

1. Title Page

Title: Alpha-Range Visual and Auditory Stimulation reduces the Perception of Pain

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Category: The manuscript is being submitted as an original article

Funding: The work was part of a self-funded PhD project

Conflict of Interest: There were no financial or other relationships that might lead to a conflict of interest

What does this study add?

This study provides new behavioural evidence showing that visual and auditory entrainment of frequencies in the alpha-wave range can influence the perception of acute pain in humans.

2. Abstract

Background: Alpha power is believed to have an inverse relationship with the perception of pain. Increasing alpha power through an external stimulus may therefore induce an analgesic effect. Here, we attempt to modulate the perception of a moderately painful acute laser stimulus by separately entraining three frequencies across the alpha band: 8Hz, 10Hz and 12Hz.

Methods: Participants were exposed to either visual or auditory stimulation at three frequencies in the alpha-band range and a control frequency. We collected verbal pain ratings of laser stimuli from participants following ten minutes of flashing LED goggle stimulation and ten minutes of binaural beat stimulation across the alpha range. Alterations in sleepiness, anxiety and negative mood were recorded following each auditory or visual alpha-rhythm stimulation session.

Results: A significant reduction in pain ratings was found after both the visual and the auditory stimulation across all three frequencies compared to the control condition. In the visual group, a significantly larger reduction was recorded following the 10Hz stimulation than succeeding the 8Hz and 12Hz conditions.

Conclusions: The present study suggests a short presentation of auditory and visual stimuli, oscillating in the alpha range, have an analgesic effect on acute laser pain, with the largest effect following the 10Hz visual stimulation. Pain reductions following stimulation in the alpha range are independent of sleepiness, anxiety and negative moods.

3. Introduction

The alpha rhythm (7-14Hz) is the most studied frequency band in the human brain, as it can be detected in 95% of healthy young adults with their eyes closed (Srinivasan, 1999). The alpha rhythm has historically been described as an 'idling' rhythm and was believed to represent low information processing. However, more recent work indicates a central role in cognitive processing, specifically the top-down control of sensory information (Klimesch et al., 2007). Suppression of alpha power in the contralateral sensorimotor and occipital cortices has been repeatedly found to be correlated with the strength of a painful stimulus (Hu et al., 2013; Mouraux et al., 2003; Ohara et al., 2004; Peng et al., 2014; Ploner et al., 2006; Raij et al., 2004). Importantly, behavioural pain intensity ratings have been directly correlated with decreases in alpha power (Babiloni et al., 2006; Gross et al., 2007; Mouraux et al., 2003).

Early work by Trifiletti et al, observing that high alpha was associated with intense analgesia, alluded to the idea that this relationship may work both ways (Trifiletti, 1984). The concurrent presence of high alpha power during analgesia could indicate a causal relationship. Alpha rhythms are believed to arise from the thalamus, and subsequently transmitted through thalamo-cortical tracts to the cortex. Alpha rhythms can hence be influenced via inputs to the thalamus, synchronising or desynchronising alpha oscillations (Schmidt et al., 1985). Recent neurofeedback

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studies have developed this idea and confirmed brain training to increase alpha power can lead to a long-term reduction in chronic pain (Jensen et al., 2013). The main disadvantage of neurofeedback is that it takes concentration and often weeks of training to be effective and thus is currently ineffective for acute pain (Kayiran et al., 2010).

Visual and auditory entrainment enables almost immediate increases in cortical alpha power through an external pulse with a consistent frequency oscillating in the alpha range (Frederick et al., 2005; Spaak et al., 2014). Entrainment occurs when other regions of the brain fall into lockstep with the stimulated cortex, eliciting a broader increase in alpha power (de Graaf et al., 2013; Halbleib et al., 2012; Spaak et al., 2014; Thut et al., 2012).

While visual alpha entrainment primarily affects the primary visual cortex with the strongest resonance at 10Hz (de Graaf et al., 2013; Herrmann, 2001), literature suggests that modulations in cortical activity are widely elicited throughout the cortex (de Graaf et al., 2013; Timmermann et al., 1999). Although resting EEG records maximal alpha amplitude over the occipital regions (Cantero et al., 2002), entrainment through auditory stimulation proves just as effective at increasing alpha power (Karino et al., 2006; Schwarz and Taylor, 2005). Both visual and auditory alpha entrainments thus prove promising candidates for effortless acute pain relief.

In the current study, we entrained three different alpha frequencies (8Hz, 10Hz and 12Hz) with the aim to achieve a meaningful level of experimental pain relief through either auditory or visual alpha entrainment. We hypothesized that we would observe the largest reduction in pain ratings after the 10Hz stimulation in both the visual and auditory studies, as this is closest in frequency to the average peak of the spectral distribution of the alpha rhythm (Klimesch, 1997; Posthuma et al., 2001).

4. Methods

4.1.1. Ethics statement

All volunteers provided written, informed consent according to the International Conference on Harmonisation Good Clinical Practice guidelines, before participating in the study. The study obtained ethical approval from the NRES Committee North West – Liverpool Central (reference number 13/NW/0007).

4.1.2. Experimental design

Participants were divided into two experimental groups: auditory and visual. Participants allocated to the auditory entrainment group were asked to visit Salford Royal NHS Foundation Trust on two separate visits. The auditory group's visits consisted of a separate entrainment visit and a control visit, the order of which was randomised. A minimum of two weeks was left between the visits to allow the skin to fully recover in case of mild sensitivity outlasting the first visit. Due to time constraints, the visual entrainment study was condensed into one visit with fewer

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overall pain sessions and a single control condition. This was deemed suitable, as participants were not aware of the purpose of the study, and because the study was not designed to make direct (quantitative) comparisons between visual and auditory entrainment. As such, we did not balance certain variables between the auditory and visual experiments, such as salience of the stimuli in each modality.

A diagram of the experimental procedure can be seen in Figure 1.

4.1.3. Participants

Sixty-four healthy, (33 male, average age 24.65 ± 8.2 SD), self-reported right-handed volunteers were invited to Salford Royal NHS Foundation Trust to participate in the study. All participants volunteered for the study after contacting the group through advertisements placed on the University of Manchester website and throughout Salford Royal NHS Foundation Trust. Volunteers in both groups were provided with a participant information sheet a minimum of 24 hours prior to the first visit. Participants were provided with a verbal and written explanation of the laser pain applied during the study, without revealing the aims and objectives of the study. All volunteers were aged 18 years or older. Participants self-reported themselves as free from a history, or family history of epilepsy, pain, morbid psychiatric illness, neurological illness, ischemic heart disease, uncontrolled high blood pressure, peripheral vascular disease, chronic skin disease (e.g. eczema, psoriasis) and hypertension not controlled by medication. After providing written and verbal consent, volunteers were randomly assigned to either the auditory or the visual entrainment group. 32 Volunteers were allocated to the auditory entrainment group (16 Male, mean age 23.25 ± 7.9 SD) and 32 to the visual entrainment group (17 Male, mean age 25.82 ± 8.6 SD).

4.1.4. Pre-experimental Psychophysics procedure

It has been demonstrated that a contactless activation of nociceptors related to A δ and C fibres can be achieved through the use of a brief CO₂ laser stimulus (Meyer et al., 1976). In this study, the pain stimulus consisted of a CO₂ laser stimulus of 150ms duration and a beam diameter of 15mm. This laser was applied to the dorsal surface of the volunteers' right forearm, firing once every 10 seconds. The laser beam was moved to a new location after every pulse to avoid sensitisation, habituation or damage to the skin. It was obligatory for participants to wear a pair of safety goggles whenever the laser was in use.

Each visit was initiated with the calculation of the participants' moderately painful level with the aid of a 0-10 numeric rating scale. Level 0 on the scale was marked as 'no sensation', level 4 represented the pain threshold, and level 10 was marked as the maximum amount of pain they believed they could tolerate. Participants were told to regard the sensation halfway between pain threshold and their tolerance level as 'moderately painful', identified as the number 7 on the pain scale. A ramping procedure with increasingly powerful laser stimuli was initiated, during which the participants were asked to verbally rate each pulse until their level 7 was attained.

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This entire procedure was repeated three times. Ratings of the laser intensity levels were then tested by repeating a series of laser pulses at the volunteers' predetermined level 7. The laser voltage was readjusted if a level 7 was not consistently attained.

4.1.5. Pre-experimental Questionnaires

After determining volunteers' level 7 on the 0-10 numeric rating scale, participants were asked to complete a set of behavioural questionnaires previously demonstrated to correlate with changes in acute laser pain (Brown et al., 2008; Morton et al., 2009) or believed to be modulated by changes in alpha power (Melzack and Perry, 1975; Ossebaard, 2000). These consisted of the Profile of Mood States (POMS), the State-Trait Anxiety Inventory (STAI), the Karolinska Sleepiness Scale (KSS), Participant Sleep Questionnaire, Pain Catastrophizing Scale (PCS), Patient Health Questionnaire - 9 (PHQ-9) and the Pain Anxiety Symptoms Scale (PASS). The Participant Sleep Questionnaire, PCS, PHQ-9 and PASS were used to determine the volunteers' quality of sleep, degree of pain related catastrophic thinking, depression and pain specific fear and anxiety respectively. These were given once at the beginning of each visit. The POMS, STAI-state, and KSS were repeated during the experiment after each of the pain assessment trials and are explained in more detail below.

4.1.6. Profile of Mood States (POMS)

Nine items representing negative moods were taken from the Profile of Mood States (POMS; McNair et al., 1971) to determine participants' degree of emotional distress, as previously described (Brown et al., 2008; Sullivan et al., 2001). The nine emotions were divided to represent three different mood categories: (1) sadness (sad, discouraged, hopeless); (2) anger (angry, hostile, irritable); and (3) anxiety (anxious, tense, worried). Participants were asked to rate the intensity of each of the 9 adjectives on a 5-point Likert scale with 0 representing 'not at all' and 4 'very much', in relation to their current emotional state. A composite score of emotional distress was computed by taking the sum of all nine items on the mood scale. Participants received the POMS before the start of the experiment and after each of the pain assessment trials.

4.1.7. State-Trait Anxiety Inventory (STAI)

The STAI consists of 20 items assessing trait anxiety and 20 items assessing state anxiety. The state and trait assessments of the inventory were presented to the volunteers separately. The state anxiety inventory evaluates temporary nervousness, fear, discomfort etc. (i.e. the arousal of the autonomic nervous system), whereas the trait inventory measures prolonged feelings of stress, worry or discomfort. The items on both inventories are rated on a 4-point Likert scale (from "Almost Never" to "Almost Always"). A higher overall score indicates greater anxiety (Spielberger et al., 1983). Participants received the trait inventory once in each visit, at the start of the experiment. The state inventory was given after each of the pain stimulus

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assessment trials. The Inventory was used to assess whether visual or auditory alpha entrainment influenced participants' present state of anxiety, and if so, whether it correlated with changes in pain perception.

4.1.8. Karolinska Sleepiness Scale (KSS)

A 9-point KSS was used where 1=very alert, 3=alert, 5=neither alert nor sleepy, 7=sleepy (but not fighting sleep) and 9=very sleepy (fighting sleep) (Akerstedt and Gillberg, 1990). Participants were asked to rate the scale once, preceding the experiment and once after each pain assessment trial. This was done to control for any changes in the volunteers' alertness, and subsequent potential influence on pain perception.

4.1.9. Pre-experimental trial

Participants were asked to rate 30 pulses on the pain rating scale, at 10-second intervals, of their predetermined 'moderately painful' level 7, in order to document their average baseline pain ratings.

4.1.10. Auditory entrainment

Volunteers allocated to the auditory stimulation group attended two visits in a randomised order, one control and one entrainment visit. During the entrainment visit, volunteers were subjected to 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz in a randomised order. Binaural Beats were employed to enable auditory alpha entrainment. The hearing range for a healthy adult is between 20Hz-20,000Hz, thus frequencies at 8Hz, 10Hz and 12Hz are not audible to the human ear. Binaural beats are produced when two tones close in frequency generate a beat frequency equal to the difference in frequency of the two tones (Wahbeh et al., 2007). For example, volunteers in the present study listened simultaneously to 445Hz played in one earphone, and 455Hz played in the other, producing a binaural beat frequency of 10Hz. Binaural beats are believed to originate in the brainstem's superior olivary nucleus, where the contralateral auditory input is integrated (Oster, 1973).

Binaural beats were produced using BrainWave Generator software version 3.1.12 (<http://www.bwgen.com/>) as used by (Goodin et al., 2012). As binaural beats are known to be entrained most readily with a carrier frequency ranging from 300Hz to 600 Hz (Reedijk et al., 2013) with the greatest effect between 450-500Hz (Oster, 1973; Perrott and Nelson, 1969), all entrainment sessions utilised a 450Hz carrier frequency. Presenting 446Hz and 454Hz, 445Hz and 455Hz, 444Hz and 456Hz tones in the left and right ears created 8Hz, 10Hz and 12Hz binaural beats respectively. All frequencies were presented to participants at 70 dB SPL, as previously done by Stevens and colleagues (Stevens et al., 2003). Participants were asked (at the end of their involvement in the study) whether they were able to differentiate between the 8Hz, 10Hz and 12Hz frequency entrainment. None claimed to be able to do so.

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After each randomised 10-minute auditory entrainment session, participants were asked to rate 30 painful heat laser pulses, and subsequently requested to complete the POMS, KSS and STAI-state, in relation to the entrainment session.

The control visit was identical to the entrainment visit, except that the volunteers listened to white noise for 10 minutes, three times, instead of 8Hz, 10Hz and 12Hz binaural beats. Binaural beats are a relatively high in saliency stimulus. As such, the carrier frequency was not used for the control stimulus: listening to a constant tone at 455 Hz is not as salient as a tone that fluctuates, and therefore would risk participants becoming habituated to the sound. This would introduce an uncontrolled, confounding variable in comparing the conditions of interest with the control condition. By contrast, the stimulation frequency when listening to white noise is constantly changing over time, resulting in a higher saliency stimulus, and therefore a better comparator to binaural beats.

4.1.11. Visual entrainment

Participants in the visual entrainment group were subjected to four randomised visual entrainment sessions at 8Hz, 10Hz, 12Hz and 1Hz (control), each lasting a total of 10 minutes. The visual stimulus consisted of a pair of in-house made flashing LED goggles. Volunteers kept their eyes closed throughout the stimulation, as this is just as effective, but more pleasant for the volunteer. After each one of the visual entrainment sessions, volunteers were subjected to 30 moderately painful laser pulses at their predetermined level 7, and asked to rate these on the 0-10 numerical rating scale. Following all four pain-rating trials, volunteers were asked to complete the KSS, STAI-state and POMS. Instructions included attempting to relate the questionnaires to the preceding entrainment session, rather than post pain rating trial. Again, on completion of the study, the aim of the study was revealed to the participants and participants were asked if they were able to differentiate between the 8Hz, 10Hz and 12Hz frequency entrainment. Again, none claimed to be able to do so.

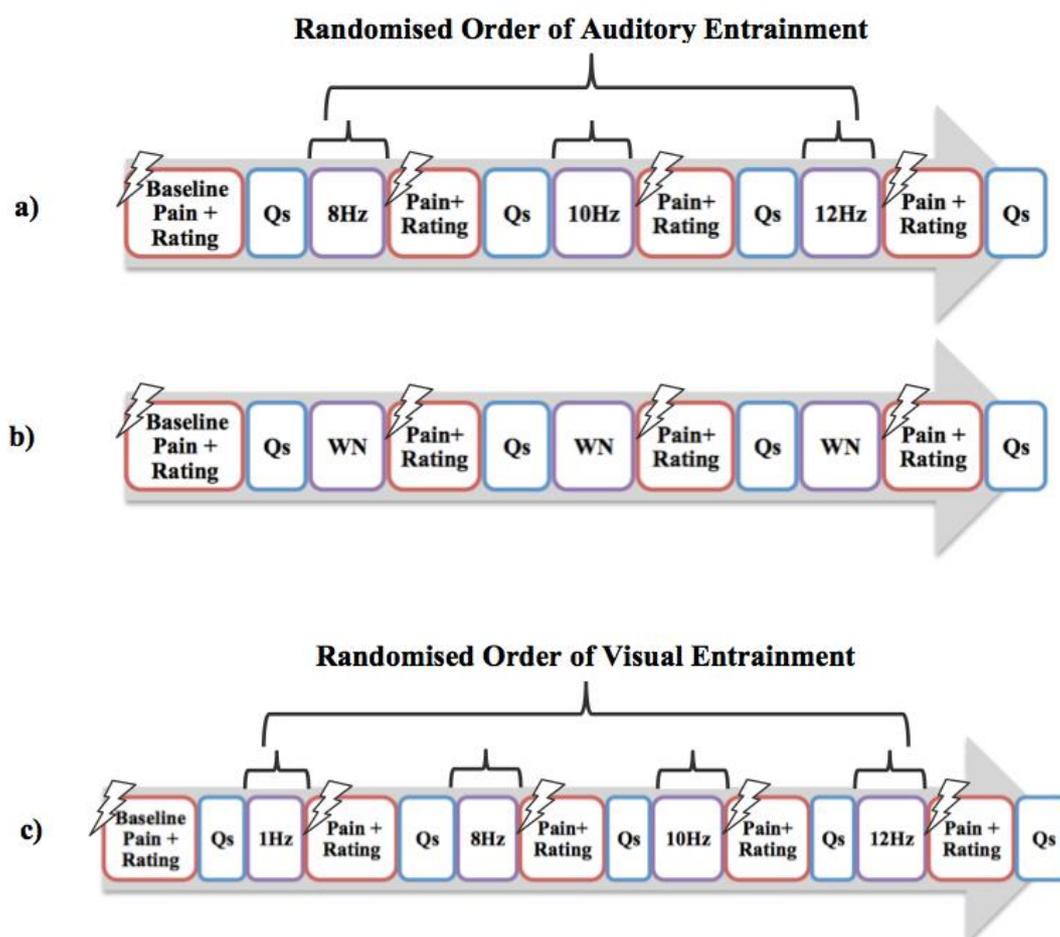


Figure 1a) (Entrainment Visit) and 1b) (Control Visit). Procedure in the Auditory Entrainment Group. Participants in the auditory group attended two visits (control and entrainment), in a randomised order. Both visits were initiated with baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS) and the rating of 30 laser pulses at ‘level-7’ pain. In the entrainment visit, participants were subjected to 10 minutes of auditory entrainment at 8Hz, 10Hz and 12Hz in a randomised order. Following entrainment, participants rated 30 pulses, and completed the POMS, KSS and STAI-state questionnaires. The control visit was identical to the entrainment visit, but the stimulus was 10 minutes of white noise, three times, instead of alpha entrainment. **1c) Procedure in the Visual Entrainment Group.** A single visit was required for participants in the visual entrainment group. Participants completed baseline questionnaires (Sleep Questionnaire, PCS, PHQ-9, PASS, POMS, STAI, KSS) and 30 pulses of their ‘level-7’ pain. Participants were subjected to four visual (flashing LED goggle) entrainment sessions at 8Hz, 10Hz, 12Hz and 1Hz (control), each 10 minutes long, in a randomised order. Following entrainment, volunteers were subjected to 30 ‘level-7’ pulses and were asked to rate these on a 0-10 numerical rating scale. Following each pain rating session, volunteers completed the KSS, STAI-state and POMS. **Qs** = Questionnaires **WN** = White Noise

4.1.12. Data Analysis

Statistical analysis was performed using SPSS version 20. A p value of less than 0.05 was considered significant. The average pain rating for each subject and trial was calculated. We applied a mixed linear model to pain ratings of the 8Hz, 10Hz, 12Hz and control condition of both groups to assess the size of the change in pain ratings compared to control. This model took into account the baseline pain ratings as a covariate and the frequency entrained ('treatment'), the order of the entrainment session ('treatment order'), and for the auditory group, the order of the control/entrainment visit ('session') as factors. Using each condition as a reference category, the model was refitted with a Bonferroni correction to assess the significant differences. The same model was applied separately to the POMS, STAI and KSS scores, taking into account the same respective covariates and factors. The baseline scores of the PHQ-9, PCS, Participant Sleep Questionnaire and PASS were correlated to the changes in pain ratings from baseline in the 8Hz, 10Hz, 12Hz and control condition.

5. Results

The largest reduction in pain ratings from the control condition could be observed after the 10Hz entrainment session in both the auditory and visual groups, followed by the 8Hz then the 12Hz condition. There were no significant changes or correlations observed in the questionnaire scores.

5.1.1. Pain Ratings – Auditory Entrainment Group

Taking into account baseline pain ratings as a covariate and the stimulation frequency, order of entrainment, and visit order as factors, the mixed linear model calculated that pain ratings succeeding the 8Hz, 10Hz and 12Hz entrainment conditions were all significantly different from all three control conditions ($t(31) = 4.90, p < 0.001$; $t(31) = 5.61, p < 0.001$; $t(31) = 4.85, p < 0.001$, respectively). Adjusted mean pain ratings following entrainment were respectively 0.51(SE 0.10), 0.58 (SE 0.10) and 0.5 (SE 0.10) points lower than the control on the numeric ratings scale (Figure 2). No significant difference was detected between the three auditory entrainment conditions when refitting the model with a Bonferroni correction.

Table 1a

| Average Absolute Pain Ratings – Auditory Condition | | | | | |
|--|----------|---------|------|-------|-------|
| Condition | Baseline | Control | 8 Hz | 10 Hz | 12 Hz |
| Absolute Values | 6.74 | 6.62 | 6.08 | 6.05 | 6.08 |

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Table 1b.

| Adjusted Pain Ratings – Auditory Condition vs. Control | | | | | |
|--|----------------------|----------------|------|-------------|-------------------------|
| Condition | Adjusted Differences | Standard Error | t | Effect Size | Significance (2-tailed) |
| 8Hz | 0.51 | 0.10 | 4.90 | 0.76 | $p < 0.001$ |
| 10Hz | 0.58 | 0.10 | 5.61 | 0.80 | $p < 0.001$ |
| 12Hz | 0.51 | 0.10 | 4.85 | 0.77 | $p < 0.001$ |

Table 1a shows the average absolute pain ratings at baseline, following white noise control stimulation, and following the 8Hz, 10Hz and 12Hz auditory entrainment. **Table 1b** reveals the adjusted pain ratings, taking into account baseline pain ratings as a covariate and the stimulation frequency, order of entrainment, and visit (control vs. entrainment) order as factors. Pain ratings succeeding the 8Hz, 10Hz and 12Hz entrainment conditions were all significantly different from all three control conditions. No significant difference was detected between the three auditory entrainment conditions when refitting the mixed linear model with a Bonferroni

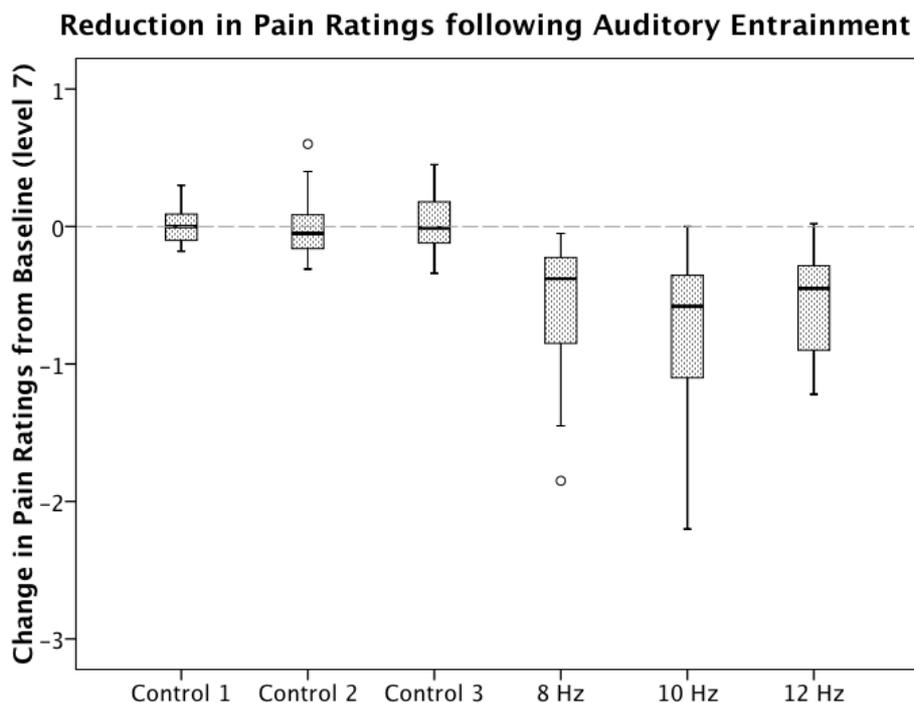


Figure 2 Auditory Group - Change in Pain Ratings. Boxplot displaying the change in average pain ratings from baseline across subjects in each condition. Applying the mixed linear model revealed that pain ratings following 8Hz, 10Hz and 12Hz entrainment were all significantly different from all three control conditions. The dark horizontal line within the box indicates the median, the box length indicates the interquartile range, the whiskers represent the highest and lowest values of the results and the outliers are represented by circles. The horizontal line at 0 indicates the level-7 baseline pain.

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5.1.2. Pain Ratings – Visual Entrainment Group

The mixed linear model established that, accounting for covariates and factors, the pain ratings in the entrainment conditions (8Hz, 10Hz and 12Hz) were all significantly different from control ($t(31)=2.28$, $p<0.01$; $t(31)=5.32$, $p<0.001$; $t(31)=2.59$, $p<0.01$ respectively). The model-corrected pain ratings of the 8Hz, 10Hz and 12Hz conditions were on average 0.6, 1.1 and 0.3 points lower on the pain rating scale than the control, respectively. Additionally, when refitting the model, pain ratings were significantly different in the 10Hz condition compared to the 8Hz ($t(31)=2.22$ $p<0.01$) and 12Hz ($t(31)=4.04$, $p<0.001$) condition. The 8Hz and the 12Hz conditions did not differ from each other ($p=0.287$) (Figure 3).

Table 2a.

| | | | | | |
|------------------------|------|------|------|------|------|
| Absolute Values | 6.68 | 6.52 | 5.94 | 5.52 | 6.17 |
|------------------------|------|------|------|------|------|

Tabel 2b.

| Adjusted Pain Ratings – Visual Condition vs. Control | | | | | |
|---|----------------------|----------------|------|-------------|-------------------------|
| Condition | Adjusted Differences | Standard Error | t | Effect Size | Significance (2-tailed) |
| 8Hz | 0.60 | 0.20 | 2.28 | 0.59 | $p<0.01$ |
| 10Hz | 1.12 | 0.18 | 5.32 | 1.01 | $p<0.001$ |
| 12Hz | 0.35 | 0.09 | 2.59 | 0.70 | $p<0.01$ |

Table 2a shows the average absolute pain ratings at baseline, following 1Hz control stimulation, and 8Hz, 10Hz and 12Hz visual entrainment. **Table 2b** reveals the mixed linear model adjusted pain ratings, taking into account baseline pain ratings as a covariate and the stimulation frequency and order of entrainment as factors. Pain ratings following all three entrainment conditions were significantly lower than control. When refitting the model, pain ratings were significantly lower in the 10Hz condition compared to the 8Hz ($t(31)=2.22$ $p<0.01$) and 12Hz ($t(31)=4.04$, $p<0.001$) conditions. The 8Hz and the 12Hz conditions did not differ from each other ($p=0.287$).

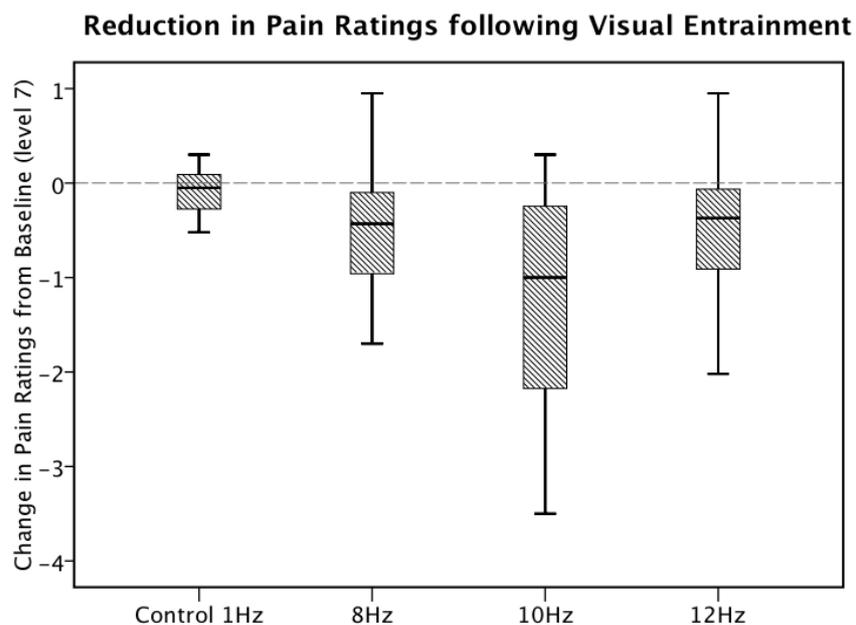


Figure 3 Visual Group - Change in Pain Ratings. Boxplot displaying the change in average pain ratings from initial baseline ratings. Applying the mixed linear model revealed that pain ratings in the entrainment conditions were all significantly different from control with the largest reduction succeeding the 10Hz entrainment. The dark horizontal lines represent the mean, the boundaries of the box indicate the 25th- and 75th-percentiles, the whiskers the 5th- and 95th-percentiles, and the horizontal line across the origin (0) indicates the level-7 baseline pain.

5.1.3. Questionnaire Results

A mixed linear model was applied to the KSS, POMS and STAI–State scores of the 8Hz, 10Hz, and 12Hz condition comparing them to the control scores. The model revealed no significant score changes compared to the control condition in both the visual and auditory groups. The pre-experimental questionnaires showed no significant correlation to the change in pain ratings from baseline across all three conditions, in both groups.

6. Discussion

The present study investigated the effect of visual and auditory entrainment (at 8Hz, 10Hz and 12Hz) on the perception of a moderately painful stimulus. In both the auditory and visual groups, all three entrainment conditions resulted in pain ratings significantly lower than control. As hypothesized, we found the largest reduction in pain ratings after the 10Hz entrainment. In both groups, the 8Hz condition was more effective than the 12Hz. However, the visual group demonstrated slightly larger reductions in pain ratings in the 8Hz and 10Hz conditions (0.6, 1.1) compared to the auditory group (0.51, 0.58).

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We did not see any significant correlations between pre-experimental questionnaires (Participant Sleep Questionnaire, PCS, PHQ-9, PASS) and the change in pain ratings. Additionally, the STAI, POMS and KSS scores did not display significant differences between conditions, suggesting the effects of alpha-entrainment were independent of effects on mood and sleepiness. Alpha entrainment may therefore affect cortical processes directly, independently of mood and sleepiness.

Entrainment Conditions

Across both modalities, the largest reduction followed the 10Hz entrainment (although inter-condition differences were only significant in visual entrainment). It could be postulated that the largest reduction in pain occurs when the frequency closest to the volunteers' individual alpha frequency (IAF) increases in power. It has been suggested that entrainment is more effective if the driving stimulus is close in frequency to the IAF (Frederick et al., 2005). The IAF is determined by the membrane properties of the thalamic neurons projecting to the cortex, laying around 10Hz (Klimesch, 1999a; Klimesch et al., 1997; Steriade et al., 1990), varying marginally between individuals (Klimesch, 1999a). IAFs are lower in children, and decrease again with normal aging (Klimesch, 1997; Kopruner et al., 1984; Posthuma et al., 2001). As the population sampled is relatively young, the IAF of a subset might be slightly lower. This could explain why 8Hz had the second-largest effect on pain reduction in both the auditory and visual groups. Further work acquiring electrophysiological data would be able to establish whether an increase in alpha power closest in frequency to the individual's IAF or an increase in 10Hz, unrelated to IAF, results in the largest alpha analgesia.

Visual vs. Auditory

As visual and auditory entrainment paradigms were not equivalent, changes in pain ratings have not been statistically compared. Nevertheless, visual entrainment resulted in a numerically larger decrease in pain ratings in the 8Hz and 10Hz conditions than the auditory entrainment. A study by Frederick et al., 1999 found auditory stimulation resulted in greater entrainment at the vertex than visual stimulation (Frederick et al., 1999). If this concept were applied to the current results, it would imply that even though entrainment is greater in the auditory group, visual entrainment is more effective at reducing pain. It must be noted that the study conducted by Frederick et al. compared entrainment at 18.5Hz, and therefore these results may not be representative of what is happening in this study. Additionally, increased alpha power at the vertex may be irrelevant to the processing of pain perception.

Binaural beats have demonstrated widespread entrainment across frontal and central sites of the scalp (Schwarz and Taylor, 2005). Visual entrainment primarily affects the primary visual cortex. Nonetheless, entrainment in the visual cortex can elicit changes in cortical activity widely distributed throughout the cortex (Timmermann et al., 1999). Furthermore, the visual cortex has the strongest resonance at 10Hz (Herrmann, 2001), where the largest analgesic effect was observed.

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Although alpha power is most commonly generated in thalamocortical feedback loops of excitatory and inhibitory nerve cells (Lopes da Silva, 1991; Steriade et al., 1990), in the visual cortex, alpha power is also believed to be generated by cortico-cortical networks (Bollimunta et al., 2008; Lopes Da Silva and Storm Van Leeuwen, 1977; Spaak et al., 2012; Steriade et al., 1990), that are modulated by attention (Bollimunta et al., 2011; Yamagishi et al., 2003). As alpha power is commonly accepted to be incompatible with high states of arousal, it could be postulated that visual alpha entrainment reduces the attention paid to the intensity of painful stimuli, making them seem less salient than following auditory entrainment. In contrast, reducing alpha activity has been suggested to reflect alertness to external inputs (Klimesch, 1999b) allowing the mind to be occupied with numerous inputs, and hence allowing the pain stimulus to feel more salient. Targeting alpha power generated by the attention modulated cortico-cortical networks by entraining the visual cortex may thus result in better-targeted analgesia than auditory entrainment.

Another reason why binaural beat stimulation was not as effective at reducing pain may have been that auditory entrainment is not very long lasting. Wahbeh and colleague saw no lasting effects 30mins after auditory alpha entrainment at 7Hz (Wahbeh et al., 2007). The 7Hz entrainment was however conducted with 133 and 140 pure tone carrier frequencies. Numerous studies have demonstrated that carrier frequencies between 450-500 are much more effective than those around 100Hz (Oster, 1973; Perrott and Nelson, 1969). Nevertheless, Wahbeh and colleagues results question the reliability of auditory entrainment.

A potential contribution to the larger effect in the visual study could be due to the effect of distraction. Distraction can have a significant influence on the perception of pain (Boyle et al., 2008). Bright flashing LED lights are potentially more distracting than the soothing sound of binaural beats. Disorientating post-entrainment effects of visual stimulation may hinder subjects from fully attending to the pain. However, it seems unlikely that the effect of distraction can produce significant differences between alpha frequencies, as found in this study. Furthermore, it is presently not possible to differentiate between the effects of alpha entrainment and distraction. They may insofar be separate mechanisms, but may also interact; increasing alpha power may increase distractibility.

Anxiety, Sleepiness and Negative Moods

STAI, POMS and KSS scores did not change after visual or auditory entrainment. This finding suggests that alpha influences pain perception independently of anxiety, wakefulness and negative moods. Ossebaard et al., 2000 demonstrated that after 35 minutes of visual alpha entrainment, a significant reduction in STAI scores could be observed (Ossebaard, 2000). In the present study, there was no evidence to suggest entrainment influenced the volunteer's anxiety. However, in the study conducted by Ossebaard et al., initial anxiety scores were on average higher than in our study, suggesting that volunteers in our study were feeling at-ease at the start of the experiment. Hence, a decrease in STAI would have been difficult to detect. Additionally, Ossebaard and colleagues entrained their volunteers for 35 minutes. A

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larger reduction in anxiety might have been observed if the entrainment sessions were longer and a more anxious population had been sampled. Whether this reduction in anxiety would have amplified the reduction in pain ratings is worth further study.

Melzack and Perry (1975) claimed that increases in alpha power could only influence the perception of pain when coupled with relaxation and suggestion (Melzack and Perry, 1975). The present study demonstrates that even without any changes in negative moods, anxiety or sleepiness, alpha power alone has the ability to alter the perception of acute pain. Reducing negative moods may amplify the analgesic effects of alpha entrainment due to ensuing increased relaxation, rather than the other way round. A recent study by Spaak et al. (2014) supports this notion by demonstrating alpha rhythms are not an epiphenomenon of attentional processes, instead, they influence perception independently (Spaak et al., 2014).

Limitations of the Design

The STAI, POMS and KSS questionnaires were always provided following the pain trials to ensure the first and last pain stimulus was delivered at identical post-entrainment times. This resulted in the participants starting the questionnaires 5 minutes post-entrainment. Although participants were asked to relate the questionnaires to the entrainment session, the entrainment effect would have diminished, and potentially contaminated by the intersecting pain trial. Furthermore, the average time to complete all three questionnaires varied significantly between volunteers, and consequentially, post-entrainment to questionnaire time window also varied accordingly.

Very little work has been completed investigating the similarity between naturally occurring alpha and entrained alpha rhythms. Whether entrained alpha accurately mimics cortical rhythms is still unknown. It is presumed they rely on the same mechanisms, as entrained activity influences subsequent spontaneous cortically generated activity, outlasting the stimulation period. Literature suggests that changes in neural activity during rhythmic stimulations are similar to the neural changes observed when the stimulation finishes (Halbleib et al., 2012). Spaak et al reported visual 10Hz entrainment outlasting the stimulation by multiple cycles. This suggests a 10Hz flicker induced alpha oscillations intrinsic to the cortex (Spaak et al., 2014).

The longevity of entrainment and analgesic effect was not considered in this study. Longer-term effects of alpha-range stimulation have been seen using other techniques, such as a repetitive Transcranial Magnetic Stimulation (rTMS) (Moisset et al., 2016). Although the analgesic effects induced by rTMS appear immediately after the stimulation, the largest effect can be delayed up to 3 days and can last up to 1 week (Andre-Obadia et al., 2008; Lefaucheur et al., 2001). Furthermore, when considering other techniques that have been used to modulate oscillatory frequencies (e.g. TMS, neurofeedback training, mindfulness training etc.), the duration of the induced effect increases after repeated sessions (Grant, 2014; Jensen et al., 2013; Mhalla et al., 2011; Moisset et al., 2016). As such, further work assessing

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the long-term analgesic effects of alpha entrainment following on-off and regular use is needed to allow us to assess the usefulness of alpha entrainment in clinical practice.

Concluding Remarks

The present study provides new evidence visual and auditory entrainment in the alpha range can influence the perception of acute pain independently of arousal and negative emotional influences. The results reveal that 10Hz stimulation has a significantly larger analgesic effect than 8Hz and 12Hz following the visual entrainment, and non-significantly following the auditory entrainment. Overall, visual entrainment produced a larger effect than auditory entrainment in the mid- and lower alpha frequencies. This provides further evidence that external stimulation can modulate pain perception and requires further study to ascertain its relevance to clinical pain states.

7. Acknowledgments:

We would like to thank Ann Lenton and Timothy Rainey, of the Human Pain Research Group, University of Manchester, for all their help with participant recruitment and technical help for this study and Jason Taylor for his guidance and analytical support.

8. Author Contributions

All authors listed have substantially contributed to the manuscript as follows:

Conceived and designed the experiments: KE CAB AKPJ.

Performed the experiments: KE

Analyzed the data: KE

Contributed reagents/materials/analysis tools: KE CAB

Discussed the results and commented on the manuscript: KE CAB AKPJ

Wrote the manuscript: KE CAB AKPJ

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