

CAFFEINE'S CONTRIBUTION TO FMRI DYNAMICS DURING REST AND MENTAL STRESS

by

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DURING REST AND MENTAL STRESS**

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ACADEMIC ETHICS AND INTEGRITY STATEMENT

I, Cem Karakuzu, hereby certify that I am aware of the Academic Ethics and Integrity Policy issued by the Council of Higher Education (YÖK) and I fully acknowledge all the consequences due to its violation by plagiarism or any other way.

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ABSTRACT

CAFFEINE’S CONTRIBUTION TO FMRI DYNAMICS DURING REST AND MENTAL STRESS

Caffeine is an adenosine receptor antagonist with known effects on arousal and cerebrovascular tone. Yet its rapid, systemic impact on fMRI signals and their coupling to physiology remains understudied. We examined how caffeine modulates BOLD responses and systemic physiology across two intake delays (10 vs. 30 minutes; Caff-10, Caff-30), and two different scanning conditions, arithmetic equation solving and resting-state. GLM analysis revealed robust activation in task-positive networks. Especially, the IPS, one of the active regions, is crucial in number processing. Relative to Caff-10, Caff-30 showed modestly greater activation in IPS and insula, alongside patterns near sulcal territories, suggesting a vascular contribution on task responses. Also, we conducted ICA, dual regression, and voxel-wise cross-correlations between BOLD-RVT, and BOLD-PPG-Amp. Resting-state ICA prioritized a WM/ventricular pattern, whereas the analogous component emerged later during task, implying stronger variance attributable to systemic processes at rest. Dual regression did not yield significant results, which is reflecting limited power and network overlap. However, cross-correlation analyses draw a consistent picture. Caffeine lengthened and broadened physiology-BOLD coupling. In rest, Caff-30 exhibited earlier negative and prolonged positive RVT-BOLD correlations. For PPG-Amp, spatial expression shifted by caffeine from more localized VIS effects (Caff-10) to widespread WM-CSF patterns (Caff-30). Together, these results indicate that on the timescale of minutes, caffeine reshapes both the temporal and spatial dimensions of systemic influences on BOLD, while modestly enhancing task-relevant activations. Recent caffeine intake can affect physiological variance and alter network engagement. Therefore, researchers must measure and control physiological signals when interpreting fMRI results.

Keywords: fMRI, Mental Stress, Caffeine, Resting State Networks.

ÖZET

DİNLENME İLE ZİHİNSEL STRES SIRASINDA KAFEİNİN fMRG DİNAMİKLERİNE KATKISI

Kafein, uyarılma ve serebrovasküler ton üzerinde etkileri olan bir adenozin reseptör antagonistidir. Ancak fMRG sinyalleri üzerindeki sistemik etkisi ve fizyolojiyle bağlantısı yeterince incelenmemiştir. Kafeinin alımdan sonraki iki zamanda (10 ve 30 dakika; Kafein-10, Kafein-30) ve aritmetik denklem çözme ile dinlenme durumu boyunca BOLD yanıtlarını ve sistemik fizyolojiyi nasıl mod üle ettiğini inceledik. GLM analizi, görev pozitif ağlarda güçlü bir aktivasyon ortaya koydu. Özellikle aktif olan bölgelerden IPS, sayı işlemede çok önemlidir. Kafein-10 ile karşılaştırıldığında, Kafein-30 IPS ve insulada orta derecede daha fazla aktivasyon gösterdi. Ayrıca sulkal bölgelerdeki örüntüler, görev yanıtlarında vasküler katkıya işaret etmektedir. Sistemik etkileri araştırmak için BBA (Bağımsız Bileşen Analizi) ve ikili regresyon gerçekleştirdik. Ayrıca, BOLD-ZSH (zamana göre solunum hacmi) ve BOLD-PPG-Amp arasındaki voksel bazlı çapraz korelasyonlar da araştırıldı. Dinlenmedeki BBA, ak madde/ventriküler örüntüyü önceliklendirirken, görev sırasında benzer bileşen daha geç sırada gözüktü ve bu da dinlenme halindeki sistemik süreçlere atfedilebilecek daha güçlü bir varyansa işaret etti. İkili regresyon anlamlı sonuçlar vermese de çapraz korelasyon analizleri tutarlı bir tablo çizmiştir. Kafein, fizyoloji-BOLD eşleşmesini uzatmış ve genişletmiştir. Dinlenmede, Kafein-30 daha erken negatif ve uzun süreli pozitif ZSH-BOLD korelasyonları sergiledi. PPG-Amp için, kafeinle mekansal ifade daha lokalize görsel ağ etkilerinden (Kafein-10) yaygın ak madde-BOS örüntülerine (Kafein-30) kaydı. Bu sonuçlar, kafeinin BOLD üzerindeki sistemik etkileri yeniden şekillendirdiğini ve görevle ilgili aktivasyonları sınırlı düzeyde artırdığını göstermektedir. Kafein, fizyolojik varyansı etkileyebilir ve ağ etkileşimini değiştirebilir. Bu nedenle, fMRG sonuçları yorumlanırken fizyolojik sinyaller ölçülmeli ve kontrol edilmedilidir.

Anahtar Sözcükler: fMRG, Zihinsel Stres, Kafein, Dinlenme Durumu Ağları.

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LIST OF SYMBOLS

β	Beta
ϵ	Residual error



LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
BOLD	Blood-oxygen-level-dependent
CBF	Cerebral Blood Flow
CEN	Central Executive Network
CMRO ₂	Cerebral Metabolic Rate of Oxygen
CSF	Cerebrospinal Fluid
DAN	Dorsal Attention Network
DMN	Default Mode Network
EPI	Echo Planar Imaging
FC	Functional Connectivity
fMRI	Functional Magnetic Resonance Imaging
FPN	Frontoparietal Network
GE	Gradient Echo
GLM	General Linear Model
GM	Gray Matter
GRE	Gradient Recalled Echo
HbO	Oxygenated Hemoglobin
HbR	Deoxygenated Hemoglobin
HPA	Hypothalamic-pituitary-adrenal
HRF	Hemodynamic Response Function
ICA	Independent Component Analysis
IPS	Intraparietal Sulcus
MRI	Magnetic Resonance Imaging
PCA	Principal Component Analysis
PPG	Photoplethysmography
PPG-Amp	Photoplethysmography Amplitude
RF	Radiofrequency
rs-fMRI	Resting-State fMRI

RSNs	Resting State Networks
RVT	Respiratory Volume per Time
SAM	Sympathetic Adrenal Medullary
SE	Spin Echo
SMN	Sensorimotor/Somatomor Network
SN	Salience Network
task-fMRI	Task-Based fMRI
TE	Echo Time
TR	Repetition Time
TNN	Task Negative Network
TPN	Task Positive Network
VAN	Ventral Attention Network
VIS	Visual Network
WM	White Matter

1. INTRODUCTION

Historically, the brain and body have been perceived differently depending on the spirit of the time. Because they were skilled at water technology, the Greeks viewed the body as a water clock. Hippocrates said that the body consisted of main humors that must be balanced, and any imbalance resulted in a disease. Aristotle thought that the brain was the cooler of the body. Galen, a Roman physiologist, said that the four ventricles of the brain might be the center for personality and the ability to think [3]. Along with the development of technology, both understanding of how brain parts work and what the brain is like have changed over the years. So, the analogies that people make for the brain have been dependent on what the most complex device was at that time.

In the modern world, after the invention of steam engines, people deemed that the human body was a hydraulic machine as blood was sent to perform movement, and likewise brain worked in this principle. The telegraph machine and telephones created a view that there were wires and cables inside the brain. It was responsible for sending the right signals to the right places, like a dispatcher [4]. When automata were on the scene, scientists tried to solve the algorithms that the brain used. Lastly, the invention of transistors made it one step closer to now, and researchers say that the brain is a computational machine. In recent years, with the rise of large language models, we now see the human mind as a natural artificial intelligence. Although all analogies are lacking, it must be said that they always increase our understanding of how the brain processes. Besides, sometimes the knowledge about the human brain inspired scientists to develop new pioneering contributions, such as neural networks.

After the Second World War, digital computers became the new superstars. Also, the terminology used in computers, such as memory, information, and storage, increased the validity of this metaphor. Even though computers are the closest analogy of the human brain, the internet has made it closer. With the development of the

internet, the network approach has been widespread. Also, more powerful and capable computers, as well as a more interconnected world by the internet, increased the studies on information theory [5]. With the rise in information theory, scientists started to see neurons as binary coders, besides our theoretical knowledge about how they work, such as action potential and its all-or-none law, supporting this view. Recent big projects like Human Brain Connectome and Blue Brain were funded, and much information is still being gathered. Today, neuroimaging with sophisticated tools, and studies with large samples bring about the picture of a connected brain. Previously, the brain used to be investigated as separate centers, regions, and locations. However, scientists now approach it like connected hubs, structurally and functionally related locations. In addition, mathematical models like graph theory are also utilized to find out connections between the regions and make a resemblance with edges and nodes. This connectionism view exploits many interdisciplinary studies from biology to engineering [6].

Of course, none of them would have been possible if the technological advancement in brain imaging had not leaped in the last 100 years. Opening a skull and inspecting the brain itself was not possible and still is not, except in medical surgeries. In fact, even brain surgeries are too limited and the scientific gain from those practices is not much. Fortunately, advancements in technology help us to derive new theories about and insights into how the human brain works [7]. Another recent breakthrough is the development of functional magnetic resonance imaging (fMRI). fMRI can measure the variations in blood oxygen level, which is named the blood-oxygen-level-dependent (BOLD) signal. Because brain activity causes metabolic activity, oxygenated blood flow increases and affects this ratio; hence, fMRI can capture these changes. Human behavior and mental functions are based on separate but interconnected brain regions, which are termed brain networks. fMRI helps us to pinpoint those networks and the relationship between them during a certain task or while resting. In medical treatment, diagnosing, or research, finding the most engaged area or network for a mental function is highly important, and fMRI is the most valuable tool for this aim in present [8]. Nonetheless, the capability of neuroimaging caused neglect. Such was the power of monitoring the brain in isolation that researchers forgot the body. They disregard

the relationship between them. In fact, stating a relationship between them creates a dualism, and this point of view results in wrongful conceptions in interpreting fMRI studies. Thus, one of the scopes of this thesis is to raise awareness of it.

In fMRI and brain network research, I believe that the essence is summarized by a quote that comes from a quite distant area of study. Waldo R. Tobler, a famous geographer, said that the first law of geography is

”Everything is related to everything else, but near things are more related than distant things [9].”

When we consider the organizational structure of the brain, the working principle is similar. Neurons spend energy to execute a task, and nourishment must be provided to the cells. Therefore, supplying the nutrition in one area is economically more efficient, and we observe this evolutionary development in the brain regions. This localization and compartmentalization are defined in the studies, and we can show functionally related areas such as language in Broca, planning in the prefrontal cortex, movement in the motor cortex, or vision in the occipital cortex.

However, the most complex known information processing system in the universe is far from this simplicity. Neuroimaging studies show that the human brain includes functional brain networks that present neural activation at the same time regardless of the anatomical connectedness. A network system in the brain diminishes the power of the localization view. Nevertheless, the first law of geography can still be a reasonable analogy, which I will explain.

1.1 MRI

Magnetic Resonance Imaging (MRI) is one of the greatest inventions of science in history. Although its invention and underlying principles require writing a book about it, I am going to explain its basics.

Logothetis (2008) neatly summarized the MRI and fMRI in his review [10]. In quantum mechanics, nuclei have spins that might be considered as turning magnets. Those spins randomly point in different ways without an external influence. The MR machine, basically a powerful electromagnet, creates a magnetic field and aligns all spins in the same direction, i.e. magnetization vector. A radiofrequency (RF) pulse is sent by the MR machine, as synchronized with the spins' precession (Larmor frequency), the magnetization vector rotates from the z direction to the transverse plane. This transverse magnetization decays quickly during relaxation, and there are different types of relaxation periods as T_1 , T_2 , and T_2^* . The image is produced according to these relaxation processes. Even though other molecules might be used, usually, hydrogen is used due to the abundance of water in the body.

A contrast mechanism is required to differentiate parts of the brain, such as gray matter (GM), white matter (WM), or cerebrospinal fluid (CSF). These contrasts are calculated for each voxel (volumetric pixel), which is 3D counterpart of the pixel. The source of contrast can be water diffusion, spin density, or relaxation times. MR scientists arrange those parameters according to the needs in the imaging. The most calibrated parameter is the relaxation time in this process. By changing the weights of relaxation times, diverse tissue types can be separated. For instance, the T_1 image uses the difference in T_1 relaxation time to separate anatomical regions of gray and white matter. In fMRI, the functional differences are acquired by T_2^* relaxation time, which is used to measure blood oxygenation disparity.

Another essential property of MRI is spatial localization. Applied small magnetic field gradients on the homogeneous magnetic field created by the scanner, using the Larmor relationship. Protons in different positions along a gradient resonate at different frequencies; therefore, their spatial localization can be encoded.

The interval between the RF excitation pulse and the recovered signal is named echo time (TE). TE and the repetition time (TR) determine the image contrast and quality. The pulse sequence is specified by the types of RF and gradient pulses to produce an MR image. The most common pulse sequences are gradient-recalled echo

(GRE) and spin echo (SE).

As mentioned previously, the produced MR image depends on the parameters. Pulse sequences that have longer TR than T1 and shorter TE than T2 are utilized for spin density images. Anatomical imaging is collected by sequences whose TR is less than or equal to T1 and TE is shorter than T2. In functional imaging, TRs that are much longer than T1 and TE that are equal to or larger than T2 and T2* are used to generate T2 and T2* weighted images. The MR images can be obtained with multiple RF excitations or with echo planar imaging (EPI), in which a single RF pulse is followed. The most used EPI methods are spin-echo (SE-EPI) and gradient-echo (GE-EPI). They diverge in how they refocus spins and can be set to produce T2 and T2* weighted images in functional imaging.

1.2 Neurovascular Coupling and fMRI

The heart of fMRI lies in neurovascular coupling and the very first law of geography by Waldo Tobler. Let us start with geography: If the temperature is high at a point on a map, a similar temperature is measured near this area. If you move away from that point, it is more likely to encounter a different temperature. In the brain, if a neuron creates an action potential, it is more likely to find a group of neurons sending signals near that neuron.

However, for example, if you find a tree in an area, the likelihood of finding another tree near that area is higher, but it still depends on the water resources. If there is a limited water resource, the likelihood decreases, but if there is enough water resource, it may increase. So, the spatial closeness is also moderated by where the water is. Therefore, the same species of plant might be found along a river. Also, if the river expands, the plants might produce as follows along the riverside.

Water is blood, rivers are vessels, plants are neurons. The same relationship is observed in the human brain. A neuron fires; it consumes energy. After the action

potential, the sodium-potassium pumps must be restored, and this process spends ATP. Since there is not enough room to store energy inside a neuron, a continuous oxygen and glucose supply must be provided by blood. This constant supply system is called neurovascular coupling. Also, the reaction of the vascular system is named hemodynamic response, and this response links neuronal activity to vascular reaction in three parameters: blood flow, blood volume, and oxygenation.

After a neuron is activated, it releases neurotransmitters into the synaptic cleft. Those released neurotransmitters bind to receptors on the postsynaptic neuron and increase the potential. Also, astrocytes, supporting cells for neurons, respond to neurotransmitters, and they release vasoactive substances that are able to dilate or constrict local blood vessels. Most of the time, vasodilation occurs with neural activation. Due to the vasodilation, cerebral blood flow (CBF) rises and fulfills, even exceeds, the oxygen demand. Even though the oxygen consumption, i.e., $CMRO_2$ (cerebral metabolic rate of oxygen), increases, the rise of CBF is much larger than it. Hence, the proportion of oxygenated hemoglobin (HbO) in venous blood goes up when compared to deoxygenated hemoglobin (HbR).

The aforementioned MRI working principles point out that a change in the magnetic field is used for imaging. Indeed, fMRI aims to catch this difference in HbO/HbR ratio. HbR is paramagnetic, but HbO is diamagnetic. Thus, the change in HbO/HbR ratio alters the local magnetic field homogeneity. Decrease in HbR causes less magnetic inhomogeneity, therefore, there is slower $T2^*$ decay and higher signal intensity with HbO increment. As a result, the BOLD effect is measured by fMRI, and this gives us information about the metabolic activity in the brain in vivo.

The middle steps from the neural activity to BOLD fMRI signal change; the neurovascular coupling, dilation or constriction of blood vessels, and change in CBF and oxygenation form hemodynamic response function. When looking at the hemodynamic response after a stimulus, the initial dip is observed in first two seconds. Because oxygen consumption increases before the rise of CBF, there is a little drop in signal. Then, in 4-6 seconds, overflow of oxygenated blood increases the signal. Lastly, in 12-20 seconds,

signal decreases a bit more before returning to baseline.

1.3 Functional Connectivity

Functional connectivity (FC) is the statistical relationship between activation patterns in different regions. This relationship is often measured as temporal correlation and shows how much these regions are communicating or activating together in time. fMRI is one of the main tools to measure these connections in the brain during rest or task [11]. When FC is measured while the subject is resting to obtain the brain's inherent BOLD fluctuations and connections without a specific task, this connectivity is referred to as intrinsic connectivity. Also, the scan is known as resting-state fMRI (rs-fMRI). Naturally, structurally connected regions have higher correlations, but those pathways are neither necessary nor sufficient when measuring FC at any random time [12].

FC is used in both clinical and research studies. For example, recent studies mentioned that people with particular brain diseases, such as Alzheimer's, show different connectivity integrity in FCs [13]. Understanding FC illuminates how the brain processes information, besides captures individual differences. It also helps us to show the effects of several intervention techniques, such as psychotherapy and neurofeedback, on the brain [14],[15]. Inversely, the resting state connectivity can predict the response to a treatment and determine its possible effectiveness on the patient [16].

After the discovery of resting state networks with spontaneous low-frequency BOLD signals in the sensorimotor system [17], researchers started to investigate these signals across the regions. Following years, a group of researchers acquired large samples and summarized the resting state networks in 7 main large-scale networks [18]. This atlas is still the most used one in FC and resting-state studies.

The Default Mode Network (DMN) is the foremost in large-scale networks. It consists of the medial prefrontal cortex, posterior cingulate cortex, precuneus, and

angular gyrus. It is active when people are not engaging in a specific task. Indeed, its connectivity is found to be higher when people are resting and mind-wandering. It is a task-negative network (TNN), because the connectivity decreases when people focus and give attention [19].

Frontoparietal / Central Executive Network (FPN/CEN) includes the lateral prefrontal cortex and posterior prefrontal cortex. Unlike DMN, it is a task-positive network (TPN), and its connectivity is higher when focusing on a task or problem-solving [20].

The Dorsal Attention Network (DAN) contains the intraparietal sulcus (IPS) and frontal eye fields. It is evidently active, while visual attention must be directed from one location to another [21]. Importantly, IPS has a key role in the sense of numbers and shows activation in any kind of number task [22]. In a case study, a deficiency in numerical tasks and arithmetic problem-solving was observed after the infarction in the left IPS [23]. Therefore, we especially focused on IPS in our study.

Ventral Attention / Salience Network (VAN/SN) mainly consists of the anterior insula and dorsal anterior cingulate cortex (ACC). It works like an allocator of attention. In many stimuli presentations, it detects which stimuli need a focus and orients our attention to it. Therefore, it is a switch between DMN and FPN [24]. Its dysfunction is related to cognitive decline in aging, and it is a suggested predictor of cognitive impairment [25].

The sensorimotor / Somatomotor Network (SMN) has precentral and postcentral gyri. It is responsible for the execution of voluntary movement and somatosensation. It manages the interaction between the supplementary and primary motor cortices [26].

Visual Network (VIS) comprises the occipital cortex. It is active during primary and higher visual processing. In addition, studies show that it is not only active in visual processing but also in mental imagery [27, 28]

The Limbic Network includes the orbitofrontal and anterior temporal cortex. Although its activation is observed during affective events and memory processing, a recent study suggested that it should have been considered as part of the extended DMN rather than a distinct network [29].

Resting state networks (RSNs) can be investigated with different methods in fMRI. Seed-based and voxel-wise correlations are the most common methods. However, in FC analysis, the outcomes can be interpreted with a flaw. Researchers want to draw inferences about how the brain works, how cognitive abilities emerge, and how people behave. In brain research, the connectivity in the brain is calculated by estimating interactions between measured signals. However, the results of those statistical techniques may be seen as mechanistic explanations of how the brain regions interact with each other, what is a consequence of an activation or inhibition in a particular area, or how behaviors are the result of activations in the brain. However, these statistical methods are useful to measure the signals that we are able to measure, and they have a low dimensionality, unlike real brain activity, which is high-dimensional. There are myriad missed variables, and their impact is higher than the captured ones. Also, the derived correlations can be consequences of any causal model regarding unknown variables. Hence, the correlations, especially when the structure is not well measured by all terms, cannot make for an explanation of the underlying working principle [30]. Therefore, implying causality from FC may be highly misleading. Also, some researchers stated that the term FC also augments what it implies in reality. Therefore, abandoning the term FC and instead using correlation networks can be seen in the papers [31]. On the other hand, methods like dynamic causal modelling try to establish a causal link and to show effective connectivity [32, 33].

1.4 fMRI and Physiology

fMRI measures the hemodynamic changes in the brain, i.e., metabolic activity; thus, changes in blood flow and pressure affect this measurement. There are two sources for this measurement, direct and indirect. Directly, neurons consume energy

for their electrochemical activity, the action potential. Any mental activity, regardless of its complexity, completes the process through neural activation. Thus, fMRI can detect the change in oxygenated blood flow, and this metabolic activity infers the neural activation in the measured region. In the fMRI experiments, baseline levels are compared with BOLD levels during task activation, and this difference can help us determine how this metabolic activity changes and which regions consume more energy. However, fMRI does not show that this activity is neuronal activation or inhibition, because BOLD signals only detect metabolic activity, and cannot differentiate between excitatory or inhibitory activation of a neuron.

fMRI is indirectly influenced by physiological changes because any factor that alters blood flow or oxygenation will affect the BOLD signal. Physiological processes influence the BOLD signal through both high-frequency and low-frequency fluctuations. High-frequency effects include cardiac pulsatility (~ 1 Hz) and respiratory motion (~ 0.3 Hz). Cardiac pulsatility produces quasiperiodic signal fluctuations tied to the heart-beat, often localized near ventricles, sulci, and large vessels, where vascular and tissue motion occur. Respiratory motion introduces similar artifacts, caused by chest and diaphragm movements that distort the magnetic field. If left uncorrected, these artifacts can contaminate both rs-fMRI and task-based fMRI (task-fMRI).

Many algorithms are developed by scientists to determine and remove physiological noise in fMRI scans. So far, the consensus is that directly monitoring physiological signals, for example, cardiac activity via photoplethysmography (PPG), respiration via pneumatic belts, provides the most reliable way to correct these effects, since it allows explicit modeling of confounds rather than relying only on statistical separation. Among such model-based approaches, Retrospective Image Correction (RETROICOR) is the most widely used method for reducing high-frequency physiological artifacts [34]. RETROICOR models cardiac and respiratory phase information with low-order Fourier series, effectively capturing the quasiperiodic fluctuations of cardiac pulsatility and respiration. Importantly, it can correct aliased artifacts even in relatively slow fMRI acquisitions and does so without applying spatial filtering, which makes it particularly suitable as an initial preprocessing step.

Low-frequency effects come from slower physiological fluctuations such as respiration depth and rate (~ 0.03 - 0.1 Hz), heart rate (~ 0.1 Hz), and photoplethysmography amplitude (PPG-Amp). Variations in respiration volume per time (RVT) change arterial CO_2 levels, leading to widespread vasodilation and delayed BOLD fluctuations that resemble neuronal signals. The relationship between RVT and the BOLD signal is well-modeled by a biphasic curve with a predominantly negative deflection [35],[36]. Increases in respiration depth or rate typically produce MR signal decreases, with these changes occurring after a delay compared to neuronal BOLD responses. PPG-Amp variations, linked to peripheral vascular tone and sympathetic arousal, strongly influence both GM and WM BOLD activity and are closely tied to the global signal [37],[38]. It has been shown to explain additional variance beyond CO_2 related measures [39].

In this thesis, RETROICOR is used specifically to remove high-frequency cardiac and respiratory artifacts, while deliberately preserving low-frequency physiological fluctuations such as RVT and PPG-Amp. By combining RETROICOR with cross-correlation analysis, the relationship between low-frequency physiological dynamics and BOLD fluctuations was investigated, providing deeper insight into how systemic physiology interacts with neural activity.

1.5 Mental Stress and Caffeine

Stress is a multidimensional concept to investigate, which can be psychological, physical, or social. A stressor causes a reaction that can affect the function and regulation of the body to protect homeostasis. When facing a stressor, automatic reactions rapidly generate sympathetic adrenal medullary (SAM) response and activate the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for cortisol release. SAM activation helps us to react to stressors related to the fight, flight, or freeze response, and it demands high energy consumption [40]. Stress hormones are crucial to humans' adaptation to the daily and substantial life stressors. This stress response is vital to maintain stability of the living system, i.e., protecting the homeostasis, and this response process is named allostasis. Cortisol and adrenaline, which are results

of the HPA-axis and SAM, are examples of mediators of allostasis. Those mediators could be secreted as a reaction to stressors or to daily behaviors like eating, sleeping, and exercising to increase the adaptation [41].

When mental stress is investigated, studies show that cognitive tasks can change sympathetic responses. Forced mental arithmetic tasks led to activation of the sympathetic nervous system by increased norepinephrine concentration and spillover, and Stroop's task caused an increase in heart rate and blood pressure [42]. However, different cognitive tasks may activate different aspects of the sympathetic nervous system [43].

fMRI is an important tool for observing the brain in vivo. It helps us to determine which regions are correlated with each other, and how the FC changes throughout the stress response. A study using aversive movies as fear-inducing stimuli shows that those stressors increased interconnectivity in a network containing cortical (e.g., fronto-insular, dorsal anterior cingulate, inferotemporal) and subcortical (e.g., amygdala, thalamus, midbrain) areas, depending on the stress response [40].

Also, using arithmetic tasks with feedback presentation is able to create psychosocial stress and cause salivary cortisol increase, and deactivation of limbic system-related brain regions [44],[45]. Another study shows mental workload heightens the heart rate and pupil diameter. Also, stressful sounds such as blackboard scratching or a dentist's drill are associated with deactivation of limbic regions of the salience network and with increased activation in executive control networks [46]. Mental stress activates the sympathetic system, and it affects the fMRI BOLD signal.

Caffeine is one of the most consumed psychoactive drugs in the world. It has hemodynamic and humoral effects in regular consumption, and it is proposed that it may amplify SAM response to the daily stressful events [47]. It acts as a nonselective antagonist of A1 and A2A adenosine receptors [48]. Increased neuronal activity, vasoconstriction, and CBF decrease are observed [49]. Caffeine increases the BOLD signal in fMRI [50]. Also, regular consumption alters its effect. For example, a study stated

that the BOLD signal change and magnitude in the visual cortex were larger in high coffee consumers in comparison to low coffee consumers [51]. In addition, fMRI studies show that caffeine can emphasize the anti-correlations between the DMN and TPN. Also, decreases in connectivity and global signal amplitude in the eyes-closed resting state, yet not in the eyes-open[52].

1.6 Caffeine, Physiology, and BOLD Signals

Neurochemically, caffeine binds to adenosine A1 and A2A receptors and leads to vasoconstriction and disinhibition that make people more alert. Besides, in systemic effect of caffeine influences the heart rate and respiration, which are observed as physiological noise in fMRI scans. It decreases resting CBF and causes a lower baseline BOLD level. In task-based fMRI studies, because the baseline is changed by the caffeine, a reduction in BOLD signals can be observed due to less task-activated vasodilation. However, the results depend on participants' consumption habits, dose of administration, and choice of physiological motion correction.

In RSNs, caffeine attenuates low-frequency BOLD signals, resulting in reduced resting state connectivity due to both less spontaneous vasodilation and neural activation. It increases the irregularity in the intrinsic network; thus, FC values are reduced. In addition, networks directly related to resting, such as the DMN, are diminished more.

Reduced resting state after caffeine intake can be vascular and/or neural; consequently, the investigation of caffeine with fMRI must be conducted with caution, and physiological effects must be considered in detail.

In our study, we aim to investigate the interplay among caffeine, physiology, and BOLD signals. Caffeine directly and indirectly affects BOLD signals through neurochemical and physiological activation. rs-fMRI and task-fMRI are used in the experiment. Therefore, we research how physiological variability has a role in brain

networks in the moderation of caffeine. Physiological signal collection leads to illumination of the intertwined structure of the brain and the body. Also, subject-level and group-level comparison in resting state and task-based experiments helps us to make meaningful inferences about systemic physiology and BOLD signals.

1.7 Introduction to Methods of Analysis

There are three main analysis methods in the study, which are General Linear Model (GLM), Independent Component Analysis (ICA) along with dual regression, and whole-brain correlations with physiological signals.

General Linear Model Analysis (GLM) shows task-fMRI signal maps. In task-fMRI, each voxel's value can be modeled with a linear combination of predicted responses. Basically, a regression that estimates a voxel along with error variance. Task predictors predict the value; also, other regressors like motion or physiology can be entered into the model. The task regressors are convolved with hemodynamic response function (HRF) because although neural activity is a brief response, BOLD is slower, and the neural activation time should be timed with the BOLD shape.

ICA is a blind source separation method to derive components of source signals. In fMRI, it helps us to produce spatiotemporal activation maps that are independent. Later, dual regression is used to make a group-level analysis to find a possible effect of caffeine. In our experiment, the experimental design is within-subjects (paired subjects). Therefore, a contrast design is created according to this design. Task data is used, and early and late caffeine intake conditions are compared.

In the study, the essential part is collecting physiological signals, PPG-Amp, and RVT. Because we are interested in how resting-state and task-based voxel activation are related to systemic physiology, cross-correlations are examined to identify this relationship. Task-based, rest, early caffeine, and late caffeine conditions' analyses are conducted for this purpose.

2. MATERIALS AND METHODS

2.1 Design and Participants

The study follows a multimodal approach and consists of fMRI and physiological signal measurement. Each modality helps us to illuminate different aspects of the brain, body, and their inherent relationship. Also, it gives us insight into how these measures interact with each other. Although investigating each modality separately keeps valuable inferences, the whole picture provides a greater understanding of the relationship between the brain and body.

The experimental method is a simple within-subjects design, i.e., repeated measures, with two independent variables. The first independent variable is time, and it has two levels, 10 minutes and 30 minutes after caffeine intake, which is designed to see the instant effect of caffeine. The second independent variable is the fMRI paradigm, which also has two levels: the arithmetic task and resting state. The dependent variables are our multimodal measurements, i.e., BOLD, PPG, and respiration signals. The arithmetic task is used to create mental stress for the participants. The equations are counterbalanced in the experiments.

The data is named as Caff-10 for 10 minutes, and Caff-30 for 30 minutes conditions. The arithmetic equation solving condition is named task, and the resting state condition is named rest.

The number of participants was 18 (20-35 age range, 7 females). All participants were healthy volunteers who wanted to take part in the experiment after an online call for participants at the university. All participants provided informed consent, and they were instructed about MRI safety warnings.

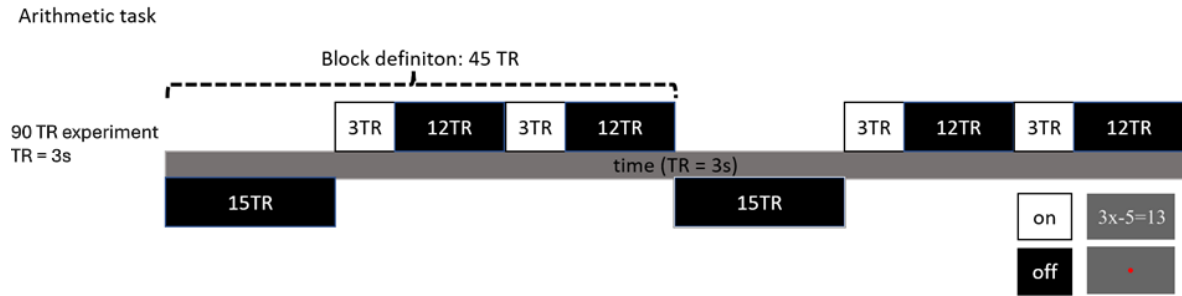


Figure 2.1 Arithmetic task design for 45 TR.

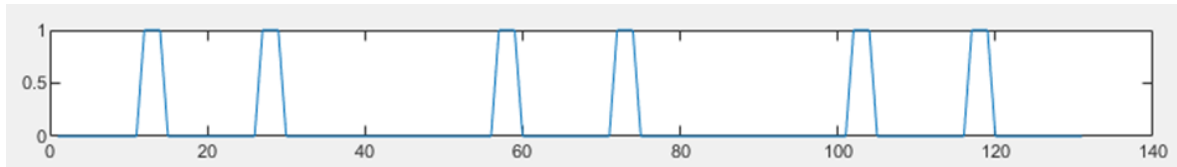


Figure 2.2 Arithmetic task design for 135 TR.

2.2 Procedure

All subjects were administered a 200 mg caffeine pill, unbeknownst to them whether it was caffeine or a placebo. 10 minutes after the intake, they underwent the fMRI scan of the first session. Each session includes two runs. The prior one is the arithmetic task, and the following one is the resting state. The order of the fMRI paradigms in the second session is the same as in the first one. The second session is initiated 30 minutes after the initial intake, with this interval including the time spent on the first session and the anatomical scan conducted between the two sessions. So, there are four functional scans in total, with conditions of 10 or 30 minutes after the intake, and an arithmetic task or resting state.

2.3 Data

2.3.1 fMRI

The scans were collected by Siemens Magnetom Prisma Fit 3 Tesla (GRE-EPI, FA = 90, TR = 3 s, TE = 36 ms, in-plane resolution = 2.5 mm isometric, number of TRs = 120/135) in Acibadem Altunizade Hospital.

The arithmetic task scans are 135 TR, and the resting state scans are 120 TR. After the removal of the first four signals in preprocessing, there are 116 TR for resting and 131 TR for the task. In the analysis, there are 18 subjects in rs-fMRI and 17 subjects in task-fMRI. One task-based scan from a subject is excluded because of the number of TR inconsistencies.

2.3.2 Physiological Signals

Peripheral physiological signals were simultaneously recorded using a pulse oximeter placed on the left index finger and a respiratory belt positioned around the abdomen, with a sampling rate of 400 Hz. They were collected during all scans; however, there was no recording for one participant in the arithmetic task due to technical issues.

2.3.2.1 PPG. PPG non-invasively measures blood volume changes in peripheral tissues, such as the fingertip, by detecting variations in light absorption caused by the cardiac cycle. Each heartbeat creates a pulsatile waveform that shows the inflow of blood into the arteries and its gradual return to baseline. The rising portion of the signal marks the systolic phase, when the heart pumps blood and produces a clear peak. A smaller secondary rise, called the diastolic peak, can also appear and is thought to reflect the return of the pulse wave from the periphery. In the following analysis, we will focus on systolic peaks, as the diastolic component will be removed during filtering.

A systolic peak in PPG approximately corresponds to a heartbeat; however, since it is a peripheral measurement capturing downstream effects of cardiac contractions, there is a slight delay compared to direct measurements such as electrocardiogram. Because these periodic fluctuations are tightly linked to the cardiac cycle, the PPG signal serves as a reliable source for constructing physiological noise regressors. In particular, it can be used to derive cardiac RETROICOR regressors, which model cycle-related physiological motion in fMRI data [34]. These correspond to the high-frequency oscillatory component of the PPG, reflecting the heartbeat-to-heartbeat dynamics.

In addition, the relative height of the systolic peak compared to the preceding trough provides an index of vascular tone, commonly referred to as PPG amplitude. This slower-varying, low-frequency modulation of the waveform is influenced by autonomic, particularly sympathetic vascular activity, and will be explained in more detail in the next section

2.3.2.2 Calculation of Physiological Measures. High frequency physiological noise was modeled with RETROICOR slice-based regressors by using the RetroTS.m function from the Analysis of Functional NeuroImages (AFNI) MATLAB library to be used in the ricor block of fMRI preprocessing and remove physiological motion artifacts [53]. RetroTs.m function uses PPG and respiration signals to estimate cardiac and respiratory phases and to model pulsatile and breathing-cycle-related effects in the BOLD time series.

PPG-Amp is defined as the peak-to-trough difference of the PPG signal. It reflects vascular compliance, peripheral perfusion, and autonomic vascular tone, with larger amplitudes indicating stronger pulsatile blood flow and vasodilation, while smaller amplitudes indicate vasoconstriction and diminished peripheral perfusion. Thus, PPG-Amp serves as a marker of sympathetic nervous system activity and peripheral vascular resistance. In fMRI research, low-frequency fluctuations in PPG-Amp have been linked to widespread changes in BOLD signals, suggesting sensitivity to systemic vasomotion and autonomic arousal [38]. In our study, the PPG signal was band-pass filtered be-

tween 0.5 and 2 Hz to facilitate systolic peak detection, and PPG-Amp was calculated by averaging the peak-to-trough distance for each TR, following Özbay et al. (2018) [37]. This measure was then used to evaluate the cross-correlations between voxel-wise fMRI BOLD signals and vascular tone.

In the present study, RVT was estimated using the RetroTS.m function as the difference between successive inspiration and expiration peaks (troughs), normalized by the breathing period, and interpolated to the fMRI TRs as in Birn et al. (2006) [35]. RVT reflects both the depth and rate of breathing, providing a combined measure of respiratory variation that has been repeatedly shown to correlate with the fMRI BOLD signal through CO₂-related fluctuations (e.g., [35],[36]). It was then used to observe the cross-correlations between voxel-wise fMRI BOLD signals and respiratory volume.

2.4 Preprocessing of fMRI

First of all, the functional and anatomical images were anonymized and transformed from DICOM to NIFTI format by dicom2nifti tool of mricon.

T1-weighted anatomical images were skull-stripped and the necessary warp files to non-linearly align them to MNI152 template were created by AFNI @SSwarper script. Later, the calculated warp files were given to the proc.py for functional alignment to the template.

Although most of the steps are the same in the preprocessing, there are some different additions in resting-state analysis, such as motion parameters. We followed afnipro.py steps to perform despiking, removal of cardiac and respiratory regressors (i.e., RETROICOR), slice-timing correction, alignment of EPI and anatomical images, warping anatomical image to MNI152, volume registration, smoothing, creating a mask, normalizing voxel values, and regressing motion parameters.

Despiking helps us to truncate spike noise in the signals, and it lowers the value

higher than normal. Although sometimes despiking is not recommended in task-based fMRI, it is generally used in both task-based and resting-state analysis. In this study, we performed despiking in both conditions.

Slice-timing correction is required because the full volume is not captured at the same time; instead, it is scanned slice by slice. Thus, there is a slight difference in time, and this correction aligns the slices with the moments they were acquired.

EPI and anatomical images are aligned to identify anatomical regions where a particular signal comes from. So, we can create masks, define regions of interest, and make inferences about relevant structures during a specific task with the help of an anatomically aligned functional image. Indeed, this alignment is the first step because we want to compare different participants, besides performing analyses with the average signal change in a corresponding region. Hence, we registered this image to a template, which is MNI152_2009, in the study to compare group differences.

Smoothing is another step in preprocessing. It averages the signal value of a voxel with its neighboring voxels. It increases the signal-to-noise ratio by reducing artifacts. Different Gaussian kernel size creates different effects, and increasing the smoothness decreases the spatial information that we obtained and the adjustment of kernel size varies in studies. To overcome this trade-off optimally, we selected the kernel size that is suitable for ICA, and followed recommendations of Candemir (2023) [54]. Therefore, we determined 6 FWHM in our study. We normalized the signal values because arbitrary values might change from scan to scan. Thus, to make inferences from the signal values, normalizing them is crucial.

Importantly, physiological motion artifacts were regressed out using slice-based RETROICOR regressors before slice-timing correction; however, slower systemic fluctuations (e.g., changes in PPG-Amp or respiratory volume) were not removed and were intentionally kept for analyses of their relationship to brain networks

The final preprocessing step was regressing out 6 rigid-body motion parameters

and motion censor regressors (0.2 mm threshold) to remove motion-related artifacts. The residual time series from this regression (errts.nii) was used for subsequent analyses.

2.5 Data Analysis

2.5.1 ICA

ICA is a data-driven method to separate the sources of signals, and it has been used in fMRI research since McKeown et al. (1998) paper [55]. ICA considers the statistical independence of the signals from each other and differentiates them according to that. Hence, we can find the fMRI signals that behave in different patterns.

The classic analogy of ICA is recording people who are talking in the same room with several microphones. Each microphone records all voices from the room, but with different quantities. ICA tries to separate those sources (talking people) by using the voice recordings. It takes the observed mixtures and creates an unmixing matrix that can estimate the observations. Unlike principal component analysis (PCA), it relies on independence, not uncorrelatedness. The assumptions of ICA are statistical independence of sources and non-Gaussian distribution of at least all but one source. The outputs (components) have an arbitrary scale and order. After a post hoc analysis, by taking each component's contribution, a components explained variance can be obtained. However, it does not mean that there is a higher signal in the first component and a lower signal in the last components. It gives information about the contribution of a particular component.

ICA assumes that the source signals are hidden, but they are non-Gaussian and statistically independent. Also, their mixing process is unknown but linear.

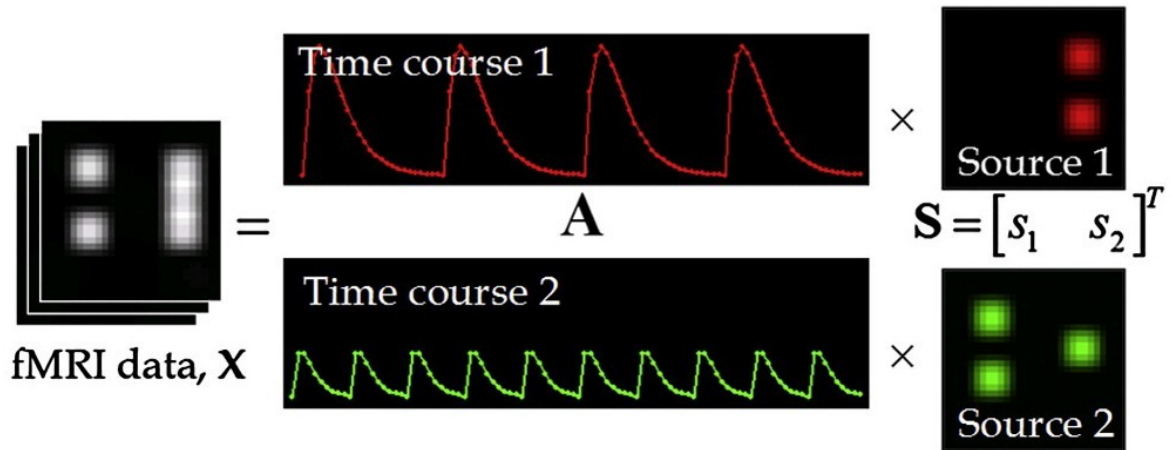


Figure 2.3 ICA fMRI signal separation figure from Calhoun et al. (2009) [1].

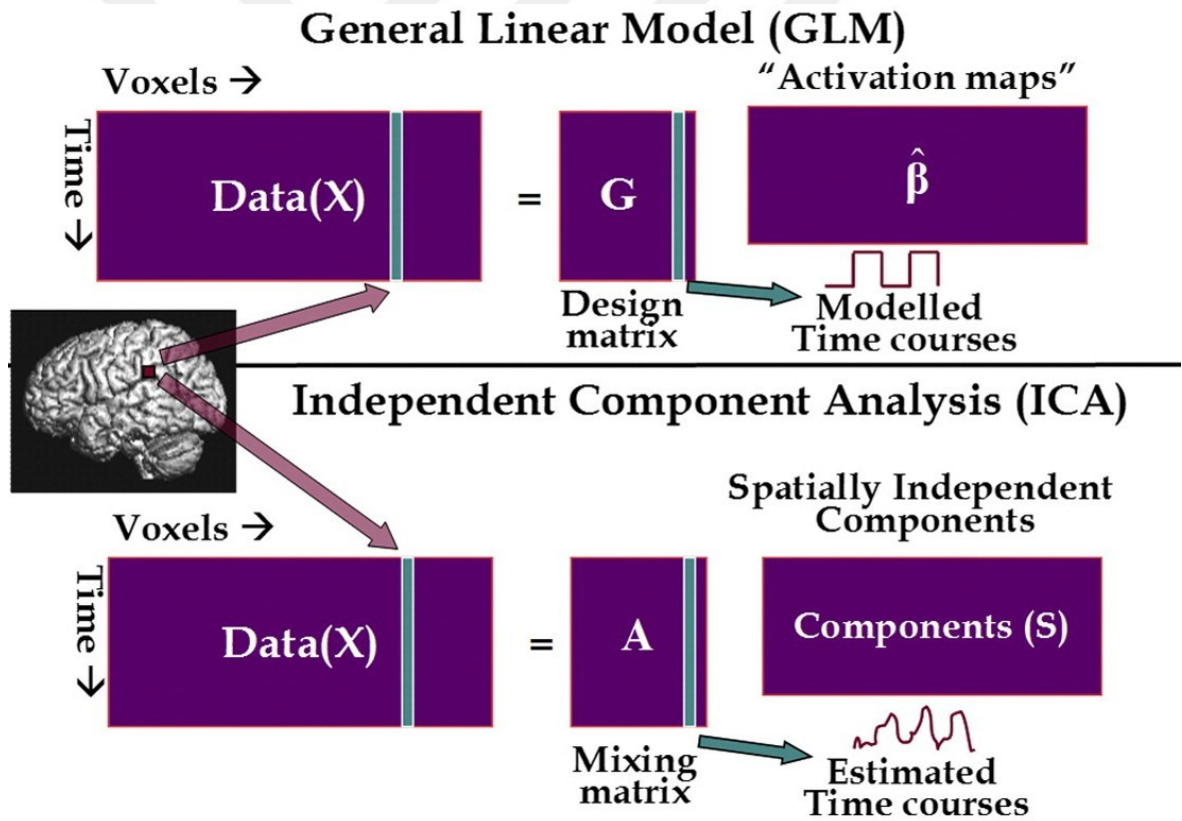


Figure 2.4 GLM and ICA comparison figure from Calhoun et al. (2009) [1].

2.5.1.1 ICA in fMRI. In fMRI, a voxel's BOLD value is determined by a combination of more than one effect, such as hemodynamic activity, motion artifacts, physiological noise, signal drifts, or even temperature noise. As long as the regressors are defined, GLM can be used in the analysis. However, model-based analysis methods

like GLM are only useful for task-based fMRI experiments whose stimulus timing is known beforehand. On the other hand, ICA is a blind source separation method and model-free. It does not need prior information about stimulus timing or expectation in response types. Thus, it is a data-driven alternative for GLM without specified regressors or HRFs, and this makes ICA a powerful tool for not only task-based but also resting state analysis.

In brain imaging, ICA can be conducted with two methods, temporal or spatial, depending on the type of independence that is assumed. While temporal ICA tries to disentangle the time courses by how the separate signals dynamically change in time, spatial ICA emphasizes how spatial patterns in signals are independent. Functional networks might be spatially distinct but can be active simultaneously, and spatial ICA groups those co-active voxels in a map. Therefore, spatial ICA is the most common method in fMRI because spatial independence is higher than temporal independence; besides, the vascular and neuronal restrictions in the brain lead to a non-Gaussian distribution in the voxel intensity. Also, obtaining a map is along with the researchers' interests in brain sciences. Thus, we performed spatial ICA in the resting state and task-based analyses [56]. Spatial ICA forms each component into a spatial map with a time course.

There are two approaches in spatial ICA: temporal concatenation and tensor-ICA. In FSL's MELODIC toolbox, it can be selected as *concat* or *tica* [2]. Temporal concatenation aggregates all runs by adding them end to end and looks at how those signals come from different regions. In contrast, it is not useful in task fMRI, because BOLD signals are not just random fluctuations, and the design influences the temporal dynamics of the signal. Besides, tensor-ICA accounts for this temporal change, and it combines the sessions while assuming all runs have the same task design, i.e., see the stimuli with the same order, instead of concatenating all of them end-to-end. It should be clarified that ICA still does not know the task design; it only knows that all runs have the same order of stimulus presentation.

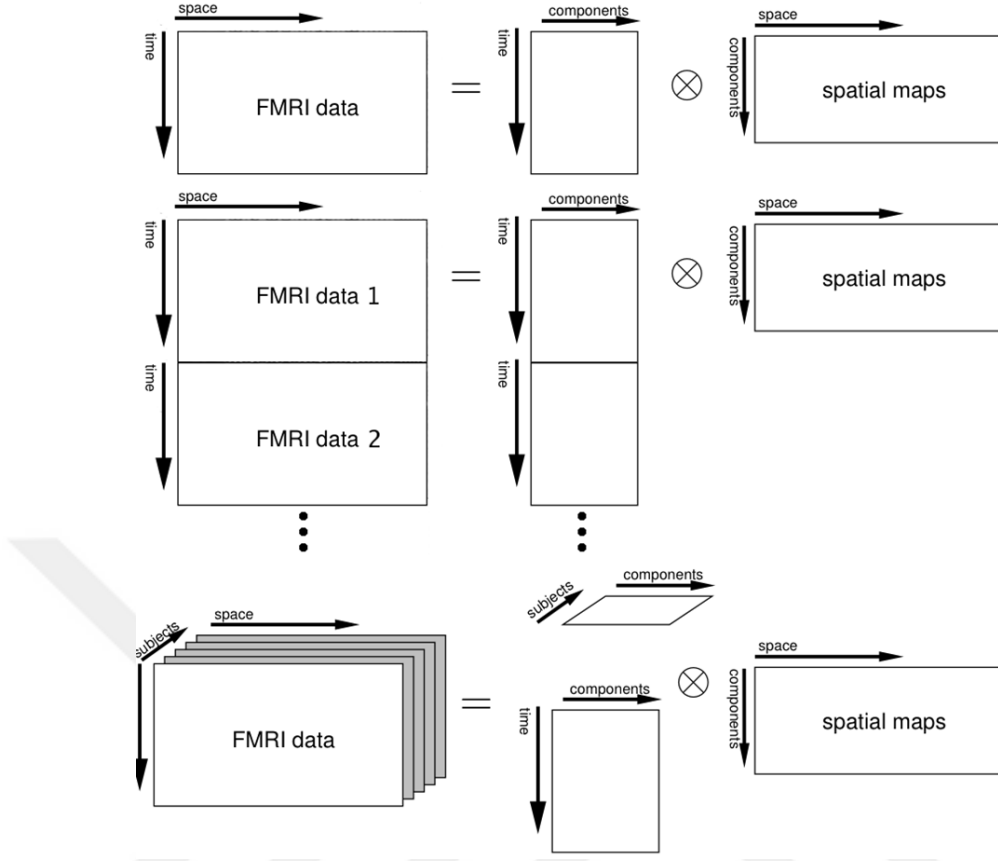


Figure 2.5 Temporal concatenation at *top* and tensor-ICA at *bottom* figure from MELODIC information page [2].

Hence, ICA provides independent spatial patterns and their time courses as components. The number of components can be determined automatically or manually. Because we have a limited number of participants, we selected 20. This number is enough to see spatial maps of the main functional networks, physiological effects, and noise. Generally, those components are used to clean the fMRI data after labelling them as signal or noise. We not only selected signals but also selected systemic effect-related areas, such as ventricles, to investigate the relationship between the body and BOLD signals.

In single-subject ICA, every subject's data is analyzed by ICA, and components, i.e., spatial maps, are obtained for each subject. This method not only gives information about a subject's brain networks but also helps to reduce dimensions for future analyses. Noise components can be identified and removed from the data. So, it is also a tool for cleaning fMRI data. On the other hand, regarding group differences, performing

ICA on each subject and obtaining spatial maps has limited interpretability due to a lack of comparability. Univariate analysis methods are useful to test group differences with ease. A researcher identifies the regressors in a GLM analysis, and all subjects in a group are analyzed by the same regressor. So, interpreting the results and capturing the group differences is possible in methods like regression. Yet, in ICA, each subject has different time courses in various orders; therefore, making group inferences by ICA is not possible. To make it possible, researchers suggested approaches for group analysis in ICA [56].

For group comparison, the researchers suggested new approaches. The essential point is preserving the subject specificity and moving from a single subject to groups. First, a method that reduces dimensions may be used, such as PCA or ICA, optionally. Second, all subject data are concatenated, and an ICA is performed at the group level. Finally, subject-specific maps and time courses are back-reconstructed by using group-ICA maps. Thus, comparability across groups will not be a problem, for instance, avoiding cases where the first component of group 1 corresponds to the fourth component of group 2 with slight variations.

In back reconstruction, there are several ways to perform it. GICA1, GICA2, GICA3, or dual regression can be used. All methods have their own advantages and disadvantages. For example, GICA3 can preserve more subject variability than dual regression, but dual regression's advantage is that it provides both time series and spatial maps at the individual level, unlike GICA3, which prioritizes either one of them. Also, it is implemented in FSL and its usage is highly compatible with the output files, and it is seemed as a standard in fMRI research [57].

2.5.1.2 Dual Regression. ICA can provide spatial maps, and we can identify those maps as an intrinsic network, physiology-related component, or noise by visually investigating the map, time-course, and power spectra. Nevertheless, the main goal in this study is to find caffeine-related effects by comparing early and late intake groups. Conducting different ICAs for each group can provide valuable information about how

caffeine affects the produced spatial maps. However, direct comparison is not possible when two different ICAs are done. To overcome this problem, dual regression is a great method to use after ICA.

First, spatial ICA is conducted on all participants' data (both 10 and 30 minutes after the intake). The temporal concatenation approach for resting state and the tensor-ICA approach for task-based design are followed. Therefore, each independent component has a spatial map and a time course. However, we need to subject-specific spatial maps and time courses to determine how a component differs in early and late intake conditions. Dual regression extracts the individual, subject-specific maps based on the already produced group-ICA component maps.

Dual regression, as the name suggests, has two regression steps. First, time courses are extracted from each subject's data. Second, each time course is used to obtain subject-specific spatial maps. In other words, it finds how much a group's independent component fluctuates in a particular subject at the first step, and places those fluctuations back into the same subject map.

To compare group or condition differences, there is a third step. To show how strongly each voxel contributes to a given network for that subject, it conducts non-parametric permutation testing. Later, a group's contribution to a map can be identified by this method. In this study, we did dual regression for both rest and task designs, and the effect of Caff-30 and Caff-10 is shown for both conditions.

2.5.2 GLM

Basically, a measured signal can be explained by the sum of predictors, their weights, as well as noise by GLM.

$$Y = X\beta + \varepsilon \quad (2.1)$$

where Y is observed data, X is the design matrix (regressors), β is the regression weight, and ε is residual error (unexplained variance).

GLM is the underlying model for regression, t-test, or ANOVA.

The design matrix consists of task regressors, nuisance regressors, and a constant. The nuisance regressors are not included in our GLM analysis, because we already used them to make physiological motion correction by RETROICOR. Therefore, we only use stimulus onset times as regressors. The contrast between the early and late caffeine groups is compared according to a paired-subjects design.

In task regressors, the stimulus events are convolved with HRF because neuronal activations are not observed directly, and the BOLD signal response lags around six seconds. Thus, task regressors are convolved with a canonical HRF to model predicted BOLD responses.

$$X_{\text{task}}(t) = s(t) * h(t) \quad (2.2)$$

where X_{task} = predicted BOLD response, $s(t)$ = stimulus function, $h(t)$ = HRF, $*$ = convolution.

Unlike ICA and dual regression, GLM requires task design priorly. Whether a voxel shows significant activity during a task condition vs baseline is investigated. Also, early vs late caffeine is tested with group-level analysis.

In our GLM analysis, we determined task stimuli onset times as the regressor.

The caffeine condition, early and late intake, is specified as a contrast. We did not include motion and other nuisance regressors in the analysis they are already corrected in the preprocessing stage. However, we still expect to see physiological effects in the results.

It is important to note that GLM, like other multivariate analyses, cannot yield a causal relationship itself. We tested how BOLD signals covariate with the task design. In other words, how BOLD signals change when participants see and try to solve arithmetic equations. Although the GLM cannot imply causation, we infer the effect of caffeine on BOLD signals because it is an experimental design that comprises independent and dependent variables.

In GLM steps, first, single single-subject (first level) analysis is conducted for every scan. Each voxels time series is estimated by GLM to get beta values. Then, group analysis (second level) is conducted by entering participants' first and second sessions to contrast design as -1 and 1. Paired-subjects t-test is used for this purpose. AFNI 3dDeconvolve is used for GLM analysis [53].

2.5.3 Voxel-Wise Cross-Correlation Analysis

Cross-Correlation analysis is a method to look at how much two time series are similar as a function of time or lag. To find the correlation between BOLD and physiological time series, their time-lagged correlations are used to not miss any possible relationship. The reason for that analysis is that examining how strongly a voxel's BOLD signal covaries with RVT, and with PPG-Amp. Also, we inspect in what lag this relationship is stronger or weaker. This analysis is done for all voxels; therefore, it is a voxel-wise correlation analysis.

In our study, whole-brain voxels and physiological signals, RVT and PPG-Amp, are analyzed. This analysis is done for each participant and condition; later, the results are averaged to infer from the results. The correlation maps are also presented as brain

maps in AFNIs visualization tool.

In our cross-correlation analysis, we used `xcorr(physio, bold)`, putting the physiological signal first and the voxel BOLD signal second; with this order, correlations that peak at negative lags mean the BOLD signal follows (lags behind) the physiological change, correlations that peak at positive lags mean the BOLD signal leads the physiological signal, and a peak at zero lag means they change at the same time.

These analyses allow us to map the relationships of BOLD and physiological fluctuations. While RVT and BOLD correlations mostly imply respiratory-related CO_2 change and its motion effects, PPG-Amp and BOLD correlations reflect the vascular tone and the effect of autonomic fluctuations. Hence, the results show the physiological variability and the contributions of systemic effects in fMRI. Also, the effect of caffeine is shown by comparing correlation maps. Task and rest conditions are analyzed separately.

3. RESULTS

3.1 GLM Analysis

As it was explained in the Methods section, the regressor of GLM analysis was the task stimulus onset times. Therefore, these beta values activation maps show how much they are associated with arithmetic equation solving. Contrasts were designed to compare Caff-10 and Caff-30 conditions.

Before the group comparison, the mean beta effect for each group was presented. The activation maps are produced with cluster-level analysis. AFNI's 3dClustSim cluster correction method was used, and the cluster size was determined as 21, while the faces or edges of voxels touch.

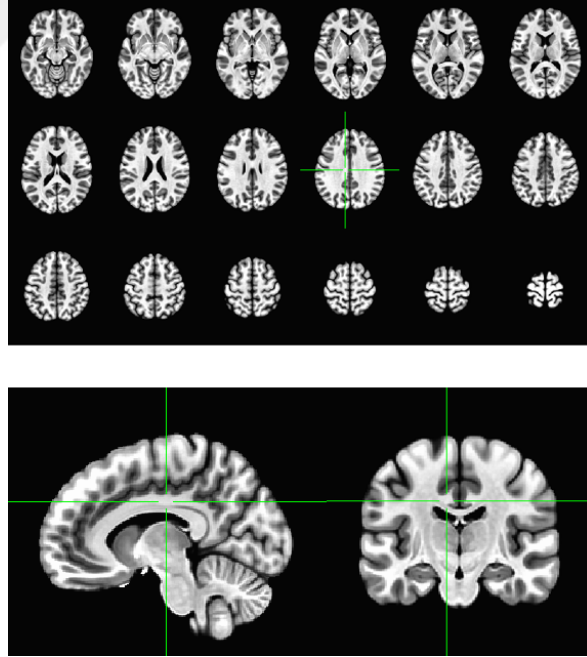


Figure 3.1 Underlying anatomical map of MNI_152 template.

3.1.1 Caff-10 Mean Effects

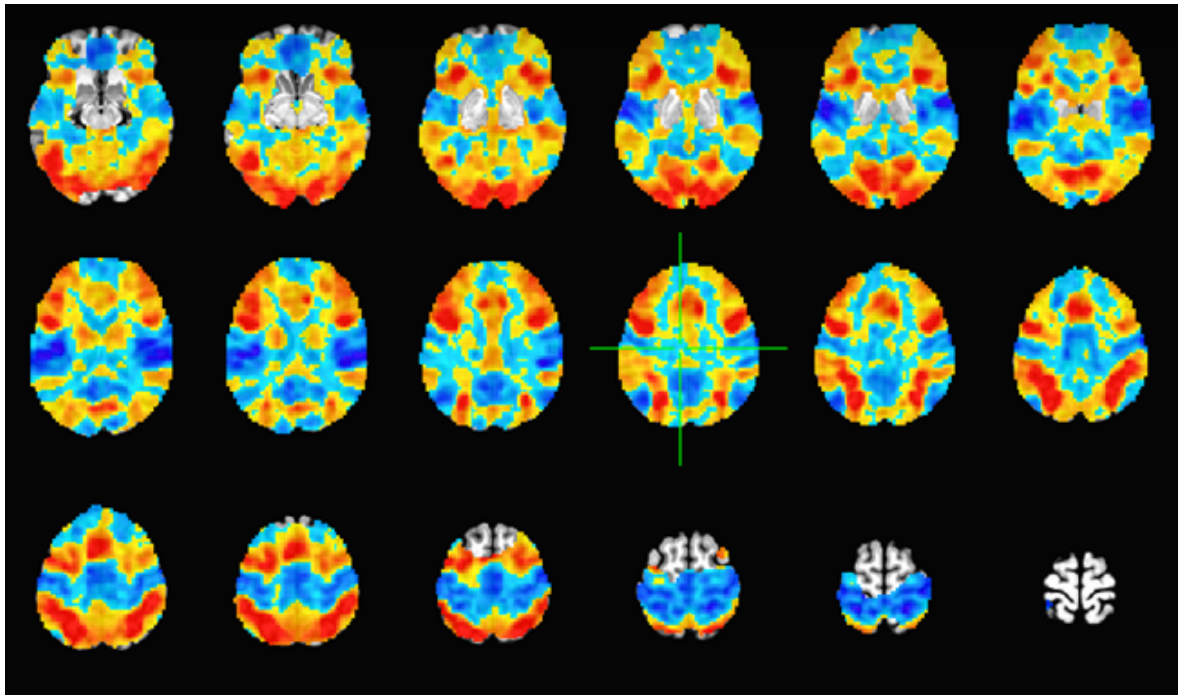


Figure 3.2 Activation map from the GLM results of Caff-10, without a threshold.

The mean beta effect of Caff-10 was investigated at cluster-level significance of $p < 0.05$, corrected.

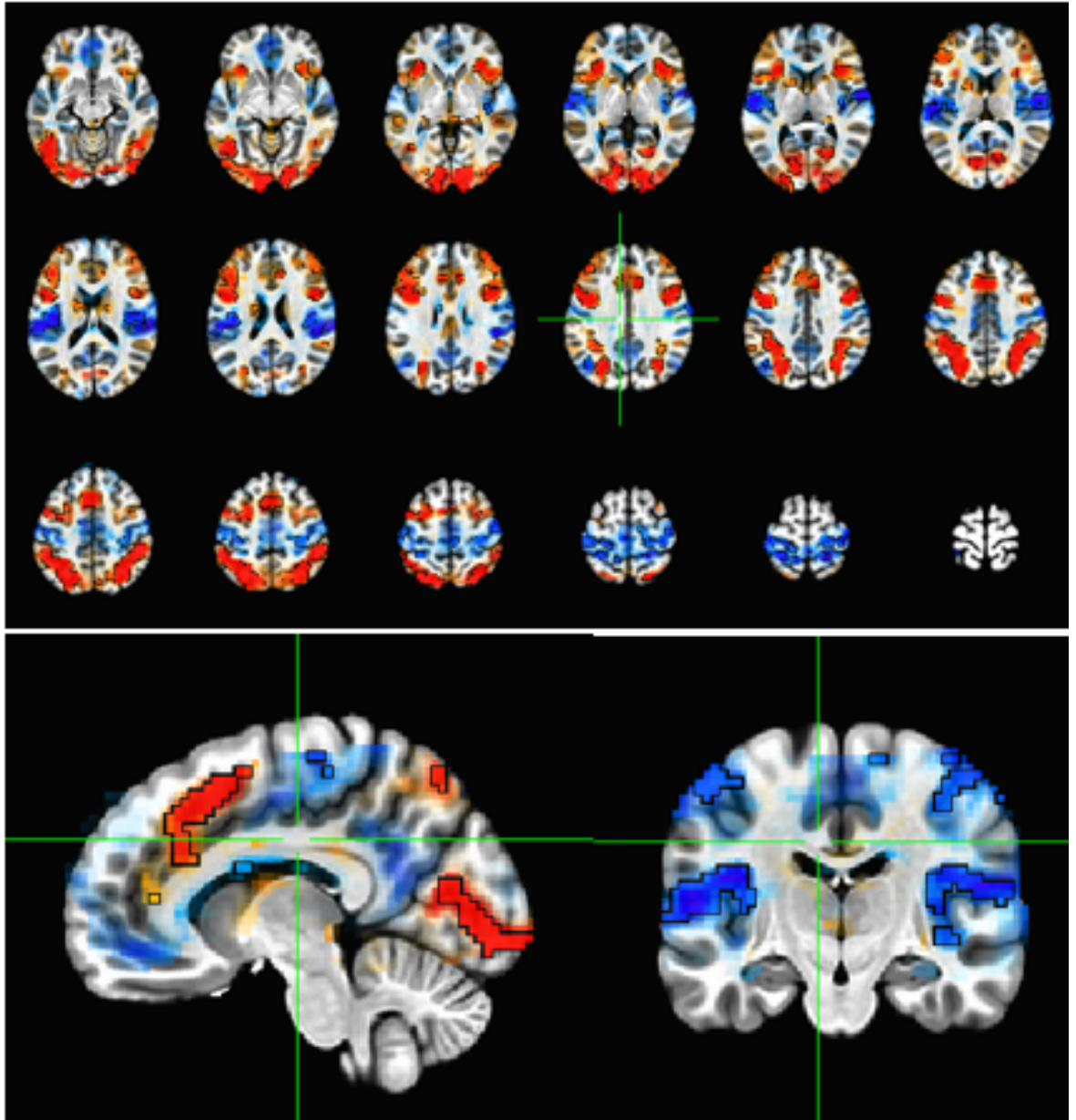


Figure 3.3 Activation map from the GLM results of Caff-10.

The GLM analysis yields that there is an activation in FPN, VIS, and DAN. Especially, there is a clear activation in IPS region. As mentioned previously, the IPS is considered the number center of the brain.

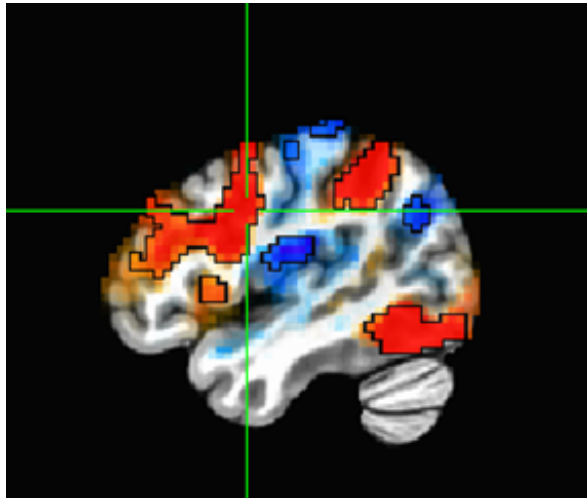


Figure 3.4 Activation in FPN from the GLM results of Caff-10.

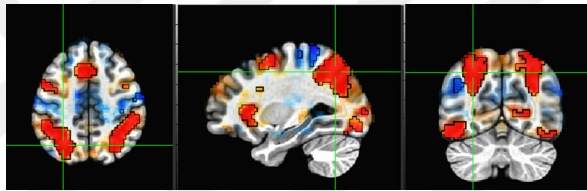


Figure 3.5 Activation in IPS from the GLM results of Caff-10.

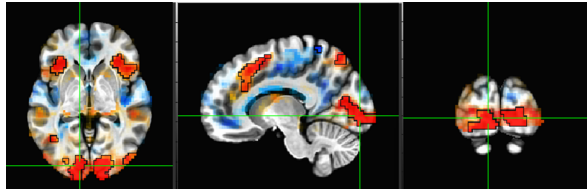


Figure 3.6 Activation in primary visual cortex from the GLM results of Caff-10.

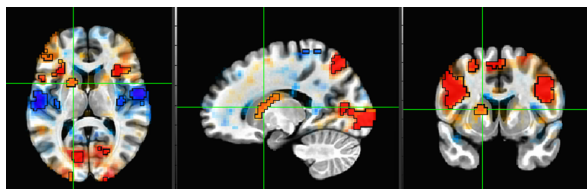


Figure 3.7 Activation in putamen from the GLM results of Caff-10.

Also, task-negative activations are seen in primary sensory and primary auditory cortex.

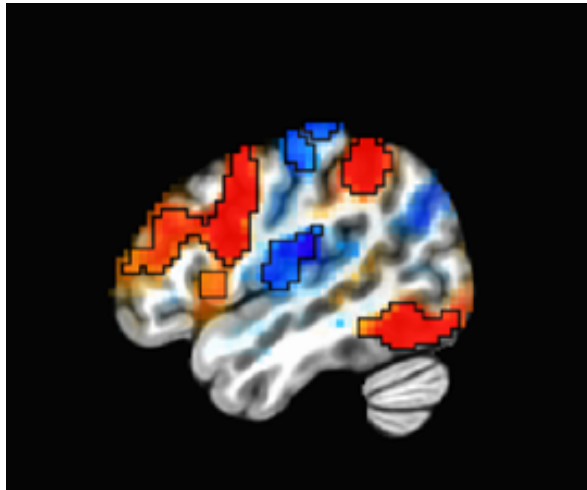


Figure 3.8 Activation in primary sensory and auditory cortex from the GLM results of Caff-10.

3.1.2 Caff-30 Mean Effects

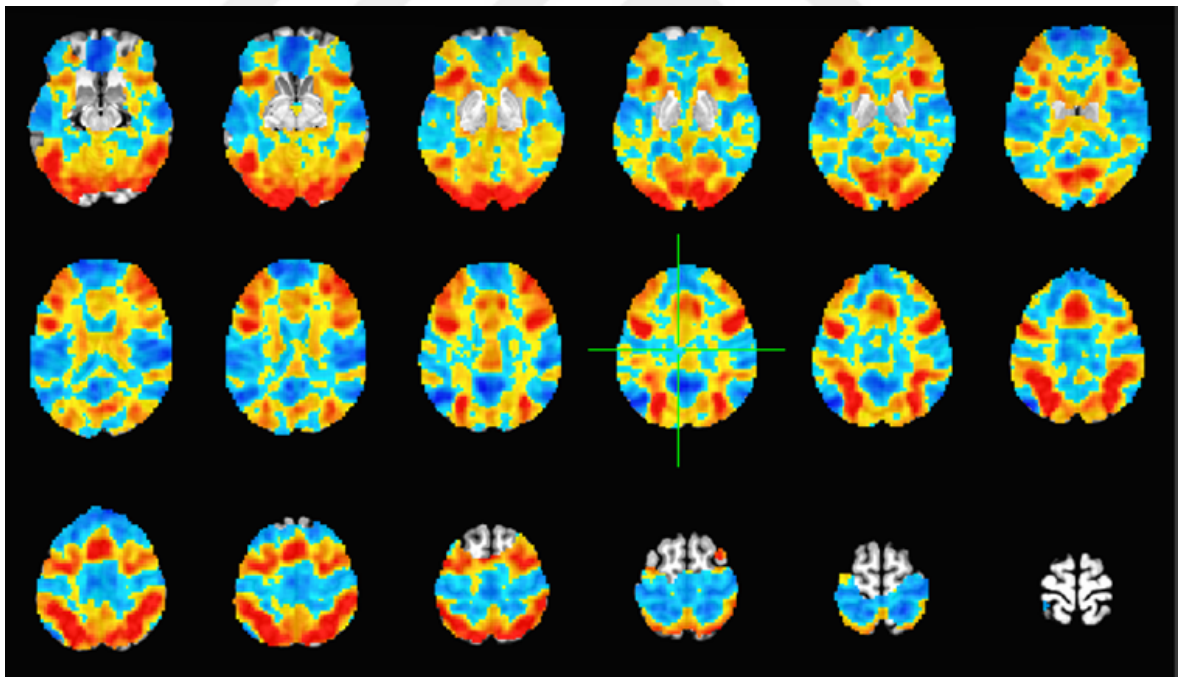


Figure 3.9 Activation map from the GLM results of Caff-30, without a threshold.

The mean beta effect of Caff-30 was investigated at cluster-level significance of $p < 0.05$, corrected.

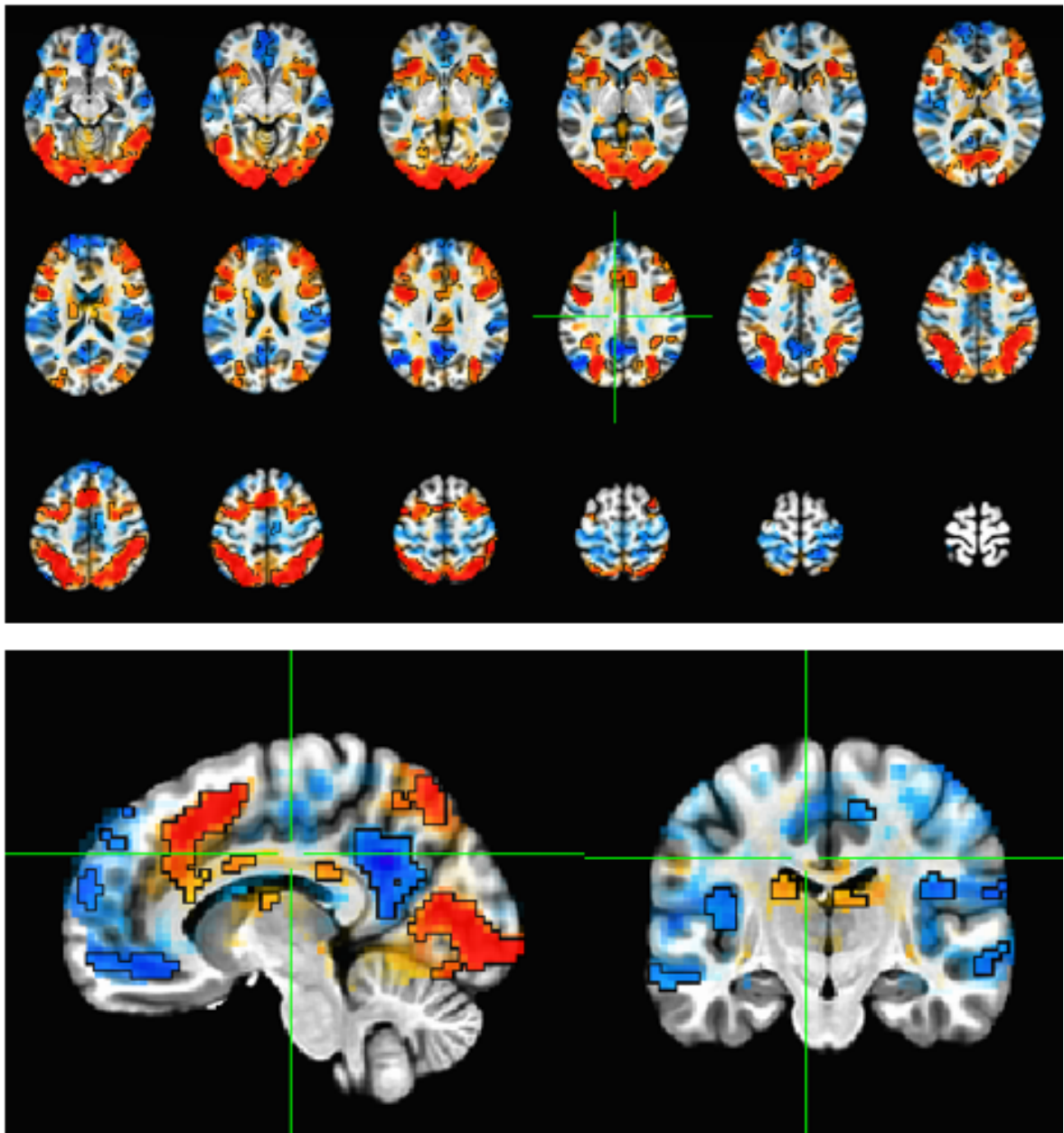


Figure 3.10 Activation map from the GLM results of Caff-30.

The GLM analysis shows that there are activations in the networks of VIS, FPN, and DAN.

There is activation in the primary visual cortex.

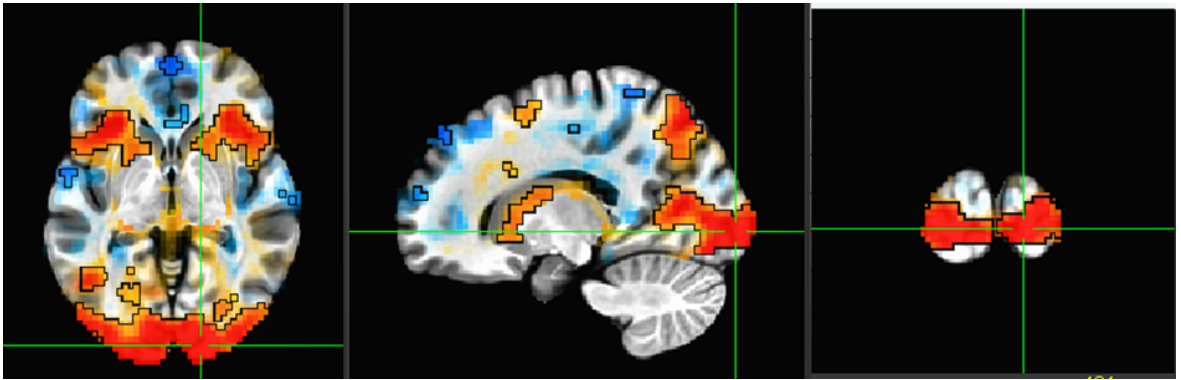


Figure 3.11 Activation map of primary visual cortex from the GLM results of Caff-30.

Like the Caff-10 group, there is an evident activation in IPS.

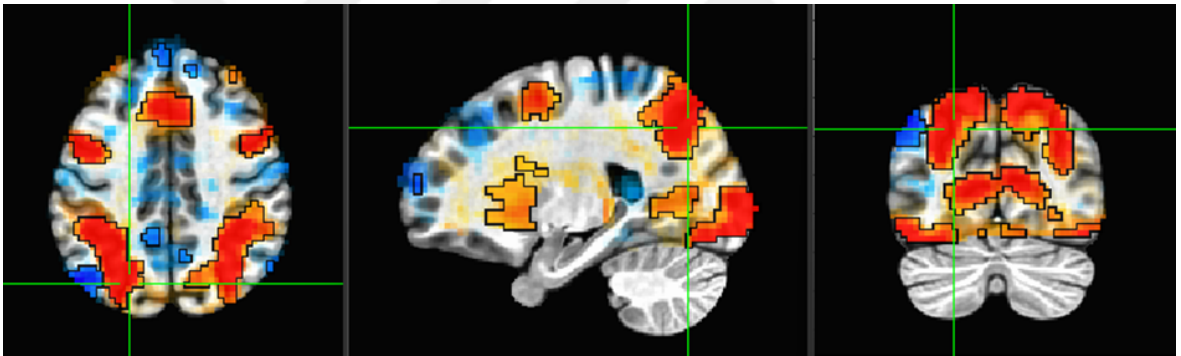


Figure 3.12 Activation in IPS from the GLM results of subject Caff-30.

Also, there are task-negative activations in precuneus and ACC, which are regions of the DMN.

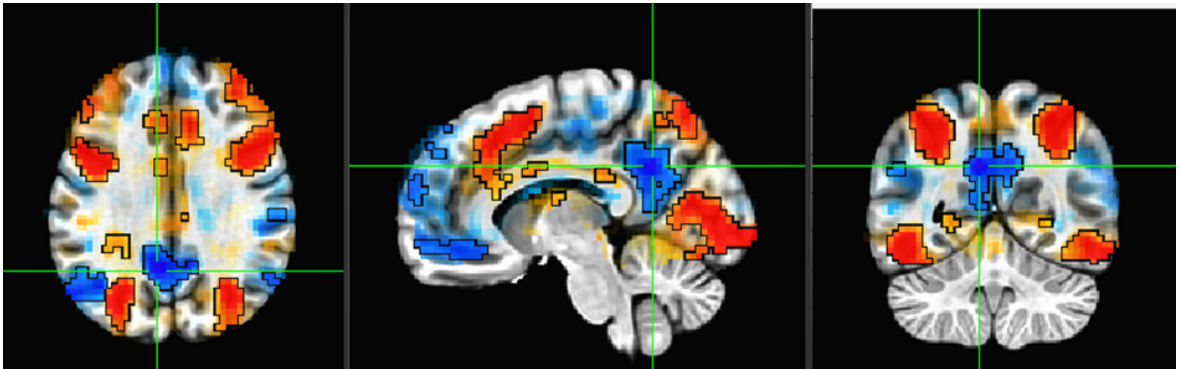


Figure 3.13 Activation in precuneus from the GLM results of subject Caff-30.

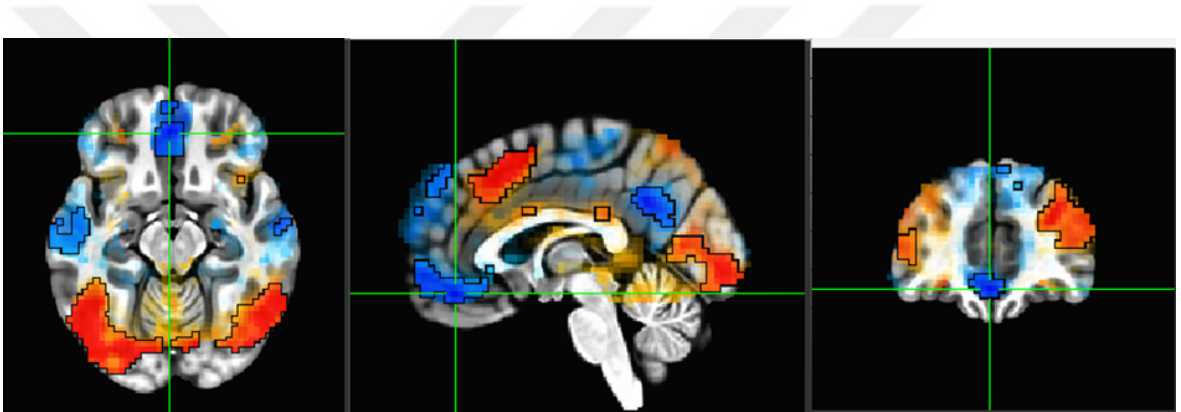


Figure 3.14 Activation in ACC from the GLM results of subject Caff-30.

On the other hand, the insula shows a task-positive activation.

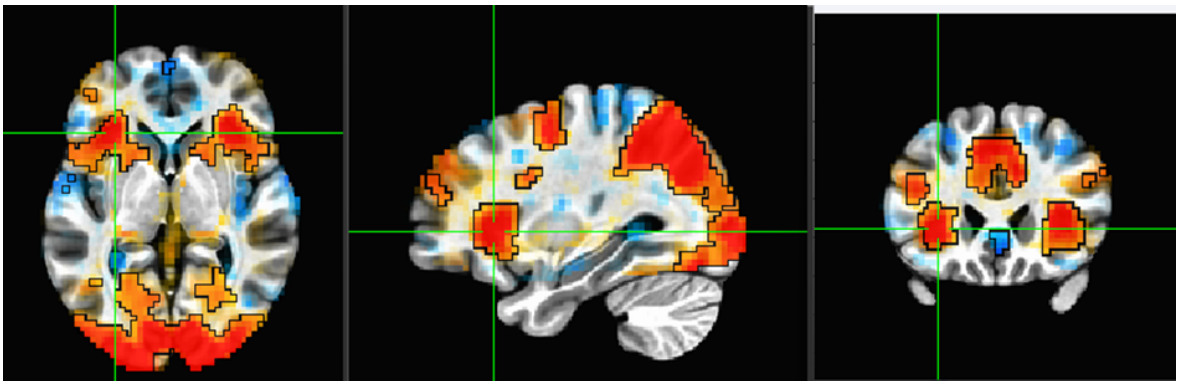


Figure 3.15 Activation in the insula from the GLM results of subject Caff-30.

3.1.3 The Difference Between Caff-10 and Caff-30

The difference between groups of Caff-30 and Caff-10 was analyzed with a paired t-test by AFNI's 3dttest script. The contrast design is Caff-30 as 1 and Caff-10 as -1. The higher activation means that Caff-30 > Caff-10.

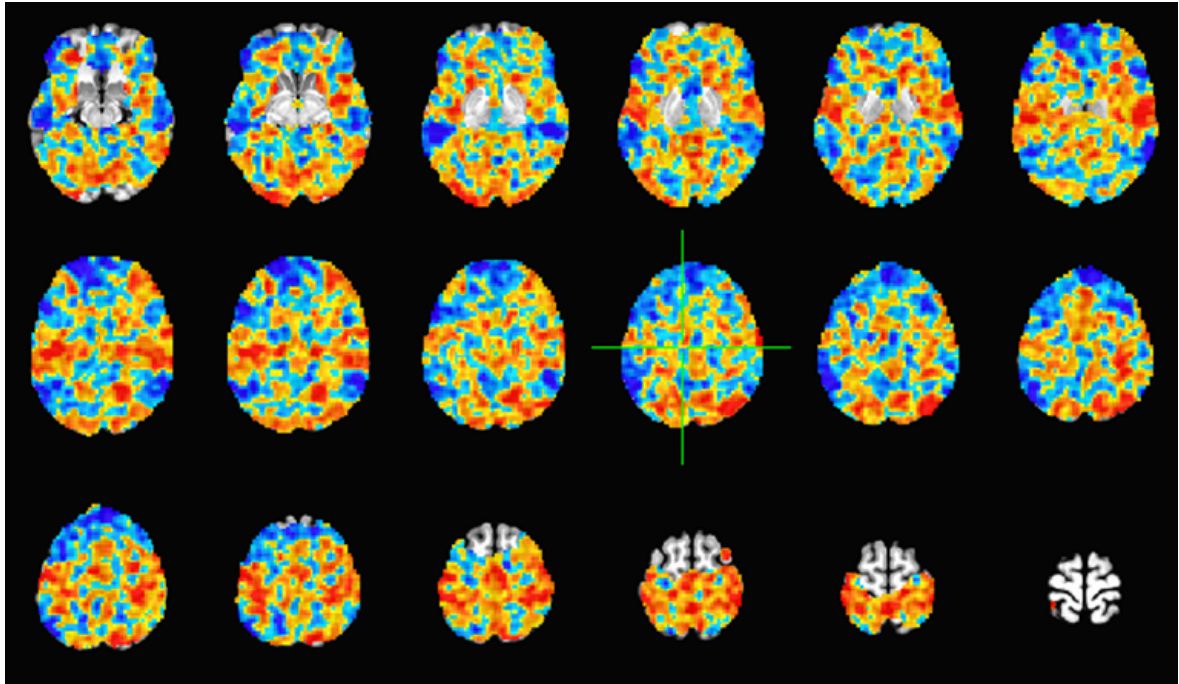


Figure 3.16 Activation map of the contrast Caff-30 > Caff-10, without a threshold.

In this group comparison, voxel-level analysis was conducted at a significance of $p < 0.05$

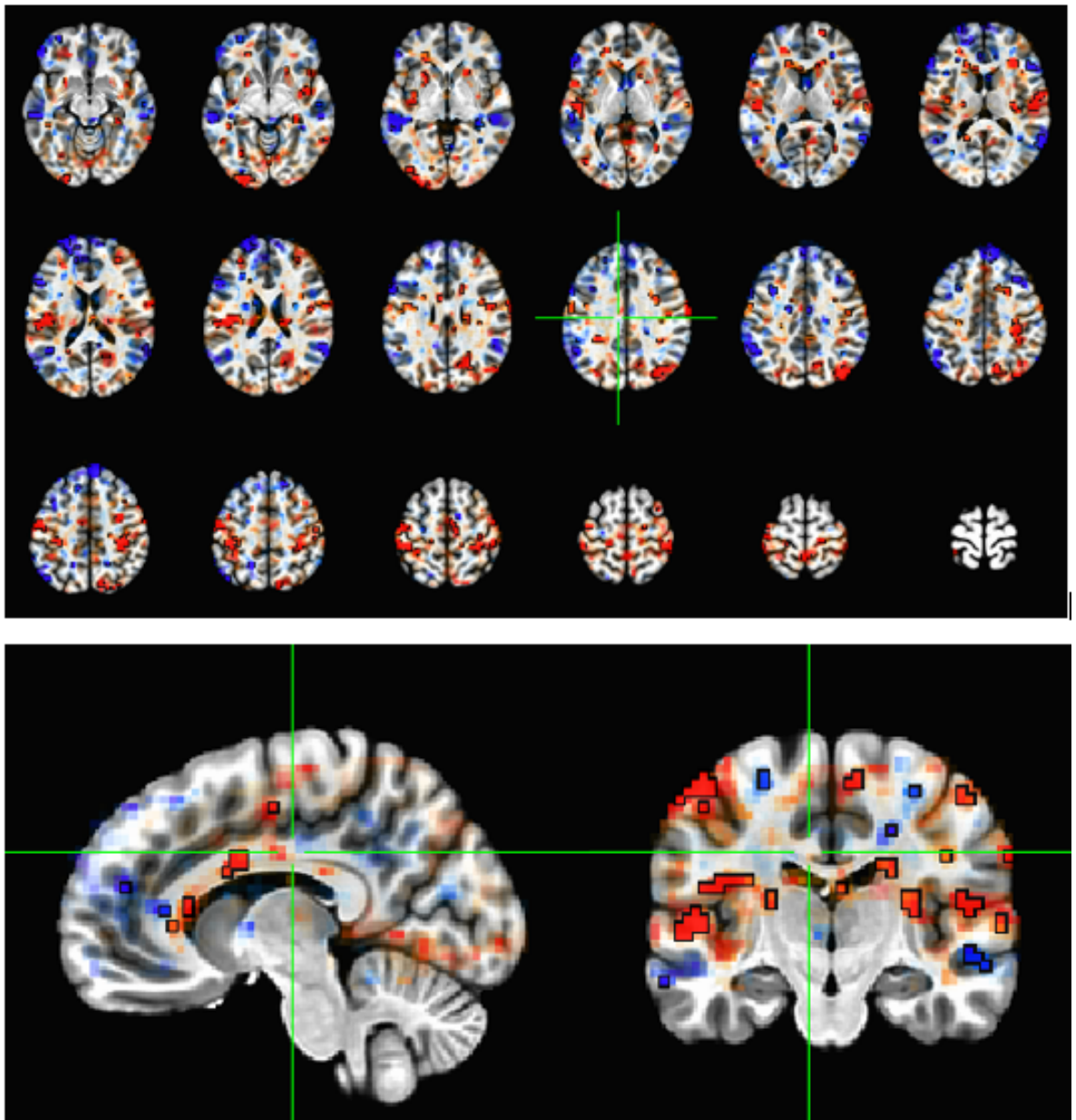


Figure 3.17 Activation map of the contrast Caff-30 > Caff-10.

There is a higher activation in the right IPS in the Caff-30 group when compared to Caff-10.

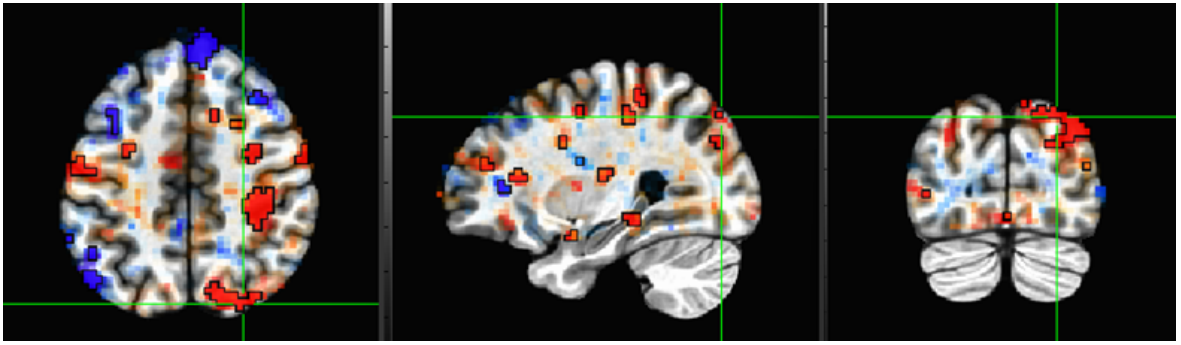


Figure 3.18 Activation in the right IPS of the contrast Caff-30 > Caff-10.

Postcentral gyrus, which is part of SMN, shows higher activation in the Caff-30 group.

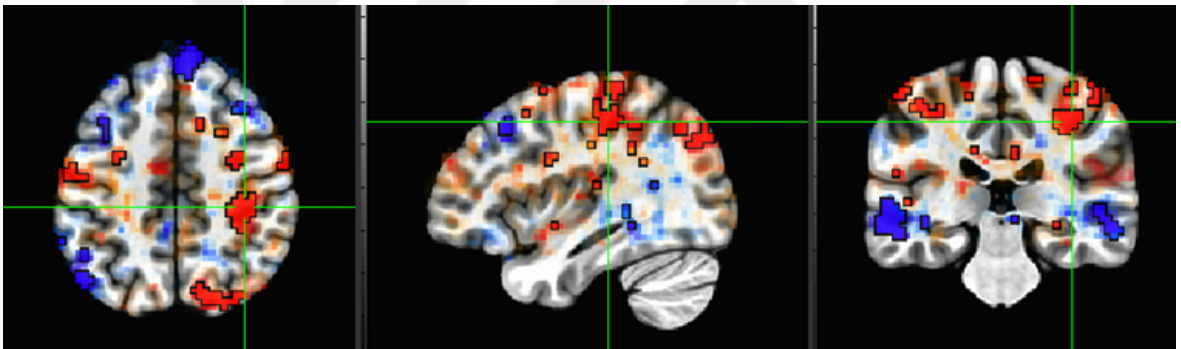


Figure 3.19 Activation in the postcentral gyrus of the contrast Caff-30 > Caff-10.

In contrast, there is a higher activation in the temporal gyrus in the Caff-10 than in the Caff-30 group.

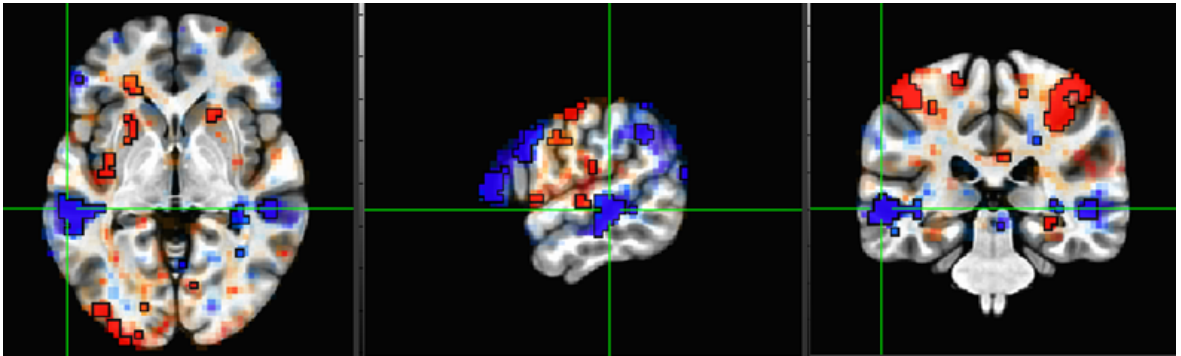


Figure 3.20 Activation in the temporal gyrus of the contrast Caff-30 > Caff-10.

Moreover, Caff-30 shows higher activation than Caff-10 in the posterior insula.

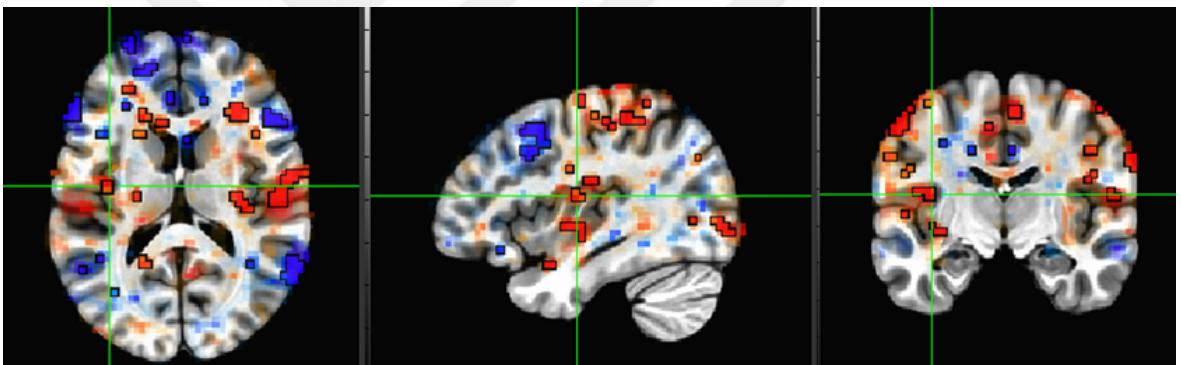


Figure 3.21 Activation in the posterior insula of the contrast Caff-30 > Caff-10.

Finally, there is a higher activation in the Caff-30 group in the ventricles and parieto-occipital sulcus.

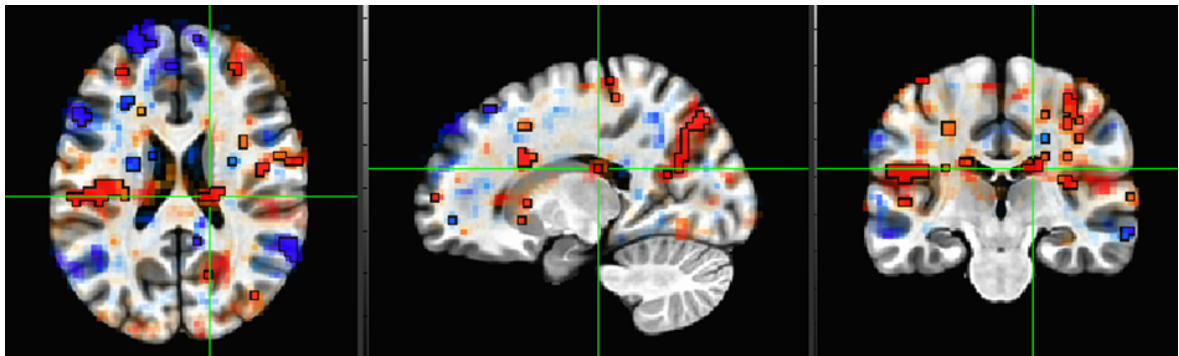


Figure 3.22 Activation in the ventricles of the contrast Caff-30 > Caff-10.

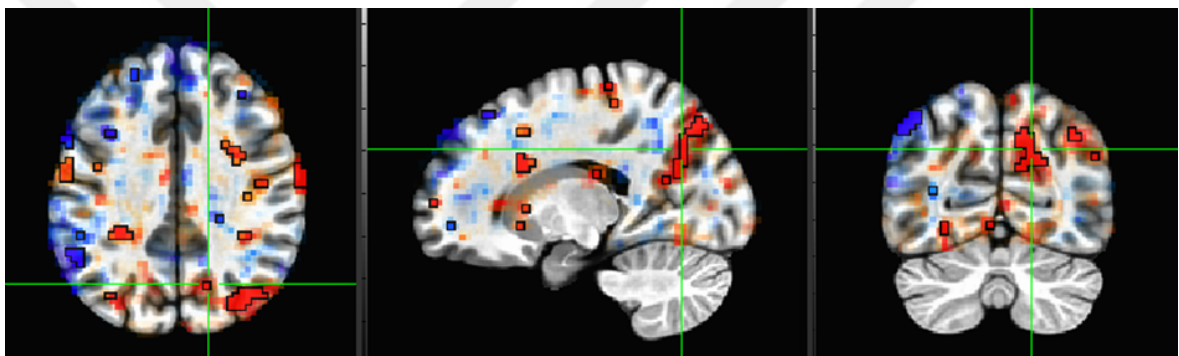


Figure 3.23 Activation in the occipital sulcus of the contrast Caff-30 > Caff-10.

These patterns may resemble cardiac activity, since venous structures typically follow the course of the cortical sulci.

When the significance is decreased to $p < 0.10$, some negative activations are seen in the ventricles.

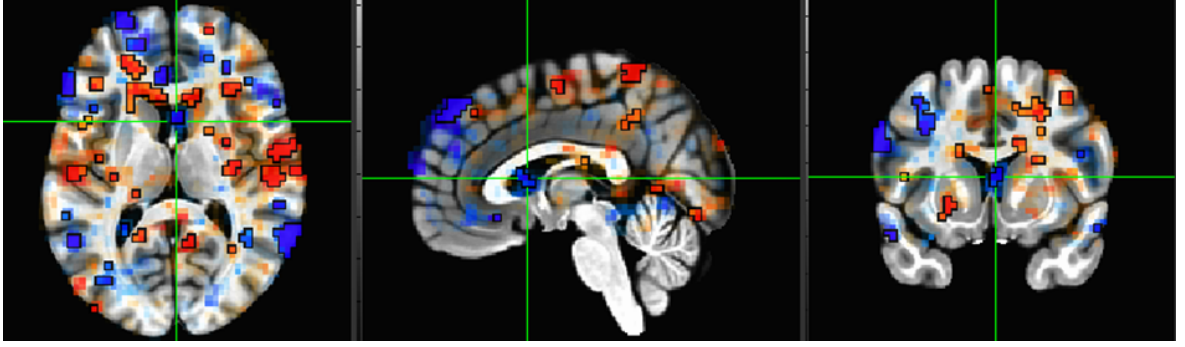


Figure 3.24 Activation in the ventricles of the contrast Caff-30 > Caff-10, $p < 0.10$.

3.2 ICA

ICA was conducted for task and rest data. In all components, the spatial maps are thresholds are set between $z = 2.3$ and 15. Griffanti et al. (2017)'s classification guideline was followed to identify the components [58]. The red color for positive patterns and the blue color for negative patterns are used to show independent component maps.

3.2.1 Resting State ICA

Component 1 shows a ventricular and WM activation pattern. Also, activation in the subependymal veins is visible. Seeing it as the first component will be discussed.

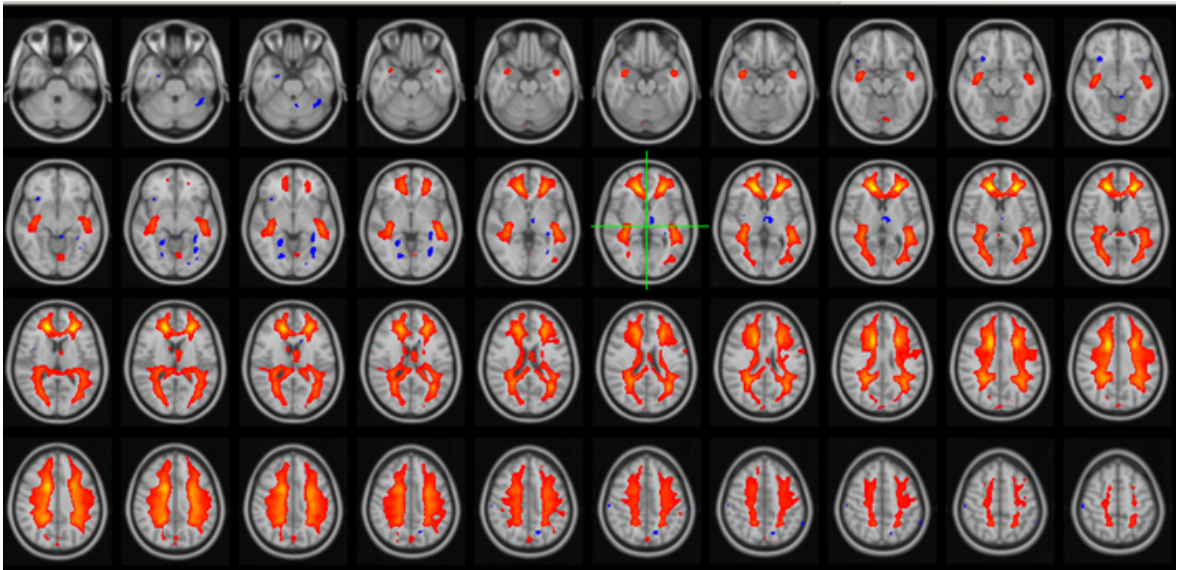


Figure 3.25 Component 1 of resting state, axial plane.

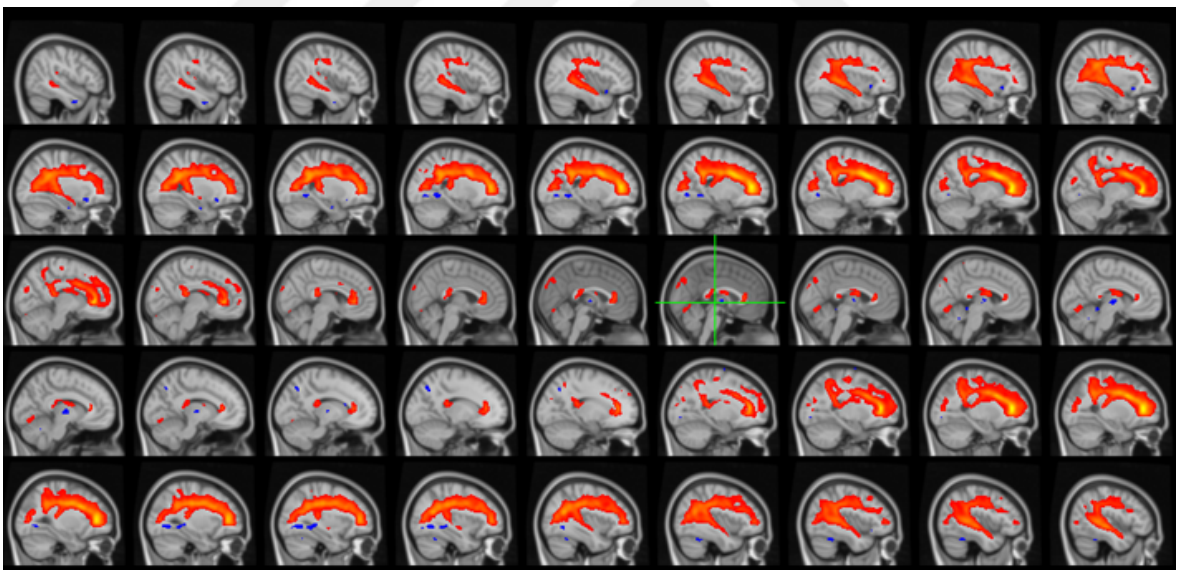


Figure 3.26 Component 1 of resting state, sagittal plane.

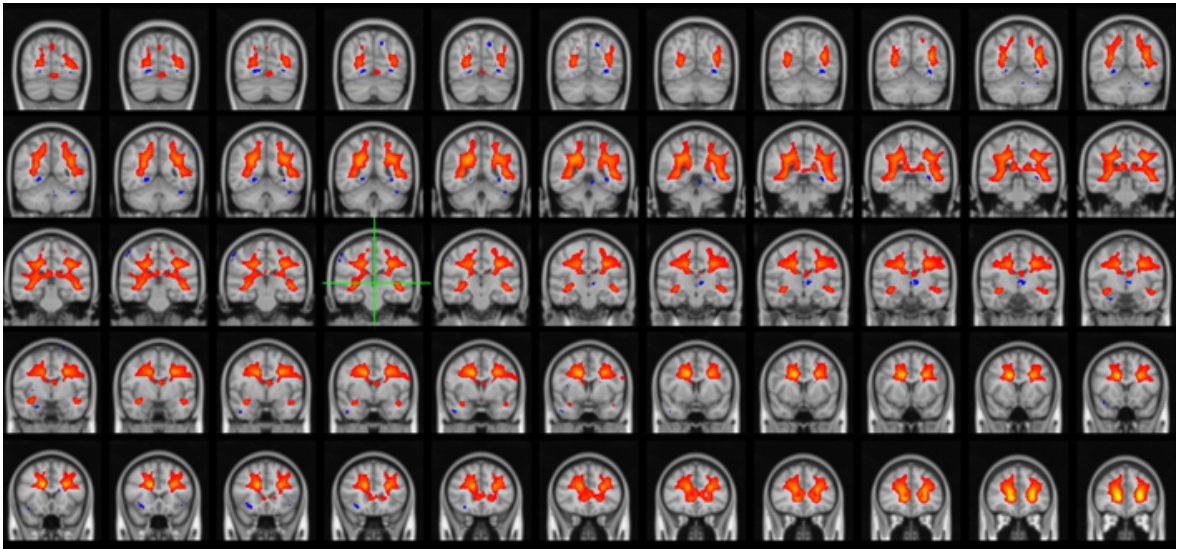


Figure 3.27 Component 1 of resting state, coronal plane.

Component 2 shows the DMN. In the resting state, seeing it in the first components was expected.

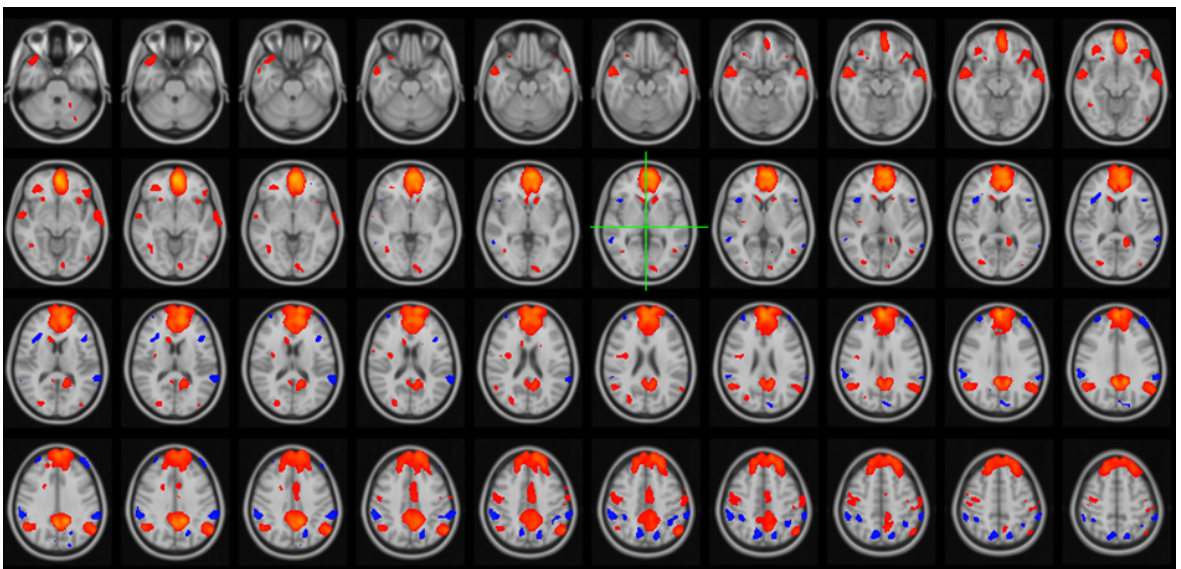


Figure 3.28 Component 2 of resting state, axial plane.

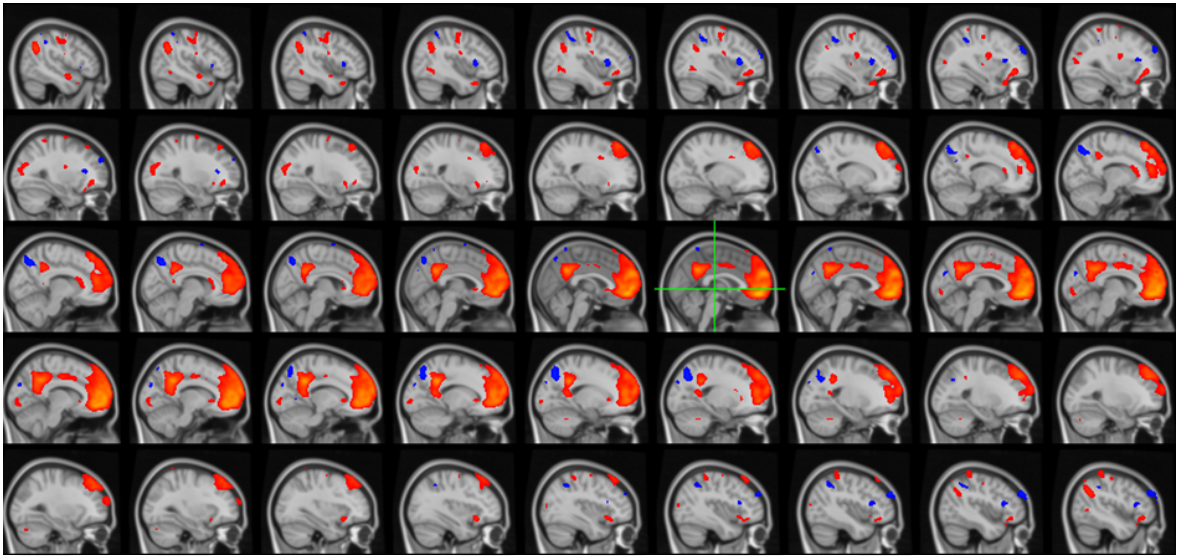


Figure 3.29 Component 2 of resting state, sagittal plane.

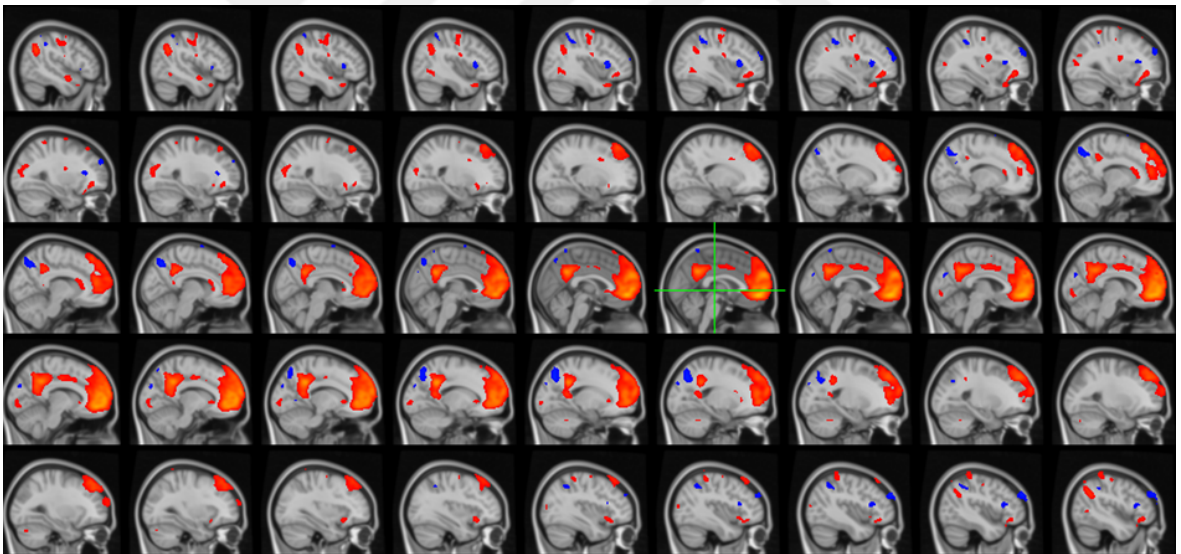


Figure 3.30 Component 2 of resting state, coronal plane.

Component 3 shows the insula along with SMN and VAN. The insula is specified in the map.

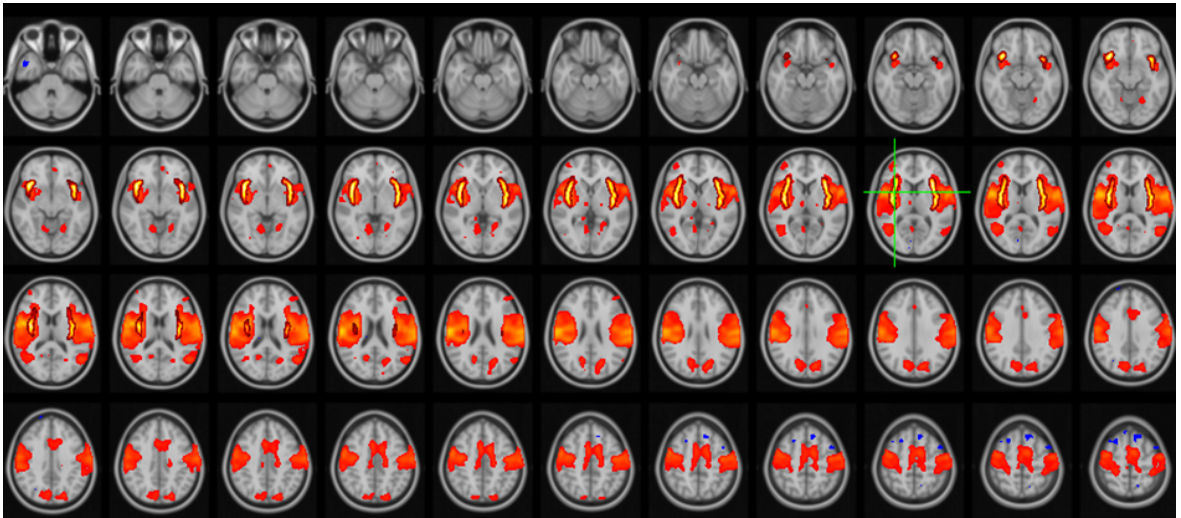


Figure 3.31 Component 3 of resting state, axial plane, insula specified.

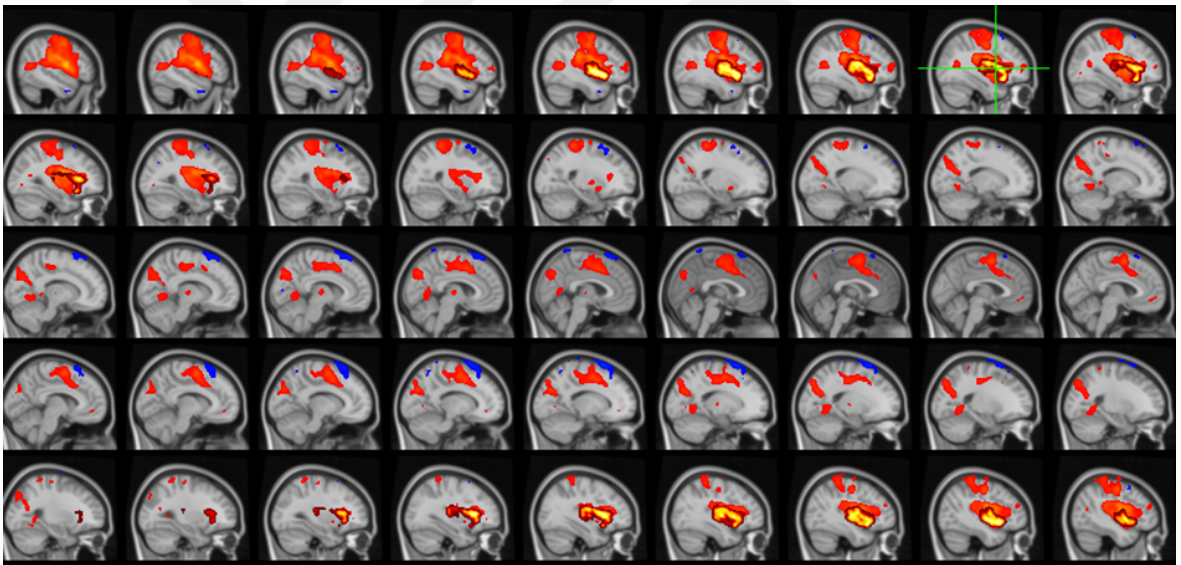


Figure 3.32 Component 3 of resting state, sagittal plane, insula specified.

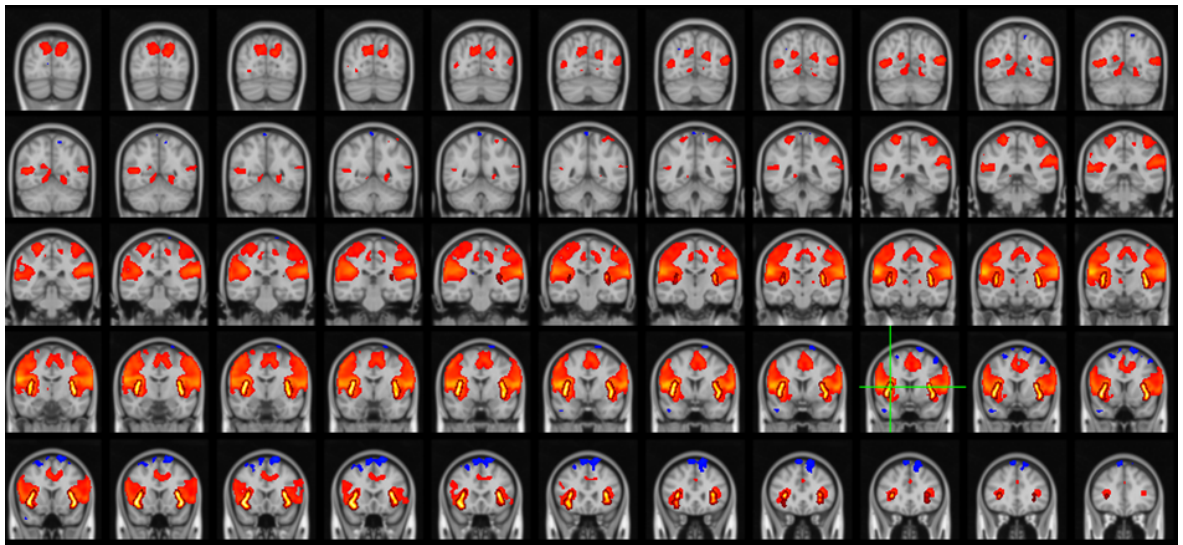


Figure 3.33 Component 3 of resting state, coronal plane, insula specified.

Component 4 shows VIS, which mostly covers the secondary visual cortex.

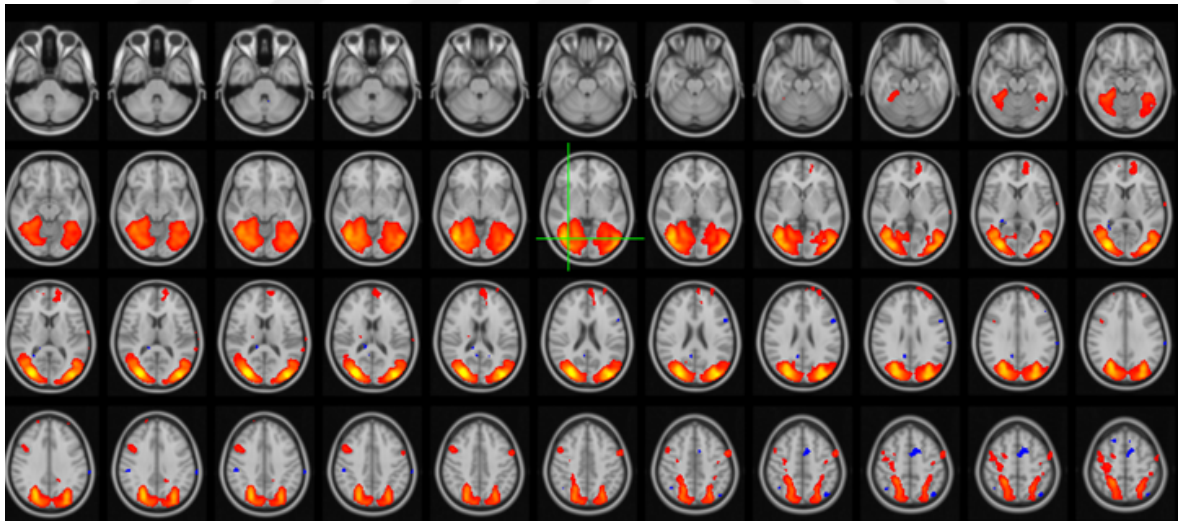


Figure 3.34 Component 4 of resting state, axial plane.

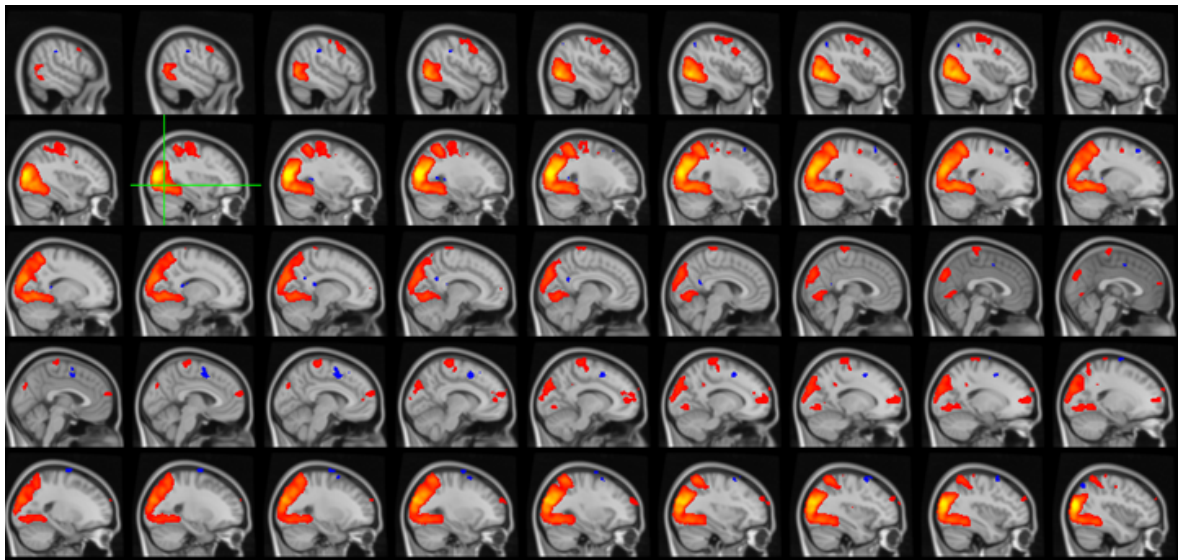


Figure 3.35 Component 4 of resting state, sagittal plane.

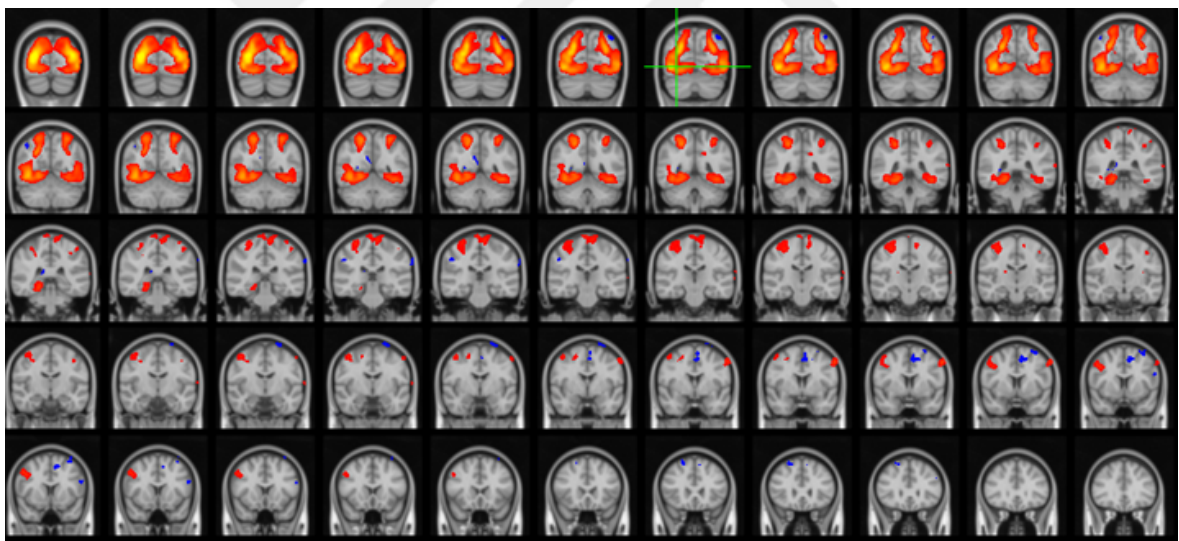


Figure 3.36 Component 4 of resting state, coronal plane.

Similarly, Component 5 is from VIS and mostly covers the primary visual cortex. Seeing two VIS related components in the first orders is a result of that resting state scans are eyes-open.

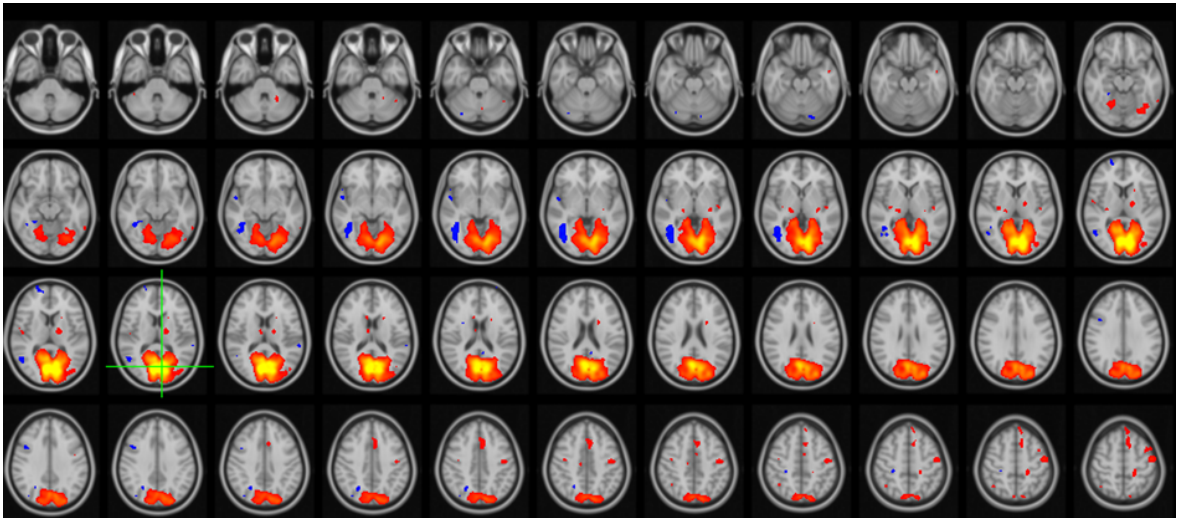


Figure 3.37 Component 5 of resting state, axial plane.

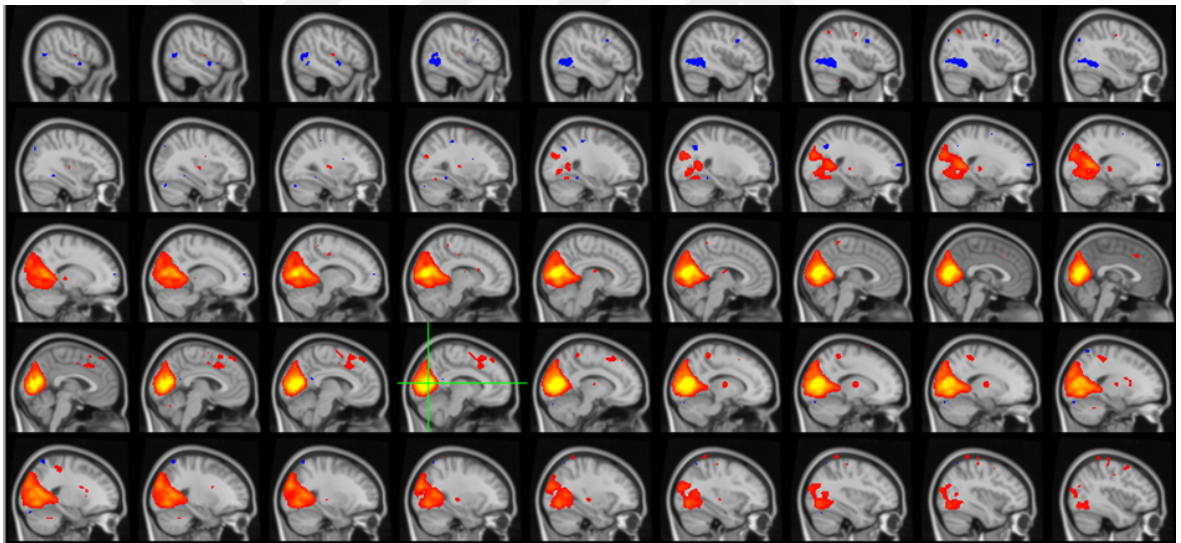


Figure 3.38 Component 5 of resting state, sagittal plane.

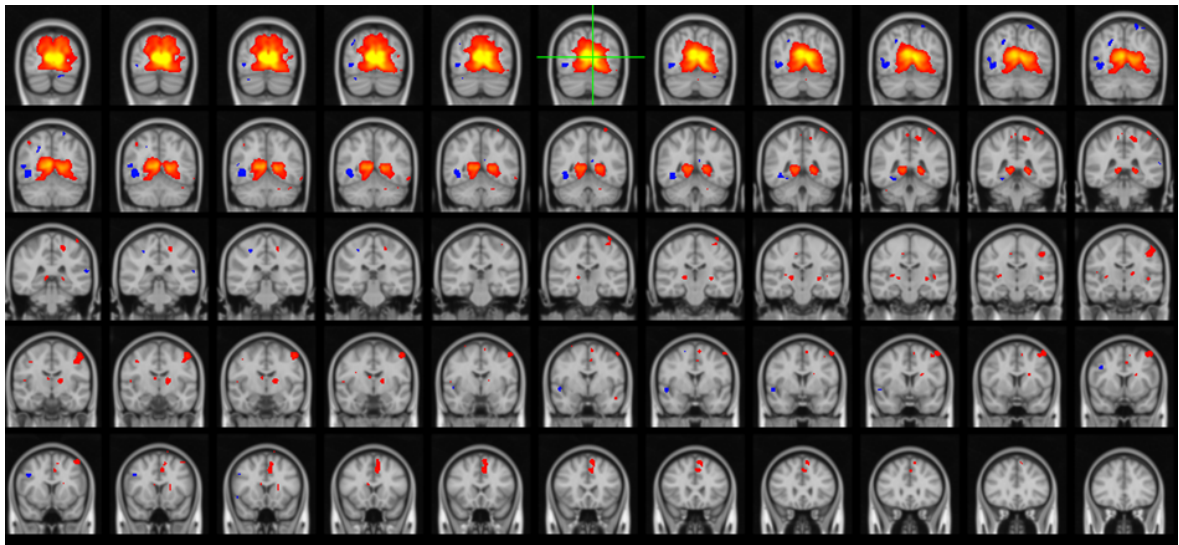


Figure 3.39 Component 5 of resting state, coronal plane.

Component 7 shows the IPS and angular gyrus, which are regions of DAN and VAN, respectively.

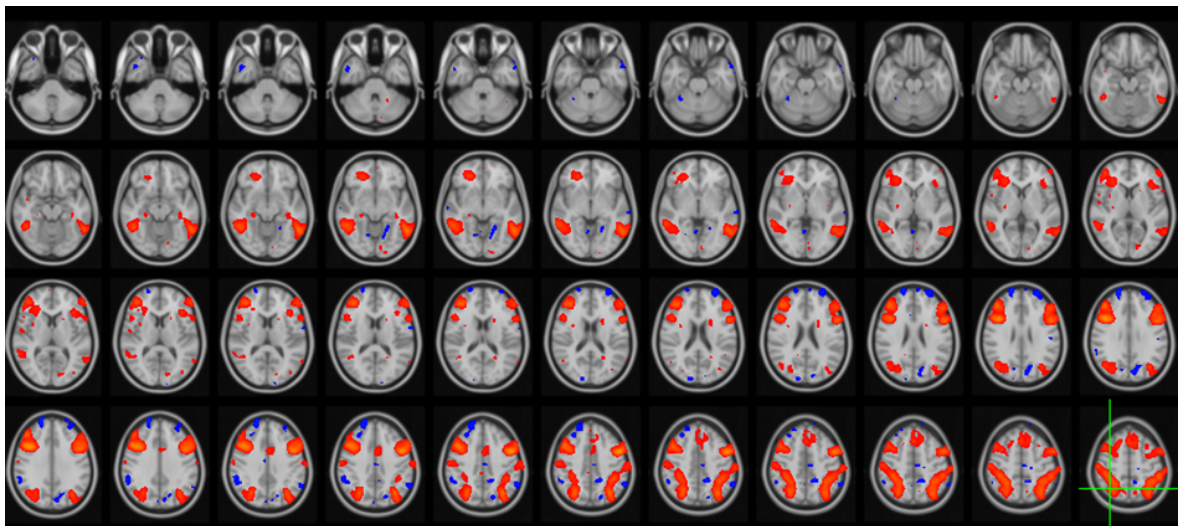


Figure 3.40 Component 7 of resting state, axial plane.

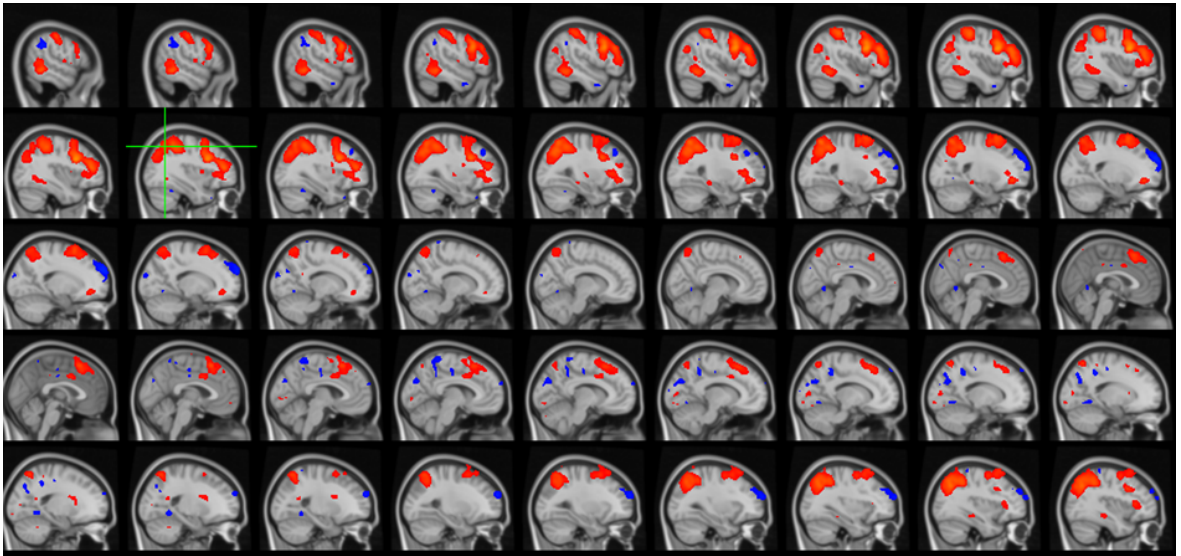


Figure 3.41 Component 7 of resting state, sagittal plane.

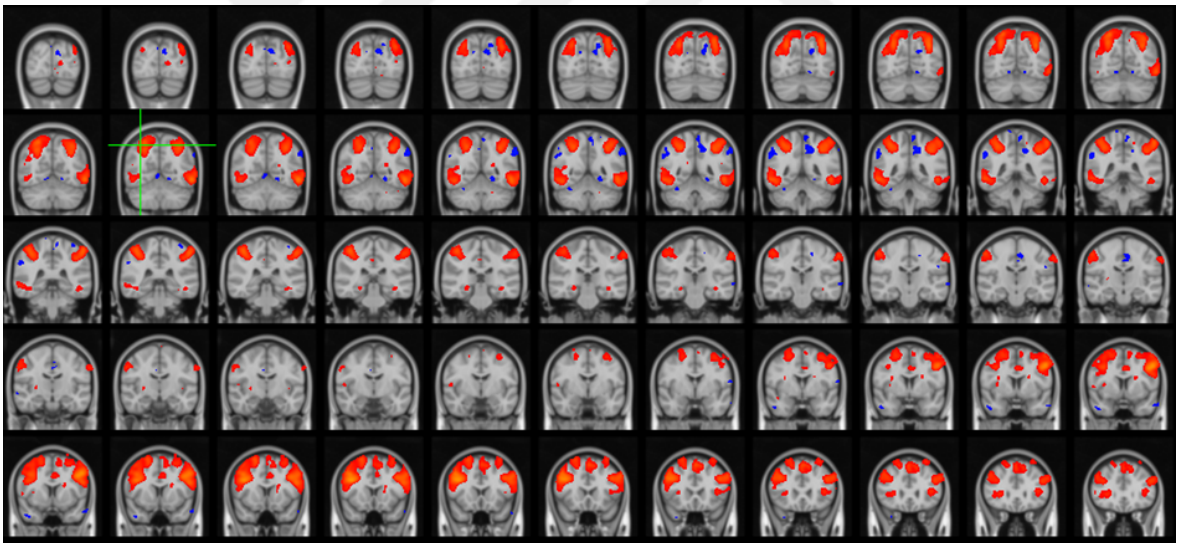


Figure 3.42 Component 7 of resting state, coronal plane.

Component 8 also shows IPS with FPN.

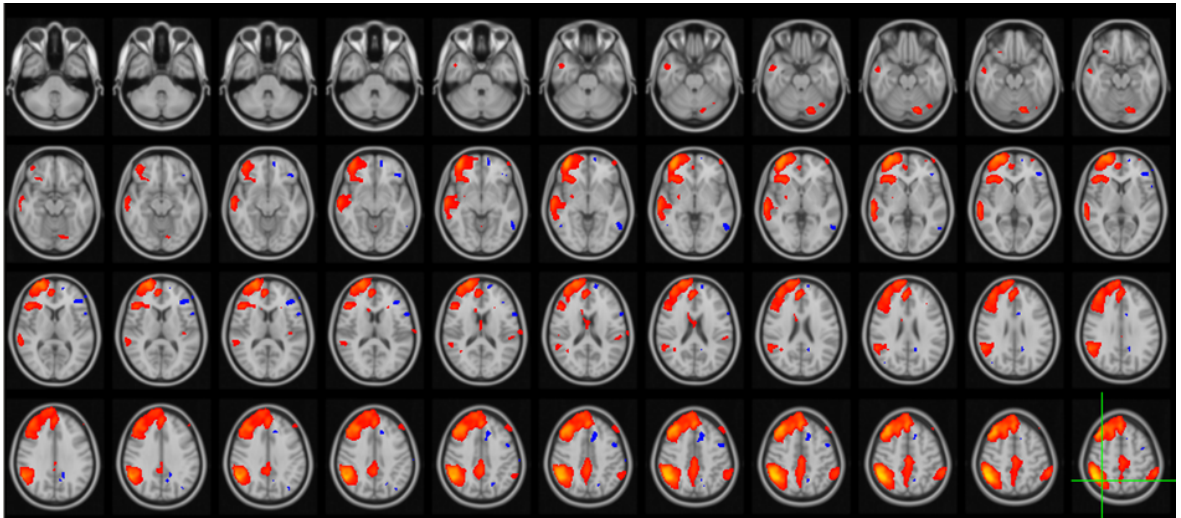


Figure 3.43 Component 8 of resting state, axial plane.

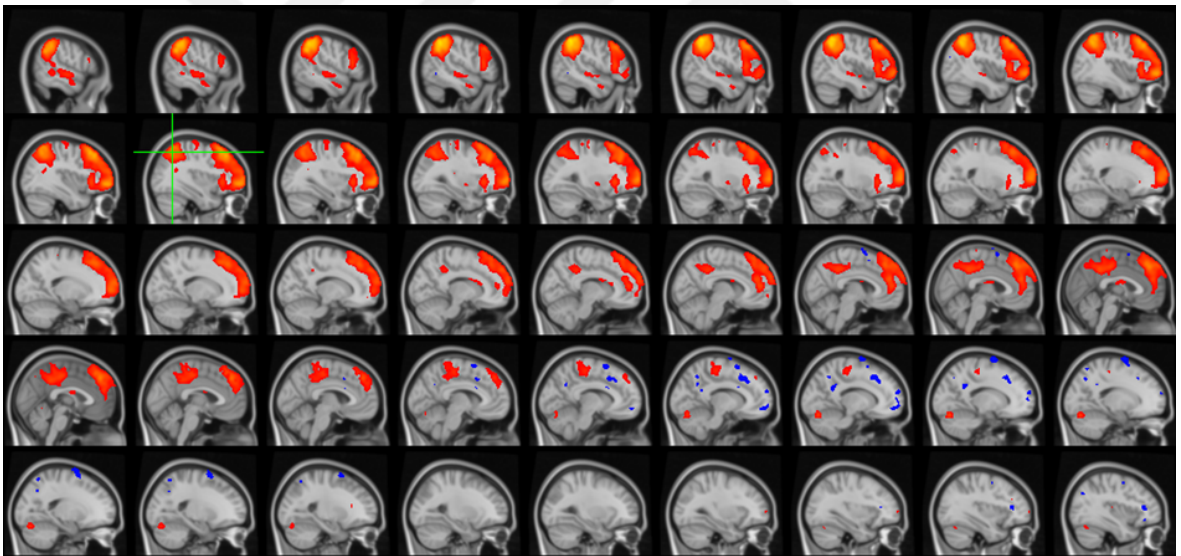


Figure 3.44 Component 8 of resting state, sagittal plane.

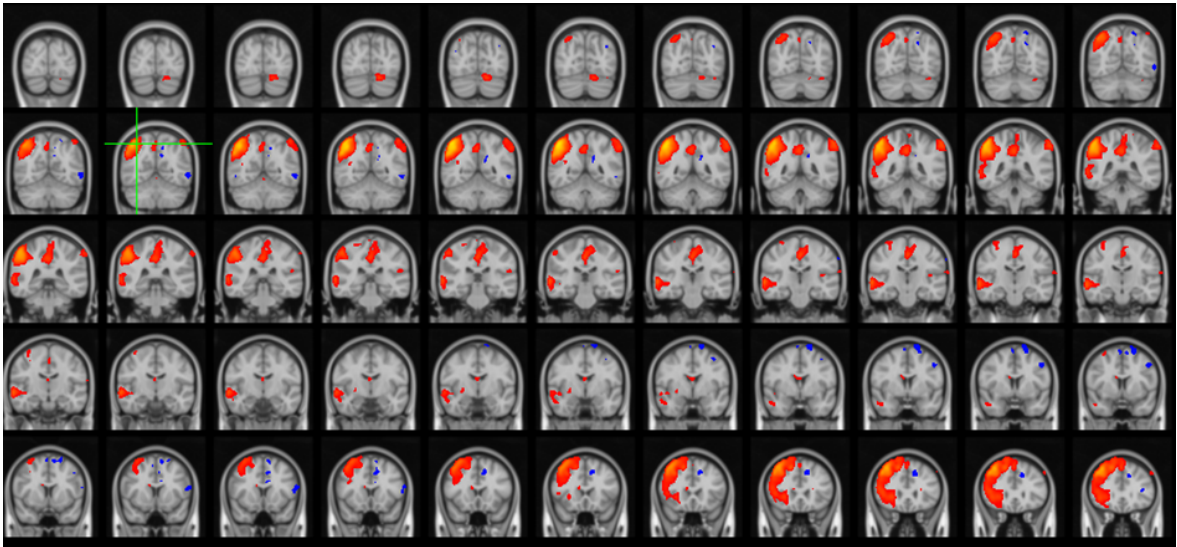


Figure 3.45 Component 8 of resting state, coronal plane.

In the latter components, there are mostly noise and random signals that show parts of other RSNs. For example, Component 15 shows the sagittal sinus.

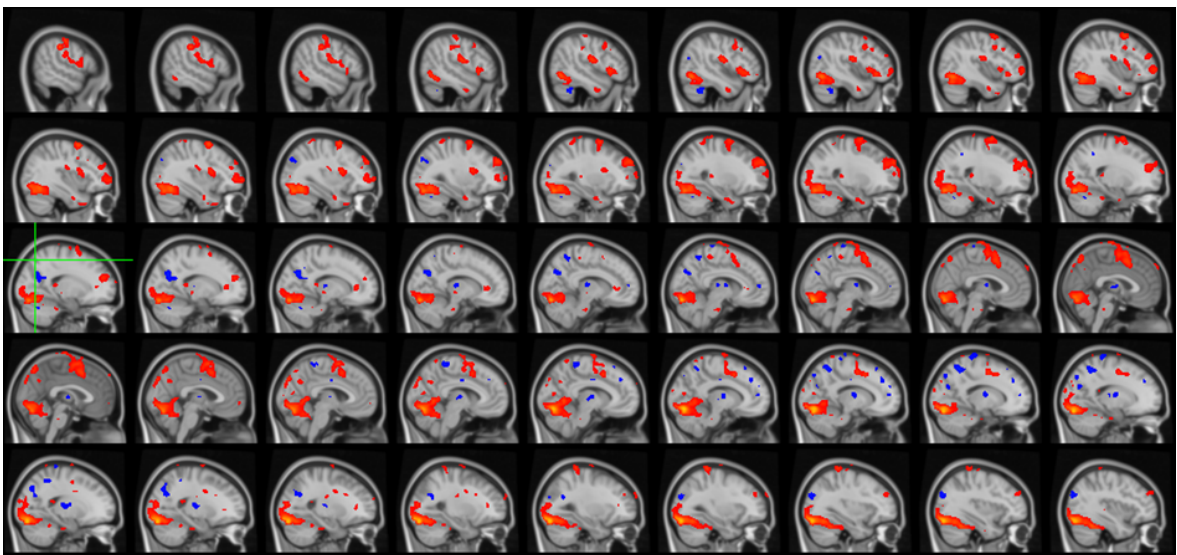


Figure 3.46 Component 15 of resting state, axial plane.

ICA shows that RSNs and non-neuronal, potentially systemic-related signals are seen as spatial maps. To investigate the effect of caffeine on these networks, dual

regression is conducted.

3.2.2 The Effect of Caffeine on Resting State with Dual Regression

According to the dual regression results of the resting state, there is no statistically significant difference between Caff-30 and Caff-10 conditions. Unfortunately, there are only marginally significant results and they are outside the corresponding component. Thus, we only reported them to present results.

One voxel in intracalcarine sulcus is marginally higher in Caff-30 group than Caff-10 group, $p < 0.07$, corrected. The voxel is outside the component, which is reflecting the DMN. Therefore, it might be a distraption effect of caffeine on the DMN through a vessel activation. However, one marginally significant voxel is not sufficient to interpret it.

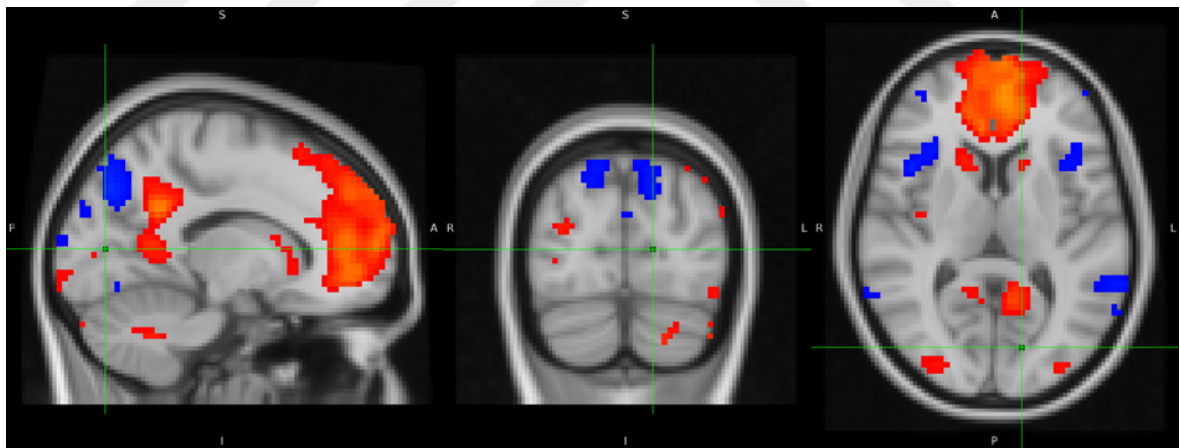


Figure 3.47 Dual regression result of resting state in component 2 for Caff-30 > Caff-10 contrast.

3.2.3 Task-based ICA

The first component in task-based ICA represents the visual cortex and IPS. When considering the task-based design, those networks and regions are the most

relevant ones, and seeing them as the first component was expected.

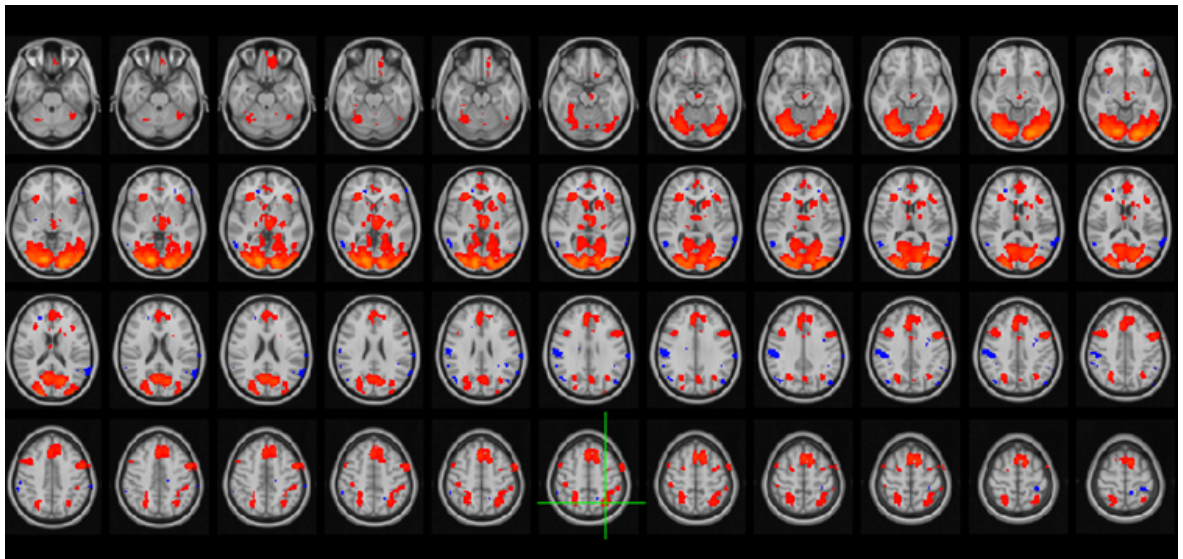


Figure 3.48 Component 1 of task-based, axial plane.

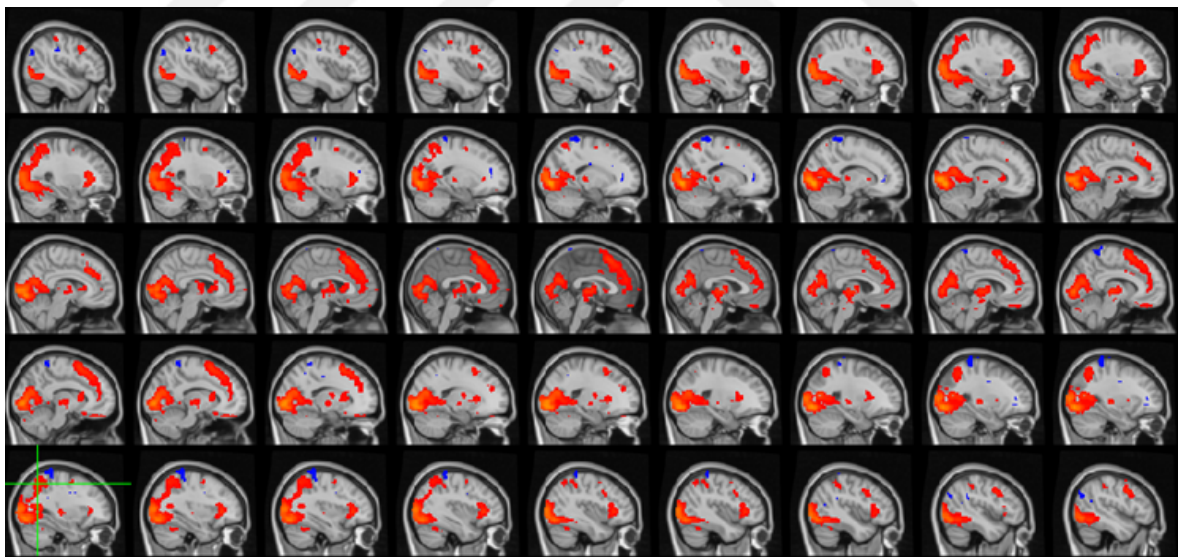


Figure 3.49 Component 1 of task-based, sagittal plane.

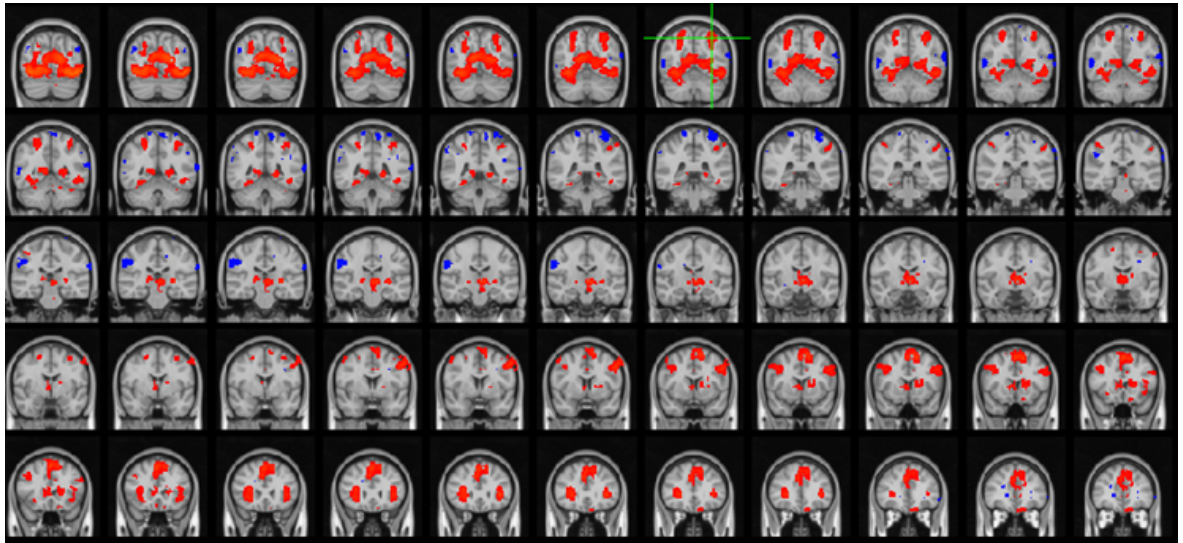


Figure 3.50 Component 1 of task-based, coronal plane.

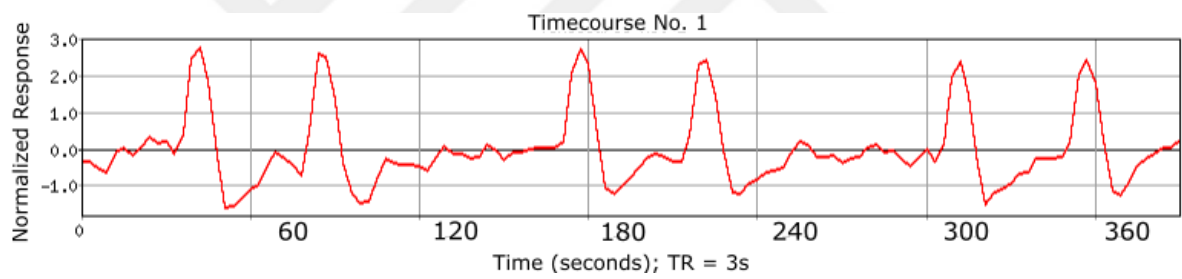


Figure 3.51 Time course of component 1 of task-based.

Component 9 shows the WM track and ventricles.

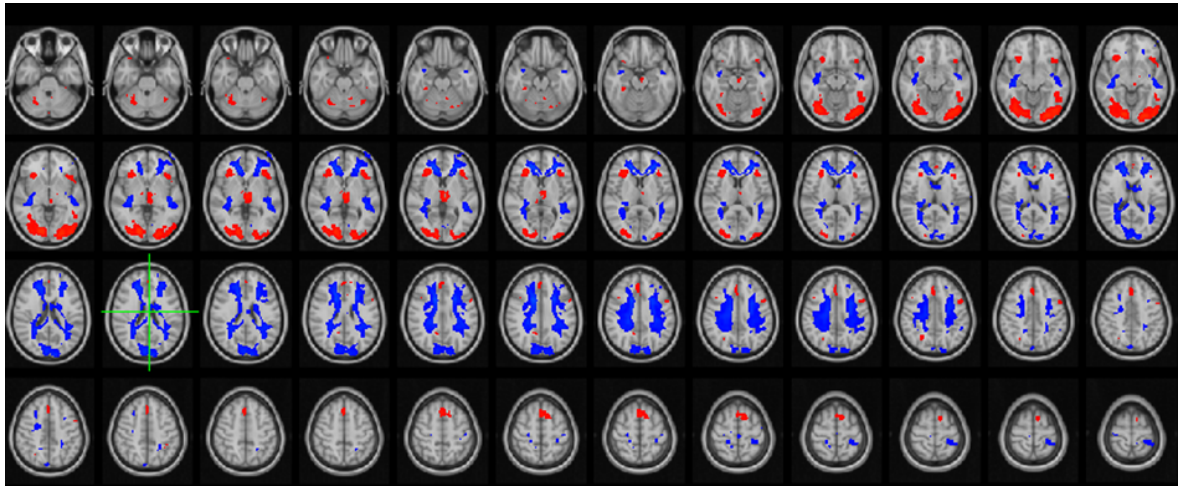


Figure 3.52 Component 9 of task-based, axial plane.

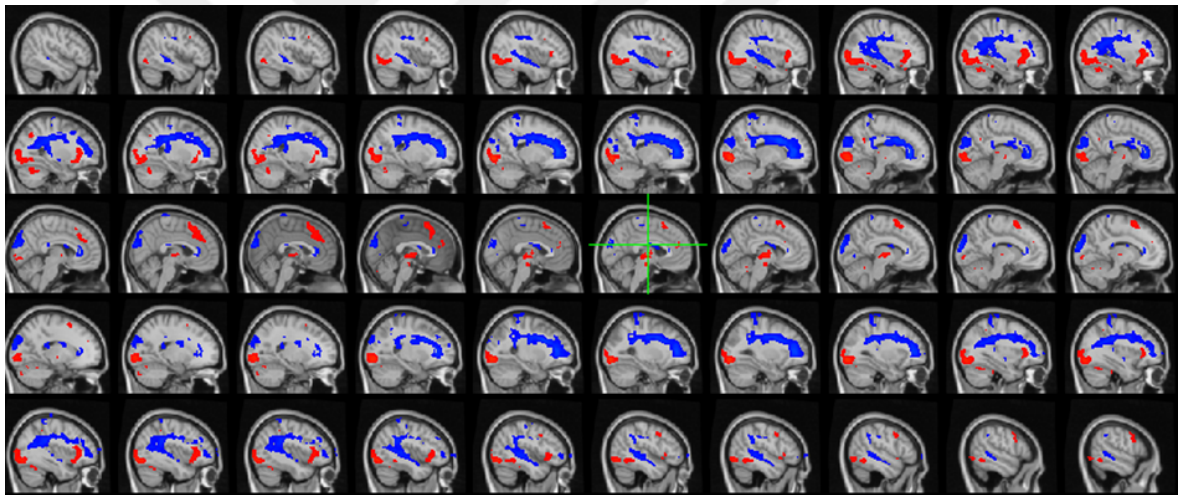


Figure 3.53 Component 9 of task-based, sagittal plane.

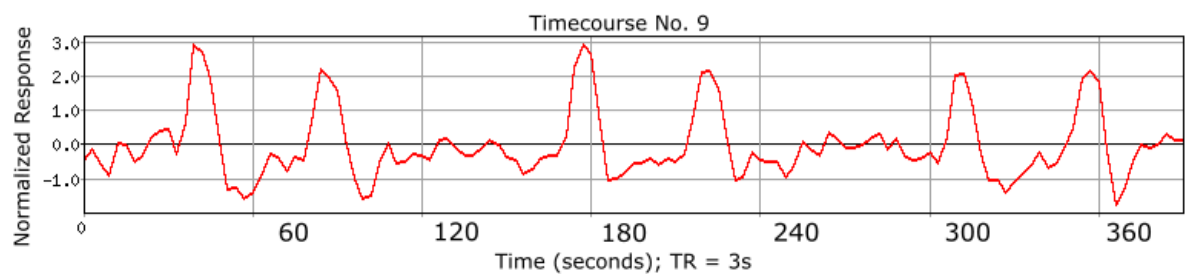


Figure 3.54 Time course of component 9 of task-based.

Component 10 shows the insula with some activation in visual and frontal cortex.

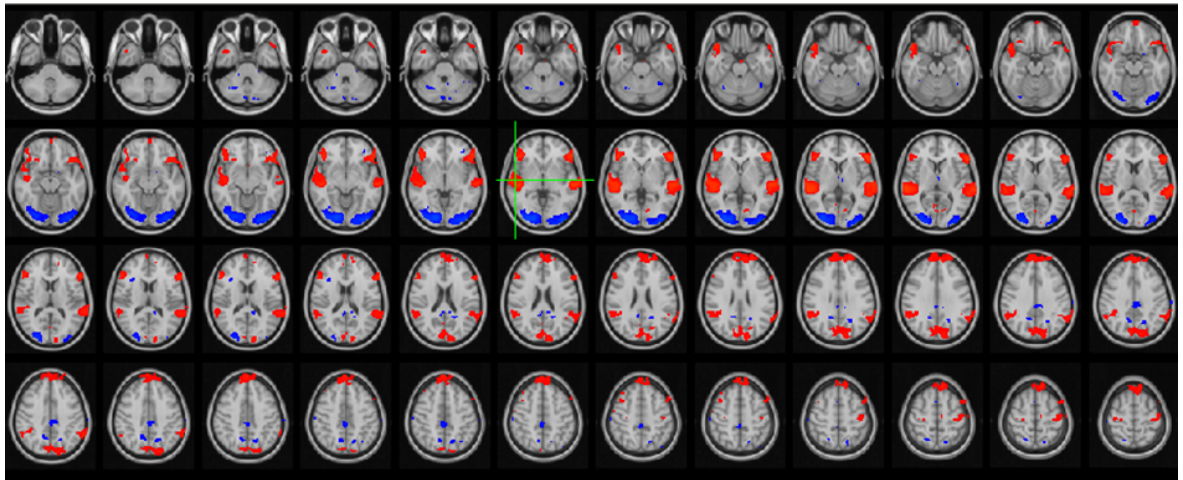


Figure 3.55 Task Component 10 axial.

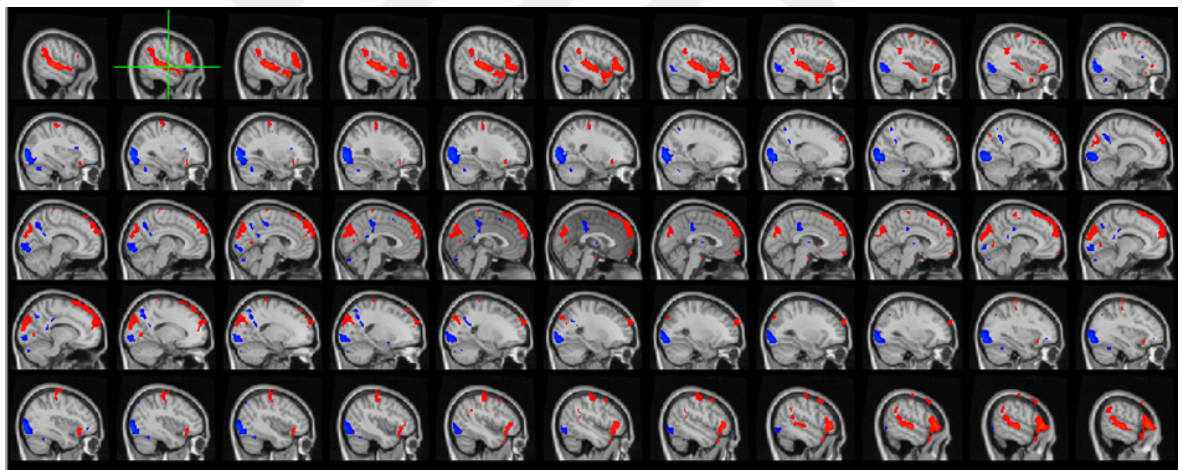


Figure 3.56 Task Component 10 sagittal.

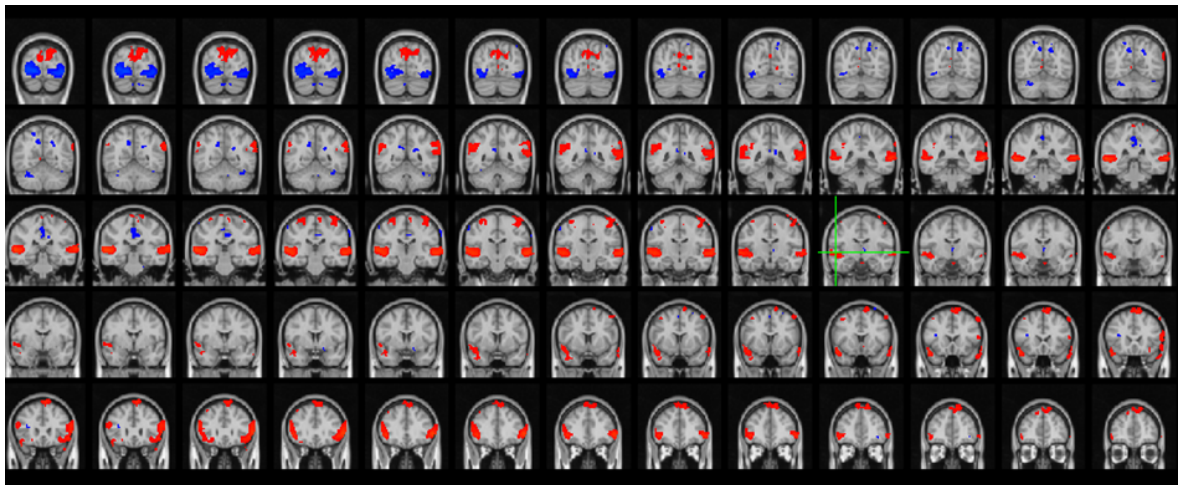


Figure 3.57 Task Component 10 coronal.

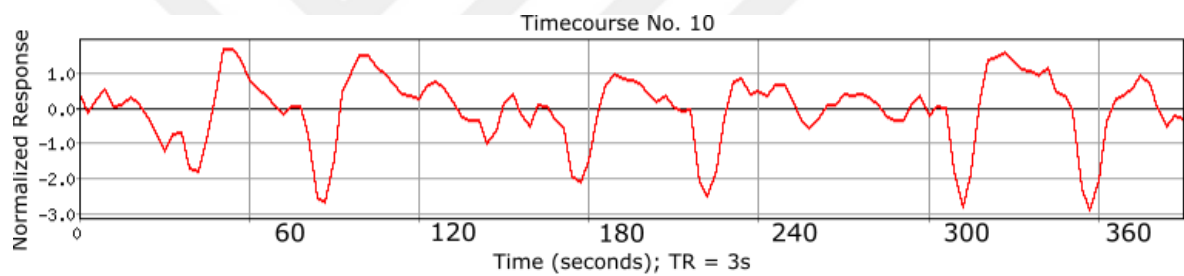


Figure 3.58 Task Component 10 time-course.

3.2.4 The Effect of Caffeine on Task-Based with Dual Regression

Likewise, there are no statistically significant differences between the Caff-30 and Caff-10 groups in terms of caffeine effect in task-based dual regression results. Nonetheless, the results are presented.

There is a marginally significant difference between Caff-30 and Caff-10 groups in component 18, which is a noise component. Thus, this is an uninterpretable result for caffeine effect.

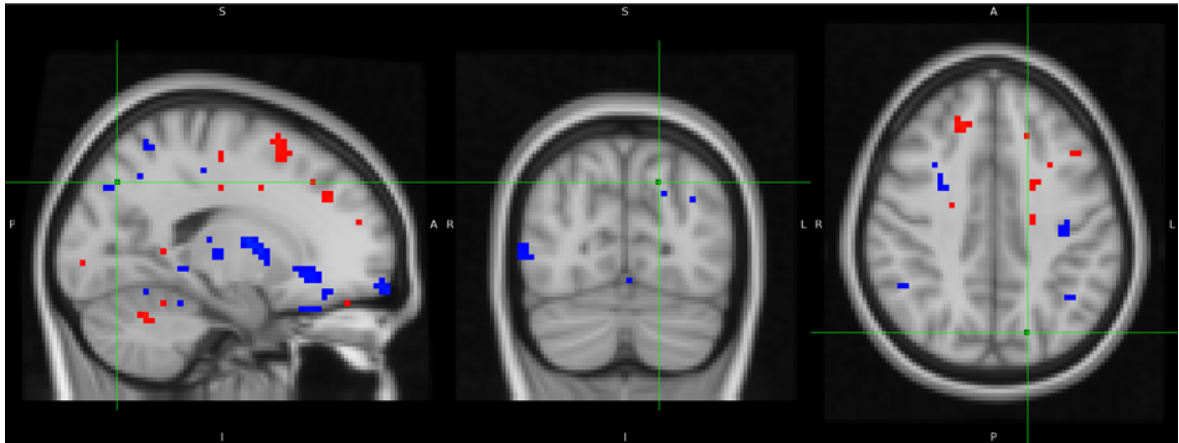


Figure 3.59 Dual regression result of task-based in component 18 for Caff-30 > Caff-10 contrast.

3.3 Cross-Correlation Analysis with RVT and PPG-Amp

The correlation of physiological signals and whole-brain voxel-wise analysis results are investigated for task and rest with time-lagged cross-correlations.

Group comparisons of average cross-correlations are provided for both RVT and PPG-Amp. Correlations at all lags are presented. For the visualization, the correlation coefficient r values between -0.2 and 0.2 are colored.

3.3.1 Rest Cross-Correlations

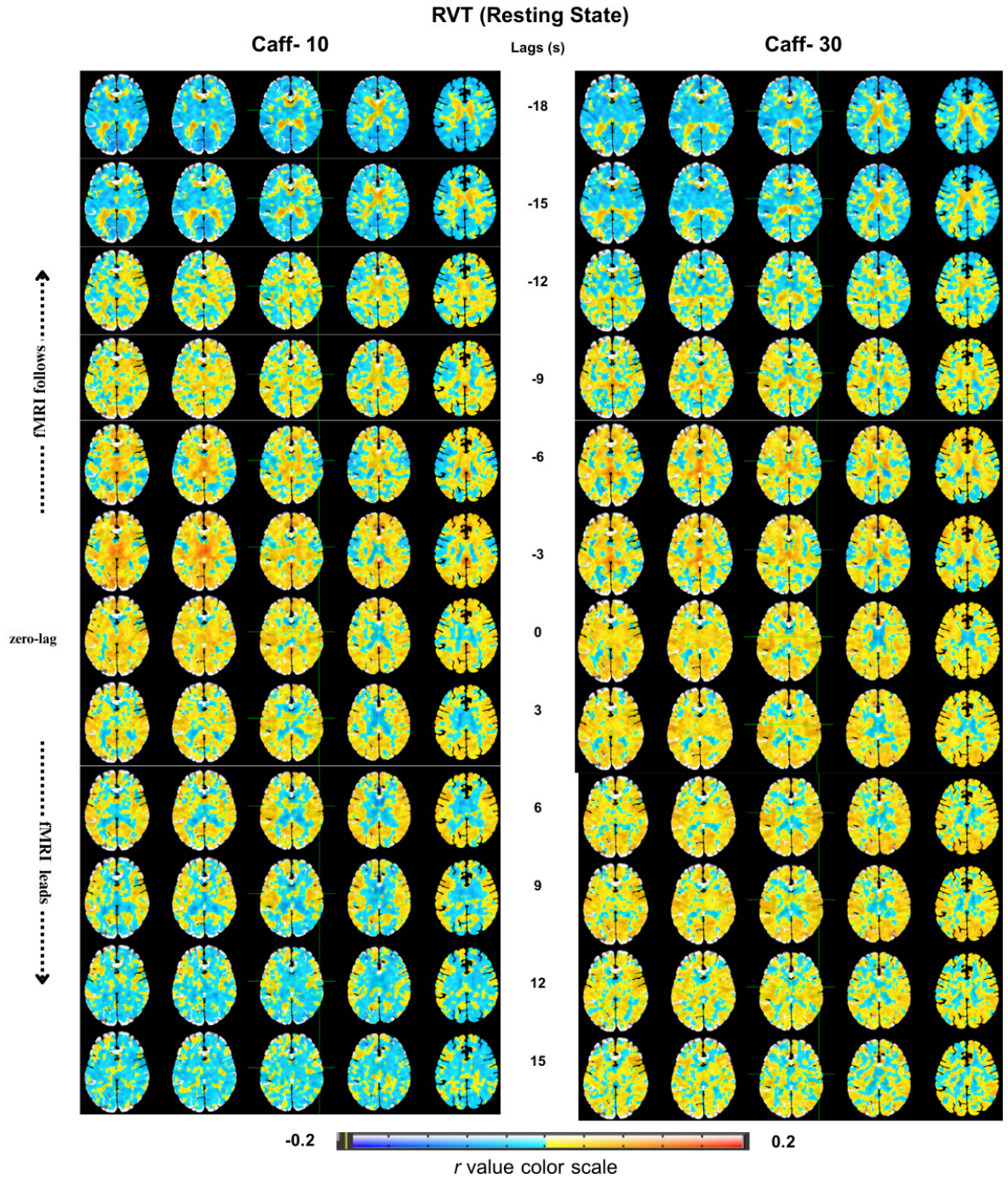


Figure 3.60 Resting-state cross-correlation between RVT and whole-brain.

When fMRI leads (bottom lags), the caffeine's systemic effect is more visible for RVT correlation in resting-state. Stronger early negative and prolonged positive correlations in Caff-30 are observed.

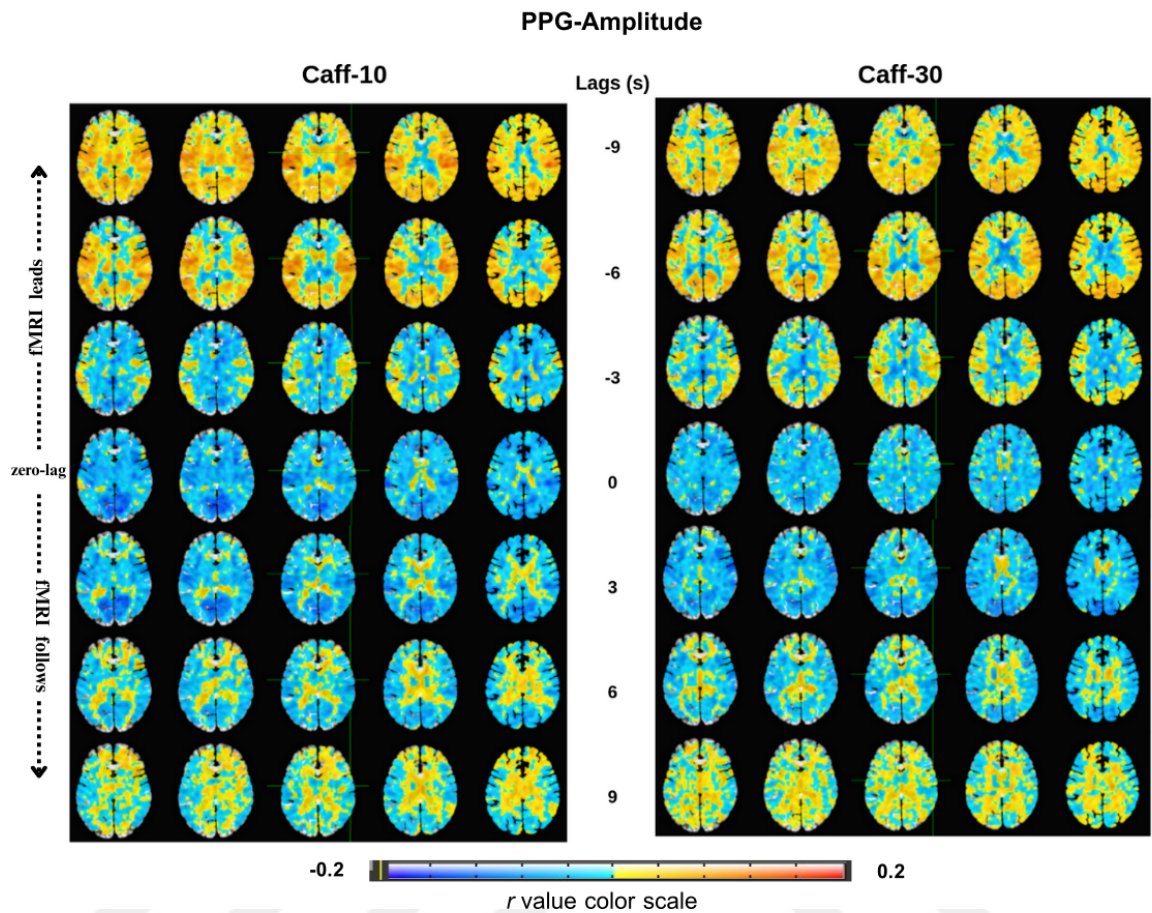


Figure 3.61 Resting-state cross-correlation between PPG-Amp and whole-brain.

At zero-lag, the caffeine effect can be seen in the correlation between PPG-Amp and whole-brain resting-state. There are prolonged positive correlations and patterns of CSF-WM in Caff-30. Also, there are stronger negative correlations in VIS in Caff-10. Instead of a focused effect in VIS, a widespread effect is seen in Caff-30, potentially due to eyes open resting-state condition.

3.3.2 Task Cross-Correlations

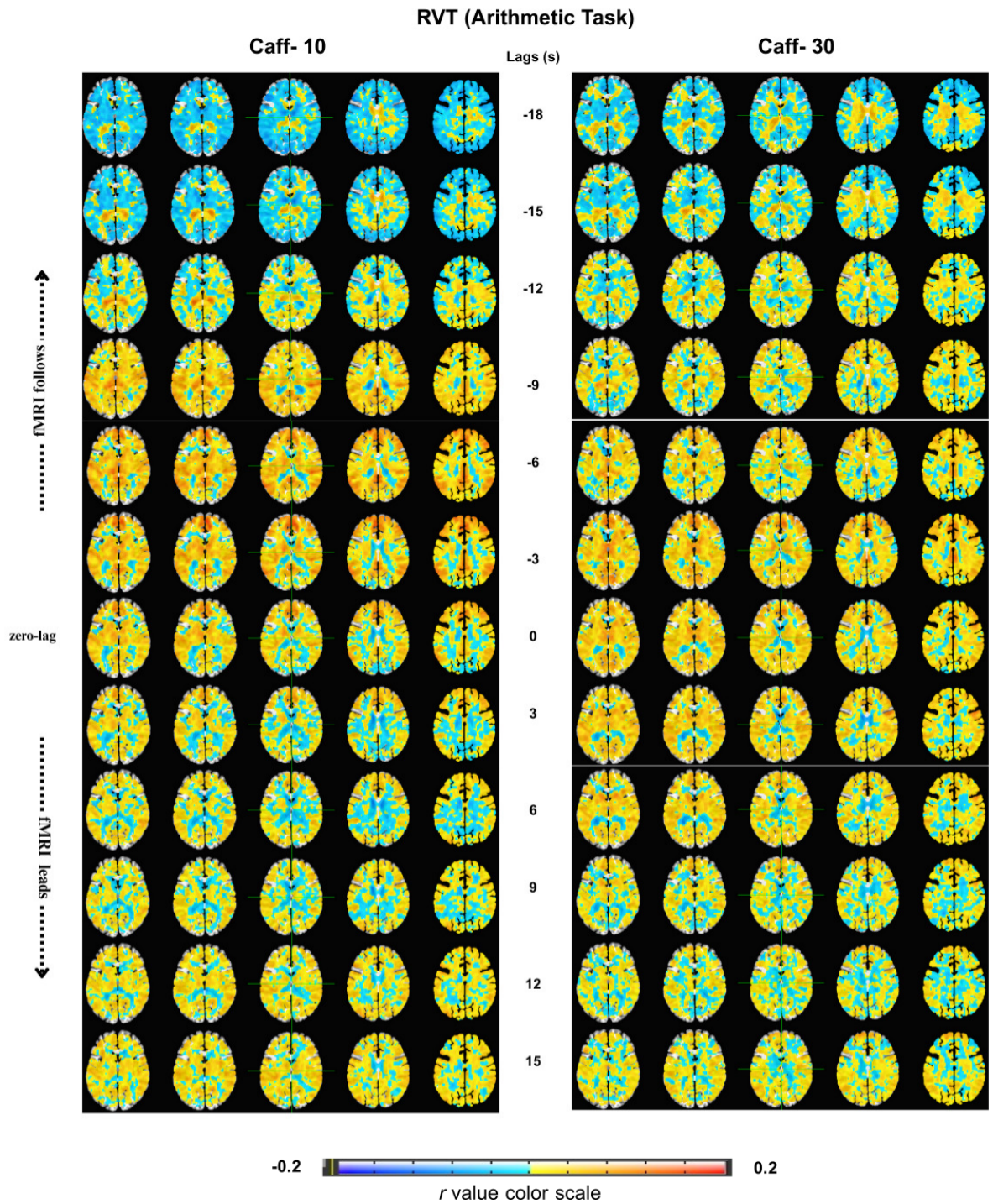


Figure 3.62 Task-based cross-correlation between RVT and whole-brain.

Although there is a diminutive difference, caffeine effect is more visible in the farthest lags for RVT and whole-brain correlation in task-based. There are prolonged

positive correlations in Caff-30. Also, larger ventricle patterns at early lags in Caff-30.

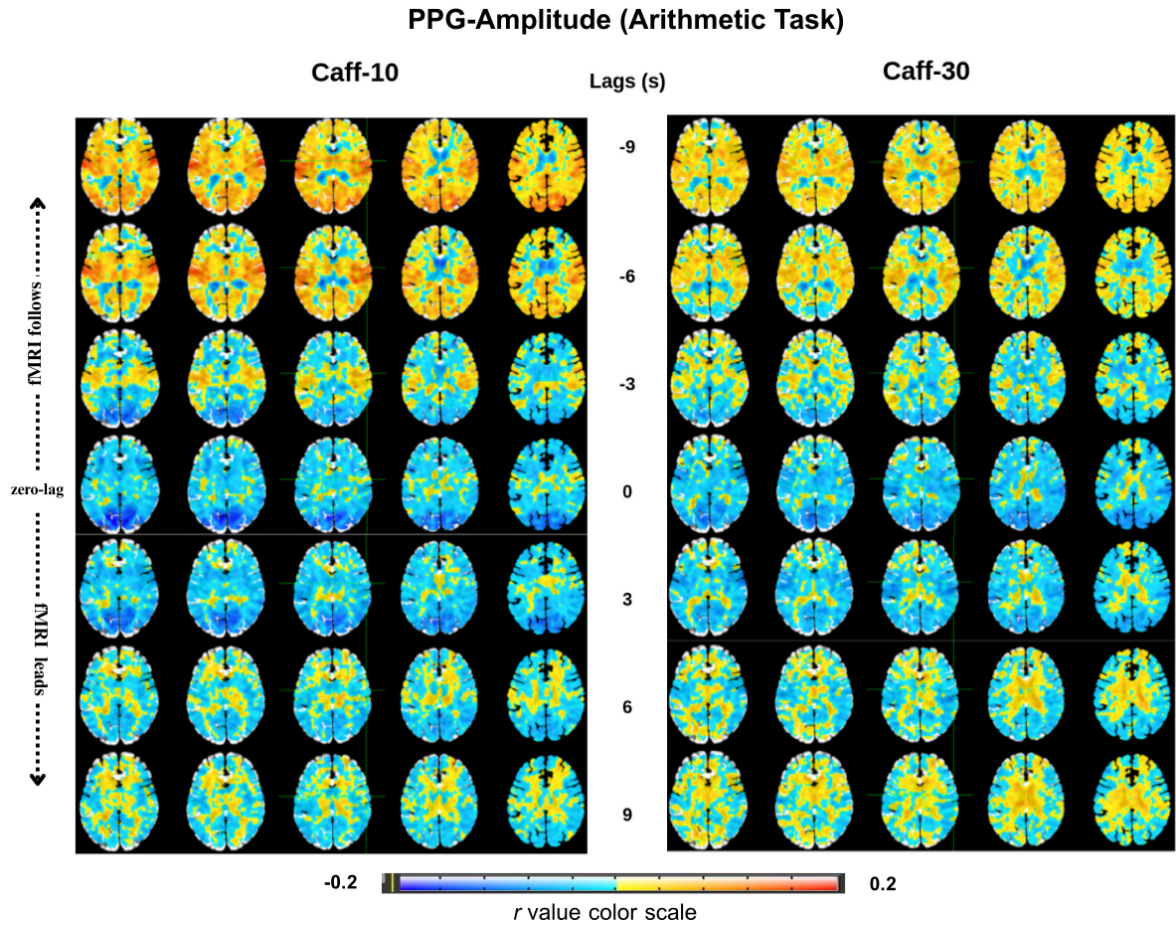


Figure 3.63 Task-based cross-correlation between PPG-Amp and whole-brain.

WM-CSF patterns are visible across lags in Caff-30 in the correlation between PPG-Amp and whole-brain in task-based. In addition, less VIS-related correlation, rather more widespread pattern are observed.

4. DISCUSSION

4.1 GLM

4.1.1 10-Min Caffeine

The mean beta effects showed task-activated BOLD response in the networks of FPN, VIS, and DAN. Those regions are task-positive, i.e., they are more active in more cognitively demanding tasks. In contrast, the DMN is considered a task-negative network due to its anticorrelations [59]. Although solving basic arithmetic equations is not highly demanding, they are sufficient to show BOLD activation in these networks, and they require selective attention, visual attention, and problem-solving, which are the main roles of the TPNs. Also, when the general activation map is inspected, negative activations in DMN-related areas are observable, even though not all regions of the DMN are statistically significant.

IPS is a region belonging to DAN. As mentioned in the introduction section, it has an exclusive place. Many studies indicated that it is responsible for symbolic number processing, active during changes in numbers, and its abnormality is associated with dyscalculia [60],[61],[62],[63],[64],[65]. In our study, it is visible, and a BOLD activation is seen in both left and right IPS.

4.1.2 30-Min Caffeine

Almost the same aforementioned patterns are traceable in the Caff-30 condition. The networks that are activated with the task are VIS, FPN, and DAN, with apparent activity in IPS. In addition, the DMN components, precuneus and ACC, are highly distinguishable. Moreover, a distinct activity in the insula, which is highly related to attention, is seen.

Despite the general similarity between the Caff-10 and Caff-30 conditions, there are small differences because of the possible effect of caffeine. Therefore, the results of the paired t-test are examined.

4.1.3 The Difference Between 10- and 30-Min Caffeine

Although we had to select a more lenient significance threshold to see the differences, there are important regions that show how caffeine interacts with the task-activated BOLD signals.

First of all, the right IPS is significantly more active in the Caff-30 condition. Both left and right IPS are related to mathematical ability. However, the right IPS is more linked to mathematics by its structural and functional connections with the frontal region in the developmental age [66]. However, it is not possible to know whether caffeine directly affects this region more or if the task activation is more visible in the right IPS, and caffeine's effect on attention increases the performance and the BOLD signal as well. Moreover, the same pattern is observed in the insula. It is highly associated with attention, and it is more active in the Caff-30 condition.

In search for the systemic effect of caffeine, it is seen that the parieto-occipital sulcus has higher BOLD signals in the Caff-30 condition. Sulci have more veins, and seeing this difference, along with this sulcus, possibly reflects a vascular effect on the BOLD and task interaction. In addition, some small activations are more visible in the Caff-30 condition. However, although the significance of the threshold needed to be increased to $p < 0.10$, some negative activation in the anterior ventricles might be seen, so there is lower activation in the Caff-30 condition. These results imply the different effects of systemic activation on BOLD signals.

As a result, in both groups, regardless of the caffeine condition, the same regions are significantly related to task design. VIS, DAN, and IPS show significant activation with the task. In group analysis, there are some regions that show differences in the

IPS and insula. Those are more active in the Caff-30 condition. When considering the ability of caffeine to increase attention and awareness, seeing this difference in a limited time window is important to present the rapid effect of caffeine. However, the other inconsistent results should not be ignored when interpreting these differences.

There are two reasons why we did not see explicit effects of caffeine. Firstly, it is task data, and the GLM analysis tries to figure out which voxels are correlated with our task design. IPS activation in both groups mentions that the interaction between arithmetic equation solving and IPS activation supports the literature. However, caffeine probably has no direct effect on mathematical operations, but an indirect effect by increasing attention and awareness. Nevertheless, someone might have predicted that there were significant systemic effects of caffeine in ventricles or sinuses, because, as we see in ICA results, task-activated regions are also highly correlated with those regions. Presumably, the low sample size resulted in low power. Thus, when we set a liberal p -value, the trending differences can be seen in the ventricles and the occipital sinus.

4.2 ICA and Dual Regression

4.2.1 Rest ICA

The first component of rest ICA shows WM and ventricular patterns. Seeing the first spatial map as a depiction of systemic effect points that vasculature is one of the main sources that form BOLD signals. In previous research, it has been shown that sympathetic activation of cerebral vasculature is involved in CBF regulation and contributes to the BOLD signal. Also, global signal changes and autonomic arousal are seen in particular sleep stages [38]. In addition, WM fMRI signal patterns are contributed to by systemic vascular influence [37]. The crucial point is that WM-ventricular patterns are seen at first in rest but at ninth in task. Indeed, although slightly different approaches were used to conduct ICA in task and rest conditions, we see similar patterns in task ICA. However, the order of the components is important

because earlier components can explain a higher variance than others. Likewise, seeing more noise components near the end is for the same reason.

Another study investigated sleep cycles with fMRI, pointing out that autonomic regulation during sleep influences CBF [67]. These autonomic arousals might be the reason for our finding of possible systemic patterns as implied in ICA components, because in the resting state, the participants tended to fall asleep, and they might fall into microsleeps. Thus, this might increase the fMRI signals that contribute to this WM-ventricle spatial map.

4.2.1.1 Dual Regression in Resting-State. The Caff-10 and Caff-30's contributions are examined. However, insignificant results do not yield a clear pictures. There is only one trending difference in intracalcarine sulcus, however it is not possible to draw an inference from it. Nevertheless, caffeine might have affected the vessel in this sulcus and diminished the DMN connectivity.

4.2.2 Task ICA

The first component consists of VIS and IPS, which are mainly related to our experiment, seeing and solving equations. Also, the time course clearly reflects our task design.

In Component 9, the WM-ventricle pattern, like Component 1 of Rest, is seen. Although it is not related to any TPN, its time course still reflects our design. Therefore, we can infer that even regions that seem unrelated to the task show similar BOLD fluctuations as other networks. The possible reason for that is that CSF flow might not be much related to TPN or general task activation. In a CSF and neural activity study, the researchers investigated visual stimuli to create hemodynamic responses and CSF flow. They proposed that neural activity can affect CSF flow through the neurovasculature [68]. As a consequence, seeing the WM-ventricular pattern corresponding to

task design could be a neurovascular modulation system of the brain. In our context, a visual and cognitive stimulus might drive diminutive CSF flow.

Component 10 includes FPN and insula. Indeed, there are other components that can be seen early with similar regions; however, mentioning Component 10 is important because it is coming from Component 9. Although the previous map should be classified as noise, seeing it earlier gives us a hint that it explains more variance than Component 10. Also, there is a negative association between the VIS and FPN. Therefore, they might be split if the number of components were determined to be higher.

4.2.2.1 Dual Regression in Task-Based. Unfortunately, dual regression did not yield clear outcomes, likewise the resting state. Because the sample size was low, the results may have been insignificant. There is only one trending difference, yet it is in component 18, a noise component. Therefore, the effect of caffeine could not be observed through dual regression in task-based.

Although there are significant effects in the GLM analysis, we cannot find in dual regression analysis. Maybe, one of the reasons for it could be the number of components. We selected the number as 20, and saw the RSNs in the spatial maps. However, some component maps also include other networks in some spatial maps. Therefore, increasing the number and obtaining more separated network maps may increase the likelihood of seeing a difference in these components. Various numbers of components are considered for future research.

4.3 ICA and RSNs

The BOLD signal changes by CBF, CBV, CMRO₂. Vascular physiology modulates CBF/CBV, especially in the resting state. Respiration alters arterial CO₂ and influences the magnetic field. Besides, cardiovascular dynamics like heart rate af-

fect pulsations and can create slower vasomotor oscillations. These processes produce BOLD fluctuations on the same 0.01-0.2 Hz timescales often analyzed as RSNs, so they can be mistaken for connectivity on the neuronal scale.

Caffeine increases the activation in task-relevant regions and networks, and it can be seen in GLM and ICA to some extent. In independent component maps, task relevant components also include physiological effects, because cardiac and respiration are regulated by task activation. In addition, the DMN was not seen as a single component, but its regions were distributed in the components. In resting state networks, those networks can be differentiated more clearly.

4.4 Whole-Brain Cross-Correlations with RVT and PPG-Amp

In cross-correlations, the relationship between RVT and fMRI signals, as well PPG-Amp and fMRI signals are clearly observable. Possible systemic related effects are modulate fMRI signals, and as a result of it, correlations with physiological signals are seen.

Across modalities, caffeine tended to prolong correlations and to extend their spatial expression from localized regions to more distributed patterns. This suggests that caffeine amplifies systemic contributions to fMRI signals, influencing not only localized neural activity but also widespread vascular and physiological fluctuations.

A consistent finding was that RVT-BOLD correlation became stronger and more temporally extended under caffeine effect, particularly during resting-state. The early negative and prolonged positive correlations in the Caff-30 condition suggest that caffeine lengthens the respiratory imprint on BOLD fluctuations. This effect may be tied to caffeine's vasoactive properties, which could alter cerebrovascular reactivity and thereby increase the influence of respiratory cycles.

The spatial distribution of correlations also shifted with caffeine. While Caff-10

emphasized negative correlations with PPG-Amp in visual areas, Caff-30 was characterized by more widespread involvement, including WM-CSF patterns. This difference implies that caffeine reduces the specificity of physiological coupling, allowing systemic influences to permeate a broader set of brain regions.

Task-based results revealed subtler differences but still supported the notion of prolonged and spatially extended correlations in Caff-30. The persistence of WM-CSF patterns across lags, alongside ventricle-related activity, reinforces the interpretation that caffeine modulates vascular and CSF-related dynamics. Although the magnitude of these effects was smaller than in resting-state, their presence even under task performance highlights the pervasive influence of caffeine on systemic-fMRI coupling. In resting-state, this relationship is a little more discernible. Because rs-fMRI allows BOLD signals to fluctuate more without regulating with a specific task, those signals can show their inherent relationships more easily. On the contrary, task-fMRI adds a new parameter to BOLD signals, and like all third variable problems, it took some explained variance from it. Therefore, due to an external moderator, inherent relationships become more latent.

5. CONCLUSION

This study provides novel insights into the effects of caffeine on brain function by combining multiple analytical approaches for the first time. Specifically, we applied GLM, spatial ICA, dual regression, and correlation analyses within the same experimental framework, allowing us to capture both task-driven and resting-state brain dynamics while integrating physiological signals. This multimodal approach is, to our knowledge, the first to systematically examine how caffeine modulates neural activation, network involvement, and vascular physiology across individuals.

Our findings demonstrate that caffeine has a dual impact: it enhances task-driven activation and network engagement while simultaneously modulating vascular physiology, reshaping resting-state connectivity. The combination of GLM, ICA, and dual regression revealed trending but robust patterns in network contributions, highlighting subtle inter-individual differences that are often missed in conventional analyses. These results underscore the importance of considering caffeine effects in both task-based and resting-state fMRI studies, particularly for investigations of neurovascular coupling.

Despite these strengths, there are several limitations. Working with physiological data is inherently challenging. Small issues, such as slight displacements of the respiratory belt, partial loss of PPG data, or motion artifacts in BOLD signals, can influence results. Careful preprocessing and artifact handling in this study helped preserve data quality and reliability. Determining an appropriate caffeine dose is also complex due to differences in habitual intake and BMI. While 200 mg may have varying effects across participants, standardizing the dose ensured experimental consistency and allowed us to study acute caffeine effects systematically.

The timing between measurements is another consideration. To capture immediate caffeine effects, intervals were kept short, which may have limited effect size in

some analyses. However, this design enabled us to observe rapid neurophysiological responses in a controlled setting. Repeated exposure to tasks in later caffeine conditions could reduce mental stress, potentially dampening BOLD responses. At the same time, habituation reduced variability unrelated to caffeine, allowing for more stable results. Finally, while a larger sample size would have strengthened the study, practical constraints on fMRI access meant that we conducted experiments at maximum feasible capacity.

Overall, this study highlights the value of integrating GLM, ICA, dual regression, and correlation analyses in a multimodal framework to capture the nuanced effects of caffeine on both resting-state and task-based brain activity. These findings emphasize the need to account for caffeine in fMRI research and illustrate the critical role of neurovascular coupling in the interpretation of the brain function.

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