Title: No meditation-related changes in the auditory N1 during first-time meditation

Author names and affiliations

Barnes, L. J.[1,2], McArthur, G. M.[1,2], Biedermann, B. A.[2,3], de Lissa, P.[2,4], Polito, V.[1,2], & Badcock, N. A.[1,2]

1. Department of Cognitive Science, Macquarie University
2. ARC Centre of Excellence in Cognition and its Disorders
3. School of Psychology and Speech Pathology, Curtin University
4. Department of Psychology, University of Fribourg (present address)

Abstract

Recent studies link meditation expertise with enhanced low-level attention, measured through auditory event-related potentials (ERPs). In this study, we tested the reliability and validity of a recent finding that the N1 ERP in first-time meditators is smaller during meditation than non-meditation – an effect not present in long-term meditators. This present study only includes first-time meditators. In the first experiment, we replicated the finding in first-time meditators. In two subsequent experiments, we discovered that this finding was not due to stimulus-related instructions, but was explained by an effect of the order of conditions. Extended exposure to the same tones has been linked with N1 decrement in other studies, and may explain N1 decrement across our two conditions. We give examples of existing meditation and ERP studies that may include similar condition order effects. The role of condition order among first-time meditators in this study indicates the importance of counterbalancing meditation and non-mediation conditions in meditation studies that use event-related potentials.

Highlights

- N1 event-related potential reliably reduced during first-time meditation
- Effect was not removed when stimulus-related instructions were uniform across conditions
- Reversing order countered the effect: N1 reduced during control condition
- Order effects a common and significant problem in meditation ERP studies

Keywords: meditation, attention, N1, N100, event-related potentials, attention

1. Introduction

Meditation – described as "paying attention on purpose, in the present moment, and non-judgmentally to the unfolding of experience" (Kabat-Zinn, 2003) – is gaining research interest as a mediator of brain processes that underlie attention. Attention is involved in a wide range of processes, from sensory processing through to response selection (Correa et al., 2006; Downing, 1988). Clinically-focused research addresses some interactions between meditation and executive aspects of attention, such as consciously directing focus away from recurring negative thoughts (Bostanov et al., 2012). However, it is possible that meditation affects attention at many levels, and in different ways.

Research suggests that meditation may influence the role of attention in both low-level (e.g., perceptual discrimination) and high-level (e.g., inhibiting an automatic response) processes (MacLean et al., 2010; Prakash et al., 2010). A meta-analysis on the psychological effects of meditation found that meditation is associated with moderate changes in high-level attention (Sedlmeier et al., 2012). These data were drawn from studies of inhibition, vigilance, and attention switching. Meditation is also associated with changes in low-level attention, as measured by long-term meditators' performance on a perceptual discrimination task and use of exogenous cues (Jha et al., 2007; MacLean et al., 2010).

The effect of meditation on attention may depend upon the nature of the meditation practice. Many practices include focus on a single sensation (e.g., the breath) and non-judgmental awareness of present experiences (e.g., noticing the content of the mind without reactivity). Focus on the breath may train one aspect of attention, such as vigilance, which may improve low-level perceptual attention; while nonjudgmental awareness may train another aspect of attention, such as the ability to remain focused in a changing environment, which may improve high-level executive attention (Valentine and Sweet, 1999). It is therefore important to investigate the effect of meditation on specific levels of attention. In the current study, we focus on the effect of meditation on low-level attention.

Low-level attention can be indexed by auditory event-related potentials (ERP; Luck et al., 2000). Auditory ERPs are produced by the synchronous activity of groups of neurons following the onset of a sound. They can be recorded actively, with attention directed toward stimuli, or passively, with attention directed away from stimuli. Passive auditory ERPs are especially useful for studies of meditation since they can be recorded during meditation without distracting a participant from their meditation. This is not the case for other methods, such as behavioral tests and active ERPs, which can only measure meditation effects after a period of meditation.

Figure 1 shows a typical auditory-elicited ERP waveform. Previous research has established that the size (amplitude) and timing (latency) of the positive and negative ERP peaks (e.g., P1, N1, P2) seen in this waveform are sensitive to particular stimulus features, task requirements, and participant characteristics. Table 1 provides a summary of processes that have been associated with auditory ERP peaks, which in turn provides an insight into the time-course of attentive processing from sensory gating to target selection. (see Key, Dove, & Maguire, 2005 for a detailed description of each peak, and Garrido et al., 2009 for a discussion of the mismatch negativity).

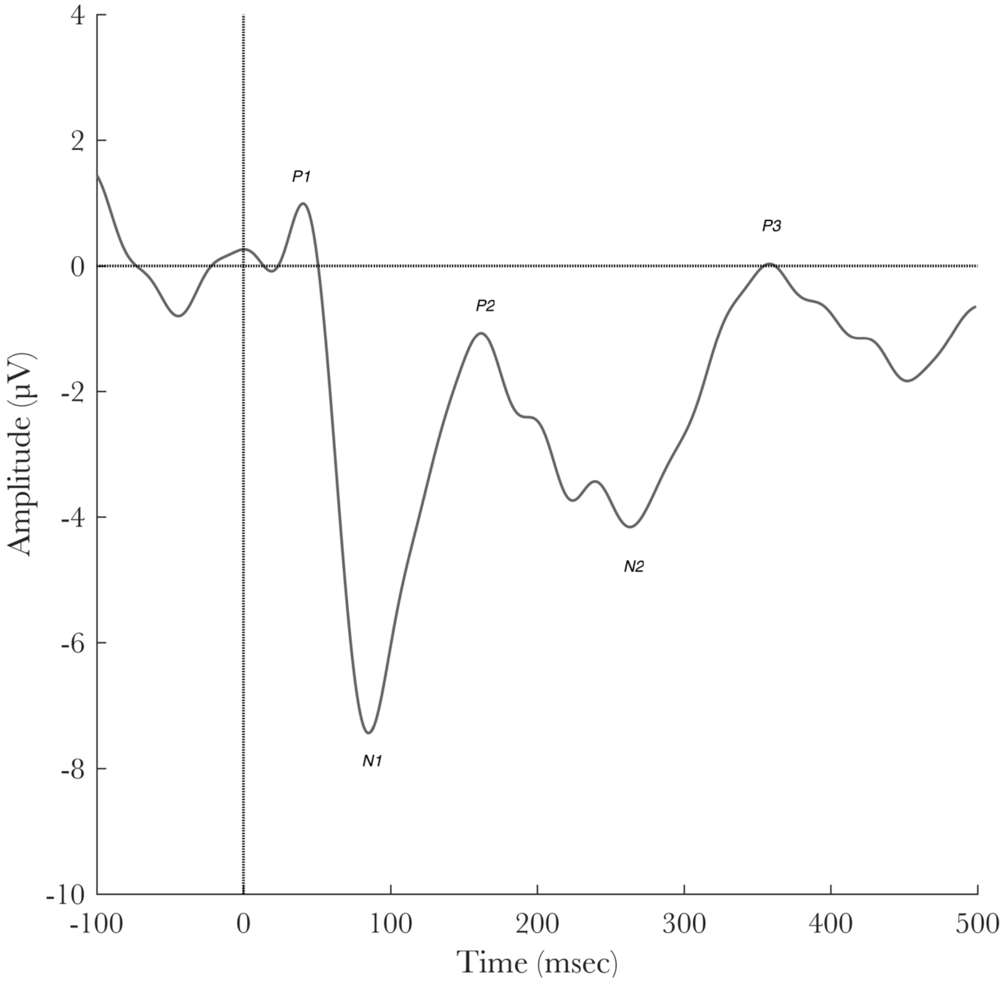

Figure 1. A typical active auditory ERP waveform for an adult.

Table 1

Processes associated with auditory ERPs

Peak	Latency (ms)	Associated processes
P1	50	Filtering sounds that repeat close together
N1	100	Discriminating different sounds
		Detecting change in sounds
P2	150-275	Detecting change in sounds
		Boosting neural activity for attended sounds
MMN	100-200	Detecting unpredicted sounds
N2	200	Detecting change in attended sounds
P3	300	Orienting attention to surprising sounds (P3a) Detecting targets among attended sounds (P3b)

To date, 14 published studies have used auditory ERPs to assess the effects of meditation on different stages or levels of attention. Overall, the outcomes of these studies suggest that the size of the passive N1, P2, N2, and P3 peaks decrease in size while long-term meditators meditate (Atchley et al., 2016; Cahn and Polich, 2009; Liu et al., 1990). Similarly, the active N2 and P3 auditory ERPs, and the passive MMN ERP, increase in size in long-term meditators after meditation, and are larger in meditators compared to non-meditators (Atchley et al., 2016; Biedermann et al., 2016; Delgado-Pastor et al., 2013; Joshi and Telles, 2009; Kyizom et al., 2010; Sarang and Telles, 2006; Srinivasan and Baijal, 2007). The latencies of the active N2 and P3 peaks, and the passive P2 peaks, appear to be earlier after long-term meditators meditate (Joshi and Telles, 2009; Kyizom et al., 2010; Telles et al., 2015).

Considered together, these findings suggest that meditation has both immediate short-term effects on low-level attention (a “state” effect) as well as long term effects from years of meditation practice (a “trait” effect). However, the strength of these suggestions is mitigated by methodological limitations that affect 11 of the 14 auditory ERP studies of meditation.

Some studies failed to include a control group, making it difficult to determine whether the ERPs were different between conditions because of the meditators' experience, or the task requirements (Barwood et al., 1978; Cahn and Polich, 2009; Chatterjee et al., 2012; Delgado-Pastor et al., 2013; Joshi and Telles, 2009; Liu et al., 1990; Sarang and Telles, 2006; Telles et al., 2015). Other studies did not include a control condition, making it difficult to determine whether the difference between groups would extend beyond a meditation task to tasks that were new to both groups (Barwood et al., 1978; Becker and Shapiro, 1981; Chatterjee et al., 2012; Joshi and Telles, 2009; Kyizom et al., 2010; Liu et al., 1990; Srinivasan and Baijal, 2007). Many studies measured auditory ERPs after a period of meditation, rather than during meditation, making it difficult to determine whether the effects were driven by meditation or reflected 'after-effects' of meditation (Chatterjee et al., 2012; Delgado-Pastor et al., 2013; Joshi & Telles, 2009; Kyizom et al., 2010; Sarang & Telles, 2006; Srinivasan & Baijal, 2007).

In contrast to these studies, three studies have compared the auditory ERPs of long-term meditators and a control group during meditation and a non-meditation control condition. Atchley et al. (2016) compared long-term meditators, short-term meditators, and first-time meditators in active and passive ERP conditions. In each condition, "oddball" stimuli were presented in blocks of 10 tones: standards (80%), pitch-deviant non-targets (low pitch; 10%), and pitch-deviant targets (high pitch; 10%). During the active task, participants were asked to press a button in response to target tones. During the passive task, participants were asked to ignore the tones and count their breaths. The active task always occurred before the passive task, so that tones which were targets in the active task were "primed" for attention in the passive task. Both long-term and short-term meditators had larger target-elicited N2 and P3 amplitudes than first-time meditators during the active task, and smaller N2 and P3 amplitudes than first-time meditators to the same tones in the passive task. This suggests that meditation increases attention to task-relevant sounds, and decreases attention to task-irrelevant sounds.

Corby, Roth, Zarcone, and Kopell (1978) compared long-term, short-term, and first-time meditators' auditory ERPs in three conditions: rest, breath awareness, and mantra repetition. Oddball stimuli (93% standard tones, 7% pitch-deviant tones) were presented in each condition. Participants were first instructed to attend to the tones (rest condition); then, to ignore the tones and attend to their breath (breath awareness condition); then, to ignore the tones and attend to their breath while repeating a word in synchrony with their breath (mantra repetition). There was no difference between groups in their ERP to the different conditions. However, across all participant groups, the N1 elicited by standards and deviants, and the P2 and P3 to deviants, decreased in amplitude across conditions, while amplitude of P2 and P3 to standards increased across conditions. The authors suggested that the reduced N1 to standard and deviant tones, as well as P2 and P3 to deviant tones, was a result of decreased attention to tones as they became more familiar; that is, they participants habituated to the tones. It is of note that the authors did not attempt to explain the increased P2 and P3 amplitudes for standard tones. However, P2 amplitude has been shown to increase with exposure to stimuli (Tremblay et al., 2014). This suggests that the increase in P2 amplitude to standard tones across conditions – like the reduction in N1, P2, and P3 to deviant tones across conditions – may have stemmed from increased exposure to stimuli. Curiously, it seems unlikely that exposure to stimuli explains the increase in P3 amplitude to standard tones across conditions. P3 amplitude typically increases with attention (Polich, 2007); but given the instructions for each condition, we would expect attention to tones to decrease, and P3 amplitude to reduce, from the "attend tones" non-meditation condition to the "ignore tones" meditation condition. Further exploration is needed to resolve this increased P3 amplitude during meditation – whether it was driven by the task, time in the testing room, or another hidden factor.

In the most recent study using auditory ERPs to examine the association between meditation and low-level attention, Biedermann et al. (2016) compared long-term meditators' and first-time meditators' ERPs elicited by a passive oddball task during a visualization control condition and a meditation condition. Stimuli were pure tones: 85% standards and 15% raised-pitch deviants. The difference between ERPs for the standard and deviant tones formed the mismatch negativity (MMN) ERP component (see Table 1). Contrasting long-term meditators and first-time meditators in both non-meditation and meditation conditions allowed measurement of "trait" effects of meditation; contrasting the non-meditation condition to the meditation condition across these two groups allowed measurement of "state" effects. Contrasting the two groups in the two conditions allowed measurement of trait-state interactions. Long-term meditators showed evidence of a trait effect in the form of a larger MMN compared to first-time meditators, regardless of condition. There was also evidence of a trait-state interaction, but in a surprising direction: the standard-elicited N1 amplitude was smaller among first-time meditators during meditation compared to non-meditation, with no such effect apparent in long-term meditators.

The effect of meditation on the passive auditory N1 ERP in Biedermann et al.'s (2016) first-time meditators deserves further scrutiny, as it raises questions about immediate effects of meditation. If these first-time meditators had a smaller N1 in the meditation condition due to meditation effects, then they are affected by the meditation state, and cannot be treated as a neutral control group for long-term meditators. However, if Biedermann's first-time meditators had a smaller N1 in the meditation condition due to non-meditation effects, then those non-meditation effects could be present in Biedermann's long-term meditators and in other meditation studies, and could change how we interpret those studies.

The specific aim of this study was to test whether first-time meditators have a smaller N1 during meditation than non-mediation because of habituation or other non-meditative effects. To this end, we conducted three experiments. The aim of Experiment 1 was to replicate the reduced N1 during meditation. The aim of Experiment 2 was to rule out attentional state, influenced by task instructions, as the reason for the reduced N1. The aim of Experiment 3 was to test whether the order of conditions produced the reduced N1.

2. Experiment 1

The aim of Experiment 1 was to test whether the first-time meditator effect reported in Biedermann et al. (2016) – that is, a smaller N1 to standard tones during meditation compared to non-meditation – would be present in a new group in first-time meditators. We used the same methods as Biedermann's study with one key difference: the use of a Bayesian analysis paradigm – rather than a frequentist analysis paradigm – to detect statistically significant effects. The Bayes factor (*B*) offers two features that orthodox statistics do not: the opportunity to make conclusive statements about the null model, and a stopping rule which terminates testing when a statistically significant outcome is reached without biasing studies towards false positive findings (Rouder, 2014; see Dienes, 2011 for a comparison of orthodox and Bayesian methods). The Bayes factor indexes evidence for the test hypothesis ($B>3$) or the null hypothesis ($B<1/3$) on a continuum, allowing us to make inferences about the reliability of null effects. A *B* between 3 and 1/3 indicates too much variability in the data, prompting further testing. We present Cohen's *d* and Student's *t*-test outcomes to facilitate direct comparison with the original study.

2.1. Methods

The methods used in this study were approved by the Macquarie University Human Research Ethics Committee (reference number 5201500921).

2.1.1. Participants

Participants were recruited from a pool of undergraduate psychology students at Macquarie University. Selection criteria were (a) no prior meditation experience, including active meditation in yoga classes; (b) normal hearing; and (c), no history of ADHD or epilepsy. Each participant took part in just one experiment. Prospective participants were informed of the selection criteria, aims of the study, and the procedure through a research participation website. Participants gave informed consent prior to data collection. They received course credit for their participation. We recruited a minimum of 8 participants. We then continued to recruit participants one by one until we achieved conclusive evidence for or against N1 attenuation during meditation, compared to the non-meditation condition. In Experiment 1, we only had to recruit the minimum of 8 participants to detect a statistically significant effect (6 females, with a mean age of 20 years; SD = 0.76, range = 19-21 years).

2.1.2. Conditions

As in Biedermann et al. (2016), there were two experimental conditions: breath-counting meditation and a visualization control condition. In line with Biedermann et al. (2016), the meditation condition was always completed after the non-mediation condition. This fixed order was used by Biedermann et al. (2016) to prevent a meditative state continuing into the non-meditation condition, which could occur if meditation was the first condition.

2.1.2.1. Visualization control condition

Participants were asked to spend 15 minutes thinking about how they would build a tree house. The pre-recorded instructions included suggestions for aspects of the tree house to consider, maximizing the chance that participants could engage in the task for the full 15 minutes. They were told that after a while, tones would be played through the headphones that they were wearing. They were instructed to ignore the tones and continue building the tree house in their minds. This condition was designed to mirror the meditation condition for posture (static, eyes closed) whilst allowing the participant's mind to imagine a setting in their mind away from the present moment. Verbatim instructions for both conditions are included in Appendix A.

2.1.2.1. Meditation condition

Participants were asked to spend 15 minutes focusing on the inhalation and exhalation of their breath, counting each exhalation from one to 10, then beginning again at one. Similar practices have been recorded as part of meditation as far back as c. 430 AD (Levinson et al., 2014), and are still common as a beginner technique (Cahn and Polich, 2006). Unlike the instructions given in the non-meditation condition (i.e., ignore the tones), participants were instructed that if they did notice the sounds, they were to gently let them go.

2.1.3. Stimuli

Auditory stimuli were blocks of 666 pure tones that were presented binaurally through Sennheiser HD 280 Pro headphones. Each block consisted of frequent 1000 Hz tones (n = 566; 85% of trials) and infrequent 1200 Hz tones (n = 100; 15% of trials), forming a passive auditory oddball paradigm. Infrequent (“deviant”) tones could not be (a) among the first three stimuli in the block, (b) separated by fewer than three standards, or (c) separated by more than 35 standards. A new semi-random tone sequence was generated for each condition and participant. Stimulus duration was 175 ms, with sigmoidal ramps over 10 ms rise and fall times. Inter-stimulus intervals (ISIs) between stimuli were jittered within a range of 925 to 1125 ms to inhibit confounding effects related to temporal expectation of sounds or artefacts from one tone consistently carrying over to the next. Stimuli were created in and presented with MATLAB R2012b (MathWorks, 2012) and Psychtoolbox version 3.0.12 (Brainard, 1997; Pelli, 1997). The stimulus presentation computer was a Dell Optiplex GX990, with a Creative Sound Blaster X-Fi Titanium HD audio card. Volume was set at the same level for all participants through the computer volume control.

2.1.4. Electroencephalogram procedure

To record brain electrical activity, electroencephalogram (EEG) electrodes were positioned according to the International 10-20 system (FP1, FP2, Fz, FCz, Cz, CPz, Pz, Oz, O1, O2, online reference M1, and ground at AFz); re-referenced to M2; with bipolar electrodes at the outer canthi (HEOG) and above and below the left eye (VEOG). Electrode sites around the eyes and mastoids were cleaned with an alcohol wipe and exfoliant. Eye and mastoid electrodes were placed on these sites. The participant's scalp was combed before fitting the EasyCap, as this reduces impedances (Mahajan & McArthur, 2010). All electrodes were connected to the skin with Signa gel, then plugged into a Neuroscan Synamps2 and Acquire software sampling at a 1000 Hz, with an online bandpass filter of 0.05-200 Hz. EEG data were recorded and stored for offline processing.

Participants wore headphones and sat in a comfortable chair. Pre-recorded instructions were presented through the headphones (see Appendix A). Participants completed the non-meditation condition and then the meditation condition. There was a short break between conditions, during which participants drew the tree house that they had imagined. Following completion of both conditions, participants were asked to answer questions regarding their focus and general awareness for each condition (Appendix B), for purposes not related to this study.

2.1.5. Analysis

2.1.5.1. EEG-ERP Processing

Each subject's EEG data was processed in MATLAB, using EEGLAB version 13.5.4b (Delorme, 2004). Each data file was high-pass filtered at 0.1 Hz and low-pass filtered at 30 Hz. Continuous data were epoched from -100 to 500 ms relative to the onset of each stimulus, and baseline corrected from -100 to 0 ms. While epochs were screened for ocular artefacts, VEOG and HEOG channels did not show disruptions in data due to eye movements. Epochs with values beyond ±150 µV were rejected. No more than 10% of the 666 epochs were rejected in any case (mean accepted = 99.70%; SD = 0.01%). Average waveforms were calculated for each condition (non-meditation and meditation) for each tone type (standard and deviant) for each individual. Data were analyzed from the frontal midline electrode (Fz), as Biedermann et al. (2016) found the clearest difference in N1 amplitude between the non-meditation and meditation conditions at this site.

2.1.5.2. N1 amplitude extraction

The N1 was identified as the first clear negative peak at Fz between 50 and 150 ms from stimulus onset. Automatic peak amplitude extraction was manually reviewed to ensure accurate selection of the N1 peak. No manual adjustments were made.

2.1.5.3. Statistics

Planned pairwise comparisons of standard-elicited N1 peak amplitude between conditions were based on the hypothesis that N1 amplitude is smaller during first-time meditation than non-meditation. The primary statistic of interest was the Bayesian measure of effect size *B*. We used a one-tailed Cauchy distribution to generate the spread of hypothesized effect sizes (“prior”), as this is more robust than a normal distribution (Gelman et al., 2008). The width of the prior distribution affects what data are judged consistent with the alternate hypothesis. Our Cauchy prior distribution width was set at 1 (as in

Wagenmakers et al., 2015). A prior width of 1 would suggest that effects further from zero are more likely, whereas a prior width of .5 would suggest that effects closer to zero are likely. We also calculated Student's *t* and Cohen's *d* to facilitate direct comparisons between this experiment and Biedermann et al. (2016). We defined conclusive evidence as B greater than 3 or less than 1/3 as suggested in Dienes (2014). We did not base decisions about sample size on Student's *t*-test outcomes as this increases the risk of Type I error. Statistical analyses were conducted using the open-source program JASP version 0.7.5.5 (Love et al., 2015).

2.2. Results and Discussion

Across participants, the mean N1 amplitude to standard tones was reduced in the meditation condition compared to the non-meditation condition. This effect was statistically reliable (B = 10.84), reflecting a 10-times better fit of the data to the hypothesized model (i.e. N1 amplitude attenuates during meditation compared to the control condition) than to the null model. Figure 2 contrasts grand mean waveforms for the two conditions. Table 2 sets out descriptive statistics for each condition, with Bayesian and Student's *t*-test outcomes for pairwise comparisons.

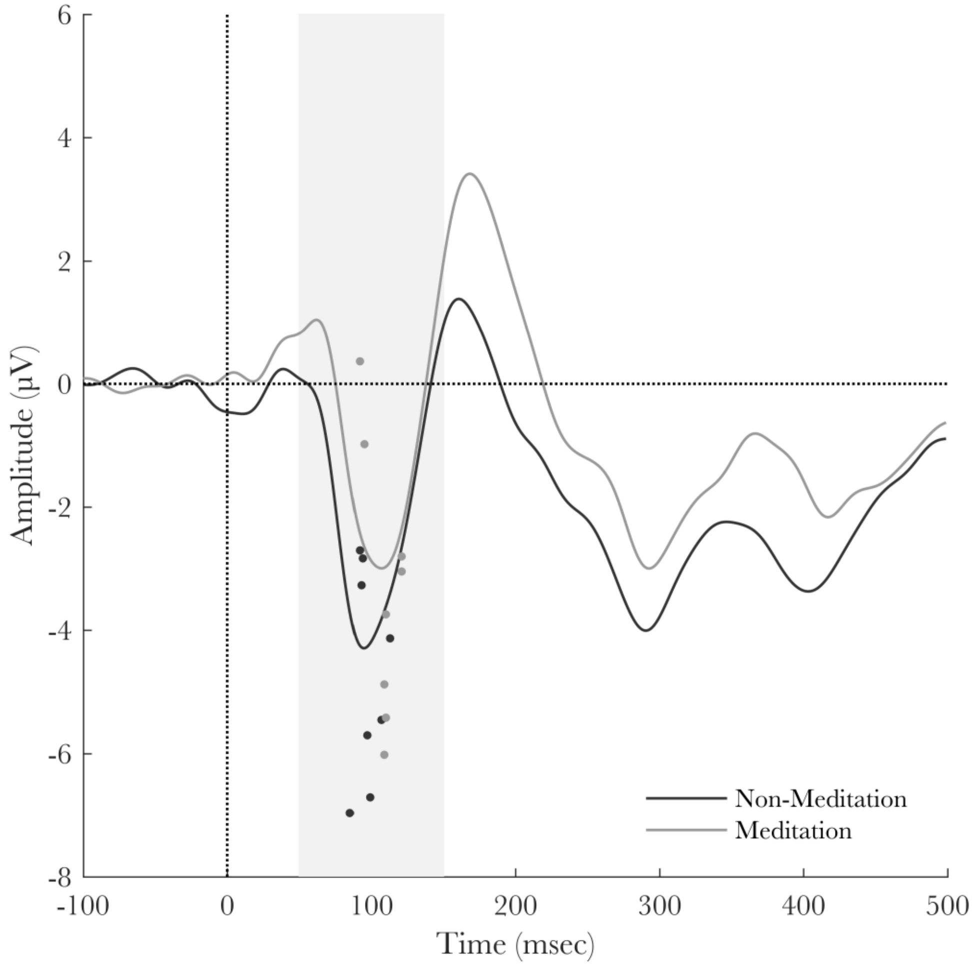

Figure 2. Amplitude (μV) across time (ms) of the average waveform elicited by standard tones at Fz for each condition (non-meditation and meditation) in Experiment 1. The vertical dotted line marks stimulus onset. The grey block marks the N1 selection range (50-150 ms). Scatter points represent individual N1 peaks for non-meditation (dark) and meditation (light).

Table 2

Descriptive Statistics for N1 Amplitude, and Inferential Statistics for N1 Difference between Non-Meditation and Meditation Conditions.

	Mean (μV)	Range (μV)	SD	*B*	*t* (df: 7)	*d*

Non-meditation	-4.721	-6.97, -2.70	1.715			
Meditation	-3.314	-6.02, 0.37	2.194	11.11	-3.34*	-1.18

Note. *=p<.05

Note. Alternate hypothesis: N1 amplitude is greater (more negative) during non-meditation than during meditation (H_A: cond1>cond2).

These findings are consistent with those reported in Biedermann et al. (2016). That is, we replicated a statistically significant effect of meditation on N1 amplitude in first-time meditators. This suggests that the first-time meditation effect reported in Biedermann et al. (2016) was not due to unique characteristics of their first-time meditator group. Rather, it appears to be a reliable effect across different samples of first-time meditators.

3. Experiment 2

Previous studies have found that N1 amplitude is modulated by direction of attention, with larger N1 ERPs elicited by attended stimuli than non-attended stimuli (Maclean, 1975). In Biedermann et al. (2016) and Experiment 1, the instructions for the direction of attention differed for the meditation and non-meditation conditions. For the non-meditation condition, participants were told to “ignore the tones”. In the meditation condition, they were told to “notice the tones; do not attend to them; gently let them go” (see Appendix A for full instructions). The different instructions may have reduced attention captured by tones in the meditation condition compared to the non-meditation condition, reducing the N1 amplitude accordingly.

The aim of the current experiment was to use uniform instructions in the meditation and non-meditation conditions to avoid inducing different attentional states. If non-uniform instructions were responsible for the first-time meditators’ reduced N1 during meditation, then we would no longer find a smaller N1 in to standard tones in the meditation condition compared to the non-meditation condition.

3.1. Methods

The methods for this experiment were identical to Experiment 1 except for the instructions. In both meditation and control conditions, participants were instructed to “notice the tones, do not attend to them; gently let them go”. The analyses were identical to Experiment 1. We tested 12 participants (9 females) with a mean age of 24 years (SD = 10.69; range = 18, 52).

3.2. Results and Discussion

The group mean N1 amplitude to standard tones at Fz was larger during non-meditation than during the meditation condition (see Figure 3 and Table 3). This effect was statistically reliable, reflecting conclusive evidence against the hypothesis of no difference. The hypothesis used in Experiment 1—N1 attenuates during meditation compared to the non-meditation condition—produced a *B* of 81.47, reflecting better fit of the data to this specific hypothesis than to the null model. These findings suggest that increased attention to tones induced by instructions to ignore the tones during the non-meditation condition cannot fully explain why first-time meditators have smaller N1 peaks during meditation compared to non-meditation.

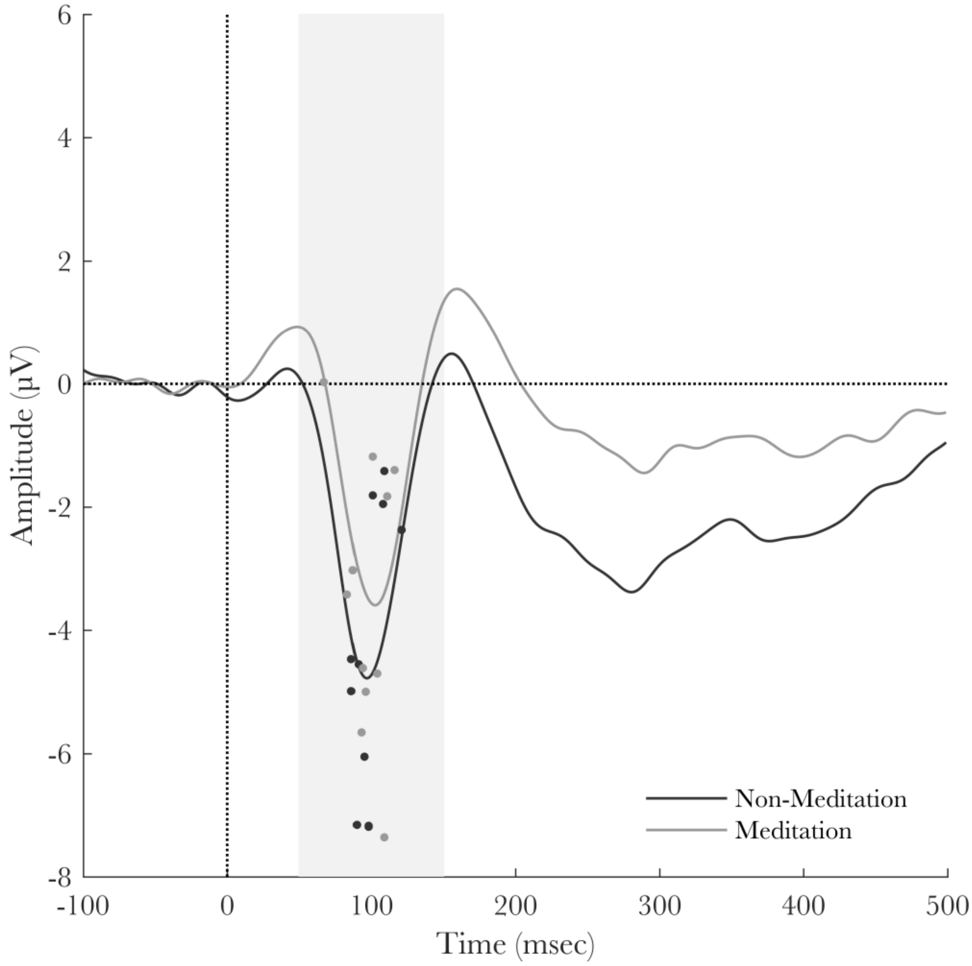

Figure 3. Amplitude (μV) across time (ms) of the average waveform elicited by standard tones at Fz for each condition (non-meditation and meditation) in Experiment 2. The vertical dotted line marks stimulus onset. The grey block marks the N1 selection range (50-150 ms). Scatter points represent individual N1 peaks for non-meditation (dark) and meditation (light).

Table 3

Descriptive Statistics for N1 Amplitude, and Inferential Statistics for N1 Difference between Non-Meditation and Meditation Conditions.

	Mean (μV)	Range (μV)	SD	*B*		$t_{\text{(df: 11)}}$	*d*
Non-meditation	-5.167	-12.88, -1.42	3.257	H_1:	H_2:		
Meditation	-2.244	-10.59, 0.03	2.017	0.03	81.47	-4.40*	-1.27

Note. *=p<.05

Note. H_1: cond1 = cond2; H_2: cond1>cond2

4. Experiment 3

The results so far indicate that first-time meditators' reduced N1 ERP during meditation compared to non-meditation was a reliable effect, and that this effect was not explained by differences in instructions to participants in different conditions. In our final experiment, we tested if the fixed order in which the non-meditation (first) and meditation (second) conditions were presented to participants in Biedermann et al. (2016), and Experiments 1 and 2 above, might explain why first-time meditators have large N1 responses during meditation than non-meditation. This fixed order is a common feature in auditory ERP studies of meditation, with 10 of the 14 studies comparing ERPs measured before meditation (during a non-meditation task or baseline rest period) with ERPs measured during or after meditation. Nearly half of the studies comparing ERPs in a meditation condition with ERPs in a non-meditation condition always presented the meditation condition second (Atchley et al., 2016; Biedermann et al., 2016; Corby et al., 1978). Seven other studies measured ERPs before and during or after meditation; that is, baseline measurement (before meditation) was always taken before the measurement of interest (during or after meditation; see Delgado-Pastor et al., 2013; Joshi and Telles, 2009; Liu et al., 1990; Sarang and Telles, 2006; Srinivasan and Baijal, 2007; Telles et al., 2015). Of these studies, four controlled for the fixed order of measurement within conditions by counterbalancing the order of conditions and basing inferences on the difference between them (Delgado-Pastor et al., 2013; Joshi and Telles, 2009; Sarang and Telles, 2006; Telles et al., 2015); though most still interpreted the within-condition change, from baseline to after meditation, as a meditation effect (Joshi and Telles, 2009; Sarang and Telles, 2006; Telles et al., 2015). Three others did not have a control condition, and used within-condition change, from baseline to during or after meditation, as their index of a meditation effect (Barwood et al., 1978; Liu et al., 1990; Srinivasan and Baijal, 2007).

As mentioned previously, the rationale behind the fixed order of conditions in Biedermann et al. (2016), as well as Corby et al. (1978), was to avoid meditation effects carrying over into non-meditation effects. The fixed order of conditions in Atchley et al. (2016) was designed to prime attention to tones during meditation by presenting them first in a tone-counting task. For the other studies with a fixed order element, the rationale is not explicit.

The fixed order of conditions in these studies, and in ours, raises the risk that changes in ERPs are driven by time-on-task or exposure to the stimuli, rather than by meditation. Specifically, the fixed order of conditions in our experiments raises the risk that first-time meditators' N1 is reduced during meditation due to factors such as N1 “habituation” or fatigue. Habituation, in its broad sense, is a reduced response to a specific repeated stimulus, and is an established feature of the N1: the N1 reduces over long series of repeated stimulation (Roth and Kopell, 1969, across 6 blocks of 115 tones; Woods and Courchesne, 1986, across 6 blocks of 72 tones). Similarly, fatigue – characterised as task-induced strain – reduces N1 amplitude (Boksem et al., 2005). Because the exposure to tones, and possibly the task-induced strain, was always greater in the meditation condition than the non-meditation condition, habituation and fatigue could drive the reduced N1. The following experiment was designed to investigate whether the attenuated N1 during meditation was due to, or mediated by, the fixed order of conditions, which increase the likelihood of effects such as habituation and fatigue. If the attenuated N1 was due to the fixed order of conditions, reversing the order of conditions will produce an attenuated N1 in the non-meditation condition (now the second condition); and counterbalancing the order of conditions will produce an attenuated N1 in the second condition, regardless of what that condition contained (using data from Experiment 2 and 3). Given the N1's susceptibility to habituation and fatigue, we predict that it will attenuate in the second condition.

4.1. Methods

In Experiment 3, we tested eight participants (6 females), with a mean age of 28 years (SD = 13.21; range = 18,52). The methods were the same as for Experiment 2, with three exceptions. First, we reversed the order of conditions so that the meditation condition preceded the non-meditation condition. Second, participants drew the tree house that they had imagined in the non-meditation control condition after both conditions were completed. Third, as conditions were no longer separated by participants drawing the tree house, participants were given a short break between conditions to keep the spacing of conditions matched across experiments.

Further, we analyzed the combined data of Experiment 2 and Experiment 3, which have the opposite condition order, but the same stimulus timing and number of participants. Together, they form a dataset counterbalanced for condition order. We used these data to test whether counterbalancing condition order produces an equivalent N1 amplitude for non-meditation and meditation conditions.

4.2. Results and Discussion

In contrast to Experiments 1 and 2, mean N1 amplitude was reduced in the control non-meditation condition relative to the meditation condition (Figure 6 and Table 5). This effect was statistically reliable ($B = 5.18$), reflecting a 5-times better fit for the hypothesis that N1 peak amplitude decreased in the second condition (now non-meditation) compared to the first condition than for the null model. Whereas the B in this experiment is smaller than that found in Experiment 3, the effect size is large ($d = -0.94$), suggesting that the difference in B between experiments reflects more noise in the Experiment 5 data rather than a smaller effect.

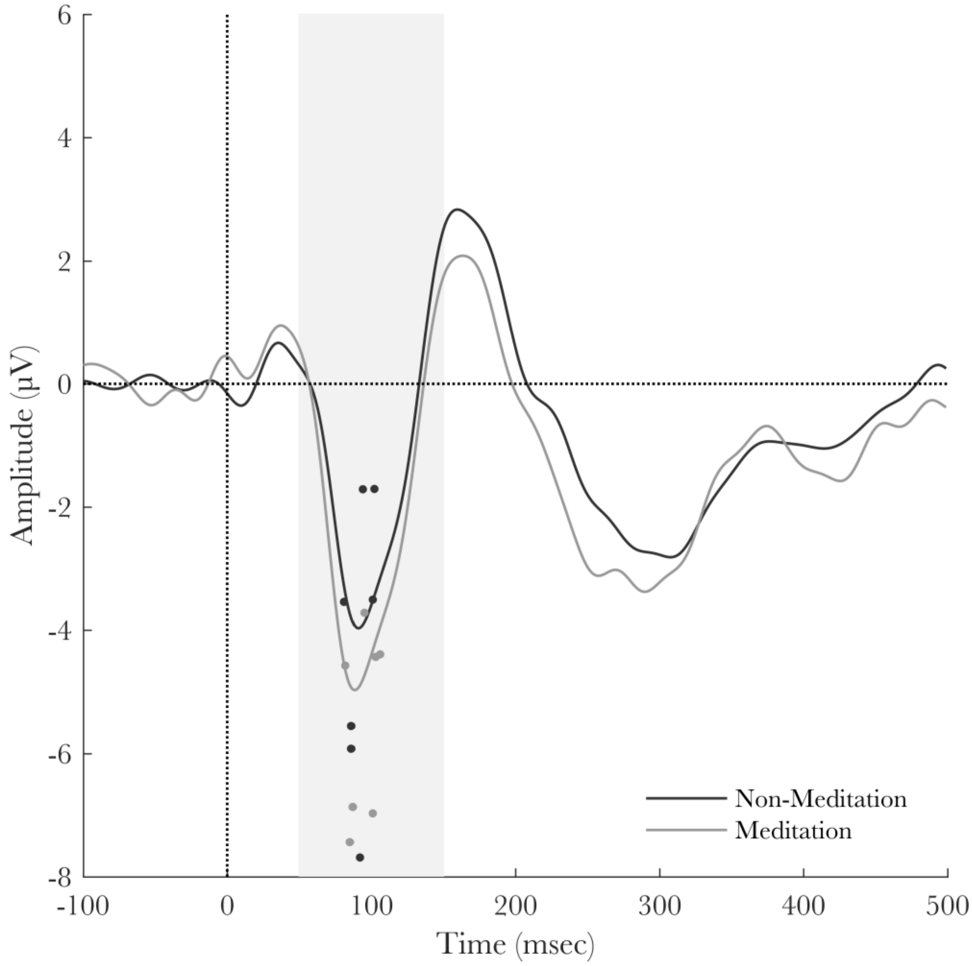

Figure 4. Amplitude (μV) across time (ms) of the average waveform elicited by standard tones at Fz for each condition (non-meditation and meditation) in Experiment 3. The vertical dotted line marks stimulus onset. The grey block marks the N1 selection range (50-150 ms). Scatter points represent individual N1 peaks for non-meditation (dark) and meditation (light).

Table 4

Descriptive Statistics for N1 Amplitude, and Inferential Statistics for N1 Difference between Non-Meditation and Meditation Conditions.

	Mean (μV)	Range (μV)	SD	*B*	*t* (df: 7)	*d*
Non-meditation	-4.747	-8.36, -1.71	2.541			
Meditation	-5.873	-8.61, -3.71	1.803	5.18	2.67	0.94

Note. *=p<.05

Note. H_A: cond1>cond2

The combined data from Experiments 2 and 3, which together have a balanced order of conditions, showed no difference in N1 amplitude between conditions (Table 5). This effect was statistically reliable; the *B* of 0.27 represents conclusive evidence that the hypothesis of no difference in N1 across conditions better fits the data than the hypothesis of a difference in N1 between conditions.

Table 5

Descriptive and Inferential Statistics for N1 Difference between Non-Meditation and Meditation Conditions, Combining Data from Experiment 2 and Experiment 3.

	Mean (μV)	Range (μV)	SD	*B*	*t* (df: 15)	*d*
Non-meditation	-4.73	-8.36, -1.71	2.09			
Meditation	-4.59	-8.61, 0.37	2.35	0.27	-0.32	-0.08

Note. *=p<.05

Note. H_A: cond1 ~ = cond2

The attenuated N1 during the second condition in Experiment 3 (the non-meditation condition), paired with the similarity of the N1 in the analysis that combined data from Experiment 2 (meditation condition presented second) and Experiment 3 (meditation condition presented first), suggest that condition order underpins the attenuated N1 during the meditation condition in Biedermann et al., as well as in Experiments 1 and 2 reported here. We conclude that the N1 attenuates in first-time meditators with repeated exposure to stimuli. The N1 is not modulated by first-time meditation versus non-meditation.

7. General Discussion

In this series of experiments, we tested whether non-meditative factors might explain why first-time meditation elicits a smaller N1 than during non-meditation. We tested whether the effect was reliable (Experiment 1); whether it was due to attention state (Experiment 2); and whether it was driven by condition order effects (Experiment 3). We observed a robust N1 attenuation during first-time meditation compared to a non-meditation condition. This effect was not explained by differences in instructions in different conditions. However, when the order of conditions was reversed, the N1 attenuated during the non-meditation condition. When the order of conditions was counterbalanced, there was no difference in N1 between conditions. The reversal of the effect with reversed condition order, and the removal of the effect with counterbalanced condition order, suggest that the effect is better attributed to the order of conditions than to the content of the meditation condition.

The findings reported here suggest that meditation effects on auditory ERPs – both state and trait – rely on long-term practice. In Biedermann et al. (2016), effects of meditation practice surfaced as a group difference (i.e., a trait effect): long-term meditators had a larger MMN response than first-time meditators, regardless of condition. In Atchley et al. (2016), effects of meditation practice surfaced in group-by-condition interactions: P3 reduced more from non-meditation (attending to tones) to meditation (attending to breath) in long-term meditators than in first-time meditators. These findings provide evidence that long-term meditation practice increases sensitivity to conflict between what was predicted and what was heard, as indexed by the MMN; and enhances the ability to selectively attend to sounds as they become relevant, as indexed by the P3. Along with our study, these studies reinforce the characterisation of meditation as a skill acquired over time.

In contrast, the reduction in N1 from an initial to subsequent condition among first-time meditators raises a new question: are the same order effects present among long-term meditators? The effects of long-term meditation could "protect" long-term meditators from the sources of order effects suffered by first-time meditators (i.e., meditators experience less N1 habituation, less fatigue, and less test anxiety), or long-term meditation could enhance other cognitive capacities (e.g., low- or high-level attention) that "compensate" for the source of these order effects. Long-term meditators in Biedermann et al. (2016) did not show a statistically significant attenuated N1 during the meditation condition even though it followed the non-meditation condition. However, failing to reach statistical significance does not rule out that there could be an effect among long-term meditators. The mean N1 at Pz in Biedermann et al. (2016) did reduce from non-meditation to meditation for long-term meditators as for first-time meditators, though the difference was very small (-3.6 mV to -3.5 mV) and not reliable. At Fz, there was a statistically significant effect of condition across groups – that is, N1 attenuated from non-meditation to meditation – with no reliable interaction between meditation experience and the attenuating N1. Atchley et al. (2016), who similarly presented the meditation condition after a non-meditation control condition, observed a reduced N1 in the meditation condition across both first-time meditators and long-term meditators. Corby et al. (1978) also used a fixed condition order, with non-meditation preceding meditation, to compare non-meditators' and meditators' ERPs. They found that the N1 attenuated across conditions regardless of meditation experience, and hence concluded that N1 attenuation from non-meditation to meditation was an order effect, not due to the content of the meditation condition. Barwood et al. (1978) elicited ERPs before, during, and after meditation. Though none of their comparisons reached statistical significance, the mean N1 reduced from -4.46 mV to -3.69 mV to -3.41 mV across the three measurements, suggesting that the N1 attenuated over time. While it is possible that long-term meditation protects or compensates for order effects, significant N1 attenuation across conditions (Atchley et al., 2016; Corby et al., 1978), and non-significant trends toward N1 attenuation across conditions (Barwood et al., 1978; Biedermann et al., 2016), suggests that long-term meditators are susceptible to order effects.

Protective or compensatory effects of meditation practice on N1 attenuation could be tested by comparing long-term and first-time meditators' N1 in a counterbalanced experiment. First, if long-term meditators are susceptible to order effects, N1 will attenuate from the first condition to the second condition, regardless of condition type. If long-term meditators are protected against order effects, their N1 will attenuate less than first-time meditators' N1. In a number of existing studies, meditation occurred second (i.e., Atchley et al., 2016; Barwood et al., 1978; Biedermann et al., 2016; Corby et al., 1978). If N1 attenuation across conditions in these studies masks a meditation effect that increases N1, in a counterbalanced experiment N1 will increase during meditation compared to non-meditation condition, regardless of condition order.

There are some reports of null or reversed effects of condition order on long-term meditators, in ERP peaks other than the N1. Telles (2015) found that P1, P2, and N2 amplitudes attenuated from rest (first condition) to non-meditation (second condition), but not from rest (first condition) to meditation (second condition). Atchley et al. (2016) found that, while N2 amplitude decreased from non-meditation (first condition) to meditation (second condition) in first-time meditators, it increased during meditation in long-term meditators. These findings could reflect that long-term meditation practice protects against decrements in low-level attention over time, or that it increases low-level attention in the meditative state.

However, the findings reported here, along with other reports of reduced N1 with condition order (Atchley et al., 2016; Corby et al., 1978), demonstrate the role that order of conditions can play in meditation research. First-time meditators, who are often included as a baseline in meditation studies, are susceptible to order effects. Long-term meditators may also be susceptible to order effects; and, to add complication, they could be affected differently to first-time meditators, so that even comparing non-meditators and meditators after the same amount of time on task will not match order effects. If a study has no control condition, or has a control condition but does not counterbalance condition order, order effects can present as meditation state effects. We cannot confidently rule out the role of order in group or condition comparisons. Thus, counterbalancing order is essential for ensuring "meditation" effects reported are truly meditation effects. Future studies should control for order effects in all participant groups to avoid making invalid conclusions about the neurophysiological and cognitive effects of meditation in both non-meditators and meditators.

The remaining question is, how can meditation studies counterbalance condition order without risking carry-over effects of meditation into a subsequent non-meditation condition? Meditation carry-over effects are the basis for studies by Chatterjee et al. (2012), Delgado-Pastor et al. (2013), Joshi and Telles (2009), Kyizom et al. (2010), Sarang and Telles (2006), and Srinivasan and Baijal (2007), which measure meditation effects after a meditation condition; and are the reason for fixed condition order in Biedermann et al. (2016). If meditation carry-over effects and condition order effects both confound comparisons between meditation and non-meditation conditions, meditation studies may need to include a gap between conditions so that meditation can occur first without affecting the non-meditation condition. Future studies should also address how far meditation carry-over effects extend. This will inform the design of new testing paradigms by showing how long a gap we need to ensure meditation does not influence a subsequent non-meditation condition. It will also validate the findings of studies that measure meditation effects after the meditation condition. These two methodological developments – controlling for both order effects and unwanted meditation carry-over effects – will strengthen meditation and ERP research.

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Appendix A. Instructions for Experimental Conditions

A.1. Experiment 1

A.1.1. Visualization. Please close your eyes, and keep them closed until I ask you to open them. Throughout this experiment, sit comfortably and relax, with your back straight and both feet flat on the floor. I would like you to think about how to build a tree house. Think about a suitable location. What type of tree might you use? Would it be in Australia, or somewhere else? How might you get to the tree house? What materials would you use? What kinds of things would you fill it with? Think about the steps involved from beginning to end. After some time building your tree house, some tones will start to play through the headphones. Just ignore them, and continue building your tree house. At the end of this task, I am going to ask you to draw or describe your tree house to me. Just keep your eyes closed, and remember: do not open them until I let you know.

A.1.2. Meditation. Please close your eyes again, and keep them closed until I let you know. Concentrate now on your breath: slowly breathing in, and slowly breathing out. With the first exhalation, count "one"; with the second exhalation, count "two"; and so on, until you reach 10. Then, start again at one. If you lose count, just start with the count of "one" on your next exhalation. Focus on your breath. When a thought arises, just notice it, let it go, and come back to your breath. After some time counting your breath, some tones will start to play through the headphones. Just notice them, do not attend to them. Gently let them go, and continue counting your breath. Please do not open your eyes until I come in and let you know, even if the tones stop.

A.2. Experiment 2

A.2.1. Visualization. Please close your eyes, and keep them closed until I ask you to open them. Throughout this experiment, sit comfortably and relax, with your back straight and both feet flat on the floor. I would like you to think about how to build a tree house.

Think about a suitable location. What type of tree might you use? Would it be in Australia, or somewhere else? How might you get to the tree house? What materials would you use? What kinds of things would you fill it with? Think about the steps involved from beginning to end. After some time building your tree house, some tones will start to play through the headphones. Just notice them, do not attend to them. Gently let them go, and continue building your tree house. At the end of this task, I am going to ask you to draw or describe your tree house to me. Just keep your eyes closed, and remember: do not open them until I let you know.

A.2.2. Meditation. Please close your eyes again, and keep them closed until I let you know. Concentrate now on your breath: slowly breathing in, and slowly breathing out. With the first exhalation, count “one”; with the second exhalation, count “two”; and so on, until you reach 10. Then, start again at one. If you lose count, just start with the count of “one” on your next exhalation. Focus on your breath. When a thought arises, just notice it, let it go, and come back to your breath. After some time counting your breath, some tones will start to play through the headphones. Just notice them, do not attend to them. Gently let them go, and continue counting your breath. Please do not open your eyes until I come in and let you know, even if the tones stop.

A.3. Experiment 3

A.3.1. Meditation. Please close your eyes, and keep them closed until I ask you to open them. Throughout this experiment, sit comfortably and relax, with your back straight and both feet flat on the floor. Concentrate now on your breath: slowly breathing in, and slowly breathing out. With the first exhalation, count “one”; with the second exhalation, count “two”; and so on, until you reach 10. Then, start again at one. If you lose count, just start with the count of “one” on your next exhalation. Focus on your breath. When a thought arises, just notice it, let it go, and come back to your breath. After some time counting your breath, some tones will start to play through the headphones. Just notice them, do not attend to them. Gently let them go, and continue counting your breath. Please do not open your eyes until I come in and let you know, even if the tones stop.

A.3.2. Visualization. Please close your eyes again, and keep them closed until I let you know. I would like you to think about how to build a tree house. Think about a suitable location. What type of tree might you use? Would it be in Australia, or somewhere else? How might you get to the tree house? What materials would you use? What kinds of things would you fill it with? Think about the steps involved from beginning to end. After some time building your tree house, some tones will start to play through the headphones. Just notice them, do not attend to them. Gently let them go, and continue counting your breath. At the end of this task, I am going to ask you to draw or describe your tree house to me. Just keep your eyes closed, and remember: do not open them until I let you know.

Appendix B. Subjective Experience Questionnaire

A.1. Task 1

Below are some statements about your experience of the first task. Please rate how much you agree with each statement, from 1, 'strongly disagree', to 7, 'strongly agree'. Bear in mind that there is no wrong answer; we are interested in your subjective experience of the task.

Please circle the number that best expresses your experience of the **first task**.

		Strongly Disagree … Strongly Agree
1.	I was fully absorbed by the task.	1 2 3 4 5 6 7
2.	I found my mind constantly wandering away from the task	1 2 3 4 5 6 7
3.	I noticed what was happening around me	1 2 3 4 5 6 7
4.	I was aware of internal sensations like my breath and heart rate	1 2 3 4 5 6 7
5.	I found myself focusing so hard on the task that I did not notice anything else	1 2 3 4 5 6 7

Using the line below as a timeline for the **first task**, try to mark out visually which segments of the time you spent focused on the task.

____

PLEASE CONTINUE OVER THE PAGE...

A.2. Task 2

Below are some statements about your experience of the second task. Please rate how much you agree with each statement, from 1, ‘strongly disagree’, to 7, ‘strongly agree’. Bear in mind that there is no wrong answer; we are interested in your subjective experience of the task.

Please circle the number that best expresses your experience of the **second task**.

		Strongly Disagree … Strongly Agree
1.	I was fully absorbed by the task.	1 2 3 4 5 6 7
2.	I found my mind constantly wandering away from the task	1 2 3 4 5 6 7
3.	I noticed what was happening around me	1 2 3 4 5 6 7
4.	I was aware of internal sensations like my breath and heart rate	1 2 3 4 5 6 7
5.	I found myself focusing so hard on the task that I did not notice anything else	1 2 3 4 5 6 7

Using the line below as a timeline for the **second task**, try to mark out visually which segments of the time you spent focused on the task.