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TITLE:

Modulation of the Neurophysiological Response to Fearful and Stressful Stimuli through Repetitive Religious Chanting

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SUMMARY:

The present event-related potential (ERP) study provides a unique protocol for investigating how religious chanting can modulate negative emotions. The results demonstrate that the late positive potential (LPP) is a robust neurophysiological response to negative emotional stimuli and can be effectively modulated by repetitive religious chanting.

ABSTRACT:

In neuropsychological experiments, the late positive potential (LPP) is an event-related potential (ERP) component that reflects the level of one's emotional arousal. This study investigates whether repetitive religious chanting modulates the emotional response to fear- and stress-provoking stimuli, thus leading to a less responsive LPP. Twenty-one participants with at least one year of experience in the repetitive religious chanting of "Amitabha Buddha" were recruited. A 128-channel electroencephalography (EEG) system was used to collect EEG data. The participants were instructed to view negative or neutral pictures selected from the International Affective Picture System (IAPS) under three conditions: repetitive religious chanting, repetitive nonreligious chanting, and no chanting. The results demonstrated that viewing the negative fear- and stress-provoking pictures induced larger LPPs in the participants than viewing neutral pictures under the no-chanting and nonreligious chanting conditions. However, this increased LPP largely disappeared under repetitive religious chanting conditions. The findings indicate that repetitive religious chanting may effectively alleviate the neurophysiological response to fearful or stressful situations for practitioners.

INTRODUCTION:

The late positive potential (LPP) has long been accompanied by emotional arousal, and it has been reliably used in emotion-related research[1,2]. Religious practice is widespread in both Eastern and Western countries. It is asserted that it can alleviate the practitioner's anxiety and stress when facing adverse events, especially during times of difficulty[3]. Nonetheless, this has seldom been demonstrated under rigorous experimental settings.

Numerous studies have confirmed that emotion regulation can be learned with different strategies and frameworks[4–6]. A few studies have shown that mindfulness and meditation can modulate the neural response to affective events[7,8]. Recently, it was found that meditation practitioners may employ emotion modulation strategies other than cognitive appraisal, suppression, and distraction[8,9]. Stimuli from the International Affective Picture System (IAPS) can be used to elicit positive or negative emotions reliably, and there are standard criteria for finding designed pictures with specified valence and arousal levels in affective research[10].

Emotional stimuli can cause early- and later- responses in the brain[3,11]. Similarly, Buddhism tradition made analogical analysis on the mind thoughts by initial and secondary mental processes[3,12]. The *Sallatha Sutta* (The Arrow Sutta), an early Buddhist text, mentions that cognitive training can tame emotion. The Arrow Sutta states that both a well-trained Buddhist practitioner and an untrained person experience an initial and negative perception of pain when facing a harmful event[13]. This unavoidable initial pain is similar to a person being hit by an arrow, as described in the *Sallatha Sutta*. Early perceptual pain is identical to the stage of early processing when a person views a highly negative picture. Early neural processing usually elicits an N1 component. Untrained persons may develop excessive emotions, such as worry, anxiety, and stress, after experiencing the initial, unavoidable painful feelings. According to the *Sallatha Sutta*, this late-developing negative emotion or psychological pain is like being hit by a second arrow. An event-related potential (ERP) experiment may capture the current design's early and later psychological processes, assuming that N1 and LPP could correspond to the two arrows mentioned above.

In this protocol, the repetitive chanting of the name "Amitabha Buddha" was chosen to test the potential effect of religious chanting when an individual is in a fearful or stressful situation. This religious chanting is one of the most popular practices of individuals with religious orientations among Chinese Buddhists, and it is a core practice of East Asian Pure Land Buddhism[14]. It was hypothesized that repetitive religious chanting would reduce the brain response to provoking stimuli, namely, the LPP induced by fearful or stressful pictures. Both EEG and electrocardiogram (ECG) data were collected to assess participants' neurophysiological responses under different conditions.

PROTOCOL:

This ERP study was approved by The University of Hong Kong Institutional Review Board. Before participating in this study, all participants signed a written informed consent form.

1. Experimental design

1.1. Recruit participants

1.1.1. Recruit participants with at least 1 year (~ 200–3,000 h) of experience in chanting the name of "Amitabha Buddha" for this study.

NOTE: In the present study, 21 human participants ranging from 40–52 years old were selected; 11 were males.

1.2. Religious chanting vs. nonreligious chanting

1.2.1. Chant the name of "Amitabha Buddha" for 40 s. First 20 s with the image of Amitabha and the next 20 s with IAPS images.

1.2.1.1. Chant only four characters of the name of "Amitabha Buddha" and imagine the Amitabha following the script in Pureland school[14].

1.2.2. Chant the name of Santa Claus (non-religious chanting condition) for 40 s. First 20 s with the image of Amitabha and the next 20 s with IAPS images.

1.2.1.2. Chant only four characters of the name of Santa Claus and imagine the Santa Claus.

1.2.3. Keep silent for 40 s. First 20 s with imagining on a blank image for control purpose and the next 20 s with IAPS images.

NOTE: No chanting.

1.3. EEG recording system

1.3.1. Record EEG data using a 128-channel EEG system that consists of an amplifier, headbox, EEG cap, and two desktop computers (see **Table of Materials**).

1.4. Stimuli presenting system

1.4.1. Use stimulus presentation software (see **Table of Materials**) to show neutral and negative pictures from the international affective picture system (IAPS) on a desktop computer.

1.5. ECG recording system

1.5.1. Use a physiological data recording system to record ECG data (see **Table of Materials**).

2. Affective modulation experiment

NOTE: The experiment had two factors with a 2 x 3 design: The first factor was the picture type: neutral and negative. The second factor was the chanting type: chanting "Amitabha Buddha", chanting "Santa Claus" and no chanting (silent view).

2.1. Use a block design, as it may more effectively elicit emotion-related components[15].

NOTE: There were six conditions, and the sequences were randomized and counterbalanced between the participants (**Figure 1**). The six conditions were as follows: religious chanting while viewing fearful pictures (RC-Fear); religious chanting while viewing neutral pictures (RC-Neut); no chanting while viewing scary pictures (NoC-Fear); no chanting while viewing neutral pictures (NoC-Neut); nonreligious chanting while viewing fearful pictures (NRC-Fear); and nonreligious chanting while viewing neutral pictures (NRC-Neut).

[Place **Figure 1** here]

2.2. Show each picture for ~1.8–2.2 s, with an interstimulus interval (ISI) of 0.4–0.6 s.

NOTE: There were 10 pictures of the same type (neutral or negative) in each session.

2.3. Allow a rest period of 20 s after each session to counter the potential residual effects of chanting or picture viewing on the next session.

2.4. Present the pictures on a CRT monitor at a distance of 75 cm from the participants' eyes, with visual angles of 15° (vertical) and 21° (horizontal).

2.5. Ask the participants to observe the pictures carefully.

2.6. Provide a brief practice run to the participants to allow them to familiarize themselves with each condition. Use a video monitor to ensure that the participants do not fall asleep.

2.7. Give the participants a 10 min rest in the middle of the 40 min experiment.

3. EEG and ECG data collection

NOTE: Before coming to the experiment, ask each participant to wash their hair and scalp thoroughly without using a conditioner or anything else that may increase the system's impedance. Collect the EEG and ECG data simultaneously by two separate systems.

3.1. Inform each participant of the experimental procedures, that is, that effective pictures were viewed under different chanting conditions.

3.2. Set the sampling rate to 1,000 Hz, and maintain the impedance of each electrode below 30 kΩ whenever possible or according to the system's requirements.

3.3. Collect physiological data, including ECG data using a physiological data recording system (see **Table of Materials**).

4. EEG data analysis

4.1. Process and analyze the EEG data with EEGLAB (see **Table of Materials**)**, Supplementary File 1–2**), an open-source software[16] following the steps below.

4.2. Use the EEGLAB function "pop_resample" to resample the data from 1,000 Hz to 250 Hz to maintain a reasonable data file size. Click on **Tools > Change sampling rate**.

4.3. Use the EEGLAB function "pop_eegfiltnew" to filter the data with a finite impulse response (FIR) filter with 0.1–100 Hz passband. Click on **Tools > Filter the data > Basic FIR filter (new, default)**.

4.4. Filter the data again with a nonlinear infinite impulse response (IIR) filter with a 47–53 Hz stopband to reduce the noise from the alternating current. Click on **Tools > Filter the data > select Notch filter the data instead of pass band**.

4.5. Visually inspect the data to remove strong artifacts generated by eye and muscle movements. Click on **Plot > Channel data (scroll)**.

4.6. Visually inspect the data again for any consistent noise generated by any channel, and the bad channels were noted.

4.7. Reconstruct the bad channels using spherical interpolation. Click on **Tools > Interpolate electrodes > Select from the data channels**.

4.8. Run independent component analysis (ICA) with the open-source algorithm "runica"[16]. Click on **Tools > Run ICA**.

4.9. Remove the independent components (ICs) corresponding to eye movements, blinks, muscle movement, and line noise. Click on **Tools > Reject data using ICA > Reject components by map**.

4.10. Reconstruct the data using the remaining ICs. Click on **Tools > Remove components**.

4.11. Filter the data with a 30 Hz low-pass filter. Click on **Tools > Filter the data > Basic FIR filter (new, default)**.

4.12. Obtain ERP data by extracting and averaging time-locked epochs for each condition with a time window of -200 to 0 ms as the baseline and 0 to 800 ms as the ERP. Click on **Tools > Extract epochs**.

4.13. Re-reference the ERP data with the average of the left and right mastoid channels. Click on **Tools > Re-reference**.

4.14. Repeat the above steps for the datasets from all participants and compare the differences between conditions using the *t*-test or repeated-measures ANOVA in a statistical analysis software (see **Table of Materials**).

4.15. Define time windows for N1 and LPP based on established theories[8,17] and the current data[3].

NOTE: In this work, N1 was defined as 100–150 ms, while LPP as 300–600 ms from stimulus onset; LPP is most prominent in the central-parietal region (**Figure 2**).

4.16. Find the neutral vs. negative picture difference at the N1 component using paired *t*-test among three conditions (**Figure 3**).

4.17. Find the neutral vs. negative picture difference at the LPP component using paired *t*-test among three conditions (**Figure 4**).

4.18. Perform region of interest (ROI) analysis on N1 and LPP components by averaging relevant channels to represent a region.

NOTE: To select ROI, the epochs of all three conditions were averaged to calculate those channels where the neutral and negative pictures had a difference that was significant in the specific time window (e.g., for N1 or LPP).

4.19. Compare the difference at N1 and LPP separately, using repeated measures ANOVA and post hoc statistics in statistical analysis software.

NOTE: Use Post hoc analysis (Bonferroni correction) and determine significant differences between the two conditions separately if the model was significant. The significance threshold was set at $p < 0.05$.

5. ERP source analysis

5.1. Perform the ERP source analysis[18] with the SPM[19] open-source software (see **Table of Materials**) following the steps below.

5.2. Link the EEG cap sensor coordinate system to the coordinate system of a standard structural MRI image (Montreal Neurological Institute (MNI) coordinates) by landmark-based co-registration. In SPM, click on **Batch > SPM > M/EEG > Source reconstruction > Head model specification**.

5.3. Perform forward computation to calculate the effect of each dipole on the cortical mesh imposed on the EEG sensors. Under the same Batch Editor, click on **SPM > M/EEG > Source reconstruction > Source inversion**.

NOTE: These results were placed in a G matrix (n x m), where n is the number of sensors (EEG space dimension) and m is the number of mesh vertices (source space dimension). The source model was X = GS, where X is an n x k matrix denoting the ERP data of each condition, k is the number of time points, and S is an m x k matrix indicating the ERP source.

5.4. Use the greedy search-based multiple sparse priors algorithm (since S is unknown) in the third step (among the many algorithms available) to perform the inverse reconstruction because it is more reliable than other methods[20]. Choose **MSP (GS)** for the Inversion type in the **Source Inversion** window.

5.5. Determine the difference between conditions using general linear modeling in SPM. Set the significance level to $p < 0.05$. Under **Batch Editor**, click on **SPM > Stats > Factorial design specification**.

6. ECG data and behavioral assessment analysis

6.1. Use physiological and data processing software to process and analyze the ECG data (see **Table of Materials**). Calculate the mean scores for each condition. In EEGLAB, click on **Tools > FMRIB Tools > Detect QRS events**[21].

NOTE: Similar to the ERP amplitude analysis, statistical software was used to further analyze the data with repeated ANOVA. Post hoc analysis was performed to determine the significant differences between the two conditions separately if the model was significant. The significance level was set to $p < 0.05$.

6.2. Ask the participants to rate their belief in the efficacy of chanting the subject's name (Amitabha Buddha, Santa Claus, etc.) on a 1–9 scale, where 1 is considered weakest and 9 the strongest.

REPRESENTATIVE RESULTS:

Behavioral results

The results for participants' belief of chanting revealed an average score of 8.16 ± 0.96 for "Amitabha Buddha", 3.26 ± 2.56 for "Santa Claus", and 1.95 ± 2.09 for the blank control condition (**Supplementary Table 1**).

ERP results

The representative channel of Pz (parietal lobe) demonstrated that the chanting conditions had different effects on the early (N1) and late (LPP) processing of neutral and negative pictures. It showed the time window of N1 and LPP, respectively (**Figure 2**).

Early perceptual stage

The ERP results showed an increased N1 while viewing the fearful pictures in three chanting conditions (**Figure 3**). It showed that negative images induced stronger central brain activities than neutral images, and the increases are comparable in three conditions.

Late emotional/cognitive stage

The ERP demonstrated an increased LPP in the nonreligious chanting and no-chanting conditions. However, the LPP induced by fearful pictures is barely visible when the participant chants Amitabha Buddha's name (**Figure 4**).

Region of interest (ROI) analysis

The three conditions were combined to estimate the regions that were generally activated at N1 and LPP components. Repeated ANOVA was performed with statistical software to calculate the difference in the N1 and LPP components between the chanting conditions (**Figure 5**).

The left three columns show the difference in the N1 component for the three chanting conditions: the silent viewing condition, the nonreligious chanting condition, and the religious chanting condition. The differences in the N1 component were similar across the three conditions. The right three columns show the difference in the LPP component for the three chanting conditions. This demonstrates that the difference in the LPP component is much smaller in the religious chanting condition than in the nonreligious chanting condition and the silent viewing condition.

Source analysis

Source analysis was applied to extract the potential brain mapping based on the LPP results (**Figure 6**). The results show that when compared with neutral pictures, negative pictures induce more parietal activation in the nonreligious chanting condition and no chanting condition. In contrast, this negative picture-induced activation largely disappears in the religious chanting condition.

Physiological results: heart rate

There was a significant change in the heart rate (HR) between the fearful and neutral pictures in the nonreligious chanting condition. A similar trend was found in the no-chanting condition. However, no such HR difference was found in the religious chanting condition (**Figure 7**).

FIGURE AND TABLE LEGENDS:

Figure 1: The experimental procedure. There were six pseudorandomized conditions, and each participant received a pseudorandomized sequence. Each condition was repeated six times in two separate sessions. This figure has been adapted from Reference[3].

Figure 2: A representative channel (Pz) showed different ERPs in six chanting conditions. The six conditions are (1) religious chanting while viewing fearful pictures (RC-Fear); (2) religious chanting while viewing neutral pictures (RC-Neut); (3) no chanting while viewing scary pictures

(NoC-Fear); (4) no chanting while viewing neutral pictures (NoC-Neut); (5) nonreligious chanting while viewing fearful pictures (NRC-Fear); and (6) nonreligious chanting while viewing neutral pictures (NRC-Neut). The channel Pz located in the mid-parietal area of the scalp.

Figure 3: The ERP results for demonstrating the N1 component in the three chanting conditions. Two-dimensional maps of the N1 component for the three conditions for each picture type. In the last column, channels with significant differences ($p < 0.05$) are shown with dots; dots that are in darker color indicate greater significance (i.e., smaller p-values).

Figure 4: The ERP results for demonstrating the LPP component in the three chanting conditions. Two-dimensional maps of the late positive potential (LPP) component for the three conditions for each picture type. In the last column, channels with significant differences ($p < 0.05$) are shown with dots; dots that are in darker color indicate greater significance (i.e., smaller p-values).

Figure 5: Region of interest (ROI) analysis. The Region of interest (ROI) analysis on the difference between negative vs. neutral picture-induced brain responses for the early component, N1, and the late component, the late positive potential (LPP).

Figure 6: Source analysis of the late positive potential (LPP) component under the three conditions.

Figure 7: The heart beat intervals under the three chanting conditions. The electrocardiogram's inter-beat intervals (RRs) under each picture type/chanting combination and the corresponding p values. Ami: Amitabha Buddha chanting condition, San: Santa Claus chanting condition, Pas: passive viewing condition, Neu: neutral picture, Neg: negative picture.

Supplementary Table 1: Rating the belief in the efficacy of the chanting subject (Amitabha Buddha, Santa Claus). It uses a 1–9 scale, where 1 indicates the least belief and 9 the strongest belief.

Supplementary File 1: Code for EEG data batch preprocessing. It removes bad channels, resamples the data to 250 Hz, and then filters the data.

Supplementary File 2: Code for ERP data repairing. It repairs bad epochs with noisy spikes.

DISCUSSION:

The uniqueness of this study is the application of a neuroscientific method to probe the neural mechanisms underlying a widespread religious practice, i.e., repetitive religious chanting. Given its prominent effect, this method could enable new interventions for therapists or clinicians to treat clients dealing with emotional problems and suffering from anxiety and stress. Together with previous studies, broader emotion regulation research should be considered in future studies[7–9,22].

There are few ERP studies on chanting, given the difficulty of constructing experiments that combine chanting and other cognitive events. This study demonstrates a feasible protocol for investigating the affective effect of chanting/praying, which is rather popular in the real world. Previous functional MRI (fMRI) studies found that praying recruits areas of social cognition[23]. One resting-state fMRI study revealed that chanting "OM" reduced outputs from the anterior cingulate, insula, and orbitofrontal cortices[24]. Another EEG study found that "OM" meditation increased delta waves, inducing the experience of relaxation and deep sleep[25]. However, these methods could not precisely investigate the specific event-related changes after religious chanting.

Researchers should control the confounding factors of language processing and familiarity to successfully investigate the potential effect of repetitive religious chanting successfully. As the participants practiced extensively and daily chanting the name "Amitabha Buddha" (Chinese characters: 阿彌陀佛; Cantonese pronunciation: o1-nei4-to4-fat6), we used the name "Santa Claus" (Chinese characters: 聖誕老人; Cantonese pronunciation: sing3-daan3-lou5-jan4) as the control condition because the local is familiar with Santa Claus. In Chinese, both names contain four characters, thus controlling for language similarity. Regarding familiarity, Santa Claus is also quite popular in Hong Kong because it is a partially Westernized city. In addition, Santa Claus is also a somewhat positive figure in Hong Kong, where there is official Christmas Holidays. Nevertheless, this control of familiarity is partial, as it is difficult to entirely match the understanding of Amitabha's name for the practitioners.

One critical step in the current study was the preparation of the fear- or stress-provoking pictures. As religious chanting may work better when threatening events occur, selecting proper stimuli from the IAPS image pool[26] was crucial. It is recommended that potential participants be interviewed and that suitable pictures be chosen to avoid too much fear or disgust. Highly negative pictures could prevent the participants from willfully averting their attention; at the same time, the fear-provoking stimuli should enable the participants to experience a sufficient threat. Another critical issue is the block design of the study. The EEG/ERP signal is sufficiently sensitive and dynamic to follow every event. However, it would be more appropriate to implement a block design with a 20–30 s viewing period because the pattern of cardiac function or emotion may not change on the order of seconds[27]. On the other hand, a 60 s block might be too long, and the neural response could become habituated in the ERP studies.

The EEG data processing stage needs to make a backup during each step, as each step alters the data and records the changes made during those steps. This can be used to track changes and make it easier to find errors during batch processing. Improving the data quality is also essential, so experience in raw data cleaning and identifying bad independent components (ICs) is needed. In the statistical analysis, comparisons were made on grand averages, and ANOVA was applied. We caution that this statistic with the fixed-effect model is susceptible to random effects[28]. Mixed-effects models can be adapted to control extraneous factors[29], and the assumption of linearity can potentially affect inferences drawn from the ERP data[30].

Several limitations are worth noting. One limitation is that the current study enrolled only one group of participants who practiced Pureland Buddhism. Enrolling a control group without any experience in religious chanting for comparison could help determine whether the effect of religious chanting is mediated by belief or familiarity. Usually, a randomized controlled trial would be more convincing to examine the impact of emotion modulation on religious chanting[31]. However, it is difficult to guarantee that any participant would repetitively chant "Amitabha Buddha" with complete willingness. Additionally, the LPP is affected by other factors, such as emotional sound or positive priming[32,33]. Thus, better-controlled experiments are needed to delineate more clearly the fundamental neuro-mechanism underlying the effect of religious chanting.

In sum, previous studies have demonstrated that the human brain is subjective to neural plasticity and swift alteration of states[34,35]; with sufficient practice and intention, the brain can reshape itself and respond differently to normally fearful stimuli. This study provides insights into the development of effective coping strategies for handling emotional distress in contemporary contexts. Following this protocol, researchers should examine the effect of religious chanting or other traditional practices to identify feasible ways to help people ameliorate their emotional sufferings.

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DISCLOSURES:

The authors declare that they have no competing financial interests.

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33 Hill, L. D., Starratt, V. G., Fernandez, M., Tartar, J. L. Positive affective priming decreases the middle late positive potential response to negative images. *Brain and Behavior*. **9** (1) e01198 (2019).
34. Belzung, C., Wigmore, P. in *Current Topics in Behavioral Neurosciences,* 1 online resource (X, 401 pages) Springer Berlin Heidelberg : Imprint: Springer, Berlin, Heidelberg (2013).
35. Denes, G. *Neural Plasticity Across The Lifespan: How The Brain Can Change*. CRC Press, an imprint of the Taylor & Francis Group (2016).

Figure 1

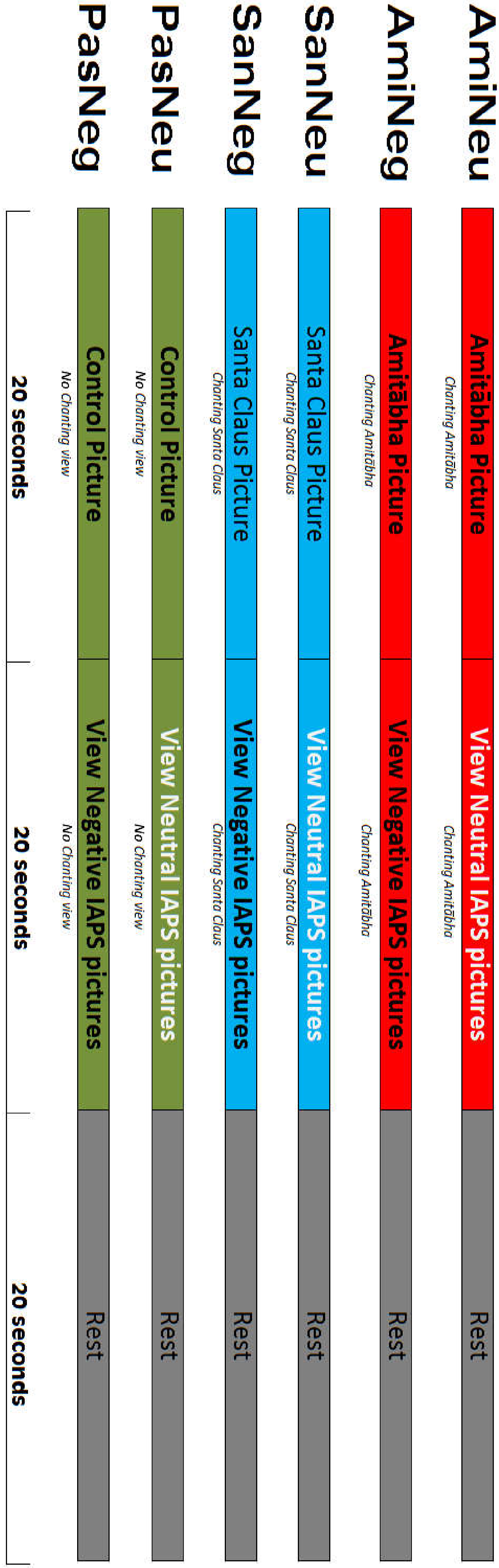

AmiNeu
Amitābha Picture
Chanting Amitābha
View Neutral IAPS pictures
Chanting Amitābha
Rest
AmiNeg
Amitābha Picture
Chanting Amitābha
View Negative IAPS pictures
Chanting Amitābha
Rest
SanNeu
Santa Claus Picture
Chanting Santa Claus
View Neutral IAPS pictures
Chanting Santa Claus
Rest
SanNeg
Santa Claus Picture
Chanting Santa Claus
View Negative IAPS pictures
Chanting Santa Claus
Rest
PasNeu
Control Picture
No Chanting view
View Neutral IAPS pictures
No Chanting view
Rest
PasNeg
Control Picture
No Chanting view
View Negative IAPS pictures
No Chanting view
Rest
20 seconds
20 seconds
20 seconds

Figure 2

Click here to access/download;Figure;fig 2.pdf

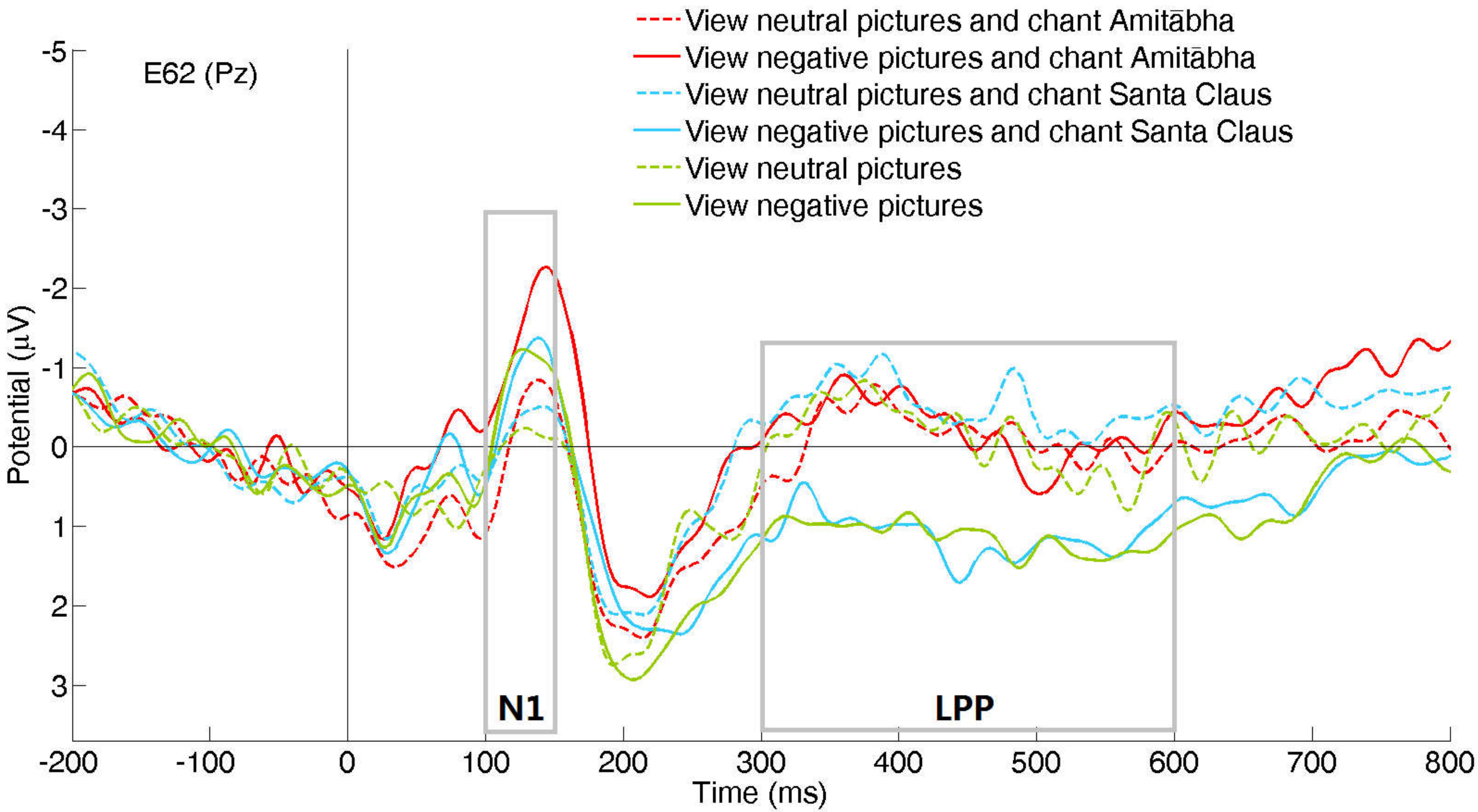


Figure 3

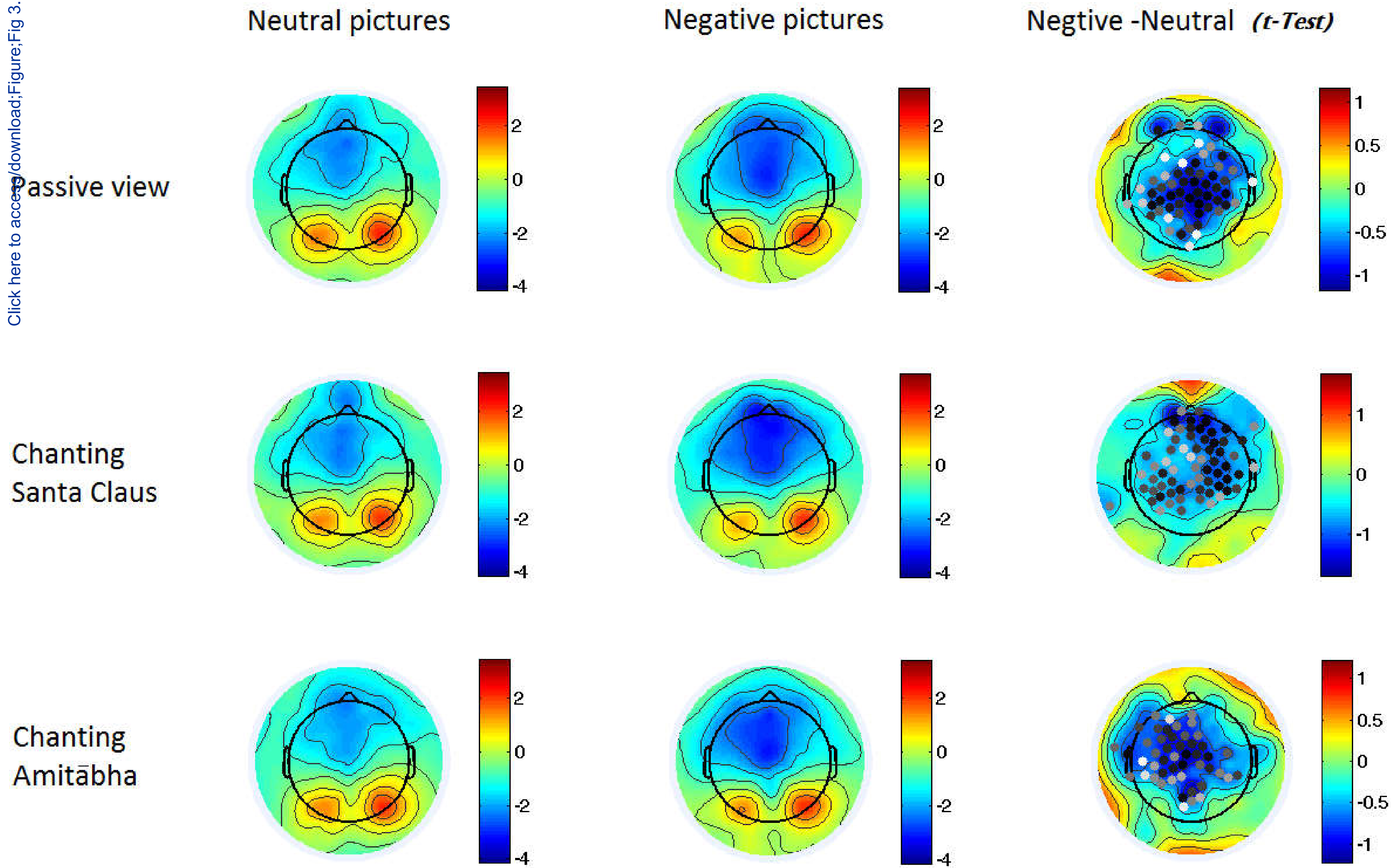

Neutral pictures
Negative pictures
Negtive -Neutral (t-Test)
Passive view
Chanting
Santa Claus
Chanting
Amitābha
2
0
-2
-4
1
0.5
0
-0.5
-1

Figure 4

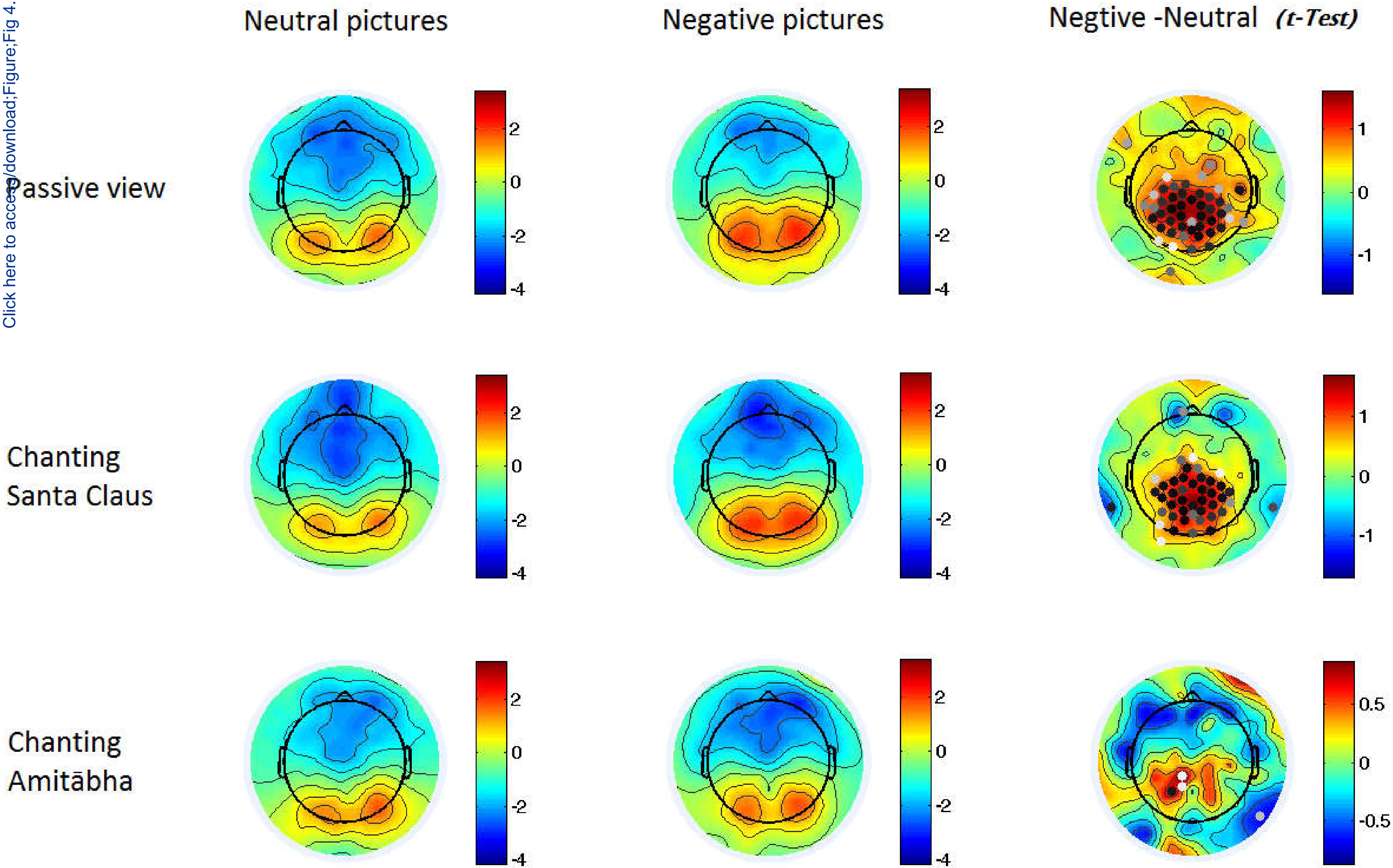
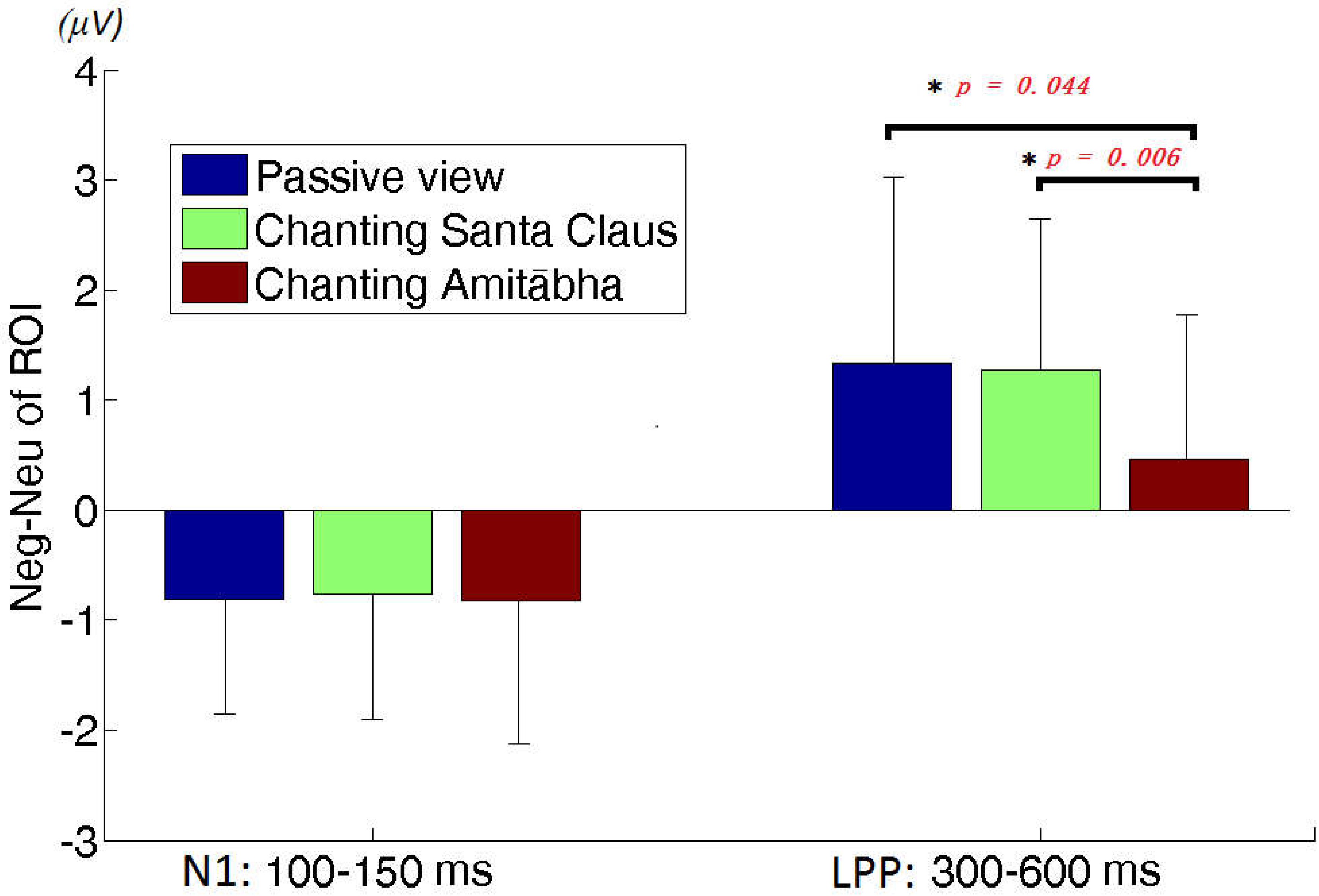

Neutral pictures
Negative pictures
Negtive -Neutral (t-Test)
Passive view
Chanting Santa Claus
Chanting Amitābha
2
0
-2
-4
1
0
-1
0.5
0
-0.5

Figure 5



(μV)
4
3
2
1
0
-1
-2
-3
Neg-Neu of ROI
Passive view
Chanting Santa Claus
Chanting Amitābha
* p = 0. 044
* p = 0. 006
N1: 100-150 ms
LPP: 300-600 ms

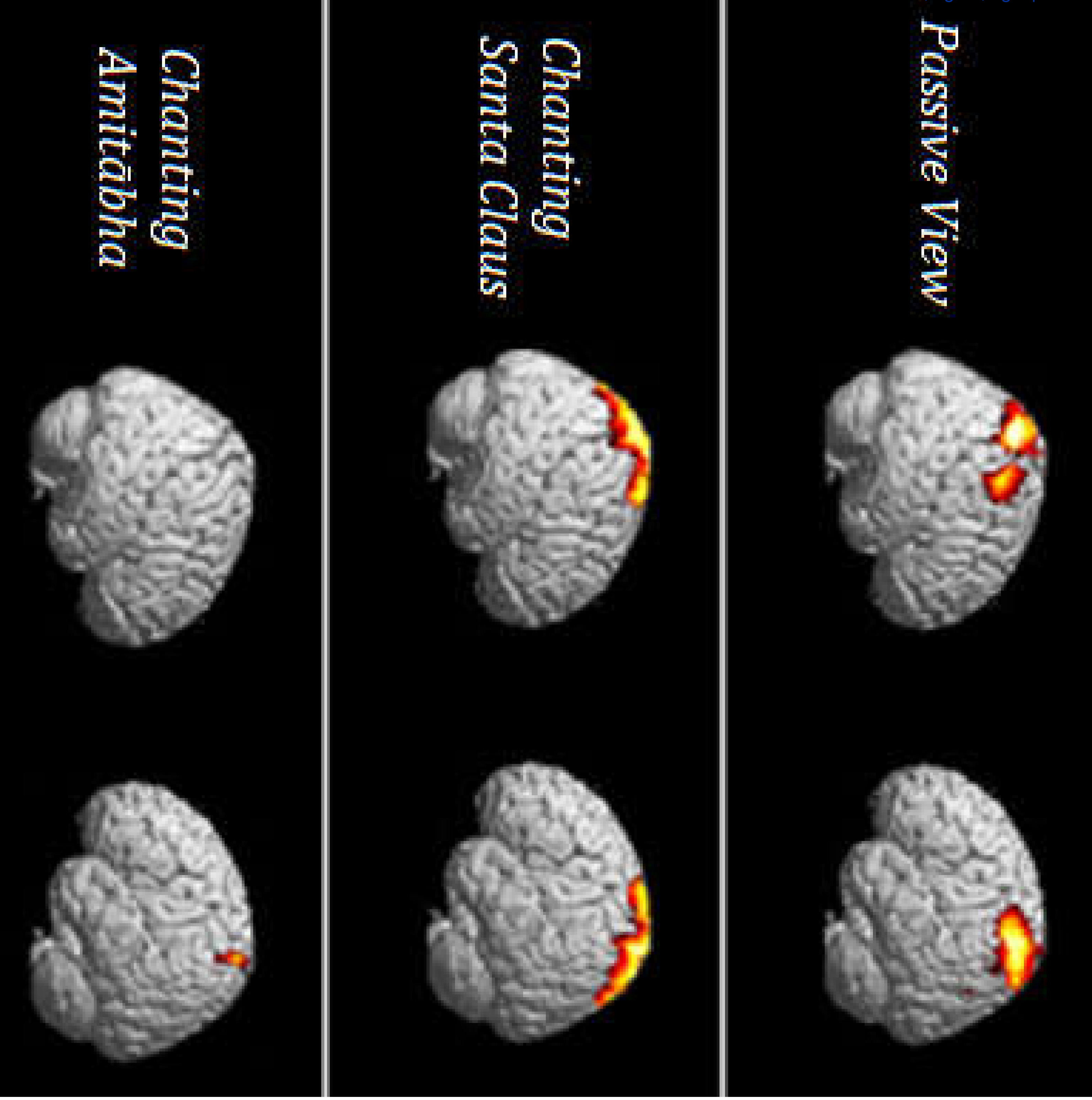
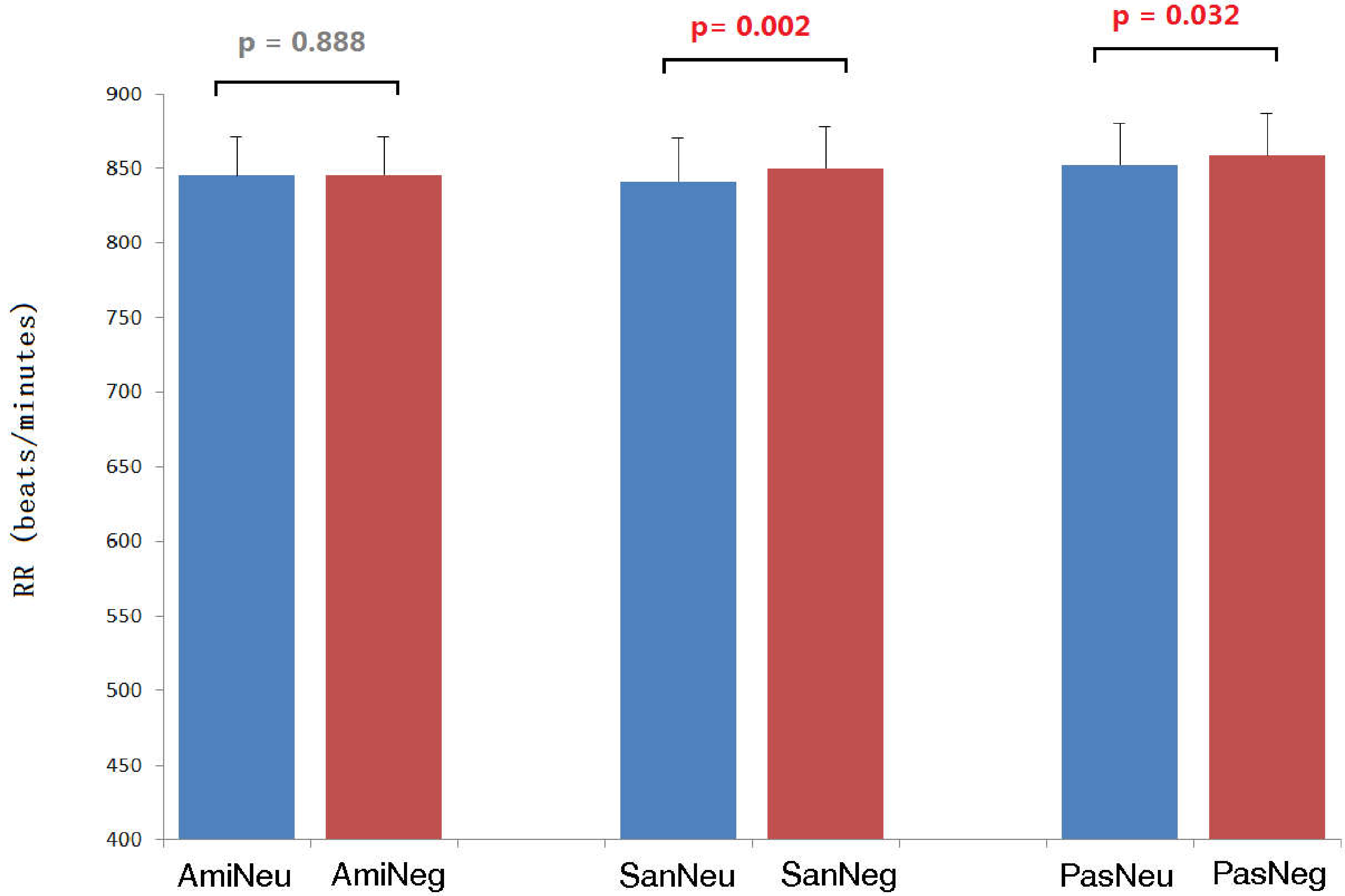

Chanting
Amitābha
Chanting
Santa Claus
Passive View

Figure 7

Click here to access/download;Figure;Fig 7.pdf



Click here to access/download
Table of Materials
62960_R2_table of materials_FINAL.xlsx

Rebuttal Letter

We'd like to thank the reviewers for their professional and constructive comments. We have spent a month time to look over the manuscript and revised all the points as commented by reviewers. Here is the point by point reply to each comment:

Do acknowledge that the reviewers spent a substantial amount of time looking over the paper – rebuttal letters that thank the referees for their time and comments

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

REPLY

Thanks for your suggestions. We have sent the manuscript for professional proofreading.

2. Please revise the following lines to avoid previously published work: 25-27, 115-123, 137-138, 144-147, 150-154, 161-175, 179-188, 250-256, 263-265, 296-298.

REPLY

We have revised these sentences accordingly.

3. Corresponding authors are different in the main manuscript (Dr. Bonnie W. Wu) and the Editorial software (Dr. Junling Gao, where the authors provide input while uploading the manuscript). Please clarify.

REPLY

Dr. Bonnie W.Wu is the corresponding author. Dr. Junling Gao helps uploading the manuscript. We have clarified this more clearly.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. For example, please generalize the terms Eprime 2.0 (Line 88), ADinstrument PowerLab systemTM (Line 91), etc. All commercial products should be sufficiently referenced in the Table of Materials. Please sort the Materials Table alphabetically by the name of the material.

REPLY

We have revised the context according to follow JOVE’s policy, and made a Table of Materials.

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

REPLY

Following your suggestion, we have changed the sentences in the protocol section to imperative tense wherever possible. We also changed the relevant texts into the form of note.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please ensure you answer the **“how”** question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. There **should be enough detail** in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. For example, please include the steps how you are performing EEG/ECG, etc.

REPLY:

We have revised the scripts according, adding steps of “how to” with more details of each procedure in EEG data collection and analysis.

We have added references to published materials with respect to the protocol action.

7. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Also, please ensure that it is in line with the title of the manuscript. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

REPLY

We have revised it accordingly, adding space and highlight up to 3 pages. Our story of the protocol is ‘how to chant’; ‘how to measure ERP related to chanting effect’ ; ‘the results of ERP’.

8. In case of software, please ensure that all button clicks and user inputs are provided throughout. Also, please ensure that the button clicks are bolded.

REPLY

We have more details at EEG analysis for every step and source analysis. More details can be shown when taking videos.

9. Please modify the Result section to include all the observations and conclusions you can derive from the Figures.

REPLY

We have modified the result section to contain more conclusions derived from the figures.

10. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol.

REPLY

We have removed the excessive descriptions to the results part.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The manuscript describes a study investigating the effects of religious chanting on event-related potential (ERP) response to negative affect stimuli before and after a chanting intervention. The study is novel and interesting in its own right and could be published in a regular (non-methods-based) journal. But the authors have chosen to present it as a protocol for the study instead. The protocol is described clearly. However, the introduction needs to be laid out a bit more scientifically with appropriate references that I list below.

Major Concerns:

1) The second para of the Introduction (line 48-61) needs to be substantially changed. Currently, the authors hypothesise an effect of religious chanting on the LPP based on a vague analogy with a Buddhist text (the Buddhist text does not specify the precise onset

times of the first and second "arrows," which the authors automatically assume correspond to N1 and LPP). I would suggest that the authors provide their justification by including 3 existing studies where meditation training or mindful strategy has been shown to corroborate the precise hypothesis of this study - that there should be a diminished LPP for the chanting condition:

1. Sobolewski, A., Holt, E., Kublik, E., & Wróbel, A. (2011). Impact of meditation on emotional processing—A visual ERP study. Neuroscience Research, 71(1), 44-48. https://doi.org/10.1016/j.neures.2011.06.002
2. Uusberg, H., Uusberg, A., Talpsep, T., & Paaver, M. (2016). Mechanisms of mindfulness: The dynamics of affective adaptation during open monitoring. Biological Psychology, 118, 94-106. https://doi.org/10.1016/j.biopsycho.2016.05.004
3. Katyal, S., Hajcak, G., Flora, T., Bartlett, A., & Goldin, P. (2020). Event-related potential and behavioural differences in affective self-referential processing in long-term meditators versus controls. Cognitive, Affective & Behavioral Neuroscience, 20(2), 326-339. https://doi.org/10.3758/s13415-020-00771-y

It is likely that the effects observed in the reported study are based on mechanisms similar to the ones underlying these previous studies.

They can then include the point about Buddhist text speculatively, by saying that N1 and LPP *could* correspond to the two arrows (rather than making it sound like they do correspond).

REPLY

Thanks for your interest and constructive suggestions. The three references you mentioned are important in linking the effect of meditation and LPP. We have incorporated these into the introduction part and substantially revised the introduction part, especially in the second paragraph, and state that 'the early and later psychological processes may be captured by an event-related potential (ERP) experiment, assuming that N1 and LPP could correspond to the two arrows aforementioned.'

2) Line 63 - Please **break the long line** into multiple lines so it sounds more coherent. Also, if possible, include a reference about the chanting practice.
REPLY

We have revised the sentence accordingly and make it more coherent.

3) Line 185 - Do the beliefs about the efficacy of chanting correlate with how much the LPP is diminished with chanting?

REPLY:
We have calculated that, and it is not significant. Partly it may be that the participants all gave a very high degree of belief in Amitabha (nearly always 9 points for most

participants). More participants in future studies may reach a stronger statistical power in this correlation.

4) Line 295 - comment 3 is relevant for this point of discussion also.

REPLY
We will list this as a limitation. Potentially, the recruitment of a control group with less belief can make the correlational analysis more powerful.

5) Since this is a methods paper, it would be good if the authors outline the challenges faced with a chanting intervention (vs. say a meditation intervention), and how the authors overcame them.

REPLY

We agree that it is difficult to control the syllabus, language, and familiarity in a chanting intervention. It is even more difficult to conduct a randomized clinical trial (RCT) on belief due to uncertainty to ensure a belief in a longitudinal experimental setting.

We have added more challenges we met in the discussion part. We have added more details of how to chant Amitabha in the manuscript so that readers can understand these difficulties better.

Minor Concerns:
1) Line 144, 151 - Please include a reference for EEGLAB and ICA correction
Delorme, A., & Makeig, S. (2004). EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. Journal of Neuroscience Methods, 134(1), 9-21. https://doi.org/10.1016/j.jneumeth.2003.10.009

REPLY

We have added this reference to the manuscript.

2) Line 231 - Please refer to the figure corresponding to the statement (Figure 5, I believe)

REPLY

We have added accordingly.

Reviewer #2:
Manuscript Summary:

This document describes the methods used in a prior study (Gao et al., 2017) which established a possible moderating effect of religious chanting on the N1 and LPP components exhibited by negative images from the International Affective Picture System (IAPS; Lang et al., 2008). The study included a 2 x 3 design (negative pictures, positive pictures; religious chanting, non-religious chanting, no chanting). The study compared Event-related potential (ERP) responses between the conditions, as well as source localisation and ECG responses to the images. The authors conclude that the results provide evidence that religious chanting may have therapeutic applications to alleviate suffering or anxiety.

Major Concerns:
Though the original study has undergone peer review, the study suffers from two major limitations that are not mentioned. First, the population appears to have consisted of experienced Buddhist religious adherents who had experience with religious chanting. The control used in the study appears to have been chanting Santa Claus, which is not mentioned in detail in the paper. It is possible that this song that was inconsequential to the target audience, where a different practice (e.g. singing one's favorite song, hearing one's favorite song before the onset, thinking happy thoughts) might have produced different results. Though the authors are right to conclude that religious chanting was demonstrated to moderate the effects, it is possible that other emotionally significant non-religious effects could influence these results, as has been suggested by other research (Brown and Cavanagh, 2017; Hill et al., 2019). Care should be taken to differentiate the Santa Claus song from other possible non-religious effects.

REPLY

We have added more details on how to chant Amitabha Buddha and Santa Claus in Section 1 to make it more clear to readers. We agree that a favorite song or broadly speaking, music can affect the emotional process, as studied by Brown et.al 2017 and Hill et. al 2019. We did consider this possibility, and thus, we did not ask the participant to sing and chant but mainly recite the names. This could partly eliminate the potential confounding effect of music or rhythmic sounds. Additionally, the chant is silent to prevent muscle movement. This may further reduce the potential effect of music. Thus said, we admit that we may not totally prevent the participants from habitual singing instead of reciting the names.

Second, the manuscript is unclear about the statistical analysis used in the ERP analysis. The manuscript mentions that the data were processed manually and with ICA, but does not mention the methods used to make comparisons between the states, nor what controls were used to control for factors between participants or electrode locations. Conventional EEG analysis is often conducted using comparisons of grand averages and ANOVA according to the methods described by Luck et al. (2014). However, the ERP community has become increasingly aware of the limitations of these techniques due to their susceptibility **to random effects** (Luck & Gaspelin, 2017). In response, many researchers have adopted linear or nonlinear **mixed effects** models, which can control for extraneous factors (Tremblay & Newman, 2015; Meteyard & Davies, 2020). This trend has emerged since the original publication, and should be communicated as a best practice in the JOVE manuscript; at very least the publication must make its ERP statistical methods much **more transparent.**

REPLY

ICA was mainly used for artefact rejection by choosing independent components (ICs) that were apparently not EEG, such as ocular and muscular artefacts. We agree that random effect is becoming more popular. We have made the analysis more transparent by clarifying that we used a fixed-effect model, a more traditional way of analysis but susceptible to the random effect. We have mentioned this as a critical point in the manuscript for clarity and future communication.

Minor Concerns:
I have also identified a number of minor issues. Some of these (e.g. **transparency** about methods) may be considered important for a venue such as JOVE, and are important to address, though can be differentiated from the deeper concerns above. These minor issues are:

* Building on Major Concern #1, the **introduction** would benefit from more details about the Santa Claus condition and the IAPS database.

REPLY
We have added more details to make the method more transparent by adding how to chant on Santa Claus condition in section 1.

* The IAPS is not cited and should be. Consider citing the IAPS in the introduction and discussing this with a couple of sentences.

REPLY

.We have described the IAPS database in the introduction part and added the related reference.

* In section 1.2, it would be helpful if **the EGI manufacturer** would be cited so that users can identify differences between the system used and their own;

* In section 1.3, the company that makes the Eprime 2.0 software should be mentioned

REPLY

Following the policy of JOVE, the manufacturer name is not allowed, and we listed it all together in the supplementary materials.

* It is unclear whether the **blocks were randomized**. This is a best practice in order to manage the effects of habituation. If so, this should be mentioned in 2.2; if not, consider mentioning this as a limitation.

REPLY:

We have stated it clearly that the blocks were randomized, and the pictures in each block were pseudo-randomized.

* It is unclear whether the data underwent **epoching (assumed) and whether they were baselined around the onset of a stimulus.** These should be mentioned in Section 4.

REPLY:

We have stated more clearly and added more details on time windows for the baseline, N1, and LPP components in section 4.

* If the authors used a protocol for **manual artifact removal**, this should be mentioned in Section 4. **Code** should be provided if possible.

REPLY:

We did this both by manual and by code. We have uploaded the code for data processing and bad epoch rejections.

* Comparison windows are not mentioned in the ERP analysis. These are very important and should be made **clear.**

We have stated this more obviously, with time windows of 100-150 ms and 300-600 ms for N1, LPP, respectively.

* Building on Major Concern #2 details of the ERP analysis should be given in much more detail in Section 4, complete with **citations**. This is the main contribution of the paper and is not mentioned substantially.

REPLY

We have added substantially more details in section 4, together with the related citations.

Dr GAO

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Best Regards,

Alfredo Ayala

Application Support Analyst

	Amitabha Figure (Religious chanting)	Santa Claus Figure (Religious chanting)
Sub1	7	6
Sub2	7	6
Sub3	9	2
Sub4	9	5
Sub5	9	2
Sub6	7	1
Sub7	9	1
Sub8	8	5
Sub9	7	1
Sub10	9	1
Sub11	9	9
Sub12	9	1
Sub13	9	1
Sub14	8	6
Sub15	7	2
Sub16	9	1
Sub17	7	1
Sub18	7	5
Sub19	9	6
Mean	8.16	3.26
SD	.958	2.557

		Paired Differences	
		Mean	**Std. Deviation**
Religious vs. Non Religious	Pair Ttest1	4.895	2.787
Religious vs. No Chanting	Pair Ttest2	6.211	2.043
Non Religious vs. No Chanting	Pair Ttest3	-1.316	2.162

Blank Figure (No Chanting)
1
1
1
5
1
1
1
1
3
1
9
1
1
2
2
1
1
0
4
1.95
2.094

t-value	Sig. (2-tailed)
7.656	0.000
13.248	0.000
-2.653	0.016

Supplementary File 1

Click here to access/download

Supplemental Coding Files

EEG_Preprocess.m

Supplementary File 2