instead of four in the D-KEFS). This increased the number of potential designs and reduced the chance of participants recalling specific designs from the first to second test conditions.

Ideational fluency. The final test used was one of ideational fluency. This is also known as the Alternative Uses Test (AUT; Christensen, Guilford, Merriefield, & Wilson, 1960). The version used in the present study was the same format and scoring methods used in a recent neuropsychological study (Dahlman, Bäckström, Bohlin, & Frans, 2013). Briefly, each trial involved the participant providing alternative uses for a common object within a time-limit of one minute. All responses were recorded and then classified into categories of unique responses. The number of unique categories was the measure of interest. The following six objects were used: pencil, brick, barrel, shoe, car tire, and coat hanger (i.e., three trials in each test condition).

Mood assessment. Transient changes in mood were assessed with the Visual Analogue Mood Scales (VAMS; Nyenhuis, Yamamoto, Stern, Luchetta, & Arruda, 1997). These scales involve the participant bisecting a 100 mm line that has a neutral face stimulus and the word *neutral* at one pole, and a face stimulus showing an emotion and the word describing the emotion (e.g., *sad*) at the other pole. The bisection point is measured in millimeters from the neutral point. The moods measured by the VAMS are sad, afraid, tired, angry, confused, happy, energetic, and tense. These have been shown to be valid and reliable measures of current mood (Kontou, Thomas, & Lincoln, 2012; Nyenhuis et al., 1997). Visual analog scales, in general, are particularly useful for the measurement of dynamic and subjective states, and are frequently used to measure pain or mood in situations where rapid changes are anticipated (Wewers & Lowe, 1990). Therefore, the present study used a standard commercially published version (Stern, 1997). However, we changed the mood state words for Spanish equivalents: *triste*, *asustado*, *cansado*, *enojado*, *confundido*, *feliz*, *energético*, and *tenso*.

Procedure

Participants were tested individually in a quiet room. Prior to participation, all participants gave written informed consent in accordance with the ethics committee approved protocol. The participants were sat at a desk and given a practice session on each of the assessments immediately prior to the start of the experiment. This was done so that there would be no misunderstanding of the rules during the actual experiment. This preexposure to the tasks was also employed to minimize the practice-effect inherent in our repeated-measures design, as practice-effects on cognitive tests are greatest between the first and second performance, and tend to stabilize after the second performance (Falleti, Maruff, Collie, & Darby, 2006). Although any practice-effect would be neutralized by the counterbalanced conditions, they would add unwanted variation to the data if present.

When the first condition of the experiment began, participants put on a pair of high-fidelity headphones connected to a PC computer, and the lighting was dimmed. In each test session, two experimenters were present. One operated the delivery of the auditory stimulation, and the other administered the cognitive and mood assessments. This arrangement was used to maintain the double-blind procedure where neither the experimenter conducting the assessments nor the participant was aware of the condition (i.e., binaural beat stimulus or control condition). Once the auditory track was started, the participant sat still in a chair listening to the music for 15 min. Then, with the stimulation continuing, the experimenter held up written instructions to start the cognitive and mood assessment. The signs written on a piece of paper instructed the participant on what to do. This was done so that the auditory stimulation could be maintained throughout the cognitive and mood assessment without any further auditory distraction via verbal instructions.

The first assessment was category fluency (i.e., three different categories) and then phonemic fluency (i.e., three different initial letters). Next was the design fluency task. The page to complete was presented in front of the participant, and they were given a pen. Next, they performed the ideational fluency task. Finally, the pages from the VAMS were given to the participant to complete. After all of these tasks were completed, the music track was stopped and the headphones were removed. The participant was then given a 15-min break in which they left the room and spent their time as they wished.

When the participant returned, the second condition was performed. They now had either the control condition or the binaural beat condition, depending on which they did first. The counterbalancing and order of conditions is shown graphically in Figure 2. The procedure in the second condition was identical to that of the first condition. Each condition took around 30 min to complete. However, the category and phonemic fluency tasks were fully counterbalanced across the conditions. The design fluency task was identical in both conditions. For the ideational fluency task, there were six exemplars, which were allocated randomly for each participant, three to each condition.

After completion of the second condition, the participant was asked to say whether they detected a rapid regular beat from either or both of the tracks. This was used to determine whether the participants remained blind to condition. The experimenter was also asked to guess which of the conditions contained the binaural beat, again to assess their blindness to condition.

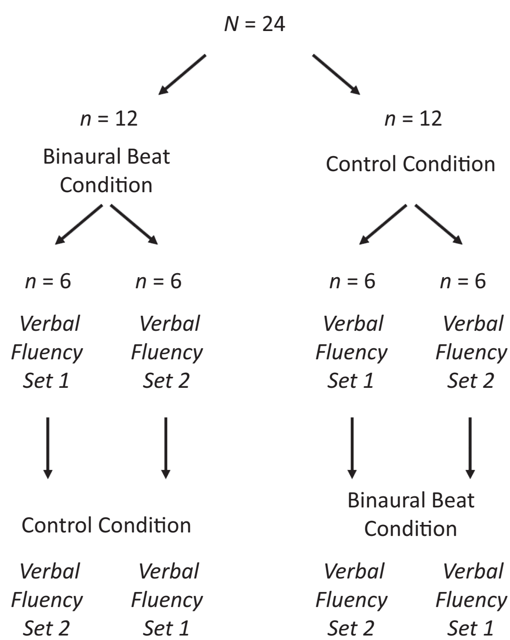

Figure 2. The design of the experiment and the counterbalancing procedures.

Results

For the category and phonemic fluency tasks, the total number of unique words were counted and a mean calculated for each task. For design fluency, the total number of unique designs was counted. For ideational fluency, the total number of unique categories of alternative uses within each trial were counted and then the total of these uses across the three trials was calculated. For the VAMS, the distance in millimeters from the neutral point was measured for each of the eight moods. The scores on all these variables were assessed for normal distributions with Kolmogorov–Smirnov one-sample tests. Where distributions in neither condition differed significantly from normal, parametric paired-sample *t* tests were used to compare the conditions. Otherwise, nonparametric Wilcoxon's sign-rank tests were employed. All analyses were two-tailed with a significance threshold of $\alpha = .05$ and a confidence interval of 95%. Estimates of effect sizes were given as Cohen's *d*.

The mean scores for the different fluency measures are shown in Figure 3. There were no significant between-condition differences for any of category fluency, $t_{(23)} = 0.254$, $p = .801$, $d = 0.052$, phonemic fluency, $t_{(23)} = -.532$, $p = .600$, $d = 0.108$, design fluency, $t_{(23)} = -.489$, $p = .630$, $d = 0.010$, or ideational fluency, $t_{(23)} = .515$, $p = .612$, $d = 0.105$. Therefore, the binaural beat appeared to have no effect on cognitive fluency performance.

The mean scores for the different mood ratings on the VAMS are shown in Figure 4. There was a significant difference between scores with and without binaural beats for the ratings of fear, participants reported more fear when listening to the binaural beats track than the control track (Wilcoxon's signed-rank test, $Z = 36.000$, $p = .017$, $d = 0.572$). However, there were no significant differences for the moods of sad (Wilcoxon's signed-rank test, $Z = 118.000$, $p = .627$, $d = 0.161$); tired, $t(23) = .479$, $p = .636$, $d = 0.098$; angry (Wilcoxon's signed-rank test $Z = 97.500$, $p = .779$, $d = 0.220$); confused (Wilcoxon's signed-rank test, $Z = 13.000$, $p = .648$, $d = 0.149$; *happy*, $t_{(23)} = .568$, $p = .576$, $d = 0.116$; energetic (Wilcoxon's signed-rank test, $Z = 129.500$, $p = .922$, $d = 0.070$); or tense (Wilcoxon's signed-rank test, $Z = 106.000$, $p = .330$, $d = 0.191$).

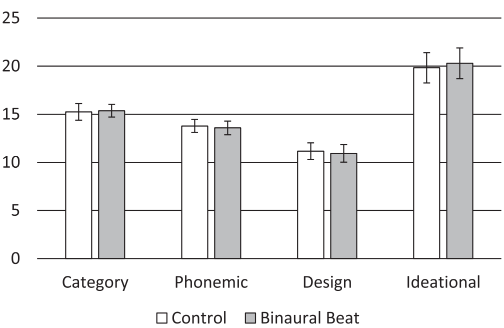

Figure 3. Mean scores (plus standard errors) for cognitive fluency performance under theta binaural beat stimulation and control conditions.

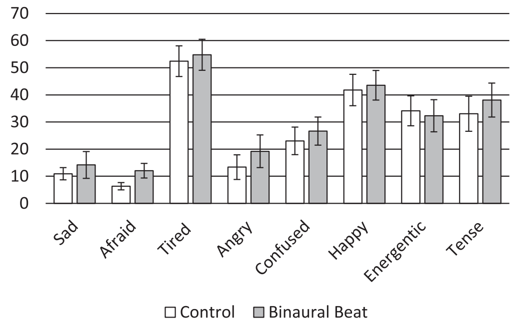

Figure 4. Mean scores (plus standard errors) for mood states under theta binaural beat stimulation and control conditions.

Finally, to establish whether the participants had been blind to condition, their responses to the question of whether they detected a rapid beat within either of the audio tracks were analyzed. Fourteen of the 24 participants reported hearing a rapid beat. However, six stated that they heard it in the actual binaural beat condition, while five stated they heard it in the control condition. The other three stated that they had detected it in both conditions. Clearly, the participants remained blind to the test conditions. Furthermore, the experimenter's ability to tell which condition contained the binaural beat was analyzed. It was determined that the experimenter was able to correctly identify the condition with the binaural beat for only 11 of the 24 participants (i.e., which was below the chance factor). It was therefore concluded that the experimenter also remained blind to conditions throughout the experiment.

Discussion

In the current research, neither the experimenter nor the participants were aware of which condition contained the experimental factor. Therefore, the double-blind procedure used in this repeated-measures experiment was well-executed. Nevertheless, there was a significant increase in participants reporting feelings of fear during the 6 Hz (i.e., theta range) binaural beat stimulation compared with the control condition. Therefore, it appears that theta frequency binaural beat stimulation can induce a mild fear-like response.

In contrast, the current study was unable to induce any changes in cognitive function, as measured by the various fluency tasks. Several other authors have reported cognitive effects. In one study, acute stimulation with binaural beats at theta frequency (i.e., 7 Hz) produced an impairment in verbal short-term memory (Wahbeh et al., 2007). In contrast, other research has suggested that gamma stimulation (i.e., 40 Hz) may enhance some aspects of episodic memory (Colzato et al., 2017). Furthermore, either gamma (i.e., 40 Hz) or alpha (i.e., 10 Hz) stimulation have been shown to enhance fluency performance in some people (Reedijk et al., 2013). The current research was exploratory and the potential reason that our stimulation had no effect may simply be that theta frequency stimulation does not affect fluency performance.

Alternatively, the present data are broadly consistent with a previous study regarding the induction of negative mood with theta frequency binaural beats. A study that used a similar design to ours reported a significant increase in depression with theta frequency (i.e., 7 Hz) binaural beat stimulation (Wahbeh et al., 2007). Although in the present study only fear was significantly higher in the binaural beat condition, it was observed in Figure 4 that other negative moods were raised, including sadness and anger, but the changes did not reach statistical significance.

The mechanism for fear induction in the current paradigm is not clear, although a possible explanation is that the lateral amygdala is thought to be a principal area involved with fear conditioning (Pape, Narayanan, Smid, Stork, & Seidenbecher, 2005; Seidenbecher, Laxmi, Stork, & Pape, 2003). As mentioned previously, binaural beats can be recorded early in the auditory pathway within the inferior colliculus (Kuwada et al., 1979), which has its main ascending connections to the medial geniculate body (MGB; Casseday, Fremouw, & Covey, 2002). Projections from the MGB are mainly to the primary auditory cortex but also to the amygdala, and there are also connections from the primary auditory cortex directly to the amygdala (Lee, 2013). There are therefore two main routes for auditory signals to reach the lateral amygdala, one directly and the other via the primary auditory cortex in the temporal lobe (LeDoux, 1995). These routes seem to subserve fear conditioning to auditory stimulation. Lesion studies with rodents suggest that either pathway can process auditory fear conditioning if the other pathway is damaged (Romanski & LeDoux, 1992). However, it seems that in the intact brain the default projection for auditory fear conditioning is the MGB-auditory cortex-amygdala route (Boatman & Kim, 2006). In fact, the primary auditory cortex may be more than a relay of auditory information to the amygdala and probably has a direct role in fear learning (Grosso, Cambiaghi, Concina, Sacco, & Sacchetti, 2015). In accordance with this, the synapses between the amygdala and primary auditory cortex show signs of long-term potentiation during auditory fear conditioning (Tsvetkov, Carlezon, Benes, Kandel, & Bolshakov, 2002).

Interestingly, normal fear processing in the amygdala is associated with theta rhythms, which may act to synchronize activity with other brain regions involved with learning (Pape et al., 2005; Seidenbecher et al., 2003). Thus, it seems likely that artificially induced theta frequency beats in the auditory pathway could reach the amygdala and perhaps stimulate theta activity. This provides a plausible mechanism as to why, in the current study, theta frequency auditory stimulation was able to induce a fear response. Nevertheless, the current research did not investigate a clear mechanism of action, and as such, the explanation of the current results is merely a theoretical possibility.

Although induction of fear is not of obvious therapeutic use, in that it would be particularly useful to be able to reduce negative moods rather than induce them, there may be some unique and novel applications that could be developed from such theoretical premises. However, the present study is limited, as its results require replication and further validation as a proof of concept. However, if they were to be replicated, then fear induction could be potentially used experimentally to study human emotions in the laboratory. Anxiety is a serious and common mental illness characterized by generalized unease, worry, and fear. Experimentally, clinical anxiety has been linked to fear conditioning that probably involves an overlapping set of underlying neural mechanisms within the ventral prefrontal cortex and the amygdala (Indovina, Robbins, Nunez-Elizalde, Dunn, & Bishop, 2011). However, current methods for studying fear conditioning in humans are limited for ethical reasons. Current methods of anxiety induction involve, for example, threat of electric shock, but this only partly models the clinical presentation of anxiety (Robinson, Letkiewicz, Overstreet, Ernst, & Grillon, 2011). As the binaural beat influenced fear responses without the stimulation being perceived, this could be a potential alternative to threat of shock in experimental anxiety induction and could help to elucidate the role of fear conditioning in real-life human problems such as the anxiety disorders.

That the binaural beat stimulation we used could induce fear without participants being able to distinguish between the binaural beat and control conditions, is consistent with past research that suggests fear-inducing stimuli can be processed unconsciously. In fact, the initial stages of threat processing are probably always automatic, involuntary and unconscious (Beck & Clark, 1997). Studies with functional MRI with pattern-masked face stimuli designed to induce fear at an unconscious level have revealed activations in the basolateral subregion of the amygdala, while unmasked stimuli that are consciously processed activate the dorsal amygdala (Etkin et al., 2004). This suggests a distinction between dorsal and basolateral amygdala fear processing depending on whether the processing is conscious or not. An earlier study with positron emission tomography had suggested that there may also be a lateralized effect related to conscious or unconscious processing. With classical conditioned fear responses to faces, exposure to the pattern-masked faces (processed unconsciously) activated the right amygdala, while exposure to faces without pattern masks (consciously processed) activated the left amygdala (Morris, Ohman, & Dolan, 1998). These imaging studies are generally consistent with the results of the current study, discussed in the preceding text, in which it is tentatively hypothesized that theta frequency binaural beats may induce fear via stimulation of the amygdala.

Nevertheless, the current results should be treated with caution until confirmed by replication and subsequent experimental validation. One issue is that this is an entirely behavioral study, in that no neurophysiological recordings were made to show that the binaural beat stimulation had a measurable influence on the brain. The conclusions concerning the effects of the stimulation were all based on the participants' self-reported feelings of fear. A further issue was the possibility of Type I errors in the results. The present study found only one statistically significant effect among 12 comparisons. The experimenters opted to keep the standard significance threshold of $\alpha = .05$ (two-tailed) rather than to use a corrected threshold in order to maximize power in this essentially small-sample exploratory study. Replication with a larger sample would provide stronger confirmation or rejection of the potential for mood induction with theta frequency binaural beat stimulation. On the other hand, the current results are from a fully controlled experimental study. Conditions and stimuli were counterbalanced, and the research was performed using a well-controlled double-blind repeated measures procedure. As an experimental procedure in which potential confounding variables were closely controlled, the most likely interpretation of the observed data is that theta binaural beats did indeed induce fear in the participants.

It is concluded therefore that theta binaural beat auditory stimulation may be able to induce a mild fear response in listeners although with no detectable changes to cognitive process as measured by fluency tasks. This finding may have implications for the understanding of fear processing in the brain and provide a potential human model of fear induction that could be used to study fear conditioning and anxiety in healthy human participants.

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