Epigenetic Changes Associated With
Two Different Conceptualisations of
Meditation- A Randomised Trial

Rohan Rapyal
BSc, MPharm

A thesis submitted in fulfilment of the requirements for the degree of
Masters Degree

Sydney Medical School
The University of Sydney
September 2016
Declaration of Original Authorship

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Rohan Rapyal
Abstract

Meditation and its related practices have become increasingly popular over the past 10 years, especially in Western society. As a result, the scientific community has focused its efforts on determining whether meditation exerts a physiological effect that is beyond that of a placebo and the exact mechanisms by which this may occur.

The relatively new-found affordability and availability of gene array technologies have provided researchers with a quick method of determining gene expression changes associated with meditation. Gene expression changes provide preliminary insights on the mechanisms by which meditation may exert any specific effects.

A literature review of the entire English-speaking database of studies that investigated gene expression changes associated with meditation, although different definitions of meditation were studied, revealed that there were no studies comparing two different definitions of meditation. I took this opportunity to conduct a randomised controlled trial consisting of 50 healthy participants (25 in each treatment arm) and comparing two different definitions of meditation, namely Sahaja yoga (mental silence meditation) and a form of mindfulness known as body scan meditation, with the intention of determining whether there are distinct biological differences between two different definitions of meditation. Also I aimed to determine whether the mental silence approach to meditation could elicit gene expression changes.

Blood samples were collected from each participant before and after a 6-week intervention period during which they were asked to attend a 1-hour intensive meditation class per week and practice for 15 minutes twice a day at home. RNA was then extracted from the whole blood using the Tempus Spin RNA isolation kit and submitted to the Ramaciotti Centre for analysis using the Affymetrix Human Genome U219 array plate. Gene data was checked and analysed using Partek Genomic Suite 6.6 and analysis of variance (ANOVA) was used to determine the significant gene expression changes.
The results suggested that mental silence and body scan meditations are able to elicit significant gene expression changes. Categories of genes that had significant changes in expression included immune system/inflammation, cancer and cell structure and function. Furthermore, it was found that mental silence and body scan meditation altered the expression of a vastly different number of genes (16 vs 48 respectively). Comparing the gene functions to the literature also led to the speculation that mental silence was associated with potentially more favourable health effects as compared to the body scan meditation group.

In conclusion, it is shown that two different definitions of meditation are able to change the expression of genes in distinct ways and that the mental silence definition of meditation may exert its biological effects by altering the expression of genes related to the immune system/inflammation, cancer and cell structure and function. Owing to the differences in gene expression changes between the two definitions of meditation, both definitions may affect the body differently. Future studies are also proposed with a strong focus on large scaled RCTs comparing different definitions of meditation.
Acknowledgement

Dedicated to Her Holiness Shri Mataji Nirmalal Devi who spent her life bringing peace and joy to every corner of the world - and inspired this work.
# Table of Contents

Declaration of Original Authorship .......................................................... ii  
Abstract........................................................................................................ iii  
Acknowledgement........................................................................................ v  
List of Tables................................................................................................ vii  
Introduction .................................................................................................. 1  
  The first question – what is meditation?....................................................... 2  
  The second question – is there a specific effect? .......................................... 4  
  Understanding the potential biological mechanisms of meditation .............. 9  
  Stress and stress reduction......................................................................... 12  
  Immune function ......................................................................................... 14  
  Immune function and meditation .................................................................. 16  
  Cell structure and function ......................................................................... 17  
  Introducing epigenetics ............................................................................. 19  
Literature Review .......................................................................................... 26  
  Cross-sectional studies .............................................................................. 29  
  Observational trials ..................................................................................... 31  
  Mixed study designs .................................................................................... 32  
  Repeated measures designs (non-RCT)....................................................... 35  
  Quasi experimental (non-RCT) .................................................................... 36  
  Randomised controlled trials ....................................................................... 37  
  Summary ..................................................................................................... 39  
  Study aims .................................................................................................. 25  
Methods ......................................................................................................... 41  
  Study design ................................................................................................ 41  
  Participants .................................................................................................. 41  
  Randomisation, blinding and controlling for non-specific effects ............... 42  
  Interventions .............................................................................................. 43  
  Measures ..................................................................................................... 43  
Results ............................................................................................................ 48  
  Gene array results ...................................................................................... 48  
Discussion ....................................................................................................... 52  
  The implications of the observed gene expression changes ....................... 53  
  Gene changes associated with mental silence ............................................. 59  
  Gene changes observed only in the body scan group .................................. 60  
  Theory, literature review and current study: gene expression changes .......... 63  
  Chronic inflammation .................................................................................. 63
List of Tables

Table 1: Summary of studies looking at gene expression changes (FC) associated with meditation ............................................................ 28
Table 2: Significant gene changes observed in the mental silence group .......... 49
Table 3: Significant gene changes observed in the body scan group ............... 50
Table 4: Gene changes categorised by: total, immune system related, cell structure and function, cancer genes ........................................... 58
Introduction

Over the past 30 years, meditation and related mind–body practices have attracted progressively greater attention from the scientific and health-care community. This has led to a growing body of evidence describing how these practices might affect mental and physical functions.

Medical practitioners and psychologists seem to be the professions demonstrating the most interest. In 2005, M. M. Cohen et al. published the results of a survey involving a representative sample of 2000 GPs across Australia that assessed their attitudes toward complementary and alternative therapies, one of which was meditation (M. M. Cohen, Penman, Pirotta, & Da Costa, 2005). The study showed that 82% of GPs rated meditation as having moderate to high potential effectiveness in the treatment of health issues. Interestingly, meditation was actively encouraged by 65% of the GPs, coming second only to “massage” at 69%. The survey also showed that despite the fact that medical practitioners were comfortable with the idea of meditation, they lacked any formal training on the topic. Furthermore, 27% (the highest percentage out of all the therapies covered in the survey) of GPs indicated that they would like further training on meditation in the form of an introductory workshop. There are two other surveys in the peer-reviewed literature describing similar patterns of attitude and perception amongst mainstream medical practitioners regarding meditation (Gryffin, Chen, & Erenguc, 2014; Pirotta, Cohen, Kotsirislos, & Farish, 2000).

With regard to consumers, in 2012 in the US the National Centre for Complementary and Integrative Health released the results of a national survey (L. I. Black, Clarke, Barnes, Stussman, & Nahin, 2015; Clarke, Black, Stussman, Barnes, & Nahin, 2015). The results indicated that approximately 18 million people had tried meditation at some stage in their lives. This may be due to the growing perception that meditation can help manage pain and reduce stress (Khoury et al., 2013; S. H. Kim, Schneider, Kravitz, Mermier, & Burge, 2013; Park, 2013; Simkin & Black, 2014; Zeidan, Grant, Brown, McHaffie, & Coghill, 2012).
The most authoritative scientific reviews, and most researchers involved in fields of research that intersect with meditation, agree that despite meditation’s substantial popularity amongst both professionals and the broader community, there is still a lack of consistent evidence to support the idea that meditation has a specific therapeutic effect beyond that of placebo. This conundrum stems from the fact that there are two fundamental questions that are yet to be satisfactorily answered about meditation. Answering these questions is essential for this conundrum to be resolved and to thus allow for significant progress in the field of meditation research. Despite the importance of answering them few researchers have attempted to develop answers that might be empirically testable let alone have been tested (Ospina et al., 2007).

**The first question – what is meditation?**

The first of these two fundamental questions is: what is the definition of meditation? Despite its origins, the definition of meditation appears to vary between authors, historical texts and spiritual philosophies. Numerous attempts have been made to create formal definitions; however there is still no consensus definition despite 40 years of Western scientific research into meditation. Some examples of attempts to define meditation include:

- a family of self-regulation practices that focus on training attention and awareness in order to bring mental processes under greater voluntary control and thereby foster general mental well-being and development and/or specific capacities such as calm, clarity, and concentration (Walsh & Shapiro, 2006).

The authoritative National Centre for Complementary and Alternative Medicine defines it as “a conscious mental process that induces a set of integrated physiological changes termed the Relaxation Response” (National Center for Complementary and Alternative Medicine, 2004).

Currently in the West meditation is commonly associated with Buddhism and hence Buddhist ideas about meditation – of which mindfulness is without doubt the most prominent. Mindfulness has been defined by Jon Kabat-Zinn, probably its most well-known proponent, as “paying attention in a particular way; on purpose, in the present moment, and non-judgmentally” (Kabat-Zinn, 1994).
However, scholars widely acknowledge that meditation pre-dated Buddhism by thousands of years. There are numerous texts describing meditation and its associated ideas in pre-Buddhist Hindu texts for example, making it obvious that the origins of meditation go as far back as 7000 BC (Feuerstein, 2006; Mascaro, 1965; Srinivasachariar & Sastri, 1946). Amongst these texts is a variety of discussions about meditation and its related philosophies. Within these texts one finds a more ancient idea of meditation that both predates mindfulness and is conceptually specific and distinct from it, as well as the other more modern concepts of meditation described above.

**Mental silence**

In the *Katha Upanishad* (thought to be written sometime during the first millennium BC) the meditative experience is described as “When the five senses and the mind are still, and reason itself rests in silence, then begins the path supreme” (Mascaro, 1965).

This notion of the “still mind” is reflected in the even more ancient texts of the Mahabharata mythology (approximately 3000 BC), where a sage describes the nature of meditation to his audience: 

“He does not hear...smell...taste...see...or experience touch...his mind ceases to imagine...He desires nothing, and like a log he does not think” (Feuerstein, 2006).

This description appears to make it even clearer that the defining feature of meditation may in fact be the experience of non-thought while remaining fully alert and aware, that is, “mental stillness” or “mental silence”. The Sanskrit terminology for this specific state is *nirvichara samadhi* (Patanjali, Prabhavananda, & Isherwood, 1991).

The concept of “mental silence” has received relatively little attention in the Western scientific literature, however there is a small but growing body of research suggesting that it is worthy of more attention. Mental silence meditation (MSM) involves a number of unique and specific experiential features:

- present moment awareness
- expanded consciousness
- positive mood
• a sense of integration/synergy of faculties
• positive health/wellness in all spheres (bio-psycho-social and spiritual)
• a sense of cosmic connection and unity
• trans cognitive/beyond thought
• specific somatic sensations/descriptors that somehow reflect intuitive knowledge.

The second question – is there a specific effect?

The second question is: does meditation have a specific effect? That is, does it have an effect above and beyond placebo, and if such an effect is present, what might its biological mechanism be?

The placebo effect is a “psychobiological phenomenon by which an improvement in health/behavioural outcomes which are not attributed to the intervention administered can be measured” (Benedetti, Mayberg, Wager, Stohler, & Zubieta, 2005). Up until recently, the scientific community has essentially been divided into several categories in regards to their attitude to meditation and its apparent effects. I summarise these below.

Sceptics

The first category argues that despite the large volume of research done on meditation there is still insufficient high quality evidence to support the notion that meditation in general has a specific effect (Manocha, 2008; Ospina et al., 2007). The facts are that only a small percentage of the thousands of studies done on meditation are actually randomised control trials (RCTs), and that within this small subset of studies only a minority use credible methods to control for non-specific (placebo) effects. Within this very small subset of rigorously designed placebo, controlled trials the evidence for an effect beyond that of placebo is at best mixed (Ospina et al., 2007).
Pragmatists

The second category acknowledges the concerns raised by the above group. However they argue that despite the absence of a clearly demonstrable specific effect, the clinical improvements regardless of the specific cause are respectable and clinically advantageous and hence should be used. To justify their position, they explain that there is a substantial body of evidence for benefit from studies with an underlying conceptual design, that is, to compare “treatment as usual” to “treatment as usual PLUS meditation”. Many of these studies demonstrate positive effects in favour of the combination approach and hence are interpreted to reflect favourably on meditation and hence its clinical potential (Barnhofer et al., 2009; Kabat-Zinn et al., 1998; Saeed, Antonacci, & Bloch, 2010; Teasdale et al., 2000; Visceglia & Lewis, 2011). The weakness is that the apparent effect may nevertheless be the result of non-specific effects overlain on top of the standard treatment regime. While this line of reasoning is pragmatically correct it ignores the fact that the same benefits may accrue from non-meditative interventions. It also assumes that meditation is not associated with a significant cost or risk.

Optimists

This third category argues that thousands of both controlled and uncontrolled studies, despite the lack of clarity afforded by these methodologies, is sufficient indication of the therapeutic potential to warrant broader adoption. The proponents argue that the combination of these low quality studies and “common sense” are sufficient justification with respect to meditation because of its apparently benign nature. This perception ignores important issues such as: the fact that some meditation techniques can be arduous, expensive and time consuming; there is a growing recognition that meditation can occasionally be associated with adverse experiences, sometimes serious ones; and that there is an opportunity cost wherein practitioners might be better off expending their time and effort on a more demonstrably effective intervention. These optimists frequently co-opt the arguments and documents from the other categories when it suits them to support their position.
Traditionalists

The herbal and traditional medicines industry, faced by demands from government regulators to justify the existence of their products, frequently fall back on the “historical evidence” argument (Micozzi, 1998; Pirotta et al., 2000). This line of reasoning posits that although there is a dearth of rigorous modern scientific evidence to support the clinical use of their products there is a considerable history of traditional generic usage that can extend for hundreds and sometimes thousands of years (Crellin, 2001). This “historical evidence”, it is argued, is sufficient to warrant modern usage. The success of the argument is proven by the fact that these products are widely available in shops with the blessing of the regulators despite the absence of modern clinical trial evidence. Meditation enthusiasts and the organisations that market meditation to them have embraced this strategy as a way of undermining the sceptics’ calls for more evidence regarding meditation.

Fanatics

This category overlaps somewhat with the traditionalists but embodies an even more extreme line of thinking. These people, who are mostly meditators, consider the need for any kind of justification or evidence as irrelevant. They embrace the idea that meditation should be universally practiced by all. They frequently ignore or play down considerations of risk or cost. The fanatics deride attempts to scientifically study meditation as attempts to confine the experience of meditation to the limitations of rationality. Much of the rhetoric used by this group is part of the larger anti-science narrative. The sceptics reciprocate by labelling the claims of the traditionalists and fanatics as spurious and that in fact the traditionalists and the fanatics are themselves a living demonstration of the dangers of irrational and indiscriminate thinking about meditation.

New wave

Finally, there is a category that in many ways is smaller than all these others but it acknowledges that each of the above categories has some, albeit variable, level of validity. It is this category to which this researcher belongs.

Most importantly, the issue identified by the sceptics, that the evidence for an effect beyond that of placebo is still equivocal is essentially true. However, one of the
reasons for this is that a fundamental error made by the sceptics and to some extent by all the above-mentioned categories, is the assumption that meditation is a single entity. Until now, meditation has been recognised as being a broad range of different practices and methods that aim to increase general wellbeing and reduce stress. Hence, it may be possible that different techniques of meditation may be invoking different effects. This is why the current broad definition of meditation must be dissected in order for a thorough investigation into whether or not different practices invoke different effects, if any. And this will occur in the Literature Review and the following sections as I discuss the various attempts at understanding the physiology of ‘meditation’.

**Evidence of a specific effect**

Difficulties in excluding placebo effects stems from the lack of appropriate control strategies, a need for randomisation or methodologically robust study designs and also our initial problem of a consensus definition of meditation.

Although the mental silence concept has not gained much traction, there is a growing body of evidence suggesting that it may have specific effects as outlined below.

In India Rai et al. conducted the first series of randomised controlled trials involving the mental silence concept by studying the clinical and physiological effects of the Sahaja yoga meditation technique (Panjwani, Gupta, Singh, Selvamurthy, & Rai, 1995; Panjwani et al., 1996; Rai, Setji, & Singh, 1988). The findings of these RCTs can be summarised as follows.

When compared to a sham meditation, Sahaja yoga was found to reduce physiological arousal and increase the activity of the parasympathetic nervous system. This led to a reduction in heart rate, respiratory rate, blood pressure, oxygen metabolism and urinary vanillylmandelic acid (Rai et al., 1988). Studies during the 1980s determined that urinary vanillylmandelic acid proved to be a useful biochemical marker of psychological stress (Fukuda et al., 1996).

In another study when comparing epilepsy patients to a control group and an exercise group which mimicked Sahaja yoga meditation, it was found that seizure frequency was decreased by 62% at 3 months and 86% at 6 months for the intervention group.
This also coincided with alterations in electroencephalogram readings (increased delta and alpha waves) (Panjwani et al., 1995).

And in another study, significant changes in galvanic skin resistance, blood lactate and urinary vanillylmandelic acid were observed at 3 months and 6 months during the intervention as compared to the control group (Sahaja yoga meditation imitation group) (Panjwani et al., 1996).

In Australia, Manocha et al. conducted a series of trials to further investigate the specific effects of mental silence and expand on the work done by Rai et al. (Manocha, Marks, Kenchington, Peters, & Salome, 2002). The RCT study designs used by Manocha et al. aimed to address the common methodological design flaws of previous studies in the hope of more reliably identifying a specific effect (Manocha, Black, Sarris, & Stough, 2011). Manocha’s first RCT looked at the effects of mental silence meditation on asthma suffers and whether it could improve mood, symptoms and airway hyper-responsiveness (Manocha et al., 2002). Manocha’s second RCT looked at the effects of the same approach to meditation on work stress (Manocha et al., 2011). Both studies showed that meditation provided significant benefits in a number of key parameters when compared to study interventions designed to control for important non-specific effects arising from factors such as credibility and expectancy, reduced physiological arousal and therapeutic contact. The RCTs appear to confirm that mental silence exerts specific effects.

Given the previous considerations, the observation of repeatable, specific effects is a remarkable finding worthy of further investigation. Importantly, neither Rai’s nor Manocha’s trials were designed to give much insight into the biological mechanisms responsible for these apparently specific effects. Additionally the trials were not designed to provide insights into any difference that might exist between different definitions of meditation and yet clearly the results of these studies point towards the notion that some definitions of meditation may be more likely to generate a specific effect than others.
Understanding the potential biological mechanisms of meditation

The most widely accepted explanation on how meditation might exert its biological impacts is that it acts via the relaxation response (Benson, Beary, & Carol, 1974a).

The relaxation response is a spectrum of psycho-physiological changes associated with reduced physiological arousal. Physiological arousal and the relaxation response occur as a result of activity converging on pathways within the autonomic nervous system. A brief explanation of the interactions between the autonomic nervous system (ANS), central nervous system (CNS) and the physiology of peripheral organs follows.

Physiological arousal

The nervous system is comprised of the peripheral nervous system and the central nervous system. The peripheral nervous system is divided into the somatic and autonomic nervous systems. The CNS is comprised of the brain and spinal cord (Brodal, 2010).

The ANS is further subdivided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS), both of which are fed by neuronal pathways stemming from the hypothalamus located in the CNS. Both the SNS and PNS regulate a range of biological functions over which we have minimal control. These include heart rate, breathing, digestion and regulation of blood flow (Brodal, 2010).

Physiological arousal occurs as a result of engagement with stimuli in the environment and has been a focus of psychophysiological research for several decades. Physiological arousal involves the activation of the SNS via hypothalamic projecting neurons from the CNS. The activation of the SNS stimulates the release of the catecholamines (epinephrine and norepinephrine) from the adrenal medulla. These catecholamines diffuse through the blood stream and act on receptors in various organs and also on blood vessels resulting in a number of effects which include: increased heart rate, increased heart contractility, increased blood pressure as a result of an increase in total peripheral resistance, increased sensory alertness, constriction of pupils, increase in electro dermal activity, increased sweating and
increased respiratory rate (Azarbarzin, Ostrowski, Hanly, & Younes, 2014; Bradley, Miccoli, Escrig, & Lang, 2008; Noteboom, Barnholt, & Enoka, 2001).

The relaxation response

The relaxation response is characterised by a decrease in SNS activity and an increase in PNS activity, essentially a reversal of physiological arousal, which leads to the following typical effects:

- slowing of heart rate
- decrease in respiration
- decrease in metabolism
- increase in salivation and digestion
- decrease in blood pressure
- increase in alpha activity in the brain
- subjective feeling of relaxation
- subjective feeling of warmth and heaviness.

The idea that meditation might work by eliciting the relaxation response has been investigated over the past few decades, with the first studies emerging in the literature in the 1970s. Since that time many studies were published that demonstrated that meditation was generally associated with physiological changes characteristically associated with a reduction in autonomic arousal. Herbert Benson’s group pioneered this line of thinking with a series of studies that appeared to demystify meditation by demonstrating that its chief physiological effect was to reduce arousal (Avorn & Benson, 1974; Benson, Beary, & Carol, 1974b; Benson, Dryer, & Hartley, 1978; Benson, Rosner, Marzetta, & Klemchuk, 1974; Benson, Steinert, Greenwood, Klemchuk, & Peterson, 1975). At first, these physiological changes were associated with Transcendental Meditation (TM), a highly commercialised form of meditation which in the 1970s and early 1980s achieved such popularity, at least in the West, was what people understood as meditation (Delmonte, 1984; Holmes, Solomon, Cappo, & Greenberg, 1983). However, Benson soon discovered that many different approaches to meditation also triggered the same process of de-arousal. These different approaches included simple relaxation methods and even practices not previously categorised as meditation such as certain forms of prayer. For example a 1977 study comparing transcendental meditation,
general relaxation training and muscle relaxation via electromyograph biofeedback demonstrated that although the TM group significantly decreased physiological arousal, the other two control groups were equally as effective (Cauthen & Prymak, 1977). Another study comparing TM to listening to music found that both groups were equally effective in decreasing oxygen consumption and carbon dioxide production (Fenwick et al., 1977).

Benson coined the term “relaxation response” to describe this set of physiological responses (Benson, 1975). These findings represented a paradigm shift in the understanding of meditation and similar practices away from mystical and spiritual ideas and towards a more secular, non-denominational conceptualisation. This led to the widespread assumption that meditation could be physiologically defined by the relaxation response. The idea has had traction ever since with many researchers and authorities (e.g. as stated earlier the US National Centre for Complementary and Alternative Medicine).

Although this way of understanding meditation was greeted with enthusiasm during the 1970s and 1980s, the fact was new studies provided evidence that many non-meditative strategies also elicited the relaxation response. Many researchers supported the notion that the definition of meditation should be allowed to expand and accommodate strategies traditionally regarded as non-meditative but that could also elicit the relaxation response. Although this seemed like the easiest solution, it meant that almost any relaxing activity could be included in the definition of meditation.

Some researchers argued that rather than dilute the concept of meditation, a different definition of meditation was required to support the perception that meditation was a specific and distinct entity (Cardoso, de Souza, Camano, & Leite, 2004).

This dilemma highlights the need to define meditation clearly. Only then can we more fully test and hence understand the biological mechanisms by which meditation may act and how this differs from other non-meditative activities. The relatively nebulous and fluctuating conceptualisations of meditation appearing in both professional journals and popular media have served to exacerbate rather than
resolve the intertwined questions about its potential mechanisms and how it might be defined.

Stress and stress reduction

More sophisticated ideas about how meditation might influence human biology developed in parallel with the emergence of fields such as psychoneuroimmunology and the growing interest in the physiology of stress. Research from these fields has provided a rich source of hypotheses about how meditation might exert its effects on mind and body.

What is stress?

The term “stress” has become common vocabulary in Western culture and has been associated with a set of negative psychological conditions. Recent studies have linked stress to common health problems include: hypertension, heart disease, substance abuse, anxiety, depression, gastrointestinal disorders, cancer, headaches and back pain (S. Cohen, Janicki-Deverts, & Miller, 2007; Conti, Maccario, & Fulcheri, 2011; Spruill, 2010; Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000). Yet, its definitions vary within different contexts and different people. The first and most generic definition of stress was proposed by Hans Selye: “Stress is the nonspecific response of the body to any demand” (Selye, 1976).

Despite the ongoing fluxes in defining what psychological stress is, its physiological impact can be characterised by short-term and sustained long-term changes. The short-term response or acute response has popularly been labelled the “fight or flight response” and is beneficial in a critical or life threatening situation. When these short-term stress events become frequent or prolonged, they result in chronic stress which over time may contribute negatively to a person’s mental and physical health.

Meditation and stress reduction

It is well established that meditation is able to reduce the negative dimensions of psychological stress (Goyal, Singh, Sibinga, & et al., 2014). The biological mechanisms by which it does so are still somewhat unclear. It is currently thought that meditation influences the body at a molecular level through the reduction in
stress biomarkers and more recently, through the decreased expression of stress related genes.

One commonly associated biomarker with stress is the steroid hormone cortisol. Researchers observed serum cortisol concentrations in meditators were significantly lower than compared to their control counter parts (Sudsuang, Chentanez, & Veluvan, 1991; Vandana, Vaidyanathan, Saraswathy, Sundaram, & Kumar, 2011). Other biomarkers for stress have also been widely accepted. Examples of these biomarkers include interleukin-6 (IL6), tumour necrosis factor a (TNF-a) and the more recently used C-reactive protein (CRP). Studies have shown that meditation also seems to influence the reduction of these markers of stress therefore giving us another possible mechanism through which it can exert its purported health effects (Creswell et al., 2012; T. W. W. Pace et al., 2010; Rosenkranz et al., 2013; Zgierska et al., 2008). It is also important to note that biomarkers and genes related to stress have a large degree of overlap with biomarkers and genes related to inflammation. Therefore, it may be possible that meditation’s biological effects are mediated through interplay between stress and inflammatory pathways.

**Chronic inflammation and meditation**

Meditation is a lifestyle modification that is commonly associated with reduction of stress. As mentioned previously, the connection between stress and the genesis of chronic inflammatory mediators is well documented (Andrew Steptoe, Mark Hamer, & Yoichi Chida, 2007). It is plausible therefore that meditation might have a positive influence on chronic inflammation by reducing stress and possibly other pathways (Chiesa & Serretti, 2009; Manocha et al., 2011). The impact of meditation on chronic inflammation, and hence the risk of developing non-communicable diseases (NCDs) (cardiovascular disease, cancer, respiratory disease and diabetes) can therefore be assessed by measuring its impact on chronic inflammation markers. Epigenetic studies offer a convenient and powerful method to assess the impact of meditation on NCD risk.

Inflammatory markers can be measured directly through serum concentrations and indirectly via gene chip array analysis. Gene expression levels can correlate, up to a certain extent, with concentrations of each inflammatory marker in the blood at the time the blood sample was taken (Vogel & Marcotte, 2012).
In 2012, Antoni et al. reported that those who participated in cognitive based stress management therapy (CBSM), displayed significant down regulation of pro-inflammatory genes including IL6, TNF and IL1 (Antoni et al., 2012).

Three separate studies exploring other various techniques of meditation including hatha yoga, Kirtan Kriya meditation and relaxation response sequence have also displayed down regulation (i.e. a beneficial reduction) of pro-inflammatory genes (Bhasin et al., 2013; D. S. Black et al., 2013; Kiecolt-Glaser et al., 2010).

These studies suggest that it would be beneficial to determine what changes to gene expression may occur as a result of the mental silence experience and whether or not any such changes may be unique to it vis-a-vis other definitions of meditation.

**Immune function**

The immune system is the collection of cells, tissues and molecules that mediate resistance to infections; its physiological function can be summarised as follows (Abbas & Lichtman, 2010):

- defence against infections
- defence against tumours
- its ability to cause cell injury and also induce cell repair
- clearance of dead cells
- its ability to induce chronic or acute inflammation
- the recognition of tissue grafts and newly introduced proteins.

There are two main branches of the immune system (innate and adaptive) and a brief summary of each is given.

**The innate immune system**

The innate immune system is the first to respond to an infection and its actions can be observed within 2-3 hours of infection. The following are key components of the innate immune system (Abbas & Lichtman, 2010):

- Epithelial barriers of the skin and mucosal tissues provide a first line of defence against the external environment. Within these epithelial tissues reside cells and natural antibiotics, which aim to block the entry of infectious agents.
• neutrophils
• monocytes/macrophages
• dendritic cells
• natural killer cells
• mast cells
• innate lymphoid cells
• the complement system.

The innate immune system has a unique response to microbes (which are microscopic living organisms capable of infection), allowing it to be distinguished from the adaptive immune system.

The innate immune system aims to eliminate microbes through the induction of an acute inflammatory response and via antiviral defence mechanisms. Inflammation increases permeability of blood vessels, which allow immune cells to access the site of injury in greater amounts. This leads to the destruction of microbes, clearance of damaged cells and the promotion of cell repair. In contrast, antiviral defence mechanisms rely on the production of type 1 interferons that are secreted by dendritic cells. Type 1 interferons include IFN-α and IFN-β, both of which are recognised by receptors on the infected cell. The binding of these interferons then leads to inhibition of viral gene expression and the degradation of the viral genome.

Overall, the innate immune system is the first line of defence against infection and the quickest to respond. In addition, it plays a crucial role in providing signals to activate the adaptive immune system and to make sure that it responds only to infectious agents and minimises the chances of an autoimmune reaction.

**Adaptive immune system**

The adaptive immune system requires the recognition of a pathogen and differentiation of lymphocytes before a significant immune response can be achieved against an infection. There are two main types of adaptive immune responses; humoral immunity and cell-mediated immunity (Abbas & Lichtman, 2010).

Humoral immunity is carried out by immune cells known as B-lymphocytes. These immune cells produce proteins called anti-bodies that bind to sections of pathogenic
microbes (also known as antigens). Anti-bodies are produced in a response to an antigen and block their ability to infect host cells and infiltrate tissues in the body. The only disadvantage of anti-bodies is that they are unable to attack microbes which have already infected cells and reside within the cell structure. This is where the second branch of the adaptive immune system plays a role.

The cell-mediated immune response is facilitated by T lymphocytes (commonly known as T helper cells). Once a cell has been infected, it displays the antigen on the cell surface. Circulating T lymphocytes recognise these antigens and either kill the infected cell or instruct the cell to destroy the microbes internally through lysosomal degradation (in the case of macrophages). The adaptive immune system has a number of key features which distinguishes it from the innate immune system:

- It is very specific meaning that each antigen elicits its own specific response through the production of specific anti-bodies and receptors.
- It is able to respond to a large diversity of antigens.
- The adaptive immune system has memory, meaning that repeated exposure of the same antigen results in a faster and more effective immune response.
- The ability to undergo clonal expansion means that once a antigen has been identified, a large number of antigen specific lymphocytes can be created from naïve lymphocytes.

**Immune function and meditation**

There is emerging evidence that mind–body related practices such as meditation may be able to modulate the immune response either through increased levels of inflammatory markers (as discussed previously) or through the increased levels of immune cells (Infante et al., 2014; Morgan, Irwin, Chung, & Wang, 2014). In my literature review there was only one study relating to immune gene expression changes (Antoni et al., 2012). This high quality study found that cognitive based stress management increased gene expression for the following genes: type I interferon response (IFIT1, IFIT2, IFIT3, IFI44, IFI44L, ISG15, MX2, OAS2, OAS3), type II interferon signalling (IFNG) and interferon signal transduction (STAT1, STAT2). As discussed previously, interferons are involved in their anti-viral activity and therefore increased expression of these genes may lead to a superior anti-viral response than in those who did not meditate.
Cell structure and function

A relatively new idea is that meditation may influence the body through its actions on cell structure and function components. Cells are the underlying basis of all living life forms including humans. “Cell Theory” is one of the fundamental basic principles of biology and states:

- All living organisms are composed of cells
- The cell is the basic unit of life
- Cells arise from pre-existing cells
- Energy flow occurs within cells
- DNA is passed on from cell to cell
- All cells have the same basic chemical composition.

Homeostasis is process by which cells maintain and regulate their internal environment in response to constant changes in the external environment. The ability to maintain homeostasis is a function of how healthy a cell is and how long it can survive. A cell maintains homeostasis by controlling what enters and leaves it through its plasma membrane and thus controlling factors essential for survival. These include:

- maintaining intracellular metal ion concentrations within a narrow range
- maintaining pH levels
- removing waste products
- regulating temperature
- producing energy
- maintaining a barrier from the external environment
- producing carrier proteins and receptors to respond to the external environment
- regulating gene expression.

The above list is by no means exhaustive and only highlights the basic processes that are required for cell homeostasis. The genes that code for proteins involved in these processes are far larger in number, however they can broadly be categorised by the following:

- heat shock proteins
- enzymes
• membrane transporters
• ion channels
• ligand gated ion channels
• G-protein coupled receptors
• cell structural proteins.

Numerous recent studies and reviews have already established that psychological stress can shorten the lengths of structures called telomeres (Epel et al., 2004; Oliveira et al., 2016; Starkweather et al., 2014; Tzanetakou, Nzietchueng, Perrea, & Benetos, 2014). Telomeres are DNA sequences at the end of each chromosome that protect it from nucleolytic degradation, unnecessary recombination, repair and inter-chromosomal fusion. Every time a cell divides, the telomere shortens and as this continues, it eventually induces cell senescence (which leads to cell death).

As discussed previously, meditative practices have been shown to reduce stress and stress biomarkers. The connection between stress and telomerase led researchers to investigate the link between mind body practices, such as meditation and whether or not they are able to influence telomerase activity. The literature surrounding this notion has grown over the past 5 years and a number of publications confirmed the idea that meditative practices might beneficially influence the activity of telomerase and thus prolong cell life (Jacobs et al., 2011; Kumar, Yadav, Yadav, Tolahunase, & Dada, 2015; Lengacher et al., 2014). Additionally, a 2013 meta-analysis of four mindfulness meditation RCTs concluded that mindfulness significantly increases telomerase activity in peripheral blood mononuclear cells (Schutte & Malouff, 2014).

Based on these studies it may be plausible to expect that mental silence may alter telomerase or the expression of its related genes and give us another possible explanation of how it might exert its biological effects. In addition, it is also possible that the two different definitions of meditation may alter the expression of other cellular structure and function genes. However, since these genes are involved in an exponential number of gene pathways, it would be impossible to determine the impact on the human body.
Introducing epigenetics

Over the last decade or so the field of epigenetics has emerged as a new and powerful way to understand the biological mechanisms that regulate health and disease. Epigenetics is a molecular biology discipline that differs from genetics because, rather than focusing on the identification of genes that lead to the expression of a certain phenotype or disease risk, or the inheritance of such genes, it examines the mechanisms that regulate the expression of genes. Epigenetics is thus predicated on a new understanding of how the genome operates – as a dynamic system that can act in very short time periods to activate or deactivate a gene and hence its ability to support the production of the proteins that it codes for (Allis, Jenuwein, & Reinberg, 2007).

In other words a single genome can have multiple, different epigenetic and hence phenotypic destinies depending on which environmental factors might act on it at crucial time points in the organism’s development. This is why the new field of epigenetics is seen in many ways to contradict the traditional view of the genome – that our genes are locked into an unchangeable pathway of development after they are produced. In traditional genetics, DNA replication and gene expression are controlled by elements such as promoters, enhancers or binding sites for repressor proteins which are present or absent in the DNA sequence itself. In contrast, epigenetic mechanisms modify the conformation of the DNA and the accessibility of other factors to DNA without altering the DNA sequence. It thus refers to a level of control external to the DNA which modifies how the DNA is read rather than to alteration of the DNA sequence itself. This external control allows for genes to be effectively switched between the “on” or active state and the “off” or inactive state, both of which can occur without causing changes to the DNA sequence (Jaenisch & Bird, 2003). This phenomenon adds an extra explanation as to why although all cells contain the same DNA, they can display different phenotypes. For example, our body contains a myriad of cells ranging from skin cells to liver cells all of which have the same DNA present. The phenotype displayed is clearly attributed to epigenetic regulation of the DNA. Another differentiating factor of epigenetics is that these gene expression changes can be inherited by the next generation (García-Giménez, 2015; Heard & Martienssen, 2014). Epigenetics therefore offers an understanding of the biological mechanisms that underlie virtually every aspect of biological function.
Most importantly, epigenetic tests have now reached a level of breadth, sophistication and affordability that allows broad insights into the full spectrum of physiological systems that might become more or less active over even short time periods in response to a given stimulus.

Previously, investigations into the biology of meditation were restricted to relatively narrow avenues of enquiry, the commonest being:

- peripheral physiological measures of autonomic activity such as: blood pressure, heart rate, skin temperature, galvanic skin resistance and more recently heart rate variability (Chatterjee, Ray, Panjwani, Thakur, & Anand, 2012; Chung, Brooks, Rai, Balk, & Rai, 2012; Yunati, Deshp, & Yuwanate, 2014)
- neuroendocrine factors such as circulating stress hormones (catecholamines, cortisol), endorphins, oxytocin, ACTH and aldosterone (Infante et al., 1998; Turakitwanakan, Mekseepralard, & Busarakumtragul, 2013)
- central nervous system assessment methods aimed at assessing brain activity such as electroencephalogram, functional magnetic resonance imaging, positron emission tomography and magnetoencephalography (Aftanas & Golocheikine, 2001; Hernandez, Suero, Rubia, & Gonzalez-Mora, 2015)

The advent of the microarray gene chip

The term microarray refers to the structured arrangement, “array”, of the probes of interest in a grid format used at a small scale, “micro”. A microarray gene chip is an apparatus where single-stranded DNA oligonucleotides are affixed to the solid surface (usually silicon or nylon). The principle of DNA microarray is based on the fact that single-stranded DNA has a natural affinity to bind to its complementary DNA or RNA strands (given the right chemical conditions). As the single-stranded DNA has a high affinity to become double stranded, when samples are added to the surface of the microarray they become hybridised to the affixed complementary oligonucleotides. These affixed oligonucleotides are created from a fragment of genomic DNA, cDNA, PCR products or chemically synthesised oligonucleotides that have already been sequenced and are available from a library.
Microarrays allow for the multiple parallel gene expression analysis of thousands of genes by binding the transcribed mRNAs of expressed genes, and are capable of covering the whole genome from a sample as little as 50–100 ng of total RNA. The advantage of this is that instead of being limited to analysing a smaller number of genes relating to specific body systems, researchers can now do a genome wide expression analysis which covers multiple body systems at once.

There are three major commercial gene expression platforms for DNA microarray. These include:

- **Affymetrix** (Affymetrix, Santa Clara, CA, USA): utilises in situ synthesis of oligonucleotides directly onto a quartz wafer (known as a gene chip).
- **Illumina** (Illumina, San Diego, CA, USA): 3-micron silicon beads are randomly distributed on either planar silicon slides or fibre optic bundles. These 3-micron beads are covered with thousands of copies of a specific oligonucleotide in order to capture complementary sequences when a sample is added.
- **Agilent** (Agilent, Santa Clara, CA, USA): Inkjet printing is used to print oligo monomers onto a special glass slide. This produces 60-mer length oligonucleotides which are printed base by base.

The gene expression studies on meditation which are discussed in the literature review (see Table 1) all use different gene array methods. For example **Affymetrix** was used by three studies (Bhasin et al., 2013; Dusek et al., 2008; Q. Z. Li, Li, Garcia, Johnson, & Feng, 2005), **Illumina** was used by 4 studies (Antoni et al., 2012; D. S. Black et al., 2013; Creswell et al., 2012; Qu, Olafsrud, Meza-Zepeda, & Saatcioglu, 2013). Overall the different platforms of microarray achieve the same purpose with differences mainly in costs and ease of use. In summary, the unique utility of the epigenetic approach is that it provides an insight into the function of multiple body systems with a single, simple battery of tests from a single sample of blood.
The biological mechanisms of epigenetic regulation

The mechanisms by which epigenetic changes occur include (but are not limited to) three main processes:

1. DNA methylation (Smith & Meissner, 2013)
2. histone modification/ chromatin remodelling (Bannister & Kouzarides, 2011)
3. RNA interactions. (Holoch & Moazed, 2015)

Methylation

The most well-known epigenetic modification is DNA methylation which results in the inhibition of transcription of the gene involved. Methylation occurs exclusively on the dinucleotide sequence which is a link between the base cytosine and the base guanine via a phosphate bond (also known as CpG). It has been noted that the majority of CpGs are methylated and those CpGs which are not methylated are usually clustered together to form CpG islands. Gene expression requires the transcription machinery to be able to easily access the gene promoter region (part of the sequence that is essential in that specific gene’s transcription). By methylating these promoter regions, it can directly obstruct the transcriptional machinery resulting in the lack of a functional gene product (Lim & Maher, 2010; Razin & Kantor, 2005).

Histone modification

In the cell nucleus DNA is wrapped around histone proteins to form units called nucleosomes. There are 5 families of histone proteins, H2A, H2B, H3, H4 (core histones) and H1/H5 (linker histones). Each nucleosome is composed of DNA wrapped around 8 core histone proteins which are linked to other nucleosomes via linker histones and linker DNA. Histone modifications affect the way DNA is wrapped around them which in turn leads to the different expression of different genes. Histone acetylation and methylation are two methods by which histones can be modified (Rice & Allis, 2001). Both these processes are reversible. Histone acetylation occurs on lysine residues on the histones and is catalysed by histone acetyltransferases. This acetylation neutralises the positive charge on the lysine residue thereby reducing affinity between the histones and DNA allowing for the
transcriptional machinery to gain access to the DNA (up regulation of DNA transcription). The opposite occurs with the assistance of histone deacytelases (HDACs), causing the DNA and the lysine to gain affinity thereby disrupting gene expression. Histone methylation involves the methylation of the lysine residue on the histones. However the methylation of different lysines can result in either suppression or expression of the genes, as compared to DNA methylation which only causes gene suppression. Methylation of the lysine can also be de-methylated by the catalyst lysine demethylase (Cheung & Lau, 2005; Imhof, 2006).

**Chromatin remodelling**

This occurs via protein complexes called chromatin remodelling complexes. They use the energy of ATP hydrolysis to change the state of the chromatin via the manipulation of the nucleosomes that the chromatin is wrapped around (Clapier & Cairns, 2009). It is also apparent that chromatin remodelling machinery works in tandem with histone modification enzymes and that both processes are essential for DNA methylation and de-methylation. Similar to histone modification enzymes, there are 4 different classes of remodelling complexes all of which share some basic properties: a higher affinity for the nucleosome than the DNA itself; domains which are able to recognise covalent histone modifications; a region for breaking histone-DNA contacts, domains/proteins that regulate the ATPase domain; domains/proteins required for interaction with chromatin or transcription factors. Although they all have these properties in common, they also have distinctive properties which differ in the composition of the complex subunits and the way they actually interact with the nucleosomes (Geiman & Robertson, 2002).

**Non-coding RNAs**

Non-coding RNA (ncRNA) molecules are transcribed from DNA that is not translated into proteins but rather plays a role in the regulation of gene expression via interactions with histones, the DNA and gene silencing. The ncRNAs are divided up into short ncRNA and long ncRNAs, with the short ncRNAs being further divided into microRNAs (miRNA), short interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs). MiRNAs bind and either degrade, cleave or block translocation of specific RNA transcripts to regulate the expression of the messenger RNA’s target. In addition to the functions of miRNA, siRNA is able to induce methylation and
chromatin condensation by binding to a RNA-induced transcriptional silencing complex. Finally piRNAs are transcribed and then bind to PIWI proteins resulting in epigenetic regulation and transposon control (a sequence of DNA that can change its position within the genome) (Esquela-Kerscher & Slack, 2006; Tollefsbol, 2014).

**Psychosocial genomics**

In recent years, it has become well established that not only physical changes in our environment but also psychological, and possibly even social and cultural changes can lead to alterations in gene expression (Provencal & Binder, 2015). Gene expression changes associated with the mind–body interaction are now encompassed by a field called “psychosocial genomics” (Rossi, 2002).

One example of psychosocial genomics occurs during pregnancy. Over the past decade there has been mounting evidence that changes in the environment during pregnancy can influence the baby in utero via epigenetic mechanisms. This has come to be known as the “developmental origins of health and disease” paradigm. The paradigm covers a number of themes including:

- the influence of diet during pregnancy and how this may affect the likelihood of the baby becoming obese during adulthood
- the consequences of mismatch between prenatal and postnatal environments and how this can lead to chronic disease later in life
- psychobiological effects of stress during pregnancy on the development of the fetus and later outcomes.

The effects of stress on prenatal development has been well documented with effects which have been observed to last into infancy, childhood, adolescence and even adulthood. Effects have included:

- impaired parts of memory and learning such as object recognition and spatial memory (prenatal stress induces spatial memory deficits and epigenetic changes in the hippocampus indicative of heterochromatin formation and reduced gene expression)
- increased risk of neurological and psychiatric disorders (stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health)
• reduction in birth weight and subsequently increasing the likelihood of disorders of cardiovascular function, glucose homeostasis, hypothalamic–pituitary–adrenal (HPA) axis activity and anxiety-related behaviours in adulthood (prenatal stress, glucocorticoids and the programming of adult disease).

This highlights the idea that stress during pregnancy can induce long lasting epigenetic changes in the fetus which result in mental and physical health issues that may last well into adulthood. On the other hand, epigenetic studies focusing on adults have established that stress, post-traumatic stress disorder and depression can alter the expression levels of stress related genes (Cole, 2010). Therefore it is reasonable to expect that meditation might also alter expression of genes that are implicated in stress.

The first study aiming to elucidate the effects of meditation on gene expression was done in 2005 by Li et al. (Q. Z. Li et al., 2005). Using a cross-sectional study design, the results suggested that genomic profiles changed in the practitioners of Falun Gong (an ancient Chinese practice of meditation). Since then other studies on the “epigenetics of meditation” have yielded similarly intriguing results. These are described in the literature review.

**Study aims**

The aim of this study was to determine firstly, whether meditation has any specific effects on the practitioner, and secondly whether there are differences between two different definitions of meditation, namely mental silence and body scan meditation (a mindfulness based technique). It was expected that both definitions of meditation are capable of invoking specific effects in the practitioner. These specific effects are thought to manifest as gene expression changes, which would be measured through microarray analysis. Additionally, it was also expected that both definitions of meditation would influence the expression of similar gene sets and particularly those that have been previously reported in the RCTs in the literature.
Literature Review

Within the field of cell biology, the field of epigenetics has particularly broad potential to provide explanatory information about the impact of meditation on the body. While there are many reviews exploring the impact of meditation, or similar behavioural therapies, on physiology, health, behaviour and wellbeing there are considerably less studies, and hence less reviews, examining the biological mechanisms by which the purported health benefits of meditation might actually occur. This is particularly true of the mechanisms that operate at the level of cell biology and the genome.

Currently, only one review examining studies of the impact of meditation on epigenetic factors has been published (Saatcioglu, 2013). In this review, Saatcioglu examined only 3 studies, each involving very different meditative practices: Qigong (Q. Z. Li et al., 2005), yoga (Sudarshan Kriya) (Sharma et al., 2008) and, in one study, a multitude of different meditative practices all thought to trigger the relaxation response (Dusek et al., 2008). These studies were diverse in their methodology, including cross-sectional studies, observational trials and controlled trials. So although this area of research is promising it is also clear that many more specific studies are needed to be done before there is conclusive understandings of what the exact influence that such interventions might have on the epigenome.

In order to develop a more thorough understanding of the extant evidence concerning meditation and its influence on the epigenome I conducted a review of the literature. Since there is currently no consensus definition of meditation this review included any study purporting to assess any kind of meditation that is, no specific definition of meditation was selected.

Computerised databases were searched, including: MEDLINE, PSYCINFO, EMBASE, Biological Reports, CINAHL, Web of Science and Scopus. In addition the general internet (Google) and paper searches were also examined. Keywords included: meditation, relaxation response, mindfulness, mbsr, transcendental, sudarshan kriya, sk, yoga, cognitive therapy, cbsm, falun gong, flg, kirtan kriya,
review, genetics, epigenetics, immune, telomerase, neutrophil, stress, transcription, inflammation. The search began in June 2013 and ended May 2015.

Publications that assessed biological markers other than epigenetic changes, such as measuring cytokines, or antibody titres et cetera, were excluded because they are not direct measures of gene activity.

The search yielded 10 articles; the details of which are summarised in Table 1. Of the 10, 3 were RCTs, 2 were non-RCTs (Quasi experimental), 1 was an observational trial, 2 were cross-sectional and 2 were of a mixed nature comprising both observational trials and cross-sectional survey components. Sample sizes varied widely from as little as 12 participants to 199 participants. Interestingly, the studies, despite considerable variation in design and intervention, have a number of similar and common findings worth discussing. Importantly, due to the small number of studies, the generally small sample sizes and the wide variation in their methodology, a quantitative meta-analysis of the outcome data was not feasible. Although a descriptive review is the only assessment currently possible, since the current body of knowledge is so early in its development, this kind of review is still valuable as it will be useful for the development of further research in this area.
<table>
<thead>
<tr>
<th>Trial design</th>
<th>Intervention</th>
<th>Period</th>
<th>Sample Size</th>
<th>Control</th>
<th>Outcomes</th>
<th>FC in Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT</td>
<td>Kirtan Kriya</td>
<td>8 weeks</td>
<td>45</td>
<td>Relaxing music</td>
<td>Increased immunity/antiviral genes. Decreased NF-κB related genes</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td></td>
<td>Black et al., 2013</td>
<td></td>
<td></td>
<td></td>
<td>Decreased NF-κB related genes and CRP</td>
<td>&gt;= 1.25</td>
</tr>
<tr>
<td></td>
<td>Mindfulness Based Stress Reduction</td>
<td>8 weeks</td>
<td>40</td>
<td>Waitlist</td>
<td>Decreased NF-κB related genes</td>
<td>&gt; 1.5</td>
</tr>
<tr>
<td></td>
<td>Mindfulness Based Stress Management</td>
<td>10 weeks</td>
<td>199</td>
<td>Psycho educational seminar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quasi Experimental (non RCT)</td>
<td>Sudarshan Kriya &amp; Related Practices</td>
<td>2 days (2 hrs each)</td>
<td>14</td>
<td>Nature walk, jazz music</td>
<td>Increased AVIL, NFE2</td>
<td>&gt; 1</td>
</tr>
<tr>
<td></td>
<td>Meditation</td>
<td>1 day</td>
<td>40</td>
<td>Read, watch documentaries or play computer games &amp; walk</td>
<td>Downregulation of HDACs, COX2, RIPK2</td>
<td>Uses adjusted differences &amp; p &lt; 0.05</td>
</tr>
<tr>
<td>Observational Trial</td>
<td>Various Meditative Techniques</td>
<td>N/A</td>
<td>2</td>
<td>N/A</td>
<td>Immune function genes up &amp; down regulated. Downregulation of DNA repair and metabolic processes</td>
<td>&gt;= 1.3</td>
</tr>
<tr>
<td>Cross Sectional</td>
<td>Various Meditative Techniques</td>
<td>N/A</td>
<td>84</td>
<td>Non-practitioners</td>
<td>Increased hTERT, BCL2, PTGS2, HSP-70</td>
<td>No cut-off, stat. sig. diff’s &gt; 2</td>
</tr>
<tr>
<td></td>
<td>Sudarshan Kriya</td>
<td>N/A</td>
<td>12</td>
<td>Non-practitioners</td>
<td>Decreased BCL2, ribosomal proteins, stress related genes. Increased HSP70</td>
<td></td>
</tr>
<tr>
<td>Mixed Design</td>
<td>Relax’n Response Techniques</td>
<td>8 weeks</td>
<td>52</td>
<td>Non-practitioners</td>
<td>Decreased immune function genes, NF-κB related genes</td>
<td>Not specified</td>
</tr>
<tr>
<td></td>
<td>Relax’n Response Techniques</td>
<td></td>
<td></td>
<td></td>
<td>Decreased immune function genes, NF-κB related genes</td>
<td>(p &lt; 0.01)</td>
</tr>
<tr>
<td></td>
<td>Dusek et al., 2008</td>
<td></td>
<td>38</td>
<td>Non-practitioners</td>
<td>Decreased immune function genes, NF-κB related genes</td>
<td>Not specified</td>
</tr>
</tbody>
</table>
Cross-sectional studies

The first study, by Li was published in 2005 (Q. Z. Li et al., 2005). It assessed a meditative practice known as Falun Gong (FLG) using a cross-sectional survey design. It compared 6 long-term FLG practitioners to 6 non-practitioners with no exposure to FLG. The study showed that practitioners had different gene expression profiles to their control counterparts. It reported significant gene changes in several different functional gene groups. Ubiquitin related genes were the first significant group which had the genes with the highest fold change. Of the 14 ubiquitin related genes identified, it was found that 13 were down regulated by more than two-fold. The second significant group of genes all coded for ribosomal related proteins. This group also had 13 out of 14 genes down regulated. The third group of genes were immunity related genes and of these 7 out of 8 were up regulated. Notable up-regulated genes included IFNG, TYK2, PSK1 and OAS1. The down-regulated gene was KRT7. The final group of genes were stress and heat shock protein related. Out of the 15 genes, 13 were down regulated and they included notable genes such as GPX1, GST, AOC and ERCC1. The two down-regulated genes were heat shock proteins HSP70B and DNAJB5.

In 2007 Sharma studied Sudarshan Kriya (SK) using the same study design as Li’s FLG study and also found that it had an impact on gene activity (Sharma et al., 2008). However, unlike Li’s study, Sharma’s study looked at a much narrower set of just 9 genes. Of the 9, there were significant changes in 3 genes: GSTP1 (glutathione S-transferase pi 1), HSP70 (heat shock protein 70) and PTGS2 (prostaglandin-endoperoxide synthase 2) – all of which were found to be significantly higher in activity in SK practitioners compared to non-practitioners. HSP70 and PTGS2 have been found to be anti-apoptotic meaning that an increased expression of these genes may prolong immune cell life (Beere et al., 2000; Chang et al., 2000). GSTP1 codes for an enzyme involved in the metabolism of xenobiotics and therefore the detoxification of cells in the body, thus increased expression of this gene would be beneficial for overall health.

The above two studies employed different measurement methodologies, each suited to the gene sample that they chose to target. Sharma’s study used PCR to measure
changes for a relatively small set of specific genes relating to stress signalling pathways, antioxidants, DNA damage, cell cycle and apoptosis. Li’s study was able to examine the impact of FLG on 12,000 genes through microarray analysis and confirm these findings with RNA protein assaying. Of these 12,000 genes it was found that 132 genes were down regulated and 118 genes up regulated in the practitioners of FLG compared to their respective controls. In contrast, Sharma’s study measured gene expression via reverse transcriptase polymerase chain reaction, a different technique to detect RNA expression. Additional parameters that were also measured included superoxide dismutase activity and glutathione concentration which were both seen to be increased in SK practitioners.

Coincidentally, there were two genes measured that are common to both studies – these were BCL2 and HSP70. BCL2 regulates programmed cell death and hence increased expression is thought to prevent premature cell death (Chao & Korsmeyer, 1998). As mentioned previously, HSP70 codes for a heat shock protein which has similar anti-apoptotic properties via a different mechanism due to its critical involvement in assisting with proper folding and formation of proteins as well as the management of cell repair systems (Mayer & Bukau, 2005). Interestingly, Li reports that BCL2 was significantly reduced in response to FLG while Sharma reports the opposite, that is, BCL2 was increased slightly in response to Sudarshan Kriya. On the other hand, HSP70 changes moved in the same direction, of the same magnitude, in both studies. This discrepancy in outcomes is most likely due to the unreliable nature of cross-sectional design and various other methodological weaknesses in the two studies. For example, Li’s study involved only 12 participants making it prone to type 2 errors (low statistical power). The two meditation techniques are very different and hence their biological effects could reflect this. The differing measurement methods (microarray vs PCR) may also introduce differences in the reliability of the outcomes.

Cross-sectional studies are relatively quick and easy to conduct when compared to other study designs mainly because only one blood sample is required per participant thereby reducing both cost and labour. However the outcomes are prone to a number of confounding effects which reduce the reliability and generalisability of their findings. This is why cross-sectional studies, especially those with small sample
sizes, are at best pilot studies whose main value is to assist in hypothesis generation for larger more thorough and hence conclusive studies to be done.

**Observational trials**

Ravnik-Glavac et al. conducted an observational study in 2012 (Ravnik-Glavac, Hrasovec, Bon, Dreo, & Glavac, 2012). Unlike other studies which tested epigenetic changes after a meditation intervention, Ravnik-Glavac et al.’s study examined epigenetic changes invoked by a change to higher states of consciousness in two long-term meditators. Higher state of consciousness was defined as being in either one of several states as defined by ancient Vedic literature. The study found that although both meditators used different meditation practices to reach higher states of consciousness, a large number of genes were differentially regulated in both.

The first meditator had been practicing for 23 years and used Zen and Kundalini meditation to experience higher states of consciousness, while the second meditator had 25 years of Buddhist meditation experience and used mental quietness and visualisations of Buddha to reach higher states of consciousness.

After taking whole blood samples from both meditators before and after they had entered a higher state of consciousness (it was unclear as to how the switch to a higher state of consciousness was determined) RNA was extracted, protected and analysed using RNA stabilisation and microarray technology. To confirm the results obtained from the microarray analysis, real-time quantitative PCR was performed. Overall 1668 unique genes were changed in meditator 1 and 608 unique genes changed in meditator 2.

Gene ontology analysis revealed that the meditator using Zen and Kundalini yoga had significant down regulation of genes relating to cotranslational protein targeting to membrane, mRNA processing, RNA splicing, RNA binding, protein serine activity, ubiquitin protein ligase activity, Golgi apparatus, centrosome and endosome proteins. Significantly up-regulated were genes involved in: erythrocyte maturation/development, induction of positive chemotaxis, haemoglobin metabolism, haemoglobin binding, anion exchanger activity, oxygen transporter activity, the cytosol, the nuclear inner membrane and the autophagic vacuole.
On the other hand, achieving a higher state of consciousness via mental quietness and visualisations of Buddha, resulted in significant down regulation of genes related to: regulation of cellular processes, regulation of biological process, biological regulation, protein binding, phosphatase binding, transcription factor binding, the cell cortex, the stereocilium, the nuclear speck and cell projection genes. The up-regulated categories included: glycerol metabolic process, alditol metabolic process, regulation of lipid catabolic process, structural constituent of ribosome, nuclease activity, racemase and epimerase activity, intracellular membrane bounded organelle, organellar ribosome, voltage gated potassium channel complex, cilium, mitochondrial respiratory chain complex 1 and VLDL particle genes.

The significant disparity in the type and number of genes differentially regulated between both meditators may be explained by a number of factors. Unlike Sharma’s study which looked at a comparatively smaller set of genes, Ravnik-Glavac et al. looked at a genome-wide change. The genome-wide analysis showed that Zen/Kundalini yoga was able to invoke a larger number of genetic changes than compared to Buddhist meditation. The importance of this outcome is that it suggests different meditations may have a varying degree of genetic impact, maybe even through different mechanisms.

The sample size of two and the lack of a control group clearly limit the generalisability of the findings. Large sample sizes and control groups are essential in attaining precise results in observational studies. Although the study does lack the appropriate sample size and control group, it raises the possibility that different practices of meditation may have different underlying mechanisms allowing each of them to invoke genetic changes to varying degrees of significance. Conversely, it may be possible that these gene expression changes are attributable to the unique nature of these two individuals and hence these changes may be invisible in the wider population.

**Mixed study designs**

Following research conducted by Sharma et al. and Li et al. a 2008 study by Dusek et al. utilised a slightly more advanced study design involving an observational trial and cross-sectional survey component (Dusek et al., 2008). The basis of the study
revolved around previously observed physiological changes in long-term practitioners of relaxation response invoking techniques such as, various forms of meditation, repetitive prayer, yoga and breathing exercises. This study further established preliminary evidence that relaxation response techniques can illicit epigenetic changes in the long term, however the authors decided to follow this up by determining if relaxation response techniques could invoke any short-term epigenetic changes in a second study (Bhasin et al., 2013). The second study uses the same hybrid study design but instead measured genetic changes over 3 time points: before the intervention, immediately after and 15 minutes after. For easier analysis both studies use Expression Analysis Systematic Explorer analysis to group the vast number of differentially expressed genes into biological themes (gene ontology categories) allowing for a discussion on what physiological changes may have occurred.

Dusek et al’s 2008 study used a sample size of 38, while Bhasin et al’s 2013 study used a larger sample size of 52, however without the inclusion of a required sample size estimation; it is hard to tell if these sample sizes were chosen arbitrarily or via a calculation. Nevertheless, participants had their blood samples drawn before and after the intervention for microarray analysis.

Dusek et al’s study revealed 2209 statistically significant genes were differentially expressed when comparing long-term practitioners to their non-practicing counterparts. Of these genes, 1275 were up regulated and 934 down regulated. When comparing samples of the novices before and after the 8 week period, it was shown that 1561 genes were differentially expressed and of these 874 were up regulated and 687 were down regulated. Gene ontology categories which had effected genes were oxidative phosphorylation, ubiquitin-dependent protein catabolism, mRNA splicing, ribosomes, metabolic processes, regulation of apoptosis, NF-κB pathways, cysteine type endopeptidase activity and antigen processing.

Bhasin et al’s study produced further supporting evidence showing that both short-term and long-term practitioners have altered gene profiles immediately after the intervention when compared to novices, with a more significant change occurring in the long-term practitioners. Progressively up-regulated genes belonged to the following categories: regulation of cell differentiation, cell adhesion, cell
communication and transport, hormone stimulus, blood pressure, cAMP, metabolic processes, biological oxidation and oxidoreductase activity. On the other hand, down-regulated genes belonged to the categories: mRNA processing, intracellular protein transport, antigen processing and presentation, immune system and primary metabolism. Genes which were differentially expressed in long-term practitioners when compared to their novice counterparts both before and after the 8 weeks intervention were described as being long-term up or down regulated. The up-regulated genes belonged to the following categories: ATP activity, protein binding, cell adhesion, defence response, amine transport, response to stress, gap junction and muscle cell differentiation. Down-regulated long-term genes were included in these categories: regulation apoptosis, nuclear transport, metabolic processes, JAK STAT cascade, T and B cell activation, regulation of cell cycle, insulin sensitivity, glucose transport, DNA replication, chemokine signalling, EGF signalling and stress response.

Both studies aimed to increase their validity by combining observational and cross-sectional study design components, however the lack of an active control and the varied methods of activating the relaxation response meant that non-specific effects could not be ruled out. This may also be able to explain why Ravnik-Glavac et al.’s study showed an up regulation of genes relating to immune function while Bhasin et al. and Dusek et al. found the opposite. Despite this, it is interesting to note that when comparing the results of both Bhasin et al. and Dusek et al.’s studies to Ravnik-Glavac et al.’s observational trial a common trend emerges. Genes regulating the NF-κB pathway are observed to be down regulated in all 3 studies, regardless of what meditation technique or study design was used.

It is noteworthy that recent studies have found that chronic psychological stress and psychiatric illness can lead to the activation of the NF-κB pathway resulting in a large number of pro-inflammatory genes being transcribed (Bierhaus, Humpert, & Nawroth, 2004; Miller et al., 2008; T. W. Pace et al., 2012). Hence it is only logical to conclude that meditation may be able to reverse inflammatory responses induced by external stressors, psychological or otherwise.
Repeated measures designs (non-RCT)

In 2013 Qu et al. published the first repeated measures design study (a type of non-randomised control trial) which furthered the notion that Sudarshan Kriya and its related practices (SK&P) have epigenetic impacts (Qu et al., 2013). Similar to Sharma’s study, it revealed that a large number of key genes were up or down regulated in the meditators and which may be linked to beneficial health effects.

The participants were all originally attending a 7-day yoga retreat in Oppenau Germany when they were recruited to the 4-day study via an announcement on the first day of the retreat. All participants were current practitioners of SK&P and had been practicing for 1.5 to 5 years. The inclusion criterion was: male, 18–50 years of age, no chronic disease and good psychological health as determined by a GHQ28 questionnaire. Only 14 participants ended up participating, of which 4 missed a session or could not donate enough blood. On days 1 and 2 all participants practiced SK&P while giving blood samples before and after the daily sessions. On days 3 and 4 participants undertook a nature walk which served as the control activity. In this design, there was no separate control group.

It was found that SK&P altered 111 genes while nature walking altered only 38, of which 14 were common in both groups. The 9 most significant genes which were regulated by SK&P only included: NFE2, RAB24, AVIL, PFKFB3, EXT1, MMP28, CCR7, CEACAM1 and SIPA1L2. NFE2 codes for a protein which is responsible for erythroid and megakaryocytic maturation and differentiation which may mean it is beneficial in increasing immune function (Andrews, 1998). It was significantly up regulated after SK&P. This is a surprising result as noted before, Bhasin et al. and Dusek et al’s studies revealed that immune function genes were down regulated (as would be expected of a mechanism of reducing inflammation). The other significant finding was the increased expression of AVIL which codes for a protein called advillin. Advillin is involved in the repair of neuronal cells in neonates and also ciliogenesis which enhances cell signalling and development (Marks, Arai, Bandura, & Kwiatkowski, 1998). On the other hand, the 7 most significant genes which were regulated by nature walking included RN7SK, SLC36A, FKBP5, IL7R, PIP3, CHN1 and ANKRD9. In general these genes do not directly pose any positive or negative effects on physiological systems; however they may affect other unknown pathways.
Repeated measure design studies are mainly designed for efficiency and convenience as the analysis strategy attempts to compensate for the absence of a control group and a smaller sample size. Most repeated measures study designs are prone to the “practice effect” which results in the participants becoming fatigued, or overfamiliar with the tests and unwilling to genuinely participate in the study. Qu et al. have minimised this effect by only running each intervention for only 2 days each, while also recruiting motivated participants from a yoga retreat. Overall this study design is superior to all other designs and is only exceeded in methodological hierarchy by randomised controlled trials.

**Quasi experimental (non-RCT)**

In 2014, Kaliman et al. conducted a quasi-experimental trial aimed at determining mindfulness meditation and related practices’ effect on inflammatory gene expression and also histone deacetylases (Kaliman et al., 2014). Decreased pro-inflammatory gene expression was found in the mindfulness meditation group. This study was also the first to specifically look at gene expression changes in histone deacetylases (HDACs), histone methyltransferases and demethylases which are all enzymes involved in chromatin epigenetic modifications. Overall the results provided an additional basis for us to hypothesis on the epigenetic mechanism by which meditation exerts its therapeutic effects.

The study consisted of 19 long-term meditators and 21 control subjects who had no previous exposure to meditation. The meditators were recruited at meditation centres throughout the US and also via mailing lists, newspaper articles and flyers. Inclusion criteria for the meditators included having at least 3 years of daily meditation practice, with a minimum of 30 minutes of meditation per day and also the attendance of at least 3 intensive retreats lasting 5 hours or more. Meditators came from a mixed background of both mindfulness based meditations and compassion-related meditations (as per Tibetan and Theravada Buddhist traditions). The control group was recruited via local advertisements which advertised the study as a non-pharmacological intervention designed to promote wellbeing.

The results of the study showed that in the mindfulness group, two inflammatory genes were down-regulated by a significant amount (PTGS2 and RIPK2). Other
genes which were suspected to be effected such as CCR7, CXCR1, TNFA and IL6 were shown to have no significant difference between groups after PCR analysis. The meditation showed a decrease in HDAC2, HDAC3 and HDAC9 gene expression when compared to controls.

Although the study demonstrated that mindfulness meditation may incur changes at the genetic level, the study’s lack of randomisation and active controls leaves some doubt as to the validity of the results. Interestingly, as opposed to previous studies mentioned before, Kaliman et al. failed to observe any significant differences in two key pro-inflammatory genes that is, TNFA and IL6.

**Randomised controlled trials**

RCTs are considered the gold standard of clinical trials as they can detect the effect of the intervention without the differences between groups being attributable to external factors. Hence, when compared to the other study designs, RCTs are far more superior in terms of internal and external validity.

Only 3 RCTs concerning meditation and genetic effects were found (Antoni et al., 2012; D. S. Black et al., 2013; Creswell et al., 2012). They involved cancer patients, dementia caregivers and lonely adults all of whom had been experiencing high levels of stress and anxiety.

These RCTs are significant as they confirm results from previous less methodologically sound study designs. All 3 studies reported decreased gene expression relating to pro-inflammatory pathways and 2 of the 3 studies observed increases in immunity related gene expression (Antoni et al., 2012; D. S. Black et al., 2013).

Two studies used active controls thus eliminating non-specific effects (Antoni et al., 2012; D. S. Black et al., 2013). In Black et al’s study there were 45 participants who were divided into the intervention (SK&P) and the active control group which listened to relaxing music for 12 minutes, while in Antoni et al’s study, which had 199 participants, the control group had to attend an educational seminar on mindfulness based stress reduction (MBSR) and its purported effects. The third study
by Creswell et al. consisted of 40 participants and used a waitlist control (Creswell et al., 2012).

Antoni et al’s results included 91 differentially expressed genes in CBSM participants of which 62 were down regulated and 29 were up regulated. A gene ontology analysis revealed that the genes encoding for pro-inflammatory cytokines IL1A, IL1B and IL6, were amongst the most significantly reduced. Prostaglandin synthesis enzymes were the second most significantly reduced followed by inflammatory chemokines and receptors. These key down-regulated genes are linked with NF-κB signalling pathways. Although genes coding for mediators of tissue remodelling and epithelial mesenchymal transition were significantly reduced, the biological implications are unclear. Type 1 interferon response genes, type 2 interferon signalling and interferon signal transduction genes were the most significantly increased, all of which enhance antiviral responses.

Similarly Black et al’s study revealed 68 differentially expressed genes of which 49 were down regulated and 19 up regulated. Immune function related genes were the most relevant to be up regulated and included IGJ and IGLL3. There were also many genes which were up regulated that had unknown functions. Pro-inflammatory cytokines and activation related immediate early genes were the most significantly down regulated. These categories included genes such as IL8, FOSB, IL6 and JUN.

Creswell et al. identified 256 differentially expressed genes of which 69 were down regulated and 74 up regulated. Although the number of differentially expressed genes was more than double that of the other two RCTs, ontology and pathway analysis revealed that the MBSR affected the transcriptional factor activity of the NF-κB pathway in a similar fashion to SK&P and MBSR.

Overall all the 3 RCTs identified similar genes were either up regulated or down regulated by 3 different meditation related practices, suggesting that they all work via a similar biological mechanism. When comparing the results of these RCTs to the other inferior study designs, it can be seen that the overall results seemed to be very similar.
Summary

The review demonstrates that meditation and its related practices do appear to have epigenetic impacts, hence giving us good reason to investigate the biological mechanism by which meditation can exert its purported effects.

The most significant group of genes were pro-inflammatory mediators that are linked to the NF-κB pathway which is involved in the inflammatory stress response (Mercurio & Manning, 1999). In 5 of the 10 studies, genes coding for pro-inflammatory mediators were down regulated significantly. These studies included several types of meditation practices, yoga and repetitive prayer (Bhasin et al., 2013; D. S. Black et al., 2013; Creswell et al., 2012; Dusek et al., 2008).

A 2007 review highlighted that psychological stress induces inflammation through the actions of pro-inflammatory mediators which are under the regulation of the NF-κB pathway (A. Steptoe, M. Hamer, & Y. Chida, 2007). Furthermore, it has been well established that a range of psychiatric illnesses, specifically clinical depression, have been linked with the chronic inflammatory state (Dantzer, O’Connor, Freund, Johnson, & Kelley, 2008; Kivimaki et al., 2014; Krishnadas & Cavanagh, 2012). The current evidence that meditation and related practices decrease the expression of genes relating to the NF-κB pathway highlights its potential as a treatment for psychiatric illnesses and also as a method of promoting general wellbeing.

Study design plays a major role in determining the validity of the results. Overall there were only 3 RCTs. None of these RCTs used controls that adequately controlled for placebo. In fact, the control methods varied considerably between studies as did the interventions themselves making inter-trial comparison and generalisation difficult. Despite these important methodological shortcomings, they all reported decreased inflammatory related gene activity. Although the other studies had similar results, flaws in their methodology meant that the validity of results were questionable.

A randomised controlled trial comparing two different definitions of meditation would therefore be the next step in the development of knowledge about the epigenetic impacts of meditation and could also be a significant contribution to the literature. By comparing two different definitions of meditation the need to control
for non-specific effects would be fulfilled by providing participants with a plausible control regimen.
Methods

Study design

The study was designed to compare the mental stillness approach of meditation with another meditation exercise, mindfulness (which is not of the non-mental stillness type). A randomised, double-blind, parallel-group, controlled trial design was adopted. Participants were recruited over a period of 1 month and randomly allocated into either the mental stillness meditation or the mindfulness group. Both groups required participants to attend a weekly 1 hour supervised session and also to practice the techniques at home on a daily basis. Participants were briefed before the commencement of the trial and advised that the trial aimed to compare the genetic changes evoked by 2 different types of meditations. Blood was taken prior to commencing the trial and again after the 4-week period had elapsed. Based on previous studies which used a wait list control, a RCT comparing two definitions of meditation would be superior to a wait list control group which has a couple of disadvantages. A disadvantage of waitlist control groups is that any gene expression changes that occur in the mental silence meditation group may be attributed to them receiving a greater clinical attention than those simply waiting in the control group. A 2013 study proved that wait list control groups may artificially inflate the effect of the intervention group (Cunningham, Kypri, & McCambridge, 2013). Additionally, participant wait time must also be considered. For example, longer wait times may cause participants to lose interest in the study and may result in increased dropout rates. The clinical trial protocol was approved by the Human Research Ethics Committee of the University of Sydney (protocol number: 2013/953).

Participants

Eligibility criteria for participants involved in the study included:

- no previous exposure to meditation a study
- no major physical or mental illness
- aged between 18–30 years
- non-smoker
- prepared to not drink alcohol for the duration of the study
• prepared to attend a 1 hour weekly session at a given location
• prepared to meditate twice daily at home
• prepared to fill out questionnaires
• prepared to keep a compliance diary
• prepared to give blood twice

Exclusion criteria for participants involved in the study included:
• major medical illness present
• have had or currently have a mental illness
• currently on medication
• no current consumption of alcohol, tobacco or illicit drugs.

Participants were recruited using flyers which were put up around the University of Sydney campus and local libraries in the surrounding area. Additionally, recruitment was also done via social media (Facebook) and word of mouth.

**Randomisation, blinding and controlling for non-specific effects**

Randomisation was performed by a research assistant, in the absence of the chief investigators, using a blindfolded lottery allocation system. Participants were then informed of their allocation at the briefing prior to the first session. This allocation was not disclosed to either of the chief investigators.

Participants were blinded to the complete hypothesis of the clinical trial and were not informed as to which methods of meditation were being used in the alternative group. They were instructed not to disclose any information regarding activities done in their groups to those in the comparison group. Data entry personnel, blood collection personnel, scorers and statisticians were also blinded to group allocation.

The two meditation interventions were structured identically so that non-specific factors such as credibility, expectation and demand characteristics were matched as closely as possible: Classes for both intervention groups were conducted in similar rooms, at approximately the same time of day, with similar support materials; instructional sessions were of equal duration with equivalent periods between interventions.
Interventions

The intervention period was 4 weeks, and involved 1 hour instructed sessions held weekly. Additionally participants were requested to practice the same technique at home twice daily for 10–15 minutes each time. Instructors for both groups were qualified meditation instructors and also health professionals.

The first group was instructed on how to enter a state of mental silence through the use of a series of silent psychological affirmations while sitting on a chair (see Appendix 2 – Mental Silence Meditation). These sessions were focused on helping the participants achieve mental silence and participants were encouraged to ask for assistance if they had any difficulty achieving this state of mind. Participants were each given a resource bag which included a CD with meditative music and the affirmations and also a book on the mental silence experience. They were advised to use these resources to perform the same mental silence exercises at home for 10–15 minutes twice a day. At the beginning of each session compliance diaries were collected (see Appendix 3 – Participant Survey Form & Diaries), and at the end of each session food and drink were provided.

The second group was structured in a similar manner to replicate the interventions as closely as possible. The sessions were held in a community centre at the same time as the other group. This group was taught a mindfulness based exercise known as “body scan meditation” which was delivered by a guided audio CD. The guided meditation was downloaded from the University of Vermont’s “The Centre for Health & Wellbeing” website and went for approximately 20 minutes. Participants were given this CD to take home and asked to practice the same technique at home twice a day for 10–15 minutes. Additionally, participants were given a handout which described the “body scan meditation” and its effects (see Appendix 1 – Body Scan Meditation). At the end of each of the weekly sessions, participants were also offered food and drink. The key difference between the two groups was that one form of meditation featured mental silence, while the other did not.

Measures

Baseline assessments were done prior to commencing the first session. All participants were invited to the same location and a briefing was held outlining the
study and what was expected from each participant. Participants were then asked to fill out the baseline questionnaire battery which also included consent and contact information forms (see Appendix 3 – Participant Survey Form & Diaries). All questions and difficulties were directed to the medical and meditation staff present at the briefing. Following this, participants were then advised of their allocation and then told to either stay or make their way to the alternate venue based on what they had been allocated. At the end of the 4 weeks the same baseline questionnaires were filled out.

**Diaries**

Participants were given a 4-week diary at the briefing and were advised to fill this out every day until they came to the next session at which the instructor would collect the diary (see Appendix 3 – Participant Survey Form & Diaries). Recorded information included: how many times did you meditate per day, did you do a foot soak (for the mental silence group only), overall level of mental silence during morning meditation, overall level of mental silence during evening meditation and also overall mood for the day.

**Collection of whole blood**

Blood sample collection took place at two time points, one before the commencement of the trial and one after the last session of the trial. Doctors and nurses carried out the extraction of whole blood from participants using a winged infusion set to draw 6 mL of blood directly into vacuette Tempus Blood RNA tubes which contained a RNA stabilisation solution. The blood tubes were then taken to a fridge for an overnight stay at 4°C and then moved to a −20°C freezer until processed as per the manufacturer’s directions (Tempus™ Blood RNA Tube, Affymetrix/Thermo Fischer scientific protocol) (Aarem et al., 2016; Häntzsch et al., 2014).

**Compliance**

Diaries were collected at the start of each session to mark attendance. Compliance during the week was assessed via the diaries that they were asked to fill out every day.
Gene arrays

The Affymetrix Human Genome U219 array plate contains over 530,000 probes which cover approximately 36,000 transcripts and variants, in turn representing over 20,000 genes mapped through Unigene (www.ncbi.nlm.nih.gov/unigene) and RefSeq annotation (http://www.ncbi.nlm.nih.gov.ezproxy1.library.usyd.edu.au/RefSeq/) . The U219 array plate is able to process up to 96 samples simultaneously, making it ideal to compare gene expression profiles in the current 50 sample sized study (Affymetrix, Santa Clara, CA, USA).

Gene arrays are able to generate thousands of data points per sample making it difficult to identify relevant information and hence specialised software is used to analyse this vast amount of data. The Partek Genomics Suite was used as it can perform advanced statistical analysis, such as ANOVA, on the raw data files obtained from the gene array chips while also providing a visual representation of the data via heatmaps and principal component analysis plots (Balding, Bishop, & Cannings, 2008). The analysis for this paper was generated using Partek® software (copyright, Partek Inc. Partek and all other Partek Inc. product or service names are registered trademarks or trademarks of Partek Inc., St. Louis, MO, USA.).

RNA extraction from whole blood

Whole blood RNA was extracted from the whole blood using the Tempus Spin RNA Isolation kit as per the manufacturer’s directions (Tempus™ Blood RNA Tube, Affymetrix/Thermo Fischer scientific protocol). The extracted RNA was verified for its quality and quantity using the NanoDrop™ 1000 Spectrophotometer (NanoDrop Technologies, Berlin, Germany) and Agilent 2001 Bioanalyser analysis (Agilent Technologies, Waldbronn, Germany) (acceptable RNA integrity numbers between 6.0–10.0) (Valletti et al., 2013). The quality of the RNA was assessed by a modified agarose gel electrophoresis method which involved detection from the Agilent 2001 Bioanalyser. After confirming the sample quality and quantity, the RNA samples were stored at −80°C until required for processing.

RNA sample submission for analysis

The Affymetrix Human Genome U219 array plate was used for the microarray analysis. Total RNA, 500 ng per sample, was submitted to the Ramaciotti Centre for
Genomics (University of New South Wales, Sydney), at which the target preparation, array washing, staining and analysis was carried out. Overall 50 RNA samples were submitted for the analysis.

**Microarray data analysis**

Raw expression values from the Affymetrix Genechip operating software (GCOS) were obtained as CEL files. These were then opened using the Partek Genomic Suite 6.6 software (Partek Inc). All samples were checked for quality and they all demonstrated characteristics of high quality cRNA (3'/5' threshold ratio of probe sets for the integral housekeeping genes of 1.5). The raw data were normalised using the robust multichip averaging algorithm which retains probe-level information and applies probe-specific background correction, compensating for any non-specific binding found in the gene chips using the perfect-match probe distribution (Rafael A. Irizarry et al., 2003).

In addition, robust multichip averaging normalisation applied perfect-match distributions across all the chips and a robust probe-set summary of the normalised probe-level data by median polishing was obtained (Beyer, 1981). Array data quality was confirmed by principal component analysis, which examined correlation between the data derived from the different arrays and identified potential outliers (R. A. Irizarry et al., 2003; Ringner, 2008). The analysis of variance (ANOVA) with a nominal alpha value set to 0.05 was then used to determine those probe sets significantly different between the mental silence meditation group and the active control meditation group within each time point (before and after) and separately across both time points. This was followed by a Benjamini and Hochberg multiple testing correction, controlled at 5%, to reduce the false positive rate (Noble, 2009). To ensure the accuracy of the results, only differentially expressed genes with a false discovery rate less than or equal to 0.05 and a fold change (FC, how many fold increase/decrease in gene expression levels) greater than or equal to 1.4 were further analysed.

Gene lists were prepared from each comparison group and identification of cellular functions of differentially regulated genes was achieved utilising the Partek pathway analysis tool as well as online bioinformatics tools including the Ingenuity®Systems Pathway Analysis (Ingenuity Systems, http://www.ingenuity.com). This allowed
identification of interrelationships of the individual groups in a network format and analysis of genes in the context of functional groups rather than as singular entities, making it easier to highlight potential mechanisms underlying the mental stillness meditation in comparison to body scan meditation. A functional analysis was performed using differentially expressed genes in order to categorise them into bio-functions and pathways. The significance of the relationship to a bio-functional category or pathway was calculated by the taking the ratio of the total number of genes or molecules from the dataset and the total number of genes or molecules from the function. Significance was also calculated by a right-tailed Fisher’s exact test with p values of less than 0.05 being deemed significant (R. A. Irizarry et al., 2003).
Results

It is generally well accepted that clinical trials should have a minimum of 30 participants per treatment arm. However, due to the exploratory nature of this study it was deemed acceptable to aim for a total sample size of 50 (with 25 in each treatment arm). Overall, 50 participants were recruited. Of these, there were 2 drop outs which resulted in 24 participants remaining in each intervention group. All of the 48 participants complied and completed the full intervention period.

Gene array results

Within group changes

The principal component analysis showed a significant separation between the samples within each group, indicating that the quality of the microarray data was acceptable. The pathways analysis did not reveal any significant results and thus was omitted.

Overall, 16 genes were changed in the meditation group when comparing blood samples from before and after the intervention period. On the other hand, 48 genes were changed in the control group when comparing blood samples from before and after the intervention period. Genes of interest were selected according to the specified criteria that they showed a fold change > 1.4 and p value ≤ 0.05. The fold change was defined as the quantity of change based on the before the intervention compared to after intervention time points. As shown in Table 2, in the meditation group, 16 unique genes (10 down regulated and 6 up regulated) were changed after the intervention period. While in the control group, as shown in Table 3, 51 unique genes (47 down regulated and 4 up regulated) were changed after the intervention period. Two genes was significantly changed and were common to both the mental silence and the body scan groups. These genes were HIRA and INPP4B. HIRA encodes a histone chaperone, which is preferential in placing the H3.3 variant in nucleosomes, and thus involved in cell senescence. INPP4B encodes an enzyme involved in phosphatidylinositol signalling pathways. HIRA was up regulated by 1.80 FC in the mental silence group and 1.55 FC in the body scan group. Conversely
INPP4B was down regulated in the mental silence group by $-1.86$ FC and $-2.32$ FC in the body scan group.

Table 2: Significant gene changes observed in the mental silence group

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC*</th>
<th>P value</th>
<th>Probe set ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGHG3</td>
<td>3.03</td>
<td>0.036</td>
<td>11761467_x_at</td>
</tr>
<tr>
<td>KIAA0101</td>
<td>2.01</td>
<td>0.007</td>
<td>11744425_a_at</td>
</tr>
<tr>
<td>HIRA</td>
<td>1.80</td>
<td>0.003</td>
<td>11720837_a_at</td>
</tr>
<tr>
<td>LMAN1</td>
<td>1.80</td>
<td>0.004</td>
<td>11747181_a_at</td>
</tr>
<tr>
<td>CLEC10A</td>
<td>1.60</td>
<td>0.023</td>
<td>11745500_a_at</td>
</tr>
<tr>
<td>TNKS2</td>
<td>1.52</td>
<td>0.009</td>
<td>11722462_a_at</td>
</tr>
<tr>
<td>WDFY1</td>
<td>-1.50</td>
<td>0.014</td>
<td>11754884_s_at</td>
</tr>
<tr>
<td>MBNL1</td>
<td>-1.52</td>
<td>0.020</td>
<td>11744794_a_at</td>
</tr>
<tr>
<td>SMARCA2</td>
<td>-1.57</td>
<td>0.017</td>
<td>11743340_a_at</td>
</tr>
<tr>
<td>PLXNC1</td>
<td>-1.59</td>
<td>0.041</td>
<td>11736207_a_at</td>
</tr>
<tr>
<td>CD84</td>
<td>-1.60</td>
<td>0.044</td>
<td>11746087_a_at</td>
</tr>
<tr>
<td>PNISR</td>
<td>-1.66</td>
<td>0.001</td>
<td>11759600_at</td>
</tr>
<tr>
<td>OSBPL8</td>
<td>-1.68</td>
<td>0.041</td>
<td>11718643_a_at</td>
</tr>
<tr>
<td>INPP4B</td>
<td>-1.71</td>
<td>0.010</td>
<td>11739840_a_at</td>
</tr>
<tr>
<td>TBL1X</td>
<td>-1.86</td>
<td>0.001</td>
<td>11740214_a_at</td>
</tr>
<tr>
<td>NOTCH2NL</td>
<td>-1.92</td>
<td>0.016</td>
<td>11719212_a_at</td>
</tr>
</tbody>
</table>

* Mean fold change in gene expression.
Table 3: Significant gene changes observed in the body scan group

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC*</th>
<th>P value</th>
<th>Probe set ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIM49D2</td>
<td>1.81</td>
<td>0.007</td>
<td>11735772_s_at</td>
</tr>
<tr>
<td>RAB2B</td>
<td>1.61</td>
<td>0.000</td>
<td>11744458_a_at</td>
</tr>
<tr>
<td>HIRA</td>
<td>1.55</td>
<td>0.035</td>
<td>11720837_a_at</td>
</tr>
<tr>
<td>KLHL18</td>
<td>1.51</td>
<td>0.005</td>
<td>11749153_a_at</td>
</tr>
<tr>
<td>PARN</td>
<td>-1.50</td>
<td>0.002</td>
<td>11750729_a_at</td>
</tr>
<tr>
<td>RNF145</td>
<td>-1.50</td>
<td>0.008</td>
<td>11747975_x_at</td>
</tr>
<tr>
<td>TRIB2</td>
<td>-1.51</td>
<td>0.044</td>
<td>11716940_a_at</td>
</tr>
<tr>
<td>RIOK1</td>
<td>-1.51</td>
<td>0.024</td>
<td>11733924_a_at</td>
</tr>
<tr>
<td>CLSTN1</td>
<td>-1.52</td>
<td>0.006</td>
<td>11715572_a_at</td>
</tr>
<tr>
<td>ETF1</td>
<td>-1.52</td>
<td>0.003</td>
<td>11721449_a_at</td>
</tr>
<tr>
<td>CHD4</td>
<td>-1.52</td>
<td>0.006</td>
<td>11743463_a_at</td>
</tr>
<tr>
<td>ITGA4</td>
<td>-1.53</td>
<td>0.028</td>
<td>11746767_a_at</td>
</tr>
<tr>
<td>CD3G</td>
<td>-1.54</td>
<td>0.045</td>
<td>11731764_a_at</td>
</tr>
<tr>
<td>SLC25A36</td>
<td>-1.54</td>
<td>0.026</td>
<td>11717498_a_at</td>
</tr>
<tr>
<td>AKAP11</td>
<td>-1.54</td>
<td>0.006</td>
<td>11763810_a_at</td>
</tr>
<tr>
<td>CLK4</td>
<td>-1.54</td>
<td>0.043</td>
<td>11759932_at</td>
</tr>
<tr>
<td>RNGTT</td>
<td>-1.55</td>
<td>0.010</td>
<td>11721329_a_at</td>
</tr>
<tr>
<td>CAMSAP1</td>
<td>-1.55</td>
<td>0.029</td>
<td>11720416_a_at</td>
</tr>
<tr>
<td>MAN1A1</td>
<td>-1.55</td>
<td>0.002</td>
<td>11752500_a_at</td>
</tr>
<tr>
<td>USP34</td>
<td>-1.57</td>
<td>0.014</td>
<td>11723681_a_at</td>
</tr>
<tr>
<td>ZSWIM6</td>
<td>-1.57</td>
<td>0.007</td>
<td>11744133_s_at</td>
</tr>
<tr>
<td>TAF15</td>
<td>-1.57</td>
<td>0.038</td>
<td>11750911_x_at</td>
</tr>
<tr>
<td>DYNC1H1</td>
<td>-1.57</td>
<td>0.035</td>
<td>11715589_at</td>
</tr>
<tr>
<td>ZNF800</td>
<td>-1.57</td>
<td>0.007</td>
<td>11759779_at</td>
</tr>
<tr>
<td>ETS1</td>
<td>-1.57</td>
<td>0.042</td>
<td>11722301_a_at</td>
</tr>
<tr>
<td>GNPNAT1</td>
<td>-1.58</td>
<td>0.005</td>
<td>11758604_x_at</td>
</tr>
<tr>
<td>ZKSCAN1</td>
<td>-1.60</td>
<td>0.026</td>
<td>11754860_a_at</td>
</tr>
<tr>
<td>TTC3</td>
<td>-1.61</td>
<td>0.026</td>
<td>11715641_a_at</td>
</tr>
<tr>
<td>ZNF644</td>
<td>-1.63</td>
<td>0.026</td>
<td>11758209_s_at</td>
</tr>
<tr>
<td>PARP14</td>
<td>-1.63</td>
<td>0.022</td>
<td>11749412_a_at</td>
</tr>
<tr>
<td>BRWD1</td>
<td>-1.65</td>
<td>0.001</td>
<td>11728439_at</td>
</tr>
<tr>
<td>PCDH9</td>
<td>-1.67</td>
<td>0.010</td>
<td>11745210_s_at</td>
</tr>
<tr>
<td>Gene</td>
<td>FC*</td>
<td>P value</td>
<td>Probe set ID</td>
</tr>
<tr>
<td>-----------</td>
<td>-----</td>
<td>---------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>ITCH</td>
<td>-1.68</td>
<td>0.027</td>
<td>11745275_x_at</td>
</tr>
<tr>
<td>BCLAF1</td>
<td>-1.68</td>
<td>0.002</td>
<td>11728219_a_at</td>
</tr>
<tr>
<td>RAD23B</td>
<td>-1.70</td>
<td>0.006</td>
<td>11745264_a_at</td>
</tr>
<tr>
<td>TMEM106B</td>
<td>-1.71</td>
<td>0.041</td>
<td>11721215_a_at</td>
</tr>
<tr>
<td>CTDSPL2</td>
<td>-1.71</td>
<td>0.005</td>
<td>11730816_a_at</td>
</tr>
<tr>
<td>P2RY13</td>
<td>-1.73</td>
<td>0.016</td>
<td>11731729_at</td>
</tr>
<tr>
<td>ARHGEF3</td>
<td>-1.74</td>
<td>0.025</td>
<td>11723491_a_at</td>
</tr>
<tr>
<td>KCNA3</td>
<td>-1.78</td>
<td>0.029</td>
<td>11731917_at</td>
</tr>
<tr>
<td>EZR</td>
<td>-1.83</td>
<td>0.023</td>
<td>11742990_x_at</td>
</tr>
<tr>
<td>CD44</td>
<td>-1.88</td>
<td>0.034</td>
<td>11715915_a_at</td>
</tr>
<tr>
<td>UFL1</td>
<td>-1.90</td>
<td>0.003</td>
<td>11751145_a_at</td>
</tr>
<tr>
<td>PRKD3</td>
<td>-1.92</td>
<td>0.002</td>
<td>11739583_s_at</td>
</tr>
<tr>
<td>UBXN4</td>
<td>-2.09</td>
<td>0.004</td>
<td>11751000_a_at</td>
</tr>
<tr>
<td>TMEM260</td>
<td>-2.19</td>
<td>0.003</td>
<td>11727474_a_at</td>
</tr>
<tr>
<td>INPP4B</td>
<td>-2.32</td>
<td>0.001</td>
<td>11739840_a_at</td>
</tr>
<tr>
<td>PTPRC</td>
<td>-2.59</td>
<td>0.001</td>
<td>11733344_at</td>
</tr>
</tbody>
</table>

* Fold change in gene expression.
Discussion

The first and most obvious observation arising from this study is that the two interventions have activated two very different sets of genes, suggesting that the impacts of the two interventions may have different biological consequences. At the inception of this project, our expectation was that the two interventions would probably be associated with changes in similar sets of genes as both interventions were ‘meditation’ techniques. We expected that differences, if any, might be seen in the extent or possibly the direction in which those up or down regulations might occur.

An important question that arises from this is whether or not these differential gene changes are beneficial, detrimental or neutral in their impact on the practitioner. Isolated changes in gene function clearly can be understood in many different ways. Although it is important to avoid over-interpretation of these changes, for the sake of discussion, I have attempted to examine each of the changes in the genes, and based on published research into these genes explore what impacts those gene functions might have on the whole person. Gene functions were obtained through either National Center for Biotechnology Information, US National Library of Medicine’s gene database (http://www.ncbi.nlm.nih.gov/gene) or through the UniProtKB (http://www.uniprot.org/).

If the understanding of the genes in the current peer-reviewed literature is a fair representation of the function of these genes and their overall impact on the practitioner, then one can argue that the pattern of gene changes in the mental silence group could be understood as potentially generally beneficial. However, the gene changes observed in the body scan group (which used a mindfulness based technique), based on the understanding of these genes in the peer-reviewed literature there is no clear pattern of benefit and in fact it can be argued that several of the gene changes might be detrimental to the practitioner.

We must address the dilemma of selecting an appropriate fold change cut-off for the gene expression changes. In general amongst the scientific community, larger powered genetic studies are considered as respectable with fold change values of
greater than 2 (with p < 0.05). As gene expression studies on meditation are still in their early stages they can be classified as being exploratory. As the description suggests, exploratory research aims to identify any subtle changes that may occur as a result of the intervention. As meditation may only evoke subtle gene expression changes, it is crucial for lesser fold change cut-offs to be used. This reduces the chances of type 1 errors therefore maximising our chances of identifying a specific effect.

The studies identified in my literature review vary in the value of the fold change cut-offs used. Of the 10 studies (see data in Table 1):

- 1 study used a fold change cut-off of > 2
- 1 study used a fold change cut-off of > 1.5
- 1 study used a fold change cut-off of >= 1.3
- 1 study used a fold change cut-off of >= 1.25
- 1 study used a fold change cut-off of > 1.2
- 1 study used a fold change cut-off of > 1
- 2 studies did not specify their cut-offs
- 1 study used adjusted differences with a p < 0.05.

My study took a modest approach and employed a fold change cut-off of > 1.4

**The implications of the observed gene expression changes**

The gene groups altered in both groups provide us with some insights into the possible biological mechanism by which meditation practices exert their clinical effects.

These findings are all the more intriguing because they contradicted our expectations. Throughout the planning and execution of the study we expected that the two interventions groups would bring about changes in activity in similar gene groups. We anticipated that the main difference between the groups would be the degree to which the gene groups were up or down regulated. So we were surprised to find that the two interventions, despite both being described as “meditation”, were associated with very different patterns of gene activity change. So much so that in fact, of the many changes in gene regulation observed in the two, there were only
two genes that changed in both groups, i.e. HIRA (increased expression in both groups) and INPP4B (decreased expression in both groups).

It is not unreasonable to conclude that despite their overt similarities, the two conceptualisations of meditation seem to be influencing the molecular biology of the practitioner quite differently. It suggests that relatively subtle differences in the way that a meditative method is defined and practiced can have implications that are significant not only in terms of semantics, philosophy and psychology but also biologically. In order to further explore the implications of the gene changes in both groups, I reviewed the available peer-reviewed literature that related to each of the gene changes that occurred in this study. The gene changes, although quite diverse, generally were for genes in three categories: (1) immune system/inflammation; (2) cancer; and (3) cell structure and function. This is described in further detail below.

Overall, it appears that body scan meditation has altered the expression of a significantly larger number of genes when compared to mental silence meditation (body scan meditation influencing the expression of 3 times as many genes than mental silence meditation). Additionally, gene expression changes relating to immune function, cancer and cellular structure and function are apparent in both body scan and mental silence meditation groups. Interestingly, in my study no gene expression changes were observed relating to chronic inflammation despite the literature review identifying 6 studies which showed the contrary.

My results (summarised in Table 5) thereby confirm the notion that the practice of meditation can induce gene expression changes specifically genes related to immune function, cancer and cellular structure and function. Previous RCTs by Manocha et al. showed that mental silence appears to have a specific effect on mood as well as some aspects of quality of life and an effect on the pathophysiology of asthma. Similarly, studies done by Rai et al. showed that mental silence was associated with improvements in quality of life, anxiety reduction, blood pressure control, beneficial electroencephalogram changes in epilepsy patients and decreased stress. The results of my study add to mental silence meditation research by providing a possible biological or molecular mechanism by which mental silence exerts its previously recorded clinical effects. For example, alterations in immune function gene expression may be a possible mechanism by which mental silence can exert an effect.
on asthma pathophysiology. It is difficult to draw definite conclusions but further and larger powered studies may be able to confirm these hypotheses.

When compared to the literature review this tells me that the two meditation techniques that I studied have commonalities and differences. For some reason both types of meditation did not evoke gene expression changes relating to chronic inflammation. This could be due to the smaller participant size. From my literature review, it can be noted that the larger sample sized studies were able to detect changes in chronic inflammation gene expression. This could mean that these gene expression changes are so subtle that only larger powered studies are able to detect them. Additionally differences in fold change thresholds varied amongst the 6 studies which showed changes in chronic inflammatory gene expression, meaning that there was no standardised reference cut-off to be used. Specifically Creswell et al. (2012) used an FC cut off $\geq 1.25$, D. S. Black et al. (2013) used an FC cut off of $> 1.2$.

Putting methodological explanations aside, perhaps another reason might be these particular two techniques of meditation influence the body in ways that are different to the techniques studies in my literature review. Even though body scan meditation is categorised as a form of mindfulness, in reality it may be different altogether. Similarly, the mental silence meditation technique may be different to the other techniques. This once again highlights the importance of a consensus definition of meditation, without which it is difficult to draw conclusions on whether or not it exerts any non-specific effects.

Immune function changes were substantial in both groups but they appeared to be potentially more beneficial in the mental silence group. A recently published review article concluded that there is some evidence that meditation has a beneficial impact on the immune system, however further studies are required to elucidate the mechanisms (D. S. Black & Slavich, 2016). The results of my study show additional evidence that meditation can have an impact on the immune system and provides a possible biological mechanism by which these immune system changes can occur that is, through epigenetic changes. Interestingly, there were differences between the groups with respect to the number of gene expression changes and the proportion of gene expression changes, which were deemed potentially beneficial. This leads me to believe that although both mental silence and body scan are considered types of
meditation, they in fact may be impacting the body in different ways. To investigate this future larger power studies comparing two active types of meditation should be considered.

Numerous studies demonstrate meditation as an effective tool for improving negative psychological and physical experiences relating to cancer patients. However, up until now there have been no studies and therefore no evidence that meditation can directly alter biological processes relating to cancer. My study shows, both mental silence and body scan meditations appear to have elicited cancer related gene expression changes. This in turn may lead to altered levels of translated proteins that then act to alter cancer pathways or processes with the possibility of ultimately altering the pathophysiology of cancer. No studies in the literature review identified cancer related gene expression changes. This could be due to a couple of reasons. Firstly, most cancer genes have a significant overlap with other gene categories. For example, a cancer gene may also be known to play a role in chronic inflammation, immune function and other cellular processes. Therefore, it is possible that previous studies have categorised cancer genes differently, that is, by allocating cancer genes to other categories. Secondly, none of the studies in the literature review studied the effects of mental silence and body scan meditations specifically. Therefore it may be possible that observed cancer gene expression changes are only associated with these two specific definitions of meditation.

As established previously, gene expression changes relating to cellular structure and function were present in 50% of the studies identified in the literature review. Both mental silence and body scan meditation showed significant changes in this category. Interestingly, this category had the largest number of gene expression changes (34 gene changes across both groups as compared to 8 immune related genes and 10 cancer related genes). This suggests that alterations in cell structure and function related genes may be another biological mechanism through which meditation exerts its purported effects.

As discussed previously, research over the past 10 years has focused around a cellular enzyme known as telomerase. In my study, I found that hTERT was significantly down regulated in the body scan meditation group (FC = −1.32). On the other hand the mental silence group appeared to have no effect on this gene’s
expression. The down regulation of hTERT is contradictory to previous results, which showed that meditation increased the activity of telomerase related genes. This may be attributed to the relatively lower sample size of the study not being able to detect subtle changes associated with telomerase activity. On the other hand there may be different underlying mechanisms of body scan meditation which may lead to a reduction in hTERT expression. To further investigate the full effect of body scan meditation a larger powered study using qPCR (quantitative polymerase chain reaction) experiments to validate the results should be considered. Although these changes were observed in the body scan meditation group, it is important to note that hTERT is only one of many subunits which comprise the telomerase enzyme. Thus, we may not fully understand the functional impact the down regulation of hTERT may have on telomerase (although logically it should decrease its function).

Mental silence meditation appeared to have no effect on hTERT, possibly because of a small sample size leading to a type 1 error. However, mental silence increased the expression of TNKS2 which has been shown to interact with telomerase. Interestingly, studies have shown TNKS2 is involved in telomere maintenance or elongation and also essential for normal growth and development in mice (Chiang et al., 2008; Hsiao, Poitras, Cook, Liu, & Smith, 2006). Overall, my results demonstrate that meditation can alter the expression of telomerase related genes; however, the biological impacts of these alterations are difficult to ascertain.

Both meditation groups also invoked gene expression changes relating to a myriad of other cell functions and to cell structure. Overall, these gene expression changes were seen as potentially more beneficial to the mental silence practitioner, however it may be difficult to conclude the exact overall biological impact it may have on the practitioner.
In order to explore the function of each of the genes implicated in my study, I carried out a function search using the Biotechnology Information, US National Library of Medicine’s gene database (http://www.ncbi.nlm.nih.gov/gene) and through the UniProtKB (http://www.uniprot.org/uniprot/). It is important to point out that the results from this gene expression study may not directly reflect the activity of the gene products, due to the numerous complex pathways that exist in the human biological system. This means that we can only speculate that these gene expression changes are categorised as being beneficial or detrimental. Although, it is possible to analyse the expression of gene products with new emerging technologies such as protein array chips, the cost of doing so is significantly larger than gene array analysis.
Gene changes associated with mental silence

Overall, of the 16 genes changed in the meditation group, 4 are related to immune system functions and 6 are involved in cell structure and function, 4 involved in cancer processes (see Table 2). The remaining 2 genes did not fit in any of these categories.

Within these 4 immune related function genes the literature suggests that 3 of the gene changes are potentially beneficial and one of them could have an undetermined effect on the practitioner. With regard to the 6 cell structure and function related genes the literature suggests that 4 of these gene changes are beneficial for practitioners and 2 have their functions understood but their impacts on the practitioner are unknown. Of the 4 cancer related gene changes, 2 were found to be potentially beneficial and 2 genes had their functions not understood yet.

Immune related genes

Potentially beneficial
- CD84 (CD84 molecule) \(-1.6\) FC
- CLEC10A (C-type lectin domain family 10, member A) \(-1.6\) FC
- IGHG3 (immunoglobulin heavy constant gamma 3) \(3.03\) FC

Impact unknown
- NOTCH2NL (notch 2 N-terminal like) \(-1.92\) FC

Cancer related genes

Potentially beneficial
- TBL1X (transducin (beta)-like 1X-linked) \(-1.86\) FC
- SMARCA2 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 2) \(-1.57\) FC

Impact unknown
- KIAA0101 \(2.01\) FC
- PXLNC1 (Plexin C1) \(-1.59\) FC
**Cell structure related genes**

**Potentially beneficial**
- TNKS2 (tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2) 1.52 FC
- LMAN1 (lectin, mannose-binding, member 1) 1.8 FC
- HIRA (histone cell cycle regulator) 1.8 FC
- INPP4B (inositol polyphosphate-4-phosphatase, type II) −1.71 FC

**Impact unknown**
- WDFY1 (WD repeat and FYVE domain containing, member 1) −1.5 FC
- OSBPL8 (oxysterol binding protein-like, member 8) −1.68 FC

**Genes with poorly understood function**
- PNISR (PNN-interacting serine/arginine-rich protein) −1.66 FC
- MBNL1 (muscleblind-like splicing regulator 1) −1.52 FC

**Gene changes observed only in the body scan group**

Overall, of the 48 genes changed in the control group, 4 are related to immune system functions and 28 are involved in cell structure and function, 6 genes are involved in cancer processes and 10 of the genes currently have no known function (see Table 3).

Within the 4 immune related function genes the literature suggests that only 1 gene change is potentially beneficial, while the other 3 genes are potentially detrimental to the practitioner. With regard to the 28 cell structure and function related genes the literature suggests that 3 of these gene changes are beneficial for practitioners, while 4 may be detrimental for practitioners and the function for 21 of these genes are unknown. Within the cancer related genes 3 of the gene changes have been shown to have a potentially detrimental impact on the practitioner while only one was shown to have a potential beneficial impact and 2 gene changes with an unknown impact on the practitioner.

**Immune related genes**

**Potentially beneficial**
- PRKD3 (protein kinase D3) −1.92 FC
Potentially detrimental
• PTPRC (protein tyrosine phosphatase, receptor type C) −2.59 FC
• KCNA3 (potassium voltage-gated channel, shaker-related subfamily, member 3) −1.78 FC
• ITCH (itchy E3 ubiquitin protein ligase) −1.68 FC

Cell structure related genes
Potentially beneficial
• TRIB2 (tribbles pseudokinase 2) −1.51 FC
• HIRA (histone cell cycle regulator) 1.55 FC
• INPP4B (inositol polyphosphate-4-phosphatase, type II) −2.32 FC

Potentially detrimental
• PARP14 (poly (ADP-ribose) polymerase family, member 14) −0.71 LOGFC
• DYNC1H1 (dynein, cytoplasmic 1, heavy chain 1) −1.63 FC
• RNGTT (RNA guanylyltransferase and 5'-phosphatase) −1.55 FC
• CD3G (CD3g molecule, gamma (CD3-TCR complex)) −1.54 FC

Impact unknown
• ARHGEF3 (rho guanine nucleotide exchange factor (GEF) 3) −1.74 FC
• PCDH9 (protocadherin 9) −1.67 FC
• UBXN4 (UBX domain protein 4) −2.09 FC
• TAF15 (TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor) −1.57 FC
• P2RY13 (purinergic receptor P2Y, G-protein coupled, 13) −1.73 FC
• BRWD1 (bromodomain and WD repeat domain containing 1) −1.65 FC
• ZNF644 (zinc finger protein 644) −1.63 FC
• TTC3 (tetra tripeptide repeat domain 3) −1.61 FC
• GNPNAT1 (glucosamine-phosphate N-acetyltransferase 1 −1.58 FC
• USP34 (ubiquitin specific peptidase 34) −0.65 LOGFC
• CAMSAP1 (calmodulin regulated spectrin-associated protein 1) −1.57 FC
• CLK4 (CDC-like kinase 4) −1.54 FC
• AKAP11 (A kinase (PRKA) anchor protein 11) −1.54 FC
• CHD4 (chromodomain helicase DNA binding protein 4) −1.52 FC
• ETF1 (eukaryotic translation termination factor 1) −1.52 FC
• CLSTN1 (calsyntenin 1) −1.52 FC
• PARN (poly(A)-specific ribonuclease) –1.50 FC
• MAN1A1 (mannosidase, alpha, class 1A, member 1) –1.55 FC
• ETS1 (V-ets avian erythroblastosis virus E26 oncogene homolog 1) –1.57 FC
• ITGA4 (integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)) –1.53 FC
• RAB2B (member RAS oncogene family) 1.61 FC

Cancer genes

Potentially beneficial
• EZR (ezrin) –1.83 FC

Potentially detrimental
• UFL1 (UFM1-specific ligase 1) –1.90 FC
• RAD23B (RAD23 homolog B) –1.70 FC
• BCLAF1 (BCL2-associated transcription factor 1) –1.68 FC

Impact unknown
• ZKSCAN1 (zinc finger with KRAB and SCAN domains 1) –1.60 FC
• CD44 (CD44 molecule) –1.88 FC

Genes with poorly understood function
• CTDSPL2 (CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase like 2) –1.71 FC
• TMEM106B (transmembrane protein 106B) –1.71 FC
• TMEM260 (transmembrane protein 260) –2.19 FC
• TRIM49D2 (TRIM49D2 tripartite motif containing 49D2) 1.81 FC
• KLHL18 (kelch-like family member 18) 1.51 FC
• RNF145 (ring finger protein 145) –1.50 FC
• SLC25A36 (solute carrier family 25 - pyrimidine nucleotide carrier, member 36) –1.54 FC
• ZSWIM6 (zinc finger, SWIM-type containing 6) –1.57 FC
• ZNF800 (zinc finger protein 800) –1.57 FC
• RIOK1 (RIO kinase 1) –1.51 FC
Theory, literature review and current study: gene expression changes

In my introduction I have described the most popular and current theories regarding meditation’s potential biological mechanisms of action and the evidence for each. In summary, they can be categorised as follows:

• the modulation of the stress and chronic inflammatory pathways (NF-κB)
• expression changes of immune function genes
• expression changes of cancer related genes
• more recently, expression changes of certain cellular structure and function genes leading to increased longevity.

For ease of discussion, the gene expression changes found in my study have been sorted into the same categories described above. However, in reality there is a certain degree of overlap between these categories. For example, CD84 is a gene which codes for a protein expressed by B lymphocytes and macrophages. A number of functional studies has confirmed this. Thus it would seem logical that CD84 would be positioned in the immune function category. However, the literature also shows that CD84 is implicated in Kawasaki disease which is characterised by inflammation of blood vessels throughout the body. So, it can also be argued that CD84 might be equally well positioned in the chronic inflammation category. For the purposes of this thesis, I chose to put CD84 in the immune function category because the majority of studies and gene banks have categorised CD84 as an immune gene. Similarly, wherever a gene might belong to more than one category I allocated it based on what the majority of the literature or gene banks categorised it as.

Chronic inflammation

As discussed previously chronic inflammation is implicated in the causation in a number of significant NCDs. Hence strategies to reduce chronic inflammation levels are regarded as key to reducing the risk of developing these NCDs at an individual and population level. Lifestyle strategies, such as the reduction of saturated fats in the diet, for example, are thought to exert much of their benefit by reducing chronic inflammation. Current understandings of chronic inflammation are increasingly also implicated in psychological stress and mental illnesses (such as depression and Alzheimer’s disease).
My literature review identified 10 studies that investigated meditations impact on gene expression. Interestingly 6 of the studies involved gene expression changes relating to chronic inflammation. Antoni et al. (2012), D.S. Black et al. (2013) and Creswell et al. (2012) all showed a reduction in NF-κB response elements. Bhasin et al. (2013), Dusek et al. (2008) and Kaliman et al. (2014) were the other 3 studies and showed a significant down regulation of chronic inflammatory genes.

In my study, contrary to what was expected, neither the mental silence group nor the body scan meditation group showed any alterations in inflammatory gene expression. Possible reasons for the absence of change in this category are discussed later in this section.

**Immune function**

In my study, both the mental silence and body scan meditations elicited gene expression changes under the immune function category. The mental silence group altered 4 genes of which 3 were down regulated (NOTCH2NL, CLEC10A, CD84) and 1 up regulated (IGHG3). IGHG3 had the largest fold change out of all the gene changes in the mental silence group with a FC of 2.0. It codes for an immunoglobulin protein, which is a key component of antibodies. Therefore, an increased expression of this gene may lead to a larger number of antibody sub-units being produced and hence a more efficient immune system. Black et al. presented similar up regulation of immunoglobulin related transcripts such as IGJ (FC 1.4) and IGLL3 (FC 1.2) but not IGHG3 (D. S. Black et al., 2013). The literature surrounding the function of some of these genes leads to the following possibilities:

- The down regulation of CLEC10A and CD84 may be beneficial to the practitioner.
- The down regulation of NOTCH2NL has an unknown impact as the function of this gene is not yet fully understood.

The body scan meditation elicited a down regulation of 4 genes: PRKD3, PTPRC, KCNA3 and ITCH. The literature reveals the following in regards to these gene expression changes:

- PRKD3 is thought to be involved in the regulation of immune functions through intracellular signalling. The down regulation of PRKD3 was
identified as being potentially beneficial as the expression of this gene has been shown to contribute to the proliferation of cancerous cells in the prostate.

- PRPRC was identified as being involved in T and B cell antigen receptor signalling and as a tumour suppressor gene. Thus, a decrease in gene expression may possibly have a detrimental impact on the immune system’s function and protection against cancer.
- KCNA3 was identified as playing a role in T lymphocyte differentiation and activation. Hence a reduction in this gene’s expression may negatively impact the adaptive immune response.
- ITCH codes of an enzyme that targets specific proteins for lysosomal degradation. It is also involved in the control of T lymphocyte differentiation and activation. Therefore, a down regulation of this gene may negatively affect the ability for T lymphocytes to perform at their maximum capabilities.

Overall, both mental silence and body scan meditations appear to alter immune related gene expression. The gene expression changes altered after mental silence and body scan meditations are primarily involved with adaptive immune function (T lymphocytes). On the other hand, genes differentially expressed in Antoni et al’s study were primarily involved with innate immune function (anti-viral proteins) (Antoni et al., 2012).

Although both the mental silence and body scan meditations altered the expression of adaptive immune function genes, they appeared to have altered the expression of genes uniquely such that:

- The mental silence group appears to have a beneficial impact on the immune system.
- The body scan group appears to have a negative impact on immune function.

These results are a step forward in understanding how meditation, specifically the technique of mental silence, is able to alter immune function. Although, the impacts of these gene expression changes are theoretical, further robust studies are warranted.
Cancer

Cancer, as discussed previously, can be associated with many modern lifestyle risk factors. Lifestyle modifications that can alter these risk factors may ultimately reduce the risk of getting cancer. Although there is a body of evidence indicating that meditation is useful in improving cancer related cognitive dysfunction, there is little evidence that meditation can alter biological processes directly related to cancer (Ando et al., 2009; Biegler, Chaoul, & Cohen, 2009; Y. H. Kim, Kim, Ahn, Seo, & Kim, 2013).

The results from my study are the first indications that meditation can influence cancer related gene expression. Mental silence altered the expression of 4 cancer related genes of which 1 was up regulated and 3 down regulated. Of the 4 cancer gene expression changes, 2 of the changes were deemed beneficial while the other 2 changes were deemed as having an unknown impact on the body.

Mental silence

TBL1X had a decreased level of expression and as its role as a possible oncogene was identified in 2014, it can be proposed a decrease in TBL1X’s expression may have a beneficial impact on the practitioner (J. Y. Li, Daniels, Wang, & Zhang, 2015). The next gene, SMARCA2, was shown to be involved in 20% of all cancer types and it is proposed that inhibitors of SMARCA2 may have a therapeutic role in treating cancers, which have loss of function mutations in its related gene (SMARCA4) (Hoffman et al., 2014). Thus it may be possible that a down regulation of SMARCA2 as observed in our study may provide a therapeutic benefit for cancers harbouring a loss of function mutation in SMARCA4.

KIAA0101 was the only up-regulated gene in the cancer category. Numerous studies have implicated KIAA0101 gene overexpression in a number of cancers such as pituitary tumours, hepatocellular carcinoma, gastric cancer, oesophageal cancer and adrenal cancer (Cheng et al., 2013; Jain, Zhang, Patterson, & Kebebew, 2011; Liu et al., 2012; Roche et al., 2015; Zhu et al., 2013). However, a study in 2009 identified its role as a regulator of DNA repair during DNA replication (Turchi et al., 2009). The differing biological roles of KIAA0101 make it hard to determine whether an increase in this genes expression will be beneficial or detrimental. Similarly,
conflicting studies make it hard to determine the effect of expression changes for the PLXNC1 gene. Whether a decrease in this genes expression is beneficial or detrimental is not fully known. PLXNC1 codes for plexin C1 that is a protein involved in inflammation and cancer cell proliferation. PLXNC1 has been shown to play a role in delaying the progression of melanoma. Chen et al. demonstrated that a loss of function of PLXNC1 increased the migration and proliferation rate of melanoma cells (Chen, Soong, Mohanty, Xu, & Scott, 2013). Our study shows a FC of −1.60 potentially making this a beneficial change for practitioners.

**Body scan meditation**

On the other hand, body scan meditation altered the expression of 6 cancer related genes, all of which were down regulated. There was only one gene change which was identified as having a potentially beneficial impact. EZR a gene coding for a protein which serves as a potential biomarker for hepatocellular carcinoma was down regulated, hence providing a potentially beneficial impact on the practitioner (Flores-Tellez, Lopez, Vasquez Garzon, & Villa-Trevino, 2015).

The down regulation of 3 of the genes regulated by body scan meditation in this category were deemed to have a possible detrimental impact on the practitioner. The first gene UFL1 is known to be a negative regulator of the NF-κB pathway, a novel regulator of C53 tumour suppressor gene and also implicated in breast cancer initiation (Wu, Lei, Mei, Tang, & Li, 2010; Yoo et al., 2014). Therefore, the decreased expression of this gene may promote chronic inflammation via the NF-κB pathway and alter cancer processes. The second gene, RAD23B, has been identified as a tumour suppressor gene which is also involved in nucleotide excision repair (Linge et al., 2014; Schärer, 2013). The third gene, BCLAF1, has been shown to act as a tumour suppressor and is also known to be involved in the regulation of DNA damage and repair genes (Lee, Yu, Gunawardena, Xie, & Chen, 2012; Savage et al., 2014). It can be thus argued that the down regulation of both RAD23B and BCLAF1 may have a detrimental impact on the practitioner.

Gene expression changes of 2 genes had unknown impacts. These included CD44 and ZKSCAN1. The literature surrounding CD44 is divided, with studies demonstrating its cancer initiating properties while others demonstrating its cancer suppressing properties. A 2011 review describes CD44 as having different roles
during different stages of cancer, hence a decrease in CD44 expression as seen in the control group may have various unknown impacts on cancer processes (Louderbough & Schroeder, 2011). Similarly, there is not enough evidence to suggest any beneficial or detrimental impacts for ZKSCAN1.

The observed cancer gene expression changes invoked by mental silence and body scan meditations give the first indications that meditation may act on cancer pathways in the body. However, it must be noted that genes involved in cancer processes are also implicated in normal cell functioning. Thus, the degree of overlap between cancer and other gene categories is enormous. Keeping this in mind, I have done my best to include genes in the category of cancer based on the number of studies implicating them in cancer. In instances where there are more studies implicating them in normal cellular processes, I have included them in other categories.

**Cell structure and function**

As mentioned previously the healthy functioning of our cells creates an opportunity for physiological homeostasis and longevity. There are a number of cellular components, which all contribute uniquely through a complex network of intracellular signalling to achieve homeostasis.

Of the 10 studies included in my literature review, 5 showed that meditation can alter the genes which code for a number of these cellular components. A summary is as follows:

- In 2005, Falun Gong practitioners were found to have:
  - down regulation of ubiquitin related proteins (E1, E2, E3), ribosomal proteins, DNA repair (RAD52, ERCC), apoptotic genes (BLC2, BCL-xl)
  - up regulation of heat shock proteins (HSP70, HSP40-3).

- In 2008, Sudarshan Kriya practitioners were found to have up regulation of glutathione S transferase (GST-P1) and PTGS2. HTERT and BCL2 were increased but were not significant.

- In 2012 Ravnik-Glavac et al. showed that switching to a higher state of consciousness could alter the expression of thousands of genes of which a
large proportion were related to cell structure and function (using a cut-off > 1.3 FC).

- In 2013 Sudarshan Kriya and related practices were shown to down regulate the expression of AVIL (a gene coding for a structural protein) and NFE2 (a gene coding for a master regulator of megakaryocyte differentiation and platelet production).
- In 2014, mindfulness meditation practitioners were found to have a decreased expression of histone deacetylases, specifically HDAC2, HDAC3, HDAC9 and histone modifiers (H4ac and H3K3me3).

My study provides additional evidence that meditation, specifically mental silence and body scan meditations, can elicit gene expression changes related to cell structure and function. The mental silence group altered 4 genes of which 2 were up regulated and 2 down regulated. The body scan meditation altered 26 genes of which only one was up regulated and the remaining 25 down regulated.

**Potentially beneficial gene expression changes – mental silence**

The mental silence group was shown to up regulate TNKS2 which has been implicated in negatively regulating telomerase through the release of endogenous TRF1 (Hsiao et al., 2006). As a deregulation of telomerase can result in a cell turning cancerous/immortal, it can be proposed an up regulation of TNKS2 as seen in mental silence practitioners may have cancer-protecting effects through its actions on TRF1.

Another gene’s expression affected by mental silence meditation was LMAN. This codes for a membrane anchor protein that recognises sugar residues of glycoproteins, glycolipids, or glycosylphosphatidyl inositol anchors. It functions to sort and recycle proteins and lipids (Duellman, Burnett, Shin, & Yang, 2015; Zheng & Zhang, 2013). Its increased expression elicited by mental silence meditation may therefore beneficially affect the cells sorting and recycling processes.

**Unknown impact of gene expression change – mental silence**

Mental silence meditation also decreased the expression 2 genes WDFY1 and OSBPL8, both of which have limited literature surrounding their function making it hard to say if a decreased expression would have a beneficial or detrimental impact on the body.
Potentially beneficial gene expression changes – body scan meditation

Body scan meditation only had one potentially beneficial gene expression relating to cell structure and function. The down regulation of TRIB2, a protein kinase with oncogenic properties, may prove to be beneficial to practitioners (Hill et al., 2015).

Potentially detrimental gene expression changes – body scan meditation

Of the 26 gene expression changes 3 were identified as being potentially detrimental. The first gene which was negatively expressed was PARP14. In 2015, it was found to be a novel factor in alleviating replication stress and enhancing genomic stability (Krishnamurthy et al., 2014). It may be possible that the decrease in expression of this gene may negatively effect the stability of cells during replication, and hence increase the rate of cell death.

Another gene found to have decreased expression was DYNC1H1. This gene codes for an ATP powered protein (also known as a motor protein) which is involved in a large number of cellular processes such as the transport of organelles and the functioning of the mitotic spindle during the cell division phase (Ayloo et al., 2014). Mutations in this gene lead to phenotypes such as Charcot-Marie-Tooth disease and mental retardation (Ding et al., 2016; Weedon et al., 2011). Due to its crucial role in cellular function, we propose that a decrease in this gene’s expression may have a detrimental impact.

The body scan meditation group also down regulated a RNGTT which functions as an mRNA and RNA capping enzyme. Capping is essential for mRNA/RNA stability, export and translation (Mukherjee, Bakthavachalu, & Schoenberg, 2014; Pillutla, Shimamoto, Furuichi, & Shatkin, 1998). A decreased expression of RNGTT may possibly reduce the capping and thus cause a loss of efficiency of mRNA/RNA.

Another gene, CD3G, is a protein that forms part of the CD3 T cell receptor complex and is involved in antigen recognition. Defects in this gene have been linked to T cell immunodeficiency and thus a decreased expression of CD3G may have a negative effect on T cell function (Fischer, de Saint Basile, & Le Deist, 2005; Gokturk et al., 2014).
Unknown impact gene expression changes – body scan meditation

Although the body scan meditation elicited one potentially beneficial and 3 potentially detrimental gene expression changes the majority of the 26 gene expression changes were classified as having an unknown effect on the body. This was mainly due to the lack of functional studies present in the literature. Within the category of cellular structure and function, these gene changes could be classified further as the following (National Center for Biotechnology Information, 2005):

- **Protein binding**
  - ARHGEF3
  - UBXN4
  - TAF15
  - BRWD1
  - TTC3
  - GNPNAT1
  - USP34
  - CLK4
  - CHD4
  - CLSTN1
  - RAB2B

- **DNA binding**
  - ZNF644
  - ETS1

- **Cell adhesion**
  - PCDH9
  - ITGA4

- **Signal transduction**
  - P2ry13

- **Microtubule/calmodulin/spectrin binding (cytoskeletal organisation)**
  - CAMSAP1

- **Protein kinase A binding**
  - AKAP11

- **RNA binding**
  - ETF1
Nucleotide binding
• PARN
Mannosidase activity
• MAN1A1

Other gene expression changes worth noting (< 1.4 FC)

Meditation has been shown to significantly alter the activity of telomerase, which has been implicated in cell longevity (Schutte & Malouff, 2014). Interestingly the body scan meditation group elicited a significant down regulation of hTERT by FC = −0.11, while there was no change in the mental silence group.

Another gene, GSTZ1 (glutathione S-transferase zeta 1) is part of the glutathione S transferase superfamily of genes which all encode for enzymes which play a significant role in detoxification of carcinogens and mutagens (Strange, Spiteri, Ramachandran, & Fryer, 2001). Interestingly, the body scan group displayed a significant (p < 0.05) down regulation of this gene by −0.11 fold while the mental silence group had no effect.

Cell structure and function genes in relation to previous studies

Meditation group

There were a number of genes which were altered in both our study and in previous studies. OSBPL8 was found to be altered in the mental silence group with a fold change of FC = −1.68. This is the opposite direction of change reported in a study by Antoni et al. (where it was up regulated instead of down regulated) (Antoni et al., 2012).

TNKS2 was a gene which was down regulated in the meditation group (FC = 1.52). In Ravnik-Glavac et al’s study it was also seen to be down regulated after the meditator had switched to a higher state of consciousness (Ravnik-Glavac et al., 2012).

Body scan group

In the body scan group, TRIB2 (FC = −1.51) had the same direction of change as Ravnik-Glavac et al’s study (Ravnik-Glavac et al., 2012). Another gene CHD4 (FC = −1.52) was found to be down regulated in the body scan group which parallels
Creswell et al’s study (logFC = −0.32) (Creswell et al., 2012). ZNF644 was found to be down regulated in the body scan group (FC = −1.63) and also down regulated in Antoni et al’s study where CBSM was the intervention. PARP14 a gene involved in regulating transcription was found to be down regulated in our control group and Antoni et al’s study (FC = −1.63) (Antoni et al., 2012). RAD23B was down regulated in the control group by FC = −1.70 and in Creswell et al’s study it was shown to be up regulated. CTDSPL2 was down regulated (FC = −1.71) by both our control group and the intervention group in Antoni et al’s study.

Categories shown to be unaffected by our study

Our study along with 7 other studies did not display any changes in ribosomal genes. These were only demonstrated to be differentially expressed in Li’s 2015 pilot study of Qigong practitioners, Ravnik-Glvac et al’s 2012 study and Dusek et al’s 2008 study (Dusek et al., 2008; Q. Z. Li et al., 2005; Ravnik-Glvac et al., 2012).

It is apparent that meditation, specifically mental silence and body scan meditation can invoke gene expression changes in a large number of cellular structure and function genes. This confirms the results of 5 previous studies which investigated the gene expression changes associated with a variety of meditations.

Strengths and weaknesses of my study

My study has a number of strengths that make it a powerful stepping stone in meditation epigenetic research. It utilised a randomised controlled trial study design making it the fourth RCT exploring gene expression changes associated with meditation. The strengths associated with the study design included:

- The participants, the head researcher and the data entry personnel were all blinded to the allocation of participants.
- The participants were blinded to the hypothesis of the study.

Additionally, it was the first gene expression study comparing two different definitions of meditation namely body scan and mental silence meditations. This also adds a new perspective to the growing body of meditation research that different definitions of meditation may have different biological impacts on the body.
Limitations of this study included the sample size of 48 participants, after taking dropouts into consideration. As a result of having only 24 participants in each intervention group, type 1 errors may have occurred especially when trying to detect possibly subtle genetic changes associated with meditation. Future studies should aim to include at least double this, that is 50 participants per intervention group to increase the statistical strength of the study. Additionally, participants could be asked to increase their face-to-face participation hours from 1 hour weekly to 1 hour every day. Similarly, the total intervention time of 5 weeks could be extended to possibly double that. Limitations surrounding the method of advertisement for the study meant that only participants from the University of Sydney would respond. Overall, this could have an impact on the external validity of the clinical trial and future studies should aim to recruit participants from all aspects of society.
Conclusion and implications for the future

My literature review found that there are 10 studies in the literature looking at the genetic impacts of meditation. Of those 10 studies, none looked at mental silence orientated forms of meditation and none compared two different definitions of meditation, despite the fact that there are many varying definitions of meditation in the literature. In addition, despite 40 years of research the scientific community is yet to come to a consensus definition of what meditation is or what it might be defined as. Even more importantly, there is no conclusive evidence of a specific effect with regards to meditation as a generic category. Having said this there is a technique of meditation called Sahaja yoga for which there is a small but emerging body of evidence for a specific effect, particularly in association with the mental silence experience associated with that technique of meditation.

In order to ascertain firstly, the mechanisms by which Sahaja yoga may exert its biological effects, secondly by which meditation might exert its biological effects and thirdly whether or not there might be differences between meditation techniques I conducted a randomised controlled trial involving 50 subjects where I compared Sahaja yoga meditation to body scan meditation assessing gene expression changes before and after. As a result of this study the between groups analysis found only one significant difference, however there were substantial between group differences that were qualitatively different between the two groups. These differences appear to indicate that the lack of between group of differences is most likely to be due to type 1 error. The within group changes nevertheless point towards the idea that the two techniques of meditation have very different biological impacts. The main effects being on immune regulation, cell structure and function, and cancer risk.

The outcomes of my study provide preliminary evidence that appears to show that different mediation techniques have different biological impacts on the body. This is based on the fact that gene expression changes were found to be unique when comparing the mental silence and body scan meditation groups. It is not unreasonable to consider the possibility that the alteration of gene expression changes in the categories of cancer, inflammation and cell structure and function may lead to different biological outcomes. This might therefore bring about different
biological outcomes in different practitioners of meditation. While purely speculative, it appears that the gene changes in the mental silence group may be more associated with favourable health effects as compared to the changes that were observed in the body scan meditation group. However, I recognise that this observation is speculative but nevertheless the implications are fascinating and warrants further research.

In conclusion, this study clearly suggests that techniques of meditation with different definitions, may well have different epigenetic implications and this supports the idea that was discussed earlier in my thesis that grouping all forms of meditation under a single rubric is an inadequate way of understanding the effects of meditation. Different approaches to meditation, because they have different biological implications, ought to be clearly distinguished from each other and studied separately. This is supported not only by the gene expression changes that were unique to each group in my study, but also the fact that some of these gene expression changes were unique when compared to previous studies.

Additionally, this study also supports the idea that the different definitions of meditation are important to understand and that there may be only some definitions, that while meditation as a generic category may not be associated with specific effect, there may be some definitions that are. The mental silence definition of meditation is one of those categories for which I have already discussed the evidence suggesting that it is a worthwhile definition to assess. In order to further explore these important findings, I suggest that the following should be done.

My study should be replicated on a larger scale in order to highlight any subtle between group differences concerning gene expression changes for both mental silence and body scan meditation. Sample sizes with 50 participants or greater in each meditation group would be appropriate. Additionally, future studies would ideally be conducted with greater levels of methodological rigor. This may involve extending the intervention time, increasing the amount of time each participant spends meditating per week and also the confirmation of any significant gene expression changes through quantitative PCR determinations. Although costly, these additional measures would not only allow us to detect subtle gene expression changes that seem to be associated with meditation, but also provide a valid means of
confirming whether or not there is a biological (measured through gene expression changes) difference between two definitions of meditation.

In addition, other definitions of meditation should be assessed in a similar way; i.e. randomised controlled trials which compare different definitions of meditation would allow us to confirm the preliminary idea that different definitions of meditation have different genetic impacts on the body. Future comparison studies may also shed light on the possibility that due to the different definitions of meditation having different biological impacts on the body, it is plausible that some of these definitions may be potentially more beneficial than others. On the other hand it may also be the case that some definitions of meditation may be detrimental to practitioners.

In order to enhance the validity of future studies it is important to establish a connection between meditation invoked gene expression changes and how these relate to physical and mental health outcomes in the practitioner. Physical and mental health outcomes could include mood, quality of life and mortality (for possible long-term cohort studies). Until now, there has been no attempt to correlate physical and mental health outcomes with gene expression changes due to meditation. Discovering the relationship between these two parameters will help us better understand what benefits are achieved through the alteration of specific genes. Therefore giving us additional insight on the biological mechanism of action through which meditation acts.

With regards to the mental silence definition of meditation (Sahaja yoga), epigenetic changes should be correlated with the degree to which practitioners are experiencing mental silence. These can then be further correlated to physical and mental health outcomes described previously. This would allow us to determine whether or not there is a connection between mental silence and enhanced physical and mental health.
References


Aftanas, L. I., & Golocheikine, S. A. (2001). Human anterior and frontal midline theta and lower alpha reflect emotionally positive state and internalized attention: high-resolution EEG investigation of meditation. *Neuroscience Letters, 310*(1), 57-60.


Appendix 1 – Body Scan Meditation

Sessions were held every Sunday for 1 hour. For the first session participants from both groups were asked to arrive at 3:00pm. A briefing was given and participants asked to fill out consent pre-questionnaires. This was followed by blood collection performed by qualified nurses. Participants were then divided into their randomly allocated groups and told to attend different venues.

At 4:00pm a guided meditation session was led by an instructor. Participants were told to sit or lie down in a comfortable position and then told to follow a 19 minute guided body scan meditation audio track which was played through a sound system. Following this participants were allowed to socialise and food was provided. Participants were also given a copy of “The relaxation and stress reduction workbook” which they were told to read at some stage throughout the study period.

A copy of that book:
Praise for The Relaxation and Stress Reduction Workbook:

“This comprehensive workbook deserves to be in the library of every active therapist, but it shouldn’t be left on the shelf! Once again, the authors have empowered the reader with straightforward instructions on every major approach to stress management known. From worry to chronic headaches to information overload, here is your one-stop guide to recovery.”

—R. Reid Wilson, Ph.D., author of Don’t Panic: Taking Control of Anxiety Attacks

“This text remains, after twenty years, the clearest, best-organized, and most readable book on stress management. It has achieved the status of the ‘classic’ self-help reference in the field.”

—Edmund J. Bourne, Ph.D., author of The Anxiety and Phobia Workbook, Coping with Anxiety, and Beyond Anxiety and Phobia

“An exemplary book on stress. It is lucidly written, rationally ordered, and comprehensive, and each section is densely packed with instructions and exercises which make the workbook easy to practice.”

—Somatics Magazine: Journal of the Mind/Body Arts and Sciences
The Relaxation & Stress Reduction WORKBOOK

SIXTH EDITION

Martha Davis, Ph.D.
Elizabeth Robbins Eshelman, MSW
Matthew McKay, Ph.D.

New Harbinger Publications, Inc.
Progressive Muscle Relaxation

In this chapter you will learn to:

- Distinguish between tense and relaxed muscles
- Progressively relax all of the muscles of your body
- Relax quickly in stressful situations

Background

You cannot have the feeling of warm well-being in your body and at the same time experience psychological stress. Progressive relaxation of your muscles reduces pulse rate, blood pressure, and the startle reflex, as well as reducing perspiration and respiration rates. Deep muscle relaxation, when successfully mastered, can be used as an antianxiety pill.

Edmund Jacobson, a Chicago physician, published the book *Progressive Relaxation* in 1929. In it he described his deep muscle relaxation technique, which he asserted required no imagination, willpower, or suggestion. His technique is based on the premise that the body responds to anxiety-provoking thoughts and events with muscle tension. This physiological tension, in turn, increases the subjective experience of anxiety. Deep muscle relaxation reduces physiological tension and is incompatible with anxiety: The habit of responding with one blocks the habit of responding with the other.

Jacobson’s original progressive relaxation procedures might take many months or even years to learn, but Joseph Wolpe (1958) developed a short form for these procedures that included verbal suggestions to relax. This abbreviated form can be mastered in a matter of days or weeks. Wolpe made this streamlined version a part of his systematic desensitization protocol for the treatment of phobias. He found that once they relaxed, clients were more capable of tolerating and responding adaptively to situations they were afraid of.

Symptom-Relief Effectiveness

Excellent results have been found with progressive relaxation techniques for the treatment of muscular tension, anxiety, depression, fatigue, insomnia, neck and back pain, high blood pressure, mild phobias, and stuttering.
Instructions

Many people do not know which of their muscles are chronically tense. When you practice progressive relaxation, you focus on the sensations of tension in one particular muscle group at a time. Then, when you release that tension, you focus on the sensations of relaxation in that same muscle group. You move progressively through your whole body from one muscle group to the next, repeating this procedure. Using progressive relaxation techniques, you learn to identify particular muscle groups and to distinguish between the sensations of tension and deep relaxation.

Progressive relaxation can be practiced lying down or seated in a chair. Each muscle group is tensed from five to seven seconds and then released and relaxed for twenty to thirty seconds. These lengths of time are simply rules of thumb and don't have to be slavishly adhered to. This procedure is repeated at least once. If a particular muscle is difficult to relax, you can practice tensing and releasing it up to five times.

Once the procedure is familiar enough to be remembered, keep your eyes closed and focus your attention on just one muscle group at a time. Another option is to purchase a professional recording such as the one listed in the Recording section at the end of this ebook.

The instructions for progressive relaxation are divided into two sections. The first part deals with the basic procedure, which you may wish to record and replay while practicing. This will familiarize you with the muscles in your body that are most commonly tense. If you do record these instructions, be sure to pause long enough for tensing and relaxing. The second section shortens the procedure by simultaneously tensing and relaxing many muscles at one time, so that deep muscle relaxation can be achieved in a very brief time period.

The Three Basic Levels of Tensing

There are three basic levels of tensing that you can use when you practice progressive relaxation. With experience, you can decide which level of tensing is most pleasant and effective for your needs.

1. Active tensing involves tensing a particular muscle group as tightly as you can without hurting yourself, studying the sensations of tension, then releasing the tension and studying the sensations of relaxation in that same area. While you are tensing one part of your body, the rest of your body is relatively relaxed. Remember to breathe diaphragmatically (it's easy to forget to breathe this way, especially during the tensing phase). Active tensing is the level of progressive relaxation described in italics below. By really exaggerating the tension, you are likely to feel where you carry chronic tension; the tense place may actually be sore. For people who have no injuries and who are not extremely tense, active tensing is recommended as the method of choice, at least for the first time you practice progressive relaxation. Some people prefer using this level every time they practice progressive relaxation, because tensing the muscles
fatigues the muscle fibers and releasing the tension feels very relaxing and good. It's a little like setting down heavy bags you've been holding while standing in a long line.

2. **Threshold tensing** is the same as active tensing except that it involves tensing a particular muscle group slightly (just enough so that you notice the tension; it's barely noticeable to the human eye). Threshold tensing should be used for areas of your body that are injured or very tense to avoid pain or injury. Many people prefer to use threshold tensing once they've become familiar with the basic muscle groups through active tensing because threshold tensing takes less effort and feels less invasive. Some people use threshold tensing from the beginning because of health issues or extreme tension.

3. **Passive tensing** is the same as active tensing except that during the “tensing phase” you simply notice any tension that is present in a particular muscle group. You can use the same basic procedure described below in italics and substitute the words “Notice the tension in your ____________” whenever the instructions call for tensing a muscle. If you feel no tension in a particular muscle, do threshold tensing or simply notice the sensations that are there. You may prefer to use passive tensing on a regular basis once you are familiar with active and threshold tensing. You will find that a round of progressive relaxation using passive tension, following a round of either active or threshold tensing, can deepen your state of relaxation.

**Verbal Suggestions**

As you are releasing tension, you may also find it helpful to say to yourself one or more of the following expressions:

- **Let go of the tension.**
- **Calm and rested.**
- **Relax and smooth out the muscles.**
- **Let the tension dissolve away.**
- **Let go more and more.**
- **Deeper and deeper.**

**Basic Procedure**

Get into a comfortable position in a quiet room where you won't be disturbed. You may want to loosen your clothing and remove your shoes. Begin to relax as you take a few slow, deep breaths.... Now as you let the rest of your body relax, clench your fists and bend them back at the wrist ... tighter and tighter ... feel the tension in your fingers and forearms.... Now relax.... Feel the looseness in your hands and forearms.... Notice the contrast with the tension.... (If you have time, repeat this, and all succeeding procedures, at least one more time.) Now bend your elbows and tense your biceps.... Tense them as hard as you can and observe the feeling of tautness.... Let your hands drop down and relax.... Feel that
difference.... Turn your attention to your head and wrinkle your forehead as tight as you can.... Feel the tension in your forehead and scalp. Now relax and smooth it out. Imagine your entire forehead and scalp becoming smooth and at rest.... Now frown and notice the strain spreading throughout your forehead.... Let go. Allow your brow to become smooth again.... Squeeze your eyes closed ... tighter.... Relax your eyes. Let them remain closed gently and comfortably.... Now, open your mouth wide and feel the tension in your jaw.... Relax your jaw.... When your jaw is relaxed, your lips will be slightly parted. Notice the contrast between tension and relaxation.... Now press your tongue against the roof of your mouth. Experience the strain in the back of your mouth.... Relax.... Press your lips now, purse them into an “O” ... Relax your lips.... Feel the relaxation in your forehead, scalp, eyes, jaw, tongue, and lips.... Let go more and more....

Now roll your head slowly around on your neck, feeling the point of tension shifting as your head moves ... and then slowly roll your head the other way. Relax, allowing your head to return to a comfortable upright position.... Now shrug your shoulders, bring your shoulders up toward your ears ... hold it.... Drop your shoulders back down and feel the relaxation spreading through your neck, throat, and shoulders ... pure relaxation, deeper and deeper....

Now breathe in and fill your lungs completely. Hold your breath. Experience the tension. ... Now exhale and let your chest become loose.... Continue relaxing, letting your breath come freely and gently.... Notice the tension draining out of your muscles with each exhalation.... Next, tighten your stomach and hold. Feel the tension.... Relax.... Now place your hand on your stomach. Breathe deeply into your stomach, pushing your hand up. Hold ... and relax. Feel the sensations of relaxation as the air rushes out.... Now arch your back, without straining. Keep the rest of your body as relaxed as possible. Focus on the tension in your lower back.... Now relax.... Let the tension dissolve away.

Tighten your buttocks and thighs.... Relax and feel the difference.... Now straighten and tense your legs and curl your toes downward. Experience the tension.... Relax.... Straighten and tense your legs and bend your toes toward your face. Relax.

Feel the comfortable warmth and heaviness of deep relaxation throughout your entire body as you continue to breathe slowly and deeply.... You can relax even more as you move up through your body, letting go of the last bit of tension in your body. Relax your feet ... relax your ankles ... relax your calves ... relax your shins ... relax your knees ... relax your thighs ... relax your buttocks.... Let the relaxation spread to your stomach ... to your lower back ... to your chest.... Let go more and more. Feel the relaxation deepening in your shoulders ... in your arms ... and in your hands.... Deeper and deeper. Notice the feeling of looseness and relaxation in your neck ... your jaw ... your face ... and your scalp.... Continue to breathe slowly and deeply. Your entire body is comfortably loose and relaxed, calm and rested.

Shorthand Procedure

Once you have mastered the basic procedure, use the following procedure to relax your muscles quickly. In this procedure, whole muscle groups are simultaneously tensed and then relaxed. As before, repeat each procedure at least once, tensing each muscle
group from five to seven seconds and then relaxing from fifteen to thirty seconds. Remember to notice the contrast between the sensations of tension and relaxation.

2. Roll your head around on your neck clockwise in a complete circle, then reverse. Relax.
3. Wrinkle up the muscles of your face like a walnut: forehead wrinkled, eyes squinted, mouth opened, and shoulders hunched. Relax.
5. Straighten your legs and point your toes back toward your face, tightening your shins. Hold. Relax. Straighten your legs and curl your toes, simultaneously tightening your calves, thighs, and buttocks. Relax.

Special Considerations

1. If you make a recording of the basic procedure to facilitate your relaxation program, remember to space each procedure so that enough time is allocated to experience the tension and relaxation before going on to the next muscle or muscle group.
2. As with all relaxation techniques, regular practice of progressive relaxation will enhance the speed and depth of your relaxation.
3. Be extra cautious when tensing your neck and back, because excessive tightening can result in muscle or spinal damage. Also, overtightening your toes or feet can result in muscle cramping.
4. People new to this technique sometimes make the error of relaxing tension gradually. This slow-motion release of tension may look relaxed, but it actually requires sustained tension. When you release the tension in a particular muscle, let it go instantly; let your muscles become suddenly limp.
5. Although initially you will learn progressive relaxation in a quiet place, eventually you will be able to use at least a shortened version of it anytime during the day when you notice you are tense.

Further Reading


Participants were also given an audio CD of the guided meditation and were advised to listen to this every day during their meditation. The transcript of the guided body scan meditation is as follows (available for download from: http://www.uvm.edu/~CHWB/psych/?Page=exercises.html&SM=mindfulnessmenu.html):

“This is the body awareness exercise. For this exercise find a position. Either seated comfortably with your neck supported or lying down. You may wish to loosen any belts. Take off your glasses. And just check in and make sure that your body is comfortable. As we go through this exercise keep in mind an attitude of accepting whatever happens. Non-striving and in other words not trying to make anything happen. But just being present with what is without judging your experience. You may find throughout this exercise that your mind wanders, that your mind is filled with thoughts. And whenever that happens, gently notice that your mind has wandered and then bring your mind back to the exercise.

To begin with find your breath. Going in and out of your body. And spend a moment. Just feeling the sensation of breath. Going in going out. Feeling your abdomen. Rising and falling feeling the contact points of your body against the floor or in the chair and beginning to draw your attention to sensations within your body. The purpose of the exercise is just to notice the feelings of your body without trying to change the and without judging.

So now we will begin going through the body with gentle awareness. First draw your attention down to your right foot. If you wish. You can use your breath. Breathing in. Imagining that your breath goes down into your right foot. Bringing with it. A gentle awareness of sensation. Noticing. Whatever it is you feel. In your toes. In the sole of your foot. And your heel. You may feel nothing at all. And that's OK. You may feel tension. Warmth coldness. Whatever it is simply noticing it. With each breath. Feeling your foot. And now letting your attention. Move through your ankle and into your right. Lower leg. Noticing sensations in your calf muscles. And your shin. Just watching whatever is going on there. Using your breath to draw your attention. Trying not to actually visualize your leg. But to feel it from the inside. Letting attention move now to your right knee. And then feeling your right thigh just noticing. Sensation in your thigh muscles. If your mind is
wandering. Let the thoughts. Float away. And bring your attention. Gently back
with kindness to the feelings in your right thigh. Now shifting attention to your left
side allowing your breath to draw attention. Down to your left foot and noticing all
the sensations in your left foot in your toes. In the sole of your foot. Your heel and
arch. The top of your foot. Allowing your awareness to take in whatever's there.
Without judging it. And then allowing awareness to move to your left ankle.
Feeling any sensations of tension or relaxation. Letting awareness move to your left
leg. Feeling the muscles in your left calf. Feeling the sensations in your left knee.
Gently drawing your attention to your left thigh and now. Noticing any sensation.
And just letting it be what it is. Bringing your attention gently back to this moment.
Into sensation feeling both of your legs. And now gently moving awareness into
Into the cradle of your pelvis. Noticing any sensations of energy and attention. Just
noticing. Just holding all sensation. In your awareness. Then moving to your lower
back. Feeling any tension in the lower muscles surrounding your spine. Any pain
any achyiness is using your breath. To surround your sensation. With accepting
awareness. And then moving your Intention to your belly. Your abdomen.
Allowing your breath. To move deeply into your abdomen. Becoming aware of the
multitude of activity within your vital organs. Just breathing. Openness and space.
Into the centre of your abdomen. Noticing. Whatever you notice. And moving your
attention up to your chest area. Feeling. Your heart and your lungs. Breathing into
your chest space with great tenderness. Just feeling. Whatever sensation is being
held in that space. Allowing for whatever sensation. Whatever emotion. You
notice. As you bring awareness. To Your Heart Centre. Now bring your attention to
your upper back into the muscles of your shoulder blades. And your shoulders. The
place where we all carry tension. And notice. What you feel your shoulders.
Without trying to change it. Just welcoming sensation. Within your awareness.
Breathing gently into your shoulders. And your upper back. And now allow
awareness to move gradually down your right arm. Feeling your upper arm. Your
tricep muscles. And moving slowly through your elbow your lower right arm.
Feeling your wrist. And the back of your hand. Feeling the palm of your right hand.
Your fingers. Allowing awareness to extend right down to your fingertips. Noticing.
There may be. Tingling. There may be warmth just noticing. Whatever you feel.
Now. Bring your awareness. To your left shoulder. Allowing awareness to
gradually move down. Your left arm. The upper arm and triceps the left elbow. The left forearm. Wrist. Feel the back of your left hand. Feeling the palm of your left hand and feeling your left fingers. Allowing sensation to reach into your left fingertips. Just gently noticing. Every sensation. Without judging. Gently guiding your mind. Back to sensation. When you've noticed that it's wandered. Now move your attention up to your neck. And your throat. Feeling. The muscles in the back of your neck feeling. The tender vulnerable area of the front of your throat. Noticing if there's any sensation any tension. And letting your awareness travel. Up the back of your neck. To your scalp at the back of your head. Feeling your ears. And then moving forward to notice your jaw. Just notice. If any tension is held there. Bring attention now to your chin. And notice the sensations in your lips. Noticing the inside of your mouth. Your tongue. Your teeth. And your gums. The inside of your throat. Now bringing attention to your nose and your cheekbones. As you bring attention to your eyes. Noticing the many tiny muscles surrounding your eyes. Just noticing all the many sensations. The Feeling of your eyeballs. Inside your eyes sockets. The feeling of your eyebrows. And then bring attention to your forehead. And finally bringing attention to the crown of your head noticing any sensations of energy. At the very top of your body. Now seeing. If you can bring awareness to your entire body at once. At this point you may not feel any sensation. Or you may feel. Sensation. Can you experience the field of energy that is your body. Can you rest in your awareness of that whole energy field. If you'd like. You can imagine. That as you breathe in the breath and your body through the crown of your head and sweeps through your body. And you exhale in the breath. Exit Through your feet. And in this way. You can imagine that you're floating on a gentle wave of breath. Gently moving in and out of your body. Holding you. You continue to rest in this posture for the next few minutes. “
Appendix 2 – Mental Silence Meditation

At 4:00pm participants began a guided meditation session which was led by a qualified instructor. Participants were instructed to sit comfortably in a chair with shoes and restrictive clothing removed. A guided meditation sequence was played out aloud for 4 minutes. Following this participants were instructed on a foot soak session where they place their feet in a bucket of salt water for 15 minutes. Participants were then told to sit in silent meditation for another 15 minutes while instrumental music was played in the background. At the end of this session food was served and participants were allowed to leave by 5:00pm.

A copy of the instruction/information pamphlet is below:
Welcome to Sahaja Yoga

True Meditation is a state of deep peace which occurs when the mind is calm and silent, yet completely alert. Sahaja Yoga is used by millions of people worldwide as a practical means to achieve a balanced state and enjoy improved mental, physical, emotional and spiritual wellbeing.

The word Sahaja means “inborn” because the purifying and healing energy that is gently awakened lies waiting within every human being from birth. Sahaja Yoga is a genuine, solid experience: an actual physical reality verifiable on one’s own nervous system. It leads to spontaneous improvements in one’s interactions and relationships with the world.

The new state transcends religious, ethnic, economic and political differences and clearly manifests the oneness of mankind. Sahaja Yoga is freely available to anyone with a genuine desire to know the true self with a quest to evolve to a higher level of awareness, understanding and enjoyment.
“Within us lies the peace, the beauty, the glory of our being. We cannot seek it outside. We have to go within.”

Shri Mataji Nirmala Devi

Who is Shri Mataji?

Shri Mataji Nirmala Devi (or ‘Mother’ as she is affectionately known) founded Sahaja Yoga in 1970. Since then she has given thousands of lectures and has taught millions around the world how to practise the very simple technique.

Born in 1923 into a Christian family in India, Shri Mataji worked with Mahatma Ghandi and was very involved in India’s successful fight for independence. She is a mother and grandmother and a descendant of an Indian royal family. Her father was a member of India’s first parliament.

Shri Mataji has dedicated the past three decades of her life to travelling tirelessly to bring her technique of Sahaja Yoga to the world. She accepts no money for her time and knowledge.

Shri Mataji is married to Sir CP Shrivastava who was formerly Secretary-General of the United Nations Maritime Organization. He has received numerous honours from many countries for his exemplary work.

Shri Mataji has been nominated twice for the Nobel Peace Prize and honoured by the US Congress and the United Nations and has received numerous awards for her contribution to the peace, health and wellbeing of humankind in almost 100 countries.

Her many talks and lectures explaining the complexities of the human systems are freely available to those wishing to pursue and grow into this new awareness and enjoyment.
Basic Techniques

**Raising The Kundalini** Commence and conclude your meditations with this and the following exercise. Place the left hand in front of your lower abdomen, palm facing the body. Raise the hand up vertically, until it reaches a position above your head. While the left hand is ascending, the right hand rotates around it clockwise, until both hands are above the head. Use both hands to tie a knot. Repeat three times. The third time, tie the knot three times.

**Bandhan** Placing a bandhan gives protection to the subtle system from attention disturbances and also protects the aura. Hold the left hand out on your lap, palm upwards. Place your right hand over your left hip and slowly raise your right hand up the left side, over your head and down the right side of your body. Then raise the right hand up the right side, over your head and down the left side. This is one bandhan. Repeat seven times.

**Balancing The Left And Right Sides** For tingling, heat or heaviness on the left hand: hold the left hand out, palm upwards and place the right hand on the earth, or direct it towards the earth. For tingling heat or heaviness on the right hand: hold the right hand out, palm upwards, bend the left arm at the elbow and direct the palm towards the back.

**Foot Soaking**
This is best done last thing at night before going to bed. Sit comfortably in a chair with your hands on your lap, palms upwards.
Place your feet in a bowl of warm water with a handful of salt.
Meditate for around 10 to 15 minutes.
Rinse and dry your feet. Then flush the water down the toilet and wash your hands.
Affirmations

With your right hand on your heart, ask your Kundalini three times
"Mother, am I the spirit?"

Right hand on your upper stomach, just below the ribs, ask three times
"Mother, am I my own master?"

Right hand on your left lower stomach, just above the left hip, ask six times "Mother, please give me the pure knowledge"

Return the right hand to the upper stomach and say confidently ten times
"Mother, I am my own master"

Right hand to the heart, say confidently twelve times
"Mother, I am the spirit"

Right hand at the point where the left shoulder meets the neck. Turn the head to the right and say sixteen times
"Mother, I am not guilty"

Place your right hand across the forehead, gently grasping the temples, and say "Mother, I forgive everyone"

Right hand on the back of the head, say "Mother, for any mistakes I have done against myself, please forgive me"

Right hand on top of the head, stretch the fingers upwards, centre of the palm on the fontanel area, slowly rotate clockwise and ask seven times "Mother, please give me my self-realisation"

These affirmations will help you to attain joyful and silent meditations.
## Chakras Chart

<table>
<thead>
<tr>
<th>Chakra</th>
<th>Qualities</th>
<th>Gross expression</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Sahasrara</td>
<td>Integration  &lt;br&gt; Mental Silence</td>
<td>Limbic area</td>
<td>Cool vibrations</td>
</tr>
<tr>
<td>6. Agnya</td>
<td>Forgiveness</td>
<td>Crossing of optic chiasma (Pineal &amp; pituitary glands)</td>
<td>Conditioning, sight, hearing, thought, “I”-ness</td>
</tr>
<tr>
<td>5. Vishuddhi</td>
<td>Collectivity  &lt;br&gt; Diplomacy  &lt;br&gt; Witness state</td>
<td>Cervical plexus (thyroid)</td>
<td>Neck, arms, mouth, tongue, face</td>
</tr>
<tr>
<td>4. Heart</td>
<td>Love  &lt;br&gt; Compassion</td>
<td>Heart Cardiac plexus</td>
<td>Heartbeat Breathing</td>
</tr>
<tr>
<td>3a. Void</td>
<td>Self-mastery</td>
<td>Abdomen</td>
<td>Liver, kidney, spleen pancreas, uterus</td>
</tr>
<tr>
<td>3. Nabhi</td>
<td>Peace  &lt;br&gt; Satisfaction  &lt;br&gt; Wellbeing</td>
<td>Solar plexus</td>
<td>Stomach Liver</td>
</tr>
<tr>
<td>2. Swadisthana</td>
<td>Creativity  &lt;br&gt; Abstract thought  &lt;br&gt; Aesthetics</td>
<td>Aortic plexus</td>
<td>Liver, kidney, spleen pancreas, uterus</td>
</tr>
<tr>
<td>1a. Kundalini</td>
<td>Motherly love  &lt;br&gt; Wisdom</td>
<td>Sacrum bone</td>
<td>Parasympathetic system</td>
</tr>
<tr>
<td>1. Mooladhara</td>
<td>Innocence  &lt;br&gt; Wisdom</td>
<td>Prostate gland  &lt;br&gt; Pelvic plexus</td>
<td>Reproduction Elimination</td>
</tr>
</tbody>
</table>
A transcript of the guided meditation is below:

“To be in meditation we begin by sitting up straight and comfortably, palms upwards in our laps and we close the eyes and take the attention inside. We begin by placing the right hand on the left hip. Pressing it. And here we ask, please give me the true
knowledge about myself. Please give me the true knowledge about myself. Then take the right hand, up under the rib cage on the right hand side, pressing it gently and here we affirm, I am my own master, I am my own master. Now we take the right hand up to the heart on the left hand side. And here we are affirm, I am the pure spirit. I am. The pure spirit. Now take a right hand up to the shoulder and neck on the left hand side reaching across. And here we affirm, I am not guilty. I am not guilty. At all. I am not guilty. I am not guilty. At all. Now take the right hand up to the forehead. Holding both the temples and here we say I forgive. I forgive everyone for everything including myself. I forgive. I forgive. Everyone. And I forgive myself. Finally we take our right hand. Raise it up as an expression of a pure desire. We place the middle of the palm of the hand. Right on the top of the head. On the fontanel or flow zone area. Here we pressed down gently. Rotating the scalp over the top of the head. Here we say, please give me the experience of mental silence and deep stillness. Please give me the experience of mental silence and deep stillness. The expression of our pure desire enables this pure energy to flow up and out the top of the head. And we just allow our attention to dissolve into the flow in silence. In meditation. In deep stillness.”
Appendix 3 – Participant Survey Form & Diaries

A participant survey form was handed out at the start of the first session and again at the end of the final session. A diary was also required to be kept by all participants. These were collected at the start of each session as a marker of attendance and compliance to the meditation schedule they were asked to follow. Both the diary and survey were given to all participants regardless of the group allocated (mental silence or body scan meditation).
<table>
<thead>
<tr>
<th>Name:</th>
<th>Date:</th>
<th>1 Mon</th>
<th>2 Tues</th>
<th>3 Wed</th>
<th>4 Thurs</th>
<th>5 Fri</th>
<th>6 Sat</th>
<th>7 Sun</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall level of mental silence during MORNING meditation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(tick box or NA=didn’t meditate)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-No thinking at all- Completely silent inside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Much less thinking than usual- In occasional thoughts, separated by longer spaces of silence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Moderately less thinking than usual- In a few thoughts, separated by brief spaces of silence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Somewhat less thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Slightly less thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-Usual rate of thinking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1-Slightly more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2-Somewhat more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3-Moderately more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4-Much more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5-More thinking than ever before</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Overall level of mental silence during EVENING meditation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(tick box or NA=didn’t meditate)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-No thinking at all- Completely silent inside</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-Much less thinking than usual- In occasional thoughts, separated by longer spaces of silence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Moderately less thinking than usual- In a few thoughts, separated by brief spaces of silence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Somewhat less thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Slightly less thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-Usual rate of thinking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1-Slightly more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2-Somewhat more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3-Moderately more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4-Much more thinking than usual</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5-More thinking than ever before</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Did you footsoak today?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Your overall mood today</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Very positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. Somewhat positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. Mildly positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. Neither positive nor negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. Mildly negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. Somewhat negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. Very negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Optional Contact Sheet

Please provide your contact information if you are willing to help us by completing this survey packet again in 4 weeks. As a token of appreciation for your effort, we will send you a report detailing your scores before and after the course.

Name

Email

Mailing address

City, state and postal code

Landline phone

Mobile phone
In what year were you born? (enter 4-digit birth year; for example, 1976)

What is the highest level of school you have completed or the highest degree you have received?
- Less than high school degree
- High school degree or equivalent
- Some college but no degree
- Associate degree
- Bachelor degree
- Graduate degree

What is your current profession or occupation?

Are you male or female?
- Male
- Female

What is your relationship status?
- Single
- In a long-term relationship (i.e. together more than a year)
- Other
Please indicate your level of agreement or disagreement.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Strongly disagree</th>
<th>Disagree</th>
<th>Neutral</th>
<th>Agree</th>
<th>Strongly agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>My attention is often focused on aspects of myself I wish I would stop thinking about</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I often seem to be &quot;re-hashing&quot; in mind recent things I have said or done</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Sometimes it is hard for me to shut off thoughts about myself</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Long after an argument or disagreement is over with, my thoughts keep going back to what happened</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I tend to &quot;ruminant&quot; or dwell over things that happen to me for a really long time afterward</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I don't waste time re-thinking things that are over and done with</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Often I'm playing back over in my mind how I acted in a past situation</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I often find myself re-evaluating something I've done</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I never ruminate or dwell on myself for very long</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>It is easy for me to put unwanted thoughts out of my mind</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I often reflect on episodes in my life that I should no longer concern myself with</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I spend a great deal of time thinking back over my embarrassing or disappointing moments</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Philosophical or abstract thinking doesn't appeal to me that much</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I am not really a meditative type of person</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I love exploring my &quot;inner&quot; self</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>My attitudes and feelings about things fascinate me</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I don't really care for introspective or self-reflective thinking</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I love analyzing why I do things</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>People often say I'm a &quot;deep&quot;, introspective type of person</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I don't care much for self-analysis</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I'm very self-inquisitive by nature</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I love to meditate on the nature and meaning of things</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>I often love to look at my life in philosophical ways</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Contemplating myself isn't my idea of fun</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
The following questions relate to your usual sleep habits during the past month ONLY. Your answers should indicate the most accurate reply for the majority of days and nights in the past month.

- During the past month, when have you usually gone to bed at night?

- During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

- During the past month, when have you usually gotten up in the morning?

- During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spend in bed.)

During the past month, how often have you had trouble sleeping because you

<table>
<thead>
<tr>
<th>Condition</th>
<th>Not during the past month</th>
<th>Less than once a week</th>
<th>Once or twice a week</th>
<th>Three or more times a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>. cannot get to sleep within 30 minutes</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. wake up in the middle of the night or early morning</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. have to get up to use the bathroom</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. cannot breathe comfortably</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. cough or snore loudly</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. feel too cold</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. feel too hot</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. had bad dreams</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>. have pain</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

- During the past month, how often have you had trouble sleeping because of some other reason?  
Please describe the reason

- How often during the past month have you had trouble sleeping because of this?  
○ Not during the past month  
○ Less than once a week  
○ Once or twice a week  
○ Three or more times a week
Please read each statement and circle a number 0, 1, 2 or 3 to indicate how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of the time
3 Applied to me very much, or most of the time

<table>
<thead>
<tr>
<th>Statement</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>I found it hard to wind down</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I was aware of dryness of my mouth</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I couldn't seem to experience any positive feeling at all</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I experienced breathing difficulty (eg, excessively rapid breathing,</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>breathlessness in the absence of physical exertion)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I found it difficult to work up the initiative to do things</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I tended to over-react to situations</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I experienced trembling (eg, in the hands)</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt that I was using a lot of nervous energy</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I was worried about situations in which I might panic and make a fool of</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>myself</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt that I had nothing to look forward to</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I found myself getting agitated</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I found it difficult to relax</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt down-hearted and blue</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I was intolerant of anything that kept me from getting on with what I was</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>doing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I felt I was close to panic</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I was unable to become enthusiastic about anything</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt I wasn't worth much as a person</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt that I was rather touchy</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt scared without any good reason</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>I felt that life was meaningless</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>
. During the past month, how would you rate your sleep quality overall?
   O Very good
   O Fairly good
   O Fairly bad
   O Very bad

   During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?
   O Not during the past month
   O Less than once a week
   O Once or twice a week
   O Three or more times a week

   During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?
   O Not during the past month
   O Less than once a week
   O Once or twice a week
   O Three or more times a week

   During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?
   O No problem at all
   O Only a very slight problem
   O Somewhat of a problem
   O A very big problem
The next set of questions are concerned with complete mental silence. To approach mental silence is to reduce mental content including re-presentations of sensory input, such as imagination. Complete mental silence (or "thoughtless awareness") is an experience in which one is no longer thinking and no longer feels the urge to continuously engage in thought. The experience occurs in a normal, lucid waking state. Thoughtless awareness is not sleep or unconsciousness. The conditions of mental silence do not exclude everything. Physical movement, sensory input, and affective states are permitted.

. How familiar are you with the notion of complete mental silence?
   ○ This is the first time I have thought about it.
   ○ The notion has crossed my mind, but I'm not sure what it means to me.
   ○ I have discussed it with friends.
   ○ I have read something about it.
   ○ I study the topic with interest.

. During the last few months, how often have you experienced complete mental silence?
   ○ Daily
   ○ Weekly
   ○ Infrequently
   ○ I haven't experienced complete mental silence.

. At some time in my life, I have experienced complete mental silence.
   ○ Agree
   ○ Agree somewhat
   ○ Not sure
   ○ Disagree somewhat
   ○ Disagree

. I believe that complete mental silence is possible for everybody.
   ○ Agree
   ○ Agree somewhat
   ○ Not sure
   ○ Disagree somewhat
   ○ Disagree

. If you do not know how to intentionally cause yourself to experience complete mental silence, do you want to learn?
   ○ No
   ○ Not sure
   ○ Yes, if it was easy to learn
   ○ Yes, I am moderately curious
   ○ Yes, I am keenly curious
   ○ I know how to cause myself to experience complete mental silence.
If you experience complete mental silence every day, skip this page and turn to the next page. Otherwise, please answer these questions.

Please indicate your agreement or disagreement with the following statements.

<table>
<thead>
<tr>
<th>Statement</th>
<th>Agree</th>
<th>Agree somewhat</th>
<th>Not sure</th>
<th>Disagree somewhat</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>It takes too much effort for me to experience complete mental silence.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I won’t feel emotionally prepared to experience complete mental silence, not currently and probably not within the next 3 months.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Descartes said, “I think therefore I am.” This is true for me.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I am afraid of what will happen if I stop thinking.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I never slow down long enough to experience complete mental silence.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>My life circumstances prevent me from experiencing complete mental silence, currently and probably during the next 3 months.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>If I experience complete mental silence then I may lose an important aspect of who I am.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
If you have never experienced mental silence, skip this page and turn to the next page. Otherwise, please answer these questions.

Of interest are your recent experiences of complete mental silence (within the last month or so).

.. How often do you try to intentionally cause yourself to experience complete mental silence?
   - Daily
   - Weekly
   - Infrequently
   - I don't try to cause myself to experience complete mental silence

.. Of the times you have tried to experience complete mental silence, about what percentage of the time are you successful?

.. Approximately how much actual time did your longest experience of complete mental silence last?
   - A moment (e.g., a second or shorter)
   - Longer than a moment but shorter than 10 seconds
   - Between 10 seconds and 1 minute
   - Between 1 minute and 10 minutes
   - More than 10 minutes

Please indicate your agreement or disagreement with the following statements.

<table>
<thead>
<tr>
<th>.. I intentionally yearn for the experience of complete mental silence.</th>
<th>Agree</th>
<th>Agree somewhat</th>
<th>Not sure</th>
<th>Disagree somewhat</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>.. I have met, in person, someone who is convincingly familiar with complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I intentionally create favorable conditions to allow myself to experience complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I can intentionally allow myself to experience complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I can intentionally cause myself to experience complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I have experienced complete mental silence with others in a shared context.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I am able to teach other people how to experience complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>.. I know how to train teachers who can teach people how to experience complete mental silence.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Participants Compliance Diary & Attendance

## CORE Flow State Scale (C FSS)

Please answer the following questions in relation to your experience in the event or activity you have just completed. These questions relate to the thoughts and feelings you may have experienced while taking part. There are no right or wrong answers. Think about how you felt during the event/activity, then answer the questions using the rating scale below. For each question, circle the number that best matches your experience.

<table>
<thead>
<tr>
<th>During the event of (name event):</th>
<th>Strongly Disagree</th>
<th>Disagree</th>
<th>Neither Agree nor Disagree</th>
<th>Agree</th>
<th>Strongly Agree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 I was ‘totally involved’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>2 It felt like ‘everything clicked’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>3 I was ‘tuned in’ to what I was doing</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>4 I was ‘in the zone’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5 I felt ‘in control’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6 I was ‘switched on’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7 It felt like I was ‘in the flow’ of things</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>8 It felt like ‘nothing else mattered’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>9 I was ‘in the groove’</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10 I was ‘totally focused’ on what I was doing</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Please put a mark on the line below to indicate your mood (how you feel) right now.

Your **WORST** mood ever

Your **BEST** mood ever
**Instructions:** We are interested in what you just experienced. Below is a list of things that people sometimes experience. Please read each statement. Next to each statement are five choices: “not at all,” “a little,” “moderately,” “quite a bit,” and “very much.” Please indicate the extent to which you agree with each statement. In other words, how well does the statement describe what you just experienced, just now?

<table>
<thead>
<tr>
<th></th>
<th>Not at all</th>
<th>A little</th>
<th>moderately</th>
<th>Quite a bit</th>
<th>Very much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. I experienced myself as separate from my changing thoughts and feelings.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>2. I was more concerned with being open to my experiences than controlling or changing them.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3. I was curious about what I might learn about myself by taking notice of how I react to certain thoughts, feelings or sensations.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4. I experienced my thoughts more as events in my mind than as a necessarily accurate reflection of the way things ‘really’ are.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>5. I was curious to see what my mind was up to from moment to moment.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>6. I was curious about each of the thoughts and feelings that I was having.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>7. I was receptive to observing unpleasant thoughts and feelings without interfering with them.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>8. I was more invested in just watching my experiences as they arose, than in figuring out what they could mean.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>9. I approached each experience by trying to accept it, no matter whether it was pleasant or unpleasant.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>10. I remained curious about the nature of each experience as it arose.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>11. I was aware of my thoughts and feelings without overidentifying with them.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>12. I was curious about my reactions to things.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>13. I was curious about what I might learn about myself by just taking notice of what my attention gets drawn to.</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>