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Brief research report

A Region of Interest Analysis Focusing on the Insular and Cingulate Cortices

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Abstract. The insula is a brain region located on the lateral part of the brain. Previous findings show that the insula serves as a multimodal hub through which information is transmitted. In a recent group level analysis the insula, the anterior cingulate and the cingulate gyrus were found to be marked with prevalent clusters. In this study an attempt is made to confirm these findings and evaluate their significance by conducting a region of interest analysis (ROI). A group of 20 participants underwent an fMRI scan while solving mathematical problems of various degrees of difficulty. After the completion of each problem, they were asked to rate the difficulty of the current task from one to four (one corresponding to "very easy" and four corresponding to "very difficult"). The fMRI scanner was used to collect brain signal during the mathematical cognition and also the metacognition phase of the experiment. Signal corresponding to brain activation for each task was analyzed from various areas by conducting an ROI analysis and a t-test was used to determine the level of significance. Brain regions that were shown to be active in the group level analysis (right insula, left anterior cingulate and left/right middle frontal gyri) were confirmed to be active by the ROI analysis.

Key words: region of interest analysis, neuroimaging, insula, cingulate, experimental error

Introduction

Historically, the insula was believed to be the cortex of the brain responsible for the sense of taste, however more recent discoveries have found the insula to also be the cortex responsible for the processing of interoceptive information (Gasquoine, 2014). The insula is composed by up to thirteen different subdivisions (Uddin et al., 2017). In general, the posterior, middle, and anterior insula are related to emotional and cognitive consolidation (Centanni et al., 2021). A meta-analysis showed that the ventral anterior insula is mostly responsible for motivation, the dorsal anterior insula for setting goals and the posterior insula for dissociating pain from affective tasks (Wager & Barrett, 2017). The flow information from the anterior to posterior parts of the insula suggests that the posterior part of the insula might serve as a hub where information gathered by all five senses are integrated (Flynn et al., 1999).There are two major networks comprising the insula. The ventral-anterior network links the anterior insula to the anterior cingulate cortex and the temporal cortex, and it seems to be related to affect whereas the dorsal-posterior network links the posterior insula to posterior cingulate and motor cortices, thus associating the insula with sensorimotor consolidation (Cauda et al., 2011). The anterior insula together with the anterior cingulate cortex form a salience circuit that is responsible for distinguishing important stimuli from non-relevant (Menon & Uddin, 2010). The ventral anterior insula is associated with the salience network and it holds connections with the frontal cortex and the anterior cingulate cortex on the right side whereas the posterior insula network holds connections with the temporal and occipital cortices (Cauda et al., 2011). Salient stimuli, such as punishment and reward activate the ventral anterior insula via the anterior cingulate cortex whereas the posterior insula activates through stimuli such as oral taste, oral texture and tactile sensations (Rolls, 2016).

Insula also seems to play a role in addiction since insula lesions result in discontinuation of smoking and deficits in insula gray matter volume is associated with addicted individuals (Droutman et al., 2015). Insula also seems to play a role in monitoring cognition, empathy and decision-making (Pavuluri & May, 2015) and that makes it a brain region is of great importance when examining neurological and psychiatric disorders (Namkung et al., 2017). The insula is known to be associated to aversive emotional states whereas the anterior cingulate cortex to cognitive conflict (Harlé et al., 2012).

The anterior insular and the anterior cingulate cortex are well known to be associated with cognitive effort (Engström et al., 2015).Cognitive effort is needed in our day to day lives and it is generally considered as repellent state(Aben et al., 2020). Cognitive effort is involved with in many theories concerning typical and atypical behaviour (Westbrook & Braver, 2015). For example, it's been hypothesized that a forthcoming task which requires cognitive effort activates a brain mechanism with similar attributes as in the case of reward anticipation and this hypothesis was validated by the use of arithmetic tasks of various difficulties (Vassena et al., 2014). Higher cognitive effort requires higher attentional demand, and the anterior insula was found to be more active when cognitive effort increases (Perri et al., 2019). The dorsal anterior cingulate cortex was found to be active mostly when the demand for investing effort is high (Aben et al., 2020). An fMRI study has shown that variability of control demand is evaluated by the anterior insula and predicted in the caudate nucleus (Jiang et al., 2015). The dorsal anterior cingulate and prefrontal cortices are responsible for the execution of attentional control (Jiang et al., 2015). There is an interplay between the insula and the default mode network which is regulated by mental effort (Brandt et al., 2015). Mental fatigue is a symptom of several neurological dysfunctions, and it is responsible for the inability to conduct daily tasks (Anderson et al., 2019). The right insula and the right putamen are involved with cognitive fatigue and specifically its subjective experience (Anderson et al., 2019).

We often evaluate the accuracy of their judgements since this helps us monitor our behaviour (Liu et al., 2024). The insula seems to play a role in the detection of errors and this is evident by the lack of volume in the right insula in participants who scored low during a metamemory task (Cosentino et al., 2015). In addition, metacognitive accuracy was associated with the thickness of the frontal and insular cortices in healthy controls, however this finding was not evident in schizophrenic patients (Alkan et al., 2020). Metamemory regulation shows improvement from childhood to adulthood and this is evident by the thickening of the prefrontal and insular cortices (Fandakova et al., 2017). Confidence rating tasks associated to decision-making were found to activate metacognition-related brain areas such as the left/right dorsal anterior cingulate cortex (Lei et al., 2020).

Any kind of measurement in nature is subjected to a certain amount of experimental uncertainty (Taylor & Thompson, 1998). The formula for calculating this uncertainty is (Kat & Els, 2012):

$$Percentage\ error = \frac{|Theoretical\ Value - Experimental\ Value|}{Theoretical\ Value} 100\%. \tag{1}$$

Sources of error can be of various kinds. When we are dealing with laboratory equipment, instrumental errors are usually the most probable cause of uncertainty. Instrumental errors can arise due to a combination of factors. This can be due to imperfections in the equipment itself or due to human factor e.g., failure of the operator of the machine to ideally calibrate it or provide an accurate zero set and setting (Bevington et al., 1993). Therefore, instrumental and human factors are a common source of errors during fMRI experiments. Ideally, before conducting an experiment possible source of errors should be spotted and eliminated in an attempt to minimize the experimental error as much as possible. For example, many biological and chemical processes depend on temperature and humidity, so many laboratories control room temperature and relative humidity with air-conditioners as ideal experimental conditions are indeed important.

All the evidence presented in the introduction are pointing to the fact that the insula and the cingulate cortices are brain areas associated with cognitive/affective interoception and mental effort, thus they are expected to activate in experiments where metacognition related to mental effort is involved. In a recent group level analysis(Kouzalis A., 2023) the right insula, the left anterior cingulate and the right cingulate gyrus were detected to be marked with prevalent clusters. It is hypothesized that activations of areas of the insular and cingulate cortices found in the group analysis will be confirmed in this study during the phase of the ROI analyses and the activations will be found to be significant. Various other brain areas that appeared to be activated in the stage of the group level analysis will also be examined. The main goal of this study is to confirm activity in the insula and cingulate cortex throughout metacognition. A secondary goal is to shed light on uncertainties found in the experimental results of researchers, in the sphere of cognitive sciences and not only.

Methods

In order to confirm activity in each brain region, an ROI analysis was performed based on the brain signal of self-assessment that was detected throughout the comparison between the self-assessment task and the control task of first level of difficulty, in the section of findings where metacognition is investigated in terms of difficulty.

The participants were right-handed non-experts in mathematics with no fMRI contraindications able to quickly follow instructions and focus on tasks. Participants were asked to fill a screening form and sign a consent form for counterindications testing. Twenty healthy adults (10 females, 20 to 30 years old) participated in the fMRI study. Participants were given mathematical problems with varying levels of complexity, involving single, double, and triple digit numbers. They were asked to provide an answer to as many trials as they could during a time block of 32 seconds. After every 32-second block, participants evaluated the difficulty of the current set within 5 seconds, which is the metacognition event. Participants self-assessed their own mental effort in this way giving a self-rating of their own metacognition by a way of objective assessment. For control blocks, three non-mathematical tasks were used, each lasting 10 seconds.

The ROI analysis was performed by generating an anatomical mask for each individual brain region based on the results of A. Kouzalis (2023). Anatomical masks were created using anatomical regions with the AFNI software. The BOLD brain signal that was extracted was used to plot the graphs depicted in Table and Figure 2.

To evaluate the significance of activity, the brain response generated from each cognitive task throughout self-assessment and also the brain response generated from each cognitive task throughout fixation were extracted, in the section of findings where metacognition is investigated in terms of operation. A two-tailed t-test was then performed on these data and a p-value was calculated. The p-value represents the average *p*-value for the four mathematical tasks and the control task compared to fixation (Figure 1).

Results and discussion

Results of *ROI analysis by-brain area* are presented in Figures 1–2 and Table.

Some highlights of the most important findings are: (a) the region of interest analysis confirmed the activity of various brain regions reported in A. Kouzalis (2023) including regions mentioned in the hypothesis such as the insula and cingulate cortex. Results are discussed, focusing mainly on brain areas (b) many of the brain areas reported in A. Kouzalis (2023) were found to have significant activation and in some cases highly significant (c) experimental errors in fMRI experiments are discussed.

A metacognitive model has been proposed where the prefrontal cortex works together with interoceptive cortices such as the cingulate and the insula to encourage the accuracy of performance self-ratings (Fleming & Dolan, 2012).

In this study the left and right middle frontal gyrus (part of the prefrontal cortex), the right insula, the left inferior frontal gyrus (part of the prefrontal cortex), the left anterior cingulate and the right cingulate gyrus were confirmed to be active throughout the metacognition task. The intensity of the brain signal did not seem to diverge considerably between mathematical operations. The control task seemed to have a heightened activity in the negative domain compared to the mathematical tasks.

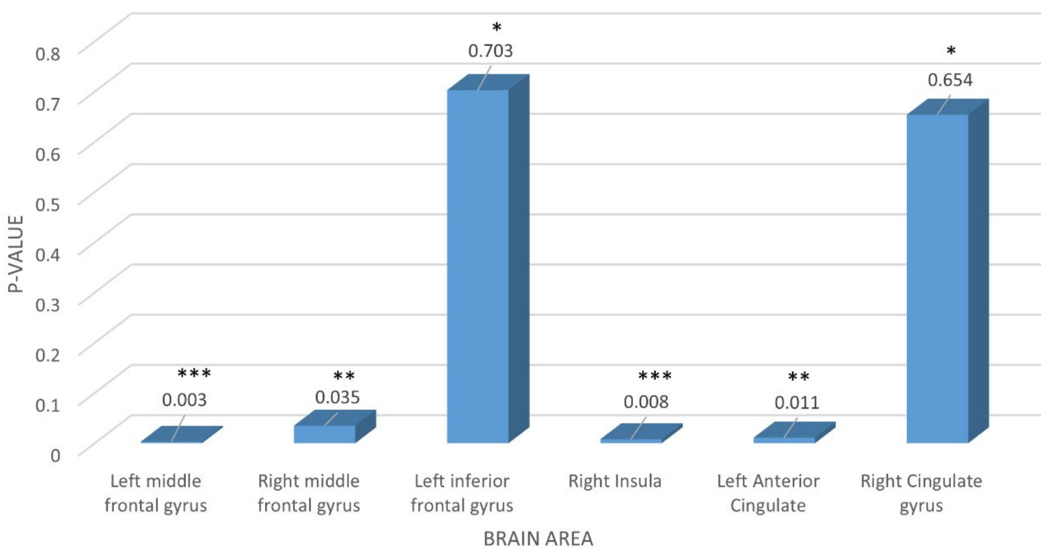

Figure 1. Significance level of brain areas that were found to be marked with prevalent clusters in A. Kouzalis (2023)

Note: $*p$-value ≤ 0.05; $**$ p-value ≤ 0.01; $***$ p-value ≤ 0.001.

Decision-making goes hand in hand with metacognition, through a process where the uncertainty regarding a decision is evaluated and the decision is revised (Qiu et al., 2018). An fMRI study found the dorsal anterior cingulate cortex to be active during metacognitive regulation processes during decision making (Qiu et al., 2018). Metacognitive sensitivity has previously been associated with the insula and findings of larger grey matter volume in the right anterior insula support this theory (Sinanaj et al., 2015). Figure 1 depicts the level of significance for brain areas reported in the group level analysis findings of A. Kouzalis (2023). The right insula, the left anterior cingulate and the right cingulate gyrus were detected to be active in the group analysis of Kouzalis A., 2023. Significant activations of the insula and the cingulate cortices were expected to be found according to the initial hypothesis. The ROI analysis conducted found the right insula to be activated significantly high. Moreover, significant activity was noticed in the left/right middle frontal gyrus and in the left anterior cingulate cortex.

During the analysis of brain responses to metacognitive processes, after solving mathematical tasks, the occurrence of a certain inconsistency was observed. It was expected that during the region of interest phase of the analysis, the contrast between the metacognition control task of level one difficulty and the font control task of level one difficulty (CON1) would have yield a brain signal equal to zero (CON1 = 0). However, after the conduction of the fMRI experiment and the analysis of the data, it was found that this was not valid. The mean brain signal for the CON1 contrast was not exactly zero, it was however the closest value to zero than any other contrast (see Table). This observation made absolute sense and it was a hint to the understanding that this inconsistency was due to an experimental error.

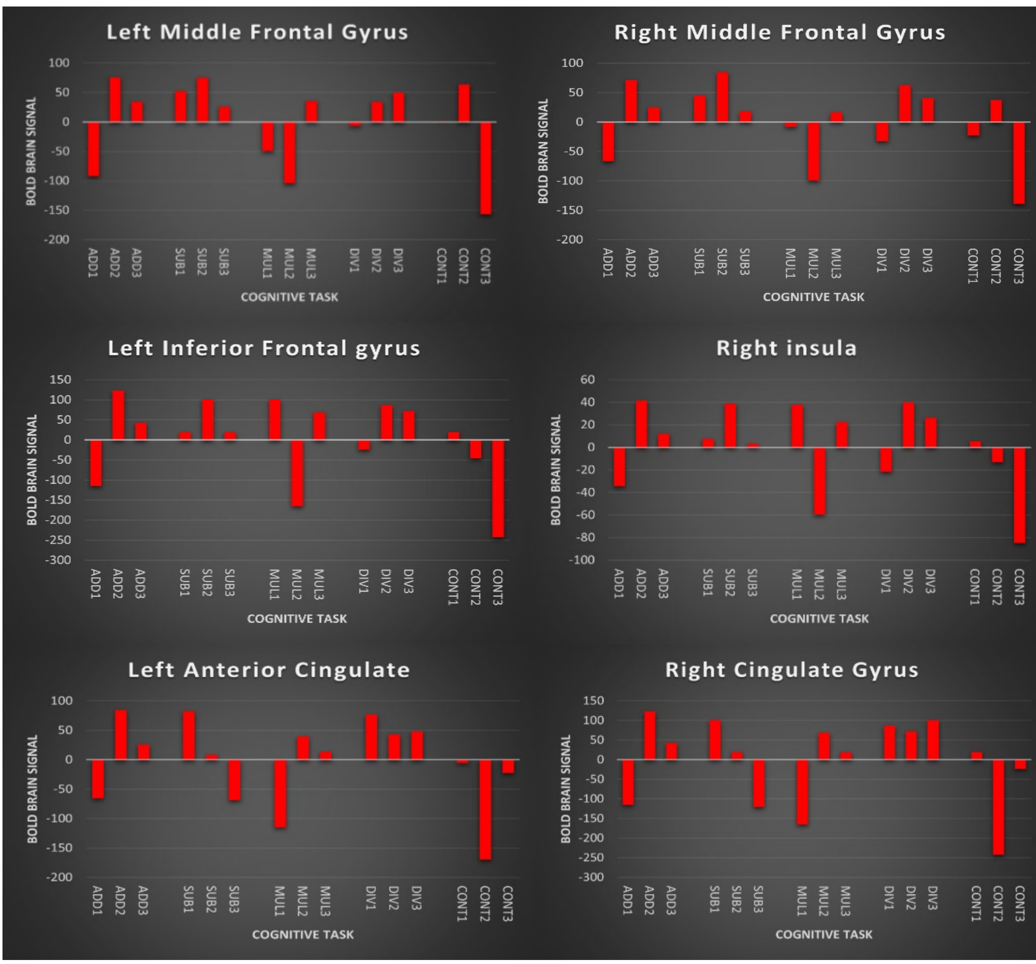

Figure 2. T the BOLD brain signal for the contrast between the metacognition task and the font control task of difficulty level 1

There are, however, two important features of experimental errors that should be considered. Firstly, experimental errors cannot be defined when the ideal theoretical value is zero in the denominator (see Equation 1). This is the case in this experiment. Secondly, experimental errors make sense when the measurement system is a ratio scale (i.e., a scale which has a true meaningful zero). In any other case experimental errors would be sensitive to the measurement units. The most popular classification (developed by psychologist Stanley Smith Stevens) has four scales of measurement: interval, ratio nominal and ordinal (Stevens, 1946). The scale in this experiment is interval (the value of zero is interval between positive and negative values), therefore making the calculation of the experimental error meaningless. For example, if an experimental value given in Celsius scale is minus four degrees Celsius and the theoretical value is plus one degree Celsius, then the absolute error is five degrees Celsius and the percentage error is five hundred percent. If now the temperature is given in Kelvin scale, the identical five Kelvin absolute error with the identical theoretical value of 274 Kelvin gives a percentage error of only 1.8%. The best estimate of brain signal is the average of all the values of the brain signal recorded for a given condition in the experiment (Taylor & Thompson, 1998). Large percentage errors are not always an indication of an experiment gone wrong. For experiments with a high potential of error a percentage error of 10% can even be considered a careful measurement (Taylor & Thompson, 1998).

Table

BOLD brain signal extracted from regions of interest

Metacognitive tasks	Left IFG	Left MFG	Right MFG	Right CG	Left AC	Right INS
ADD1	–114.82	–91.90	–66.45	–114.82	–65.59	–34.33
ADD2	123.19	75.48	71.54	123.19	84.23	41.42
ADD3	42.39	34.30	24.98	42.39	26.00	12.00
SUB1	19.68	52.47	44.86	100.66	82.75	7.60
SUB2	100.66	74.78	84.75	19.73	8.13	38.59
SUB3	19.73	26.17	18.16	–120.38	–68.61	3.26
MUL1	100.67	49.54	–7.97	–165.10	–114.82	37.80
MUL2	–165.10	–103.97	–99.71	69.13	39.78	–59.46
MUL3	69.13	35.30	16.98	19.68	13.36	22.53
DIV1	–23.93	–6.21	–32.43	86.57	77.01	–21.58
DIV2	86.57	33.68	62.42	72.05	43.01	39.77
DIV3	72.05	50.04	40.74	100.67	48.37	26.20
CON1	19.65	0.63	–22.77	19.65	–5.41	5.24
CON2	–46.08	63.52	37.24	–242.79	–169.71	–13.01
CON3	–242.79	–156.83	–139.34	–23.93	–22.60	–84.67

Note: IFG – Inferior Frontal Gyrus, MFG – Middle Frontal Gyrus, CG – Cingulate Gyrus, AC – Anterior Cingulate, INS – Insula.

Conclusion

The role of the insula and the cingulate cortex in metacognition was explored. ROI findings shed more light on this role and specifically on the role of the insula as a moderator of interoception in a cognitive task. A forward step that can be made is the limitation of the brain regions under exploration to more specific areas. The way to achieve this is to perform a spherical ROI analysis utilizing specific coordinates that are based on the coordinates of peak activity of the most important clusters. Furthermore, to test for significance, regions of interest can be contrasted with control regions. The non-occurrence of errors in data sets is practically impossible. Even under the very best experimental conditions errors will still occur, on a much lesser degree of course than if the experimental conditions were left uncontrollable. This abides not only for fMRI experiments but for any experiment with analogous data sets and analysis procedures.

Limitations. In this study an ROI analysis was performed by the use of anatomical masks. Brain regions such as the cingulate occupy large portions of the cortex and self-assessments may induce activity in distinct areas in a certain brain region under investigation. The anatomical mask that was utilized in this experiment did not differentiate between these areas. Brain areas that are found to be active during self-assessment are typically found to be deactivated throughout cognitive performance tasks (Chua et al., 2006). Deactivated areas can be utilized as control areas. These are brain areas such as i.e., the auditory cortex, the supplementary motor area, the motor cortex, the basal ganglia, the amygdala, and the cerebellum. In the current study, regions of interest were evaluated for significance according to the contrast of the metacognition task versus the time of fixation. Brain signal from ROI's was not contrasted with brain signal from control regions.

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Author's contribution:
Alexios Kouzalis – the concept and design of the study, investigations and data analysis, writing and editing the text. *Regina V. Ershova* – scientific supervision, writing and editing the text.

Conflicts of interest:
The authors declare that there is no conflict of interest.

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Краткое сообщение

Анализ области интереса, сфокусированный на островковой и поясной коре

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Аннотация. Островок – это участок мозга, расположенный в его латеральной части. Результаты предыдущих исследований показывают, что островок служит мультимодальным узлом, через который передается информация. В проведенном нами ранее групповом анализе было обнаружено, что островковая доля, передняя поясная извилина и поясная извилина являются преобладающими кластерами в метапознании. В настоящем исследовании предпринята попытка подтвердить эти результаты и оценить их значимость с использованием метода анализа области интереса (region of interest analysis, ROI). Группа из 20 участников прошла фМРТ-сканирование при решении математических задач различной степени сложности. После решения каждой задачи им предлагалось оценить сложность текущего задания от одного до четырех (один соответствует «очень легко», а четыре — «очень сложно»). Сканер фМРТ использовался для сбора сигналов мозга во время математического счета, а также на этапе решения метапознавательных задач. Сигнал из различных областей, соответствующий активации мозга для каждой задачи, анализировался путем проведения ROI, а для определения уровня значимости различий использовался t-тест. Активность областей мозга, обнаруженных при групповом анализе (правая островковая доля, левая передняя поясная извилина и левая/правая средняя лобная извилина), была подтверждена с помощью анализа ROI.

Ключевые слова: анализ области интереса, нейровизуализация, островок, поясная извилина, экспериментальная ошибка

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