The Neuroimaging and Genetics of Emotion Perception in Schizophrenia

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A thesis submitted for the degree of Doctor of Philosophy at The University of Queensland in 2017
Queensland Brain Institute
Abstract

Impaired emotion perception is a core deficit in schizophrenia (SCZ) that is robustly associated with poor functional outcome. Studies to date have failed to delineate the critical cognitive, neural and genetic factors that underlie ‘naturalistic’ emotion perception deficits in SCZ. Recently it has been proposed that in SCZ reduced precision in prior expectations impairs perception. Normal perception relies on predictive processing from precise prior expectations, which prioritise attention to context-relevant stimuli. However, no studies to date have explored the aberrancy of prior expectations during naturalistic emotion perception in SCZ and the underlying abnormal neural circuitry or involvement of SCZ genes. The present thesis aims to develop a novel dynamic emotion perception (DEP) task, in order to examine (1) the influence of prior expectations on naturalistic emotion perception in healthy individuals, and (2) emotion perception impairment in SCZ by investigating aberrancy in prior expectations, neural circuitry, and genetics.

The aim of Study 1 was to create a novel functional magnetic resonance imaging (fMRI) paradigm to examine the mechanisms of prior expectations and delineate the spatiotemporal activity of brain regions that contribute to emotion perception. The newly developed and validated fMRI DEP task has improved ecological validity, using dynamic, audio-visual videos, instead of static pictures as stimuli. The improved method provided new behavioural and neuroimaging insights into the influence of prior expectations on naturalistic emotion perception. Prior expectations facilitated emotion perception after repeated exposure and learning over the course of the experiment, engaging prefrontal regions and right amygdala. In contrast, emotions incongruent with prior expectations engaged neural correlates of automatic change detection, which in a complex dynamic environment allows for adaptive behaviours in potentially advantageous or threatening situations.

In Study 2, the DEP task was utilised to delineate, in the healthy brain, the functional networks that subserve rapid recognition of threatening emotions in situations either congruent or incongruent with prior expectations. The results showed that fast detection of threat congruent with prior expectations engaged a right amygdala subcortical network, which has been previously implicated in fear conditioning and coarse processing of emotion. In contrast, there was a trend association between fast detection of threat incongruent with prior expectations and activity in inferior frontoparietal network connected to the right temporoparietal junction; this has been previously implicated in rapid reorienting of attention to
unexpected change. These results have implications for understanding psychopathologies, such as SCZ, which are characterised by impaired processing of threatening emotions. Furthermore, this research may have applications in new interventions, such as neuromodulation, which relies on isolating and modifying abnormal neuroanatomical networks.

**Study 3** aimed to investigate the perception of dynamic emotion in SCZ and the influence prior expectations have on brain activity and functional networks. The behavioural findings revealed that patients with SCZ had lower accuracy and response speed over time, specifically for emotions that were congruent with prior expectations. At the neural level, compared to healthy controls, SCZ patients had less engagement of inferior frontal cortex, parietal cortex, insula, and right amygdala-subcortical dysconnectivity while viewing emotions congruent with prior expectations. Conversely, patients improved in their speed to detect emotions *incongruent* with prior expectations and had similar functional connectivity with right amygdala as healthy controls. These results suggest that in a dynamic environment, individuals with SCZ have weaker prior expectations, resulting in inefficient perception of context-congruent emotion.

Finally, in **Study 4**, the association between SCZ polygenic risk score (PRS) and brain activity during the DEP task in healthy adults was investigated in order to assess emotion perception as an endophenotype for SCZ. The results demonstrate that PRS is associated with higher recruitment of regions involved in emotion regulation during threatening emotion perception. These findings suggest that over-recruitment of emotion regulation neural correlates might be related to neural inefficiency in individuals who have higher susceptibility to schizophrenia. As such, over-recruitment of emotion regulation brain regions may function as a compensation for this inefficiency during emotion perception. These findings have important implications for understanding neurophysiological biomarkers relevant for patients with SCZ.

In summary, this thesis provides novel insights into the mechanisms of prior expectations during dynamic emotion perception, the underlying healthy neural circuitry, and the aberrant cognitive and neural mechanisms in SCZ. The study using PRS provides preliminary evidence for the importance of incorporating emotion perception into our understanding of the pathway from genotype to clinical phenotype. Overall the findings have broad implications for understanding naturalistic emotion perception in a healthy brain, with implications for pathologies, such as SCZ, and
application to novel interventions which aim to improve emotion perception and the underlying circuitry.
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Publications during candidature

Peer-reviewed papers


Conference abstracts


The neuroimaging and genetics of emotion perception in schizophrenia. *Ipswich Hospital Research Day 2014. Poster Presentation.*


Dynamic emotion perception in schizophrenia: fMRI and genetics. Institute of Cognitive Neuroscience, University College London 2016. Invited speaker.

Dynamic emotion perception in schizophrenia: fMRI and genetics. Wellcome Trust Centre for Neuroimaging, University College London 2016. Invited speaker.


Publications included in this thesis

Incorporated as Chapter 2


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**Contributions by others to the thesis**

As primary and associate advisors on the present research, Prof Bryan Mowry and Dr Hana Burianová provided guidance on study design and data analysis, and reviewed and commented on the thesis. In addition, Dr Andrew Martin reviewed and commented on the thesis.

**Statement of parts of the thesis submitted to qualify for the award of another degree**

None.
**Acknowledgements**

First, I would like to acknowledge all participants, particularly those who have suffered from schizophrenia, for their time and energy taking part in this project. I sincerely hope that collectively the research that is being conducted will create changes in diagnosis and treatment, and improve the lives of those suffering from this debilitating illness.

I would like to thank my supervisors Bryan Mowry and Hana Burianová for their guidance, advice, and mentorship throughout the course of my PhD. Thank you, Bryan, for giving me a chance, for your unwavering assistance, and also for opening great opportunities for me. I am particularly grateful for your encouragement and support to go overseas and present my work to some of the most prestigious international institutes, allowing me to experience diverse research environments and establish international collaborations with leading neuroscientists. Thank you, Hana, for your endless support and friendship during the PhD. You helped me grow as a researcher and acquire new skills in writing and analysis, but most importantly you helped me build confidence in myself and my work, which in the dog-eat-dog world of science is imperative!

To both of my supervisors I would like to thank you for your valuable advice and support during the difficult times in the PhD, which not only ensured I complete the work but also gave me piece of mind knowing that you were on my side.

Thank you to all members of the Burianová group, Garrido group and Mowry group (particularly Heather Smith and Cheryl Filippich), for your feedback on aspects of the project, for the (very necessary) morning tea chats/coffee breaks, and for your friendship throughout the PhD.

A special thanks to: Sathish Periyasamy for your assistance and advice on the genetic aspects of the project; Deborah Nertney for your assistance with collecting the neuropsychological and neuroimaging data; and the radiographers at the CAI (Aiman Al-Najjar and Nicole Atcheson) for your neuroimaging support and the great conversations.

Finally, I would like to thank Andrew Martin for his tireless assistance with three of the manuscripts in the thesis and for his invaluable editing and proof reading of all work- I couldn’t have done it without you!
Last but not least, I would like to thank my family, my mother- Malina Fielder, father- Nedzad Dzafic, sister- Sabina Dzafic and grandmother- Sadeta Hadzimujic, for their enduring support and love. You instilled in me the determination and grit to be able to complete this PhD, and therefore I dedicate this work to you.
Keywords
emotion perception, prior expectations, schizophrenia, functional magnetic resonance imaging, threat, functional connectivity, amygdala, polygenic risk, emotion regulation.

Australian and New Zealand Standard Research Classifications (ANZSRC)
ANZSRC code: 170205 Neurocognitive Patterns and Neural Networks, 50%
ANZSRC code: 170112 Sensory Processes, Perception and Performance, 50%

Fields of Research (FoR) Classification
FoR code: 1701 Psychology, 50%
FoR code: 1109 Neurosciences, 40%
FoR code: 0604 Genetics, 10%
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Anterior cingulate cortex (aCC)
Brodmann area (BA)
Blood-oxygen-level dependent (BOLD)
Best Estimate Final Diagnosis (BEFD)
Dorsomedial prefrontal cortex (dmPFC)
Dorsolateral prefrontal cortex (dIPFC)
Diagnostic Interview for Genetic Studies (DIGS)
Dynamic Emotion Perception (DEP)
Echo time (TE)
False alarm (FA)
Functional magnetic resonance imaging (fMRI)
Family Interview for Genetic Studies (FIGS)
Genome-wide association study (GWAS)
Hit rate (HR)
Intelligence quotient (IQ)
Inferior frontal gyrus (IFG)
Latent variables (LVs)
Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT)
Mean (M)
Mini International Neuropsychiatric Interview (M.I.N.I.)
National Health and Medical Research Council (NHMRC)
Sample size (N)
Partial least squares (PLS)
Polygenic risk score (PRS)
Psychiatric Genomics Consortium (PGC)
Queensland Centre for Mental Health Research (QCMHR)
Reaction times (RTs)
Repetition time (TR)
Right amygdala (rAMY)
Right anterior insula (rAI)
Right temporoparietal junction (rTPJ)
Schizophrenia (SCZ)
Singular value decomposition (SVD)
Standard deviation (SD)
Standard error (SE)
Statistical Parametric Mapping 8 (SPM8)
Theory of Mind (ToM)
Transcranial direct current stimulation (tDCS)
Ventrolateral prefrontal cortex (vlPFC)
Ventromedial prefrontal cortex (vmPFC)
Wechsler abbreviated scale of intelligence (WASI)
CHAPTER 1

General Introduction
Aims and Significance

Schizophrenia (SCZ) is a devastating disorder associated with high suicide rate (Hor and Taylor, 2010), homelessness (Folsom et al., 2005), and incarceration (Bradshaw et al., 2013), ranking eleventh in the global burden of disease (Vos et al., 2015). Individuals with SCZ may suffer from hallucinations, delusional beliefs, disorganised thought and behaviour, and a range of negative symptoms, such as unchanging facial expression, lack of vocal inflections, or decreased spontaneous movements (Minas et al., 1992; Mowry and Gratten, 2013). Additionally, in recent years it has been established that (social) cognitive deficits are a core feature of SCZ with the most robust association with functional outcome (Green et al., 2004; Green et al., 2005). A key aspect of social cognition is emotion perception, which is the ability to recognise how another person is feeling. Impaired emotion perception is a well-established deficit in SCZ (Chan et al., 2010; Kohler et al., 2010) and has a significant impact on overall daily functioning (Irani et al., 2012). Deficits in processing emotion are stable throughout the course of illness (Green et al., 2012; Horan et al., 2012; McCleery et al., 2016) and resistant to current pharmacological treatment (Mueser et al., 1996; Penn et al., 2009). Emotion perception is a fundamental skill required in social interactions and impaired emotion perception in SCZ impacts on community functioning and the ability to form quality relationships (Couture et al., 2006; Hofer et al., 2009; Hooker and Park, 2002; Poole et al., 2000), resulting in smaller social networks and leading to further mental and physical health issues (Cechnicki and Wojciechowska, 2006; Horan et al., 2006).

Studies to date have failed to delineate the underlying factors, which drive emotion perception deficits in SCZ. Furthermore, the majority of these studies have relied on less ecologically valid stimuli, such as static emotional faces, to study emotion perception in SCZ, possibly misrepresenting the difficulties faced by patients in “real-world” interactions. Consequently, there may be inadequate and possibly misleading knowledge pertaining to the underlying processing and biological factors involved in emotion perception deficits. Recent efforts at disentangling the underlying processes necessary for perception have characterised SCZ as a disorder of reduced precision in prior expectations (Adams et al., 2016b; Adams et al., 2013). Normal perception relies on predictive processing, in which associations that are relevant in a specific context are established, forming predictions of what to expect (Bar, 2007). Prior expectations enable efficient directing of attention to relevant incoming sensory information and have been found to facilitate emotion perception, improving response
time and accuracy (Barbalat et al., 2013). However, to-date there is no research that has examined whether aberrant prior expectations in SCZ are at the core of impairment in naturalistic emotion perception.

The underlying biological factors driving emotion perception difficulties in SCZ have been investigated with neuroimaging and genetic approaches. Neuroimaging studies utilising functional magnetic resonance imaging (fMRI) have identified regional brain activation abnormalities in SCZ, with the robust finding of altered activity in frontal regions, amygdala, and insula (Li et al., 2012; Li et al., 2010; Taylor et al., 2012). Furthermore, functional connectivity studies in patients have highlighted dysconnectivity in these areas during emotion perception (Bjorkquist et al., 2016; Potvin et al., 2017). Meanwhile, genetic studies have identified SCZ common risk variants to be related to emotion perception deficits across development (Germine et al., 2016a) and to abnormal emotion perception neural circuitry (Curcic-Blake et al., 2012; Mothersill et al., 2014). In this thesis, I expand on the current literature by investigating naturalistic emotion perception and the underlying aberrant neurophysiological factors in SCZ. In addition, I provide novel insights on the influence of prior expectancy and the expectancy-driven neural circuitry. Furthermore, I provide preliminary insights on the cumulative influence of SCZ common risk variants on emotion brain networks in a healthy unaffected sample, providing evidence for dynamic emotion perception as a viable endophenotype.

The aim of the following chapter is to provide an overview of emotion perception impairment in SCZ at the behavioural, neural, and genetic levels. I will highlight the significance of investigating (1) naturalistic displays of emotion rather than static displays, and (2) prior expectations as the underlying impaired process in emotion perception. I will review the fMRI findings related to emotion perception impairment in SCZ. Moreover, I will discuss the benefit of using multivariate, functional connectivity, and brain-behaviour analytical approaches to examine the underlying neural circuitry of emotion perception. Finally, I will cover the genetic basis of emotion perception impairment in schizophrenia; in this section, I will discuss the polygenic risk model and imaging genetics.

**Social Cognition and Emotion Perception in Schizophrenia**

**Social Cognition**

The symptoms and the cognitive deficits experienced by patients with SCZ impact daily functioning adversely. As a result, patients struggle in interpersonal relationships,
maintaining employment, and functioning in the community (Bellack et al., 1990; Couture et al., 2006; Horan et al., 2012). Social cognitive impairment is robustly associated with functional outcome, beyond the contributions of symptoms (Horan et al., 2012) and neurocognitive ability (Vauth et al., 2004). Social cognition entails perceiving and interpreting the intentions and dispositions of others and using this knowledge to guide behaviour in a particular social context (Adolphs, 2001; Green and Horan, 2010; Kunda and Sinclair, 1999). Although impairments in neurocognition have been a focus in SCZ research, it has been determined that social cognitive impairments are distinct (Sergi et al., 2007; Skoff et al., 1986) and have a higher and mediating impact on daily functioning of patients with SCZ (Addington et al., 2010; Pijnenborg et al., 2009; Vauth et al., 2004). The expert panel from the initiative for the Measurement and Treatment Research to Improve Cognition in SCZ (MATRICS) deemed social cognition to be ecologically important in evaluating overall cognition in SCZ and an integral ability to consider when evaluating clinical treatments, which has led to a focus on determining which factors in social cognitive ability contribute to functional outcome and can be targeted with intervention. The factors underlying social cognition involve higher-level analytic processing of social information and lower-level perception of emotional and social cues (Mancuso et al., 2011; Ziv et al., 2011). Emotion perception (or affect perception) is the initial skill in social cognition (Mayer et al., 1990) and is a building block of other social cognitive domains, such as theory of mind (ToM) and attribution bias, which are also impaired in SCZ (Couture et al., 2006; Mancuso et al., 2011; Nuechterlein et al., 2008; Ochsner, 2008; Penn et al., 2006), see Figure 1. Thus, it is essential to consider emotion perception deficits when considering social cognition in patients with SCZ.
Emotion Perception

Emotion perception involves deducing emotional information from both nonverbal and verbal cues, such as those in facial expressions and speech prosody (Couture et al., 2006). The authors Kohler et al. (2010) found a robust impairment of emotion perception in SCZ, during both identification and differentiation of emotion via a comprehensive meta-analysis. Importantly, this impairment remained after controlling for education level and neurocognitive functioning. There is a debate about whether impaired emotion perception in SCZ relates to a global face-processing deficit or a specific emotion perception deficit. Evidence shows that in SCZ there is a general impairment in facial processing, as patients have a tendency to divert focus from facial cues that are relevant in conveying emotional information (Clark et al., 2013; Sasson et al., 2007). However, there is also evidence supporting a more specific impairment in emotion perception in SCZ, as patients have deficits across other channels of emotion, such as interpreting the emotion in speech (Hoekert et al., 2007). Therefore, it is crucial that future studies incorporate multimodal stimuli when investigating emotion perception difficulties in SCZ. Individuals with SCZ also have difficulty in higher-level emotion processing, such as predicting emotional events and integrating...
emotions within certain contexts (Kring and Elis, 2013). More specifically, there is an explicit impairment in SCZ during the processing of negative emotions (Comparelli et al., 2013; Giannitelli et al., 2015), such as sadness, anger (Goghari and Sponheim, 2013; Pinkham et al., 2014a), fear, and disgust (Barkl et al., 2014a; Barkl et al., 2014b). SCZ patients also misattribute negative emotions onto neutral expressions (Pinkham et al., 2011; Potvin et al., 2016). This may be related to emotion dysregulation, as Strauss et al. (2013) found that SCZ patients had an inability to regulate their emotional response for unpleasant stimuli, leading to higher negative emotional experience of events.

The majority of emotion experiments have utilised static pictures of faces (Ekman et al., 1975; Izard, 1971) to examine emotion perception; for example, the Facial Emotions for Brain Activation Test (Gur et al., 2002) and the Emotion Identification and Discrimination Test (Kerr and Neale, 1993). Other studies have incorporated more naturalistic stimuli, using audio-visual video displays of emotion (Goldberg et al., 2014) in order to improve the ecological validity. In real-life, emotional expressions are in constant motion combined with speech prosody; therefore, images of static emotional faces are potentially inadequate, non-canonical stimuli (Kilts et al., 2003a). Consequently, there has been a significant shift to using stimuli with greater ecological validity when studying the underlying processes of emotion perception. Audio-visual, ‘dynamic’ video displays of emotion present more resemblance to real-life emotional expressions and elicit enhanced psychological responses (Collignon et al., 2008; Yoshikawa and Sato, 2006) and neural activity in the emotion brain network (Arsalidou et al., 2011; Robins et al., 2009) compared to static displays of emotions. Another important set of findings shows that there are significant differences in the neural and cognitive processes engaged during perception of dynamic versus static emotion stimuli. For example, Kilts et al. (2003) found that dynamic emotion perception recruited neural correlates implicated in the analysis of emotional information in the animated facial expressions, whereas static emotion perception recruited neural correlates implicated in motor imagery. In addition, compared to static emotional expressions, the animated and changing cues in naturalistic emotional expressions have been found to stimulate greater predictive processing (Kaufman and Johnston, 2014; Palumbo and Jellema, 2013). This is due to the greater uncertainty involved in perceiving dynamic emotions, compared to static emotions, as there is a larger influx of sensory information that is continually evolving and changing over time. Greater perceptual uncertainty relies on up-to-date predictions from precise prior expectations.
to reduce the incoming sensory information by directing attention to cues that are relevant, i.e., aligned with valid expectations (Friston et al., 2006; Frith and Frith, 2006). As prior expectations are a fundamental process in naturalistic emotion perception, it is therefore of paramount importance to investigate the role of prior expectancy in emotion perception deficits experienced by patients with SCZ.

Prior Expectations

Recently, SCZ has been conceptualised as a disorder of reduced precision in prior expectations (or prior beliefs; (Adams et al., 2016b; Adams et al., 2013), which results in inefficient prediction and aberrant directing of attention to incoming sensory cues. The empirical basis of this conceptualisation has been highlighted in a number of studies, showing processing differences in SCZ patients when compared to healthy individuals. For example, in SCZ, there is evidence of abnormal smooth pursuit eye movement (Hong et al., 2008), the ability to track a moving object based on the prediction of that object’s motion. Individuals with SCZ tend to fall behind in their tracking of the moving object and need to saccade in order to keep track of the object. In addition, individuals with SCZ have a resistance to visual illusions, which normally cause perception to differ from objective reality. For example, in the hollow-mask illusion, healthy individuals tend to perceive a concave face as a normal convex face, due to our strong prior knowledge that a face is not inverted. Therefore, firm prior expectations of what faces look like create a perceptual illusion (Tsuruhara et al., 2011). In contrast, patients with SCZ are more likely to perceive the concave face, correctly - as an inverted face, due to reduced precision in prior expectations (Dima et al., 2009), see Figure 2.

![Figure 2: Effects of a hierarchical precision imbalance in schizophrenia. A loss of precision encoding in higher hierarchical areas would bias inference away from prior beliefs.](image)

**Figure 2. Effects of a hierarchical precision imbalance in schizophrenia.** A loss of precision encoding in higher hierarchical areas would bias inference away from prior beliefs.
beliefs and towards sensory evidence (the likelihood), illustrated schematically in the middle panel. This single change could manifest in many ways. On the left: a loss of the ability to smoothly pursue a target moving predictably. On the right: a loss of the precision of prior beliefs can cause a resistance to visual illusions that rely on those prior beliefs for their perceptual effects. From Adams et al. (2016).

A number of studies have demonstrated that aberrancy in prior expectations influences social cognitive ability in SCZ. Chambon et al. (2011b) examined the influence that prior expectations have on the ability to infer social intentions. In their study, the authors (2011) induced expectations by selectively increasing the likelihood of a particular human movement to occur within a sequence of different types of movements. In the social conditions, participants were required to infer the direction in which a person would move an object with his/her hand while interacting with another person. The authors found that SCZ patients had difficulty inferring social intentions and this was associated with a weaker dependence on valid prior expectations. However, there are some inconsistencies in the prior expectancy literature regarding the nature of aberrancy in SCZ. For example, a study by Barbalat et al. (2012) examined the influence that prior expectations have on emotion perception. In their paradigm, expectations were induced by using cues to instruct participants to identify a specific emotion in a sequence of static emotional faces. The authors found that individuals with SCZ were particularly impaired when perceiving an angry or fearful face that was preceded by an incongruent angry or fearful cue. Barbalat et al. (2012) argued that this was associated with an overdependence on prior expectations for threat in SCZ. The aforementioned study’s paradigm relied on static representations of emotion. In comparison, Chambon and colleagues (2011) utilised dynamic representations of social intention. Thus, it is possible that the difference in stimulus type and representation may have resulted in the contradictory findings. These inconsistent findings highlight the need to investigate and specify the influence of prior expectancy on perception in SCZ and lend further support to the use of dynamic rather than static displays of emotion.

Thus, in Study 1 (presented in Chapter 2), I present a novel fMRI paradigm designed to examine the influence of prior expectations on naturalistic emotion perception. The newly developed fMRI DEP task has improved ecological validity from previous studies using static imagery, because it uses audio-visual videos. In the DEP task, prior expectancy was induced by selectively increasing the likelihood that a
particular emotion occurs in a given sequence and by using emotion instruction cues. The DEP task is utilised in all studies that comprise the thesis to gauge the influence of prior expectations on dynamic emotion perception in healthy individuals and to investigate the nature of aberrancy of prior expectations in patients suffering from SCZ. The improved method provides new behavioural and neural insights into the influence of prior expectations on naturalistic emotion perception.

**Neuroimaging of Emotion Perception in Schizophrenia**

**Task-related Activations of Emotion Perception**

The neural correlates underlying deficits in emotion perception in SCZ have been investigated using the fMRI method, which measures changes in blood flow (hemodynamic response) associated with brain activity using a blood-oxygen-level-dependent (BOLD) contrast (Ogawa et al., 1990). A number of neuroimaging meta-analyses have investigated the functional activation differences in SCZ during emotion perception (implicit and explicit emotion processing, and emotion identification and discrimination) (Delvecchio et al., 2013; Li et al., 2010; Taylor et al., 2012). In summary, decreased activation for patients, compared to healthy controls, was found in a wide distribution of regions including bilateral frontal regions, bilateral amygdala, right insula, bilateral anterior cingulate cortex, right thalamus, bilateral temporal regions and right basal ganglia. Areas with increased activation were the bilateral cuneus, left insula, left parietal lobule, right precentral gyrus, and left superior temporal gyrus. In general, the consistent finding from these meta-analyses in patients with SCZ is decreased activity in frontal regions and amygdalae, and increased activity in the cuneus, highlighting that activity in these areas might have most robust aberrancy in SCZ (see Figure 3).

![Brain activity differences between schizophrenia patients and healthy control subjects during emotion perception](image)

**Figure 3. Brain activity differences between schizophrenia patients and healthy control subjects during emotion perception.** Results of meta-analysis with meta-foci overlaid on a Montreal Neurological Institute template brain. Yellow indicates healthy control subjects > schizophrenia patients, and light blue indicates schizophrenia patients > healthy control subjects. From Taylor et al. (2012).
Emotion perception fMRI studies have commonly utilised univariate fMRI analysis, rather than multivariate techniques, to summarise brain activity during emotion perception. Univariate fMRI analysis assesses the significance of each brain region separately; in addition, this method involves a-priori contrasts to disentangle the different processes in a cognitive ability. The benefit of using the univariate approach is its ability to delineate the regionally specific responses during functional processing. However, these aspects of the univariate analysis also limit our understanding of the underlying neural circuitry involved in emotion perception, because a higher order cognitive function, such as emotion perception, requires a large-scale neural network with distributed and interactive activity (Adolphs, 2001; Arsalidou et al., 2011; Beer and Ochsner, 2006; Vuilleumier and Pourtois, 2007). Dynamic interactions within the brain may not be fully captured by examining activation changes within specific brain regions; furthermore, the a-priori contrasts may result in forced cognitive subtraction, which emphasise differences in activations and do not capture the overlaps (Amaro Jr and Barker, 2006). A critical advantage of using a multivariate analytic approach is that it is designed to identify groups of brain regions distributed over the entire brain. In addition, it does not constrict the brain activation based on researcher-defined contrasts; instead, it identifies the fundamental relations between brain activity and experimental conditions that account for maximum covariance in the data (McIntosh et al., 2004; McIntosh and Lobaugh, 2004). The multivariate method has been used widely to answer questions about the relationship between brain activity and emotion perception (Grady et al., 2013; Keightley et al., 2011; Keightley et al., 2007; Keightley et al., 2003; Talmi et al., 2008). Therefore, in the current thesis, in addition to a univariate analysis in the main comparison between SCZ and HC; I use an established multivariate analytic technique, partial least squares (PLS; (McIntosh et al., 1996), to examine the brain activity during emotion perception.

**Task-related Functional Connectivity Analysis of Emotion Perception**

The complexity of neural interactions in emotion perception can be delineated with functional connectivity analysis and, importantly, the breakdown or disconnection in brain networks brought on by illness can be examined. Functional connectivity analysis is used to detect the correlations or covariance between different brain regions over time (Friston, 1994). SCZ has been characterised as a disorder of connectivity (Friston and Frith, 1995) and numerous functional connectivity studies have identified aberrant intrinsic integration of the brain in SCZ (Anticevic et al., 2013;
He et al., 2013; Huang et al., 2010). In a number of studies, the SCZ-related disconnection during emotion perception has been explored, with effects across different channels of emotions, and conscious/unconscious processing of emotion. For example, Bjorkquist et al. (2016) investigated processing of emotional images and found that SCZ individuals have less right amygdala-prefrontal connectivity during viewing of negative emotional images, compared with healthy individuals. Lack of connectivity was associated with poorer social functioning in SCZ. Research examining the auditory channels of emotion have found that SCZ individuals have less insula/inferior frontal-superior temporal connectivity and this is associated with impaired processing of emotional prosody (Kantrowitz et al., 2015; Leitman et al., 2011). In a study investigating consciousness of emotion, Das et al. (2007) found that for unconscious emotion processing, patients had overall hypo-connectivity between the amygdala and the frontal and visual regions. During conscious emotion perception, patients had aberrant hyper- and hypo-connectivity between the amygdala and medial prefrontal cortex and regions. In summary, the amygdala (in particular the right amygdala) and the insula/inferior frontal seem to be key regions of altered functional connectivity during emotion processing in SCZ.

The relation of network activity to behaviour, and importantly deficits in abilities, can be examined by incorporating behavioural data in parallel with functional connectivity analysis. The advantage of assessing brain-behaviour relations is that we can elucidate the underlying functional connections directly related to individual differences in performance. The results from brain-behaviour analyses can specify the aberrant or compensatory circuitry related to performance, the later may reveal how an individual may compensate at the neurophysiological level in order to maintain normal performance. In a recent study, Martin et al. (2016) examined ToM performance in SCZ and the related neural circuitry. The authors identified that increased functional connectivity between the left and right inferior frontal gyri was associated with greater accuracy during ToM, specifically for patients with SCZ. Martin and colleagues (2016) concluded that poorer ToM performance in SCZ may arise due to inefficient frontal network activity. To the best of our knowledge, this is the only study that has investigated brain-behaviour relations during social cognition in SCZ; there is no study to date that has applied this analysis technique during emotion perception. In the current thesis I investigated response speed in emotion detection and the underlying circuitry, as patients with SCZ show marked deficits in processing speed (Kalkstein et al., 2010).
Thus, the primary aim of studies 2 and 3 was to delineate the functional networks that underlie emotion processing in different expectancy contexts; first, in the healthy brain and then in patients with SCZ. In Study 2 (presented in Chapter 3), I identify the functional circuitries that facilitate rapid recognition of threatening emotion in healthy individuals. I focus on threat processing in healthy individuals in order to relate the findings to SCZ, as individuals with SCZ have particularly impaired processing of threatening emotions. Next, in Study 3 (presented in Chapter 4), I examine the neural connections that influence impaired emotion perception in SCZ and disentangle the effect of prior expectations. The goal was to delineate the critical changes/differences in neural connectivity in the key region of emotion, the right amygdala, and how aberrant connectivity may influence deficits in recognising emotion in SCZ.

** Genetic Association of Emotion Perception in Schizophrenia

*Emotion Perception as an Endophenotype*

SCZ is a highly heritable disorder with genetic factors explaining 81% of the variance (Wahlstrom et al., 1986). To date, large Genome-wide association studies (GWAS) have identified 108 genome-wide significant and independent loci with common variants significantly associated with SCZ (Ripke et al., 2014a). However, of the variants identified, together they only explain a small amount of variation in liability to SCZ (7%) (Lee et al., 2012), leading studies to employ an endophenotypic approach to identify genetic effects on intermediate traits on the pathway to disease, as they are closer to the genome than the phenotype. Endophenotypes are measurable, heritable, and state independent, with neurophysiological and neurocognitive qualities. Corne et al. (1986) and, more recently, Cannon and Keller (2006) propose these features for an endophenotype:

i. Endophenotypes should be heritable. The differences in genes should affect the variation in that endophenotype.

ii. Endophenotypes should be associated with causes rather than effects of disorders. The disorder or treatments for the disorder should not cause the endophenotype.

iii. Numerous endophenotypes should affect a given complex disorder.

iv. Endophenotypes should vary continuously in the general population. It should be possible to measure the endophenotype on a continuous scale in the non-
affected population in order to increase the power of statistical analyses and simplify the sampling process.

v. Endophenotypes should optimally be measured across several levels of analysis. Convergence in behavioural, neuroanatomical, neurophysiological and neurochemical levels of analysis.

vi. Endophenotypes that affect multiple disorders should be found for genetically related disorders.

Emotion perception and the brain networks involved are considered viable endophenotypes for SCZ, encompassing all the features above (Bediou et al., 2007; Germine et al., 2016a; Lavoie et al., 2013; Martin et al., 2014). A meta-analysis by Lavoie et al. (2013) has shown evidence for emotion perception ability to be genetically related to SCZ and state-independent. In addition, findings from 29 studies examining different abilities in healthy first-degree relatives of patients with SCZ have determined worse performance compared to the general population in ToM, emotion processing, and social perception. Furthermore, the Consortium on the Genetics of SCZ (Greenwood et al., 2011) have investigated the association of 94 candidate genes on various endophenotypes and found a number of genes significantly associated with emotion recognition and emotion memory such as DRD3, AKT1 and COMT. Therefore, emotion perception deficits are proposed to be on the causal pathway from genotype to the clinical phenotype SCZ, rather than a consequence of the illness, see Figure 4.

![Figure 4](image)

**Figure 4. Emotion perception on the pathway from genotype to clinical phenotype.** Deficits in emotion perception ability are proposed to lie on the causal pathway from schizophrenia genes to illness.
Imaging genetics is a method that is used to map the neural structures and activity as a function of genotype. This method is useful to gauge potential neurophysiological endophenotypes, which are theoretically closer to the genome compared to neurocognitive traits, and therefore have a stronger relationship to genetic risk variants. (Mier et al., 2010). Recent investigations using this technique have examined a number of previously mentioned genome-wide significant risk variants for SCZ and their association to the social-emotion network. For example, during emotion processing and ToM tasks, researchers have found reduced connectivity in those with SCZ risk alleles: MIR137 (Mothersill et al., 2014), ZNF804A (Esslinger et al., 2011; Hargreaves et al., 2012; Linden et al., 2013; Walter et al., 2011), COMT (Lo Bianco et al., 2013; Surguladze et al., 2012), 5-HTTLPR (Surguladze et al., 2012), and PPP1R1B (Curcic-Blake et al., 2012). However, as SCZ is a polygenic disorder, risk profiles combining the effects of these risk alleles should be evaluated on their influence on the social-emotion network.

**Schizophrenia Polygenic Risk and Emotion Perception**

Collectively, the variants mentioned above explain only a proportion of disease risk for SCZ, new directives in research are quantifying the effects of variants into a polygenic disease risk score. This is an important trend for future imaging genetic studies (Birnbaum and Weinberger, 2013) as taking into account the additive effects of risk variants is more reflective of the complex genetic architecture of SCZ. Recent studies examining the polygenic effect of SCZ have reported association with white matter volume (Terwisscha van Scheltinga et al., 2013) and left dorsolateral prefrontal cortex inefficiency during working memory (Walton et al., 2013b). However, no imaging polygenic study to date has explored the association between polygenic risk and neural correlates of emotion perception. Notably, a recent longitudinal polygenic study (Germine et al., 2016a), examining the association between SCZ polygenic risk and neurocognition in over 4000 healthy individuals, found the most significant and reliable relationship to be with emotion recognition ability. Germine and colleagues (2016) concluded that emotion recognition represents a potential link between genetic risk for SCZ and the development of psychosis, highlighting the significance of emotion perception as an endophenotype. Therefore, in the current thesis I investigate the influence of SCZ polygenic risk on brain activity underlying emotion perception.

In Study 4 (presented in Chapter 5) I examined the association between polygenic risk of SCZ and functional networks underlying emotion perception. Specifically, SCZ polygenic risk scores were created for a sample of healthy individuals and used as
regressor scores in the neuroimaging analysis. The results from this study add to the imaging genetics literature by assessing how cumulative genetic susceptibility for SCZ influences emotion perception and the related neural architecture in healthy individuals. Furthermore, preliminary evidence is provided for the importance of incorporating emotion neural circuitry into our understanding of the pathway from genotype to clinical phenotype.

**Thesis Plan**

Given the complexity of schizophrenia, it is essential to have an integrated research approach. In the thesis, I consider the cognitive processes, neural circuitry, and genetics underlying emotion perception impairment in SCZ. In the first two studies, I delineate processes and neural circuitry involved in healthy emotion perception in a group of healthy individuals. Specifically, in study 1, I develop a novel fMRI paradigm, which is able to gauge the influence of prior expectations on dynamic emotion perception and disentangle the expectancy-driven brain activity. In study 2, I delineate the expectancy-driven functional networks that subserve rapid detection of threatening emotion. In studies 3 and 4, I gauge impairment in SCZ at the behavioural, neuroimaging, and genetic level of emotion perception. Specifically, in study 3, I investigate SCZ related aberrancy in prior expectations during emotion perception and the associated abnormal neural dynamics; and study 4, I map the neural connections underlying emotion perception as a function of genotype using SCZ polygenic risk models in HCs. A summary of the findings, including the implications and future directions, can be found in the last Chapter of the thesis.
CHAPTER 2
Dynamic emotion perception and prior expectancy
Preface

In Chapter 1, I discussed the current knowledge and findings pertaining to emotion perception impairment in SCZ and outlined the limitations in the current literature and methodology. Specifically, the current methods used to investigate emotion perception in SCZ have relied on non-canonical, static, and unimodal emotional stimuli. In addition, there is limited understanding about the underlying factors, which drive emotion perception deficits in SCZ, such as the influence of reduced precision in prior expectations. In the following chapter the objective was to improve the methodology currently used to investigate emotion perception by developing a novel paradigm, which displays emotion using dynamic, audio-visual emotional stimuli. Moreover, this newly developed dynamic emotion perception (DEP) task is able to disentangle the effect of prior expectancy on emotion perception. Therefore, the aims of this chapter were twofold: to (1) develop and validate the DEP task in a group of healthy individuals and (2) administer the DEP task during functional magnetic resonance imaging (fMRI) experiment, in order to elucidate the prior expectancy effect on naturalistic emotion perception in the healthy brain.

Note. This chapter has been published in Neuropsychologia (see Appendix A).

Abstract
Social interactions require the ability to rapidly perceive emotion from various incoming dynamic, multisensory cues. Prior expectations reduce incoming emotional information and direct attention to cues that are aligned with what is expected. Studies to date have investigated the prior expectancy effect using static emotional images, despite the fact that dynamic stimuli would represent greater ecological validity. The objective of the study was to create a novel functional magnetic resonance imaging (fMRI) paradigm to examine the influence of prior expectations on naturalistic emotion perception. For this purpose, we developed a dynamic emotion perception task, which consisted of audio-visual videos that carry emotional information congruent or incongruent with prior expectations. The results show that emotional congruency was associated with activity in prefrontal regions, amygdala, and putamen, whereas emotional incongruency was associated with activity in temporoparietal junction and mid-cingulate gyrus. Supported by the behavioural results, our findings suggest that prior expectations are reinforced after repeated experience and learning, whereas unexpected emotions may rely on fast change detection processes. The results from the current study are compatible with the notion that the ability to automatically detect unexpected changes in complex dynamic environments allows for adaptive behaviours in potentially advantageous or threatening situations.

Keywords: Dynamic emotion perception; Prior expectations; functional magnetic resonance imaging (fMRI); Partial Least-Squares (PLS); Audio-visual videos.
Introduction

The cognitive processes involved in emotion perception are of significant interest as they provide insights into how humans make sense of complex social interactions. Social situations can rapidly change, requiring a fluid and adaptable system able to recognize and predict another’s emotions (Palermo and Rhodes, 2007). Neuroimaging studies have found that recognition of emotion is influenced by ‘prior expectations’ about which emotions are likely to arise in certain contexts (Barbalat et al., 2013; Dieguez-Risco et al., 2015); however, the behavioural and neural influences of prior expectations on perception have been examined using static representations of emotion. Static images only represent a single facial pattern within a single modality and fail to capture the dynamic complexity of emotion perception relevant in everyday life. In addition, it is the dynamics of emotion that preferentially incite predictive mechanisms, leading to a greater reliance on prior expectations (Kaufman and Johnston, 2014; Palumbo and Jellema, 2013). Thus, it is of paramount importance that functional Magnetic Resonance Imaging (fMRI) studies, which investigate the influence of prior expectations on emotion perception, incorporate tasks that rely on multimodal sensory information and dynamic emotional presentations. Therefore, the first aim of the study was to develop and validate a novel task that is suitable for use in neuroimaging experiments and that incorporates the abovementioned elements of emotion perception.

To study the underlying processes of emotion perception it is essential that stimuli represent natural emotional expressions, composed of facial patterns in constant motion combined with speech prosody. Static emotional images are inadequate, non-canonical stimuli; i.e., non-moving and devoid of auditory information (Kilts et al., 2003). However, audio-visual displays of dynamic emotion present more resemblance to real-life emotional expressions and elicit enhanced psychological responses (Collignon et al., 2008; Lambrecht et al., 2014; Yoshikawa and Sato, 2006) and neural activity in the emotion brain network (Arsalidou et al., 2011; Robins et al., 2009), and, as such, provide greater ecological validity. Direct comparisons of dynamic and static emotion perception provide evidence for significant differences in cognitive processes. For example, Kilts et al. (2003) found that dynamic emotion perception recruited neural correlates implicated in the evaluation of emotional messages in the constant changing of facial patterns. More importantly, a number of studies have demonstrated that, compared to static emotional expressions, the changing dynamics in naturalistic emotional expressions stimulate greater predictive
processes (Kaufman and Johnston, 2014; Palumbo and Jellema, 2013). An explanation for this phenomenon is that dynamic emotions evolve over time and involve greater input of sensory information. Due to the greater sensory input, cognitive efficiency relies on predictive mechanisms from prior expectations, which reduce the incoming sensory information by directing attention to cues that are aligned with expectations (Friston et al., 2006). As prior expectations are a vital process in dynamic emotion perception, the second aim of the study was to examine the behavioural and neural correlates of the effect that prior expectancy has on perception of dynamic emotion.

Previous emotion perception studies investigating the effect of prior expectancy have induced expectations with a preceding emotion instruction cue or a sentence describing an emotionally relevant context. For example, Barbalat et al. (2013) manipulated prior expectations by instructing participants to identify a specific emotion in a sequence of static emotional faces, and decide whether the emotional faces were congruent or incongruent with the emotion in the instruction. It is argued that the instruction cue created an internal “template” against which to match the following stimuli (Summerfield and Koechlin, 2008), and in this way directed attention and facilitated response to emotional faces, which were congruent with the instruction cue. In Dieguez-Risco et al.’s (2015) study, on the other hand, prior expectations were induced by sentences describing different emotionally-relevant contexts, prior to the presentation of static emotional faces. Participants were instructed to indicate whether the expression shown in the face matched the context of the situation described in the preceding sentence. Interestingly, in this task, congruency with prior expectation did not facilitate responses to angry faces, whilst responses to angry faces were faster on incongruent trials. The authors explained that slower response times during the congruent angry conditions occurred due to greater uncertainty in evaluating whether a specific negative emotion matches a specific negative context, as a variety of different negative emotions may be related to different negative contexts. However, incongruent trials would be comparatively easier, because when a negative face is presented after a positive context there is a clear difference in valence. Notwithstanding the valuable findings reported by Barbalat et al. (2013) and Dieguez-Risco et al. (2015), only static displays of emotion were explored, despite the fact that facial motion preferentially invokes predictive mechanisms leading to prior expectations (Kaufman and Johnston, 2014). As such, utilization of static stimuli in experimental paradigms limits the scope of these results and, more importantly, may
confound the predictive processes that are naturally involved in dynamic emotion perception.

Studies using dynamic social stimuli have induced expectations by increasing the occurrence probability of a particular stimulus. For example, Chambon et al. (2011a) examined the influence that prior expectations have on the ability to appreciate other people’s intentions. Prior expectations were induced by selectively manipulating the likelihood of a particular intention to occur within a sequence of different types of intentions. The intentions were communicated with an actor’s hand, which was presented in a video format. The authors found that when the intentions were social and more complex, prior expectations were more likely to bias the inference of intention type. In a different study investigating change detection in dynamic presentations of emotional expressions, the likelihood of a particular emotional expression to occur was manipulated (Kreegipuu et al., 2013). This emotional oddball paradigm involved a sequence of standard expressions (such as neutral), infrequently interspersed with a deviant emotional expression (such as angry). The perception of the incongruent emotional expression was found to rely on ‘visual automatic change detection’ processes, which automatically reorientate attention to changes in the environment (Clery et al., 2013). Unfortunately, there are notable differences between oddball paradigms and tasks that involve a dynamic flow of emotion displayed in naturalistic emotion expression, and, as such, ecological validity of oddball paradigms is limited, as it fails to reflect natural change detection in emotion perception.

The aims of the current study were thus twofold: (1) to design and validate a novel dynamic, audio-visual emotion perception task, suitable for the fMRI environment; and (2) to investigate the differences in brain activity underlying perception of dynamic emotions, which are congruent as opposed to incongruent with prior expectations. In order to induce prior expectations we employed emotional instruction cues (Barbalat et al., 2013) and we increased the likelihood for a congruent emotion to occur (Chambon et al., 2011a). Furthermore, as the consensus is that emotion perception requires a large-scale neural network with distributed activity (Vuilleumier and Pourtois, 2007), we used a multivariate, Partial Least Squares (PLS; McIntosh and Lobaugh, 2004) analytic approach to investigate the coordinated activity of brain regions. We expected to find activations in brain regions consistent with previous prior expectancy literature, such as the ventromedial prefrontal cortex (vmPFC), during conditions congruent with prior expectations (Barbalat et al., 2013;
Summerfield and Koechlin, 2008), and brain regions associated with prediction error and visual automatic change detection (Clery et al., 2013) during incongruent conditions. In addition, we hypothesized that due to the greater complexity of naturalistic emotion, key regions of the ‘social brain’ would be involved during emotion perception (Kennedy and Adolphs, 2012; Van Overwalle, 2009). Specifically, we predicted that: (1) conditions congruent with prior expectations would engage the amygdala, an area critical in emotion learning (Hamann, 2001; Hooker et al., 2006), as prior expectations are reinforced by learning from past experience (Chambon et al., 2011a; Dolan, 2007); and (2) conditions incongruent with prior expectations would engage the right temporoparietal junction (rTPJ), an area known to be important in efficient detection and reorienting towards unexpected change in a social environment (Decety and Lamm, 2007; Geng and Mangun, 2011).

Methods

Participants

Twenty-eight healthy, right-handed males (age range = 23-46; mean age = 31.79, SD = 4.95) were recruited through on-line advertising to staff and students across the University of Queensland. We recruited only males in order to reduce heterogeneity; it has been found that males and females may differ in their emotion perception (Lambrecht et al., 2014; Stevens and Hamann, 2012). Screening was conducted over the phone prior to the recruitment, to confirm that participants had no eye disease, were not currently taking medication, and were without a history of neurological disorders or metal implants in their body. The Mini International Neuropsychiatric Interview version 5.0.0 (Sheehan et al., 1997), was used to ensure that participants did not have current alcohol dependence and were not experiencing a major depressive episode. Intelligence quotient (IQ) was estimated using 2 subsets (vocabulary and matrix reasoning) of the Wechsler abbreviated scale of intelligence (WASI; Wechsler, 1999). On the WASI, the mean standard score was 107.33 (SD = 11.79), indicating that the sample displayed, on average, normal IQs.

Participants were provided with an information sheet, which included a full description of the study and Magnetic Resonance Imaging (MRI) procedure. After reading the document, written informed consent was obtained. This research was approved by the Medical Research Ethics Committee of the University of Queensland. Participants received $30 as reimbursement.
Materials

The novel “Dynamic emotion perception” (DEP) task used in this study involved viewing of audio-visual video clips that carry emotional information congruent or incongruent with prior expectations. Prior expectations were induced by displaying an emotional instruction cue (Barbalat et al., 2013) before the video clips, and by increasing the occurrence likelihood of emotions congruent with the emotion in the instruction (Chambon et al., 2011b), for example by increasing the occurrence of ‘anger’ within a block where an angry instruction cue was displayed.

Video Clips

The emotional video clips were recorded by a Canon EOS 70D video camera and edited (size, audio, and luminance correction) in iMovie on a MacBook Air laptop. The video clips presented a 38-year old Caucasian female actor, wearing a black shirt. The recording captured the head, neck, and shoulders of the actor, against a beige background. The actor had consistent direct gaze towards the camera and was seated in the same position throughout filming.

The actor was instructed to speak a list of 24 sentences whilst acting three different emotions; each sentence was first spoken in a happy expression, next a neutral expression and last an angry expression. The actor repeated each sentence five times in each emotion (i.e., a sentence was spoken in a happy expression five times, next in a neutral expression five times and lastly in an angry expression five times). Before the sentences were spoken, the actor was asked to think of a situation where she would speak the sentence in that certain emotion; she was instructed to convey the emotion through tone of voice and facial expressions. The sentences spoken by the actor were emotionally ambiguous (i.e., the semantic content made sense for multiple emotions), and had consistent level of difficulty, as quantified by word length, frequency, reaction time, and accuracy (The English Lexicon Project; Balota et al., 2007). The context of the sentences related to everyday familiar activities, such as dining (e.g. “You are eating the steak”), transport (e.g. “We'll catch the ferry”), cleaning (e.g. “You are cleaning the kitchen”), outdoors (e.g. “We'll sit under that tree”), mass media (e.g. “We will watch this TV show”) and school (e.g. “Let’s start our homework”).

A total of 360 video clips were recorded. We attempted to keep the video clip recordings as close to 3 seconds each. All video clips obtained were later edited to 3 seconds; any video clips with a duration greatly deviating from 3 seconds were discarded. The video clips were assessed by the researchers for quality, and video clips with visual or sound issues were discarded, this resulted in a total of 98 edited
video clips. We conducted a pilot study with thirteen healthy participants (10 males, 3 females), in order to rate the 98 edited video clips. Participants viewed the video clips and labelled the emotion using a Likert scale: 5 = angry, 4 = slightly angry, 3 = neutral, 2 = slightly happy, and 1 = happy. The final 48 video clip stimuli obtained the highest emotional ratings, for happy and angry these were the videos that were rated as close to 1 and 5, respectively; for neutral videos this was closest to the average rating. There were 16 examples of happy, angry and neutral each, all three emotions were expressed with the same 16 sentences spoken.

The baseline video clips were created from three of the final video clips described above. The baseline video clips had scrambled visual and auditory features to control for low-level perception and motor movement. We scrambled the video clips using Matlab 7.14 (The Mathworks Inc.) by first extracting frames from the original video clip, next converting the frames from RGB images into indexed images, then scrambling the pixels in each frame and lastly combining the scrambled frames into a video clip. For audio scrambling, waveforms were extracted from the original video clip and scrambled in Matlab, and then re-inserted into the scrambled video clip.

**Cues** The emotional cues were created in Adobe Photoshop CS5.1; the cues presented a still picture of the actor expressing an emotion, with the expressed emotion written in white text underneath the picture; the picture and the writing were presented in the centre of the cue and overlaid on a black background. The still pictures of the actor were screenshots from discarded video clips (discarded due to sound issues), there were 3 still pictures of the actor, and in each the actor expressed either an angry, happy or neutral emotion. The baseline cue was the same design as the emotional cue, with a picture and writing in the centre of the cue, overlaid on a black background. However, the baseline cue contained a scrambled still picture of the actor; we scrambled the picture using Matlab by converting the RGB image into an indexed image, and then scrambling the pixels in the image. The writing underneath the baseline picture was “baseline”. At the start of each block, the emotional or baseline cue contained white text above the picture, instructing to either make an “index finger press” or “any finger press”, respectively.

The emotion cue displayed the emotion both visually (static facial expression of the actor), as well as verbally (written emotion word). The addition of visual features was done to aid memory for the emotion, as there is evidence that males, compared to females, have a verbal disadvantage, which extends into decreased memory for verbal content (Andreano and Cahill, 2009). The emotion cue containing the
instruction, presented at the beginning of each emotion block, was used to induce the prior expectation (as per Barbalat et al. (2012)), thereafter the emotion cue (without the instruction) was displayed before each video clip, to reinforce the instruction and to aid memory and expectation for the particular emotion.

**Design**

The experimental procedure consisted of three runs of the DEP task and its nine experimental conditions; 3 emotional cues (happy, angry or neutral) x 3 emotional videos (happy, angry or neutral). When the emotions in the instruction cue and video matched, this was a ‘congruent’ condition, whereas when the emotions in the instruction cue and video did not match, this was an ‘incongruent’ condition. Please note that we included neutral video clips to increase the difficulty of the task by increasing the variety of video stimuli. In the Emotion task by Barbalat et al. (2013), the authors also included 3 emotional stimuli, reasoning that a choice of 3 rather than 2 alternatives would produce an additional cognitive constraint, which would increase the congruency effect. Neutral conditions were removed from the final analysis, as neutral emotion is ambiguous and contentious in the emotion literature (Kesler et al., 2001; Cooney et al., 2006; Sabatinelli et al., 2011). Likewise, we did not use the neutral video clips as the baseline task, as neutral faces may elicit activation in social-emotion regions (Blasi et al., 2009) and are sometimes evaluated as emotional (Lee et al., 2008).

Within each run there were nine experimental blocks and three baseline blocks. Each experimental block began with an instruction emotional cue (3 sec), followed by six or nine sequences of trials consisting of an emotional cue (1 sec), a black screen as the inter-stimulus interval (ISI; mean duration of 1 sec) and an emotional video clip (3 sec) (see Figure 1). The ISI was jittered within a block, with a uniform distribution between 500 ms and 1500 ms, of either 6 x 200 ms intervals (during blocks of 6 video clips) or 9 x 125 ms intervals (during blocks of 9 video clips). The experimental blocks contained either six or nine video clips; this reduced the predictability of how many video clips each block contained, and thus the ability of participants to predict the number of incongruent or congruent video clips. Alternating the number of video clips was also done to eliminate repetitiveness, as this may result in fluctuations of attention. Within a block of six video clips, there were four congruent and two incongruent video clips (66.67% expectancy bias); and within a block of nine video clips, there were five congruent and four incongruent video clips (55.55% expectancy bias). The baseline blocks involved the same procedure as the experimental blocks, such that the block
began with an instruction baseline cue (3 sec), followed by six sequences of trials consisting of a baseline cue (1 sec), a black screen as the ISI (mean duration 1 sec, jittered) and a baseline video clip (3 sec).

**Figure 1. Dynamic emotion perception task.** A schematic diagram showing an example of a happy emotion block. Participants were asked to press a button with their index finger for happy videos (congruent trial) and press a button with their middle finger for every other emotion video (incongruent trial).

The experiment was a mixed design; meaning we used both blocked (emotional cues) and event related (video clips). Thus, within an experimental block, the cue was always one emotion (e.g., happy block: a happy instruction cue followed by a happy video (congruent), then a happy cue followed by, for example, another happy video (congruent), then a happy cue followed by an angry video (incongruent) etc.). However, video clips within a block would alternate in emotion (e.g., a happy block of six video clips, would contain four happy video clips, one angry video clip and one neutral video clip). The video clips within a block were randomized in Microsoft Excel, so that the appearance of congruent or incongruent video clips could not be predicted. The emotion blocks were counterbalanced between runs, as were the runs between participants, using the Balanced Latin Squares method.

**Procedure**

The participants were asked to respond to the video clips to indicate if the emotion presented in the instruction cue matched the emotion expressed in the video. Specifically, participants were told to press the button with their index finger when the video clip was concordant with the emotion in the instruction cue and press with their
middle finger when it was not. In the baseline condition, the participants were told to press any button during the baseline video clips. Participants were instructed to respond as quickly and as accurately as possible during the video clip. Accuracy and reaction times (RTs) were recorded for each trial.

Prior to the fMRI experiment, participants were trained with a practice task outside the MRI scanner. Both the practice task and fMRI task were presented using E-Prime 2.0 software (https://www.pstnet.com/eprime.cfm, 2013; Schneider et al., 2012) on a Windows computer screen. The practice task consistent of 9 blocks and feedback was given if the correct/incorrect button was pressed. The goal was to ensure that participants understood the aim of the task and that the finger response became automated outside the scanner. During the fMRI experiment the DEP task was seen by participants through a tilted mirror attached to the head coil on the MRI scanner. Responses were made on a custom-built MR-compatible response box.

After the fMRI experiment participants completed the two questionnaires: WASI (Wechsler, 1999) and Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT; Mayer et al., 2003), in a testing room outside the MRI scanner. The practice task, fMRI task, and questionnaires were completed at the Centre for Advanced Imaging, University of Queensland 3T scanner facility.

**MRI Procedure and Preprocessing**

Structural and functional MRI images were acquired by a 3T Siemens Magnetom TrioTim system using a 12-channel head coil. The scans collected for each participant were as follows: localizer, T1-weighted anatomical image MP2-RAGE sequence (repetition time (TR): 1900 ms, echo time (TE): 2.32 ms, resolution: 1 mm³, FoV= 230 mm, 192 slices, inversion time (TI): 900 ms, flip angle: 9 degrees), whole-head T2*-weighted echo-planar sequence (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm³, slices: 46, FoV: 192 mm, flip angle: 90 degrees), DWI (TR: 8400 ms, TE: 100 ms, resolution: 2.3 mm x 2.3 mm x 2.5 mm, slices: 60, FoV: 300 mm, b-value: 2000 s/mm², directions: 64), and resting-state (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm³, slices: 46, FoV: 192 mm ). The total scanning time per session was 45min.

Standard preprocessing of the images was carried out using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8, 2013; Friston, 2003). The preprocessing steps were as follows: slice timing on the functional images, to correct for differences in slice acquisition times within each volume using the middle slice as reference; realignment (estimate and reslice) on the functional images, to correct for inter-scan movement within each run (no participant was excluded for excessive
movement (defined as >3 mm translation, >2 degrees rotation); co-registration of the functional and structural images; segmentation of the structural image, with heavy regularisation (0.1) recommended for MP2-RAGE sequence; normalization of the resliced images into a standardized, stereotaxic space (according to the Montreal Neurological Institute template); and smoothing of normalized images with a 6mm full-width-at-half-maxim isotropic Gaussian kernel.

Data Analysis

Preliminary Analysis

Convergent validity was assessed by examining the correlation between the new DEP task and the MSCEIT (Mayer et al., 2003). The MSCEIT remains the flagship test for emotional intelligence, consistently providing high validity and reliability (Mayer et al., 2012). We chose two components of the MSCEIT, Perceiving Emotions and Understanding Emotions, as these components aim to measure the perception and interpretation of emotion, similarly to the DEP task (Kee et al., 2009). Within the two components of the MSCEIT were subcomponents (i) Perceiving Emotions, which included identifying emotions conveyed in ‘faces’ and ‘pictures’ of paintings and landscapes, and (ii) Understanding Emotions, which included understanding the ‘blends’ of emotions and recognizing how emotions may ‘change’ and develop (Mayer et al., 2003). Significant correlations were observed between RTs and accuracy percentages within the DEP task and components of the MSCEIT. Specifically, both RTs and accuracy had significant correlations with the ‘blends’ MSCEIT subcomponent ($r = -.34, p = .04; r = .55, p = .002$, respectively). The ‘faces’ subcomponent of Perceiving Emotions, which particularly converges with the DEP task, had a significant correlation with DEP accuracy ($r = .34, p = .04$). Finally, DEP accuracy was also significantly correlated with both Perceiving Emotions ($r = .38, p = .03$) and Understanding Emotions ($r = .49, p = .01$).

We also assessed discriminability in the new DEP task by computing $d'$ scores (Macmillan and Creelman, 1990) for each participant for the different emotion videos (angry, happy and neutral). The $d'$ scores indicate ability to discriminate the congruent and incongruent emotion videos, by taking both hits and false alarms into account. Please note that when the rate of false alarms was zero or the rate of hits was one a correction was applied, as per Corwin (1994). The mean $d'$ scores for each emotion video are presented in Figure 2, $d'$ varies from zero (chance performance) to 4.30 (perfect performance). The results show that discriminability did not significantly vary
for the different emotions (Ms (SD): Angry = 3.98 (0.33); Happy = 4.12 (0.29); Neutral = 4.03(0.31)), $p = 0.12$, indicating high discriminability for all emotion videos.

![Figure 2. Discriminability index (d') for emotion videos: angry, happy and neutral. Error bars show the standard error of the mean.](image)

**Behavioural Analysis**

Mean RTs and percentage accuracy from all responses acquired during scanning were calculated for each participant, across 12 conditions (3 runs x 2 emotion x 2 video). These analyses were conducted using the SPSS package (SPSS Inc.). We conducted a factorial ANOVA with a 3 (experimental run) x 2 (emotion) x 2 (video) within-subjects design to assess the effect of congruency on RTs and accuracy with factors: experimental run (Run 1, Run 2, Run 3), emotion (happy and angry) and video (congruent and incongruent). Specifically, congruent video indicates the pairing of happy cues with happy video clips and angry cues with angry video clips, whereas incongruent video indicates the pairing of angry cues with happy video clips and happy cues with angry video clips. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity where the assumption of sphericity had been violated. Significant interactions were further analyzed using Bonferroni-corrected simple contrasts and paired-samples t-tests, after running 1000 permutations.

**fMRI Analysis**

In the present study, we employed a multivariate approach PLS, which investigates the distributed patterns of neural activity rather than the independent activity of a single brain region, as we believe that this analysis is more appropriate for
our data as naturalistic emotion perception engages a widespread and interactive brain network (Arsalidou et al., 2011; Vuilleumier and Pourtois, 2007). In addition, we wanted to avoid a-priori contrasts and forced cognitive subtraction, as it assumes that there are no interactions among cognitive components in a task (Amaro Jr and Barker, 2006). PLS does not constrict the brain activation based on researcher-defined contrasts; instead, it identifies the fundamental relations (latent variables: LVs) between brain activity and experimental conditions that account for maximum covariance in the data. Similar to principal component analysis, PLS decomposes the data into orthogonal dimensions by conducting singular value decomposition (SVD). Furthermore, PLS provides enhanced statistical power and sensitivity for paradigms such as ours, which involve event-related features (McIntosh et al., 2004).

Because our interest was in brain activity during the emotional video clips, not activity during the emotional cues, we isolated activity during the video clips by conducting the analysis across five TRs (TR 0 – TR 5) starting at the onset of the video clips. Activity at each time point was normalized to the first TR (labelled TR 0 in the figures) and thus, our measure of dynamic emotion perception activity was relatively uninfluenced by cue activity. In an event-related paradigm, PLS provides a set of brain regions related to the experimental conditions for each TR on each LV. At each TR, for each participant a ‘brain score’ is calculated by multiplying the ‘salience’ (i.e., the degree of covariance of activity with the task condition on each LV) of each voxel by the signal of each brain voxel, and summing these across the entire brain. We plotted the mean brain scores at each TR to show overall brain activity fluctuations across the different conditions expressed over the 15 sec period, which is analogous to hemodynamic response functions that are typically plotted for individual brain regions.

For all analyses, we ran 500 permutations, consistent with the literature (Burianova and Grady, 2007; McIntosh and Lobaugh, 2004), to determine significant LVs at p < 0.002. In addition, we ran 100 bootstraps, estimating the standard errors of the salience for each voxel in order to assess the reliability and robustness of each voxel’s contribution to a pattern of brain activity. We used the mean-centering approach in PLS, which involves subtracting the grand mean of the data matrix from the task means. We restricted the bootstrap ratio threshold to ± 3 (p < 0.0001) and reported areas with a cluster size of 100 or more voxels. Confidence intervals (95%) were calculated from the bootstrap; for the mean brain scores in each condition across the five TRs, significant differences between conditions were determined by a lack of overlap in the confidence intervals.
Results

Behavioural Findings

We assessed the effect of congruency on RTs across different emotional valences and across experimental runs. A three-way analysis of variance yielded a main effect of run, \( F(1.61, 43.42) = 5.31, p = 0.01, \) RTs in the first run (Mean \( M \) = 864.45; Standard Deviation \( SD \) = 196.12) were slower than run 2 (\( M = 796.22; SD = 189.55 \)), \( p = 0.02 \). No significant difference was found between the first and third run (\( M = 805.90; SD = 227.64 \)) or second and third run. Additionally, a main effect of emotion was found, \( F(1, 27) = 11.38, p < 0.0001 \). Angry videos (\( M = 802.34; SD = 189.47 \)) were faster to detect than happy videos (\( M = 842.05; SD = 201.47 \)), \( p = 0.002 \). There was also a main effect of video, \( F(1, 27) = 24.71, p < 0.0001 \), such that videos congruent with the instruction cue (\( M = 858.59; SD = 219.29 \)) obtained significantly slower RTs compared to videos incongruent with the instruction cue (\( M = 785.80; SD = 171.65 \)).

There was a significant interaction between experimental run and video (\( F(1.61,43.53) = 10.43, p < 0.0001 \)), indicating that the difference between RTs for video clips congruent and incongruent with the instruction cue was depended on which experimental run was being performed. To break down this interaction, simple contrasts were conducted comparing experimental runs and videos, an RT difference was revealed between run 1 and both run 2 and run 3, for congruent versus incongruent videos, (Run 1 vs Run 2: \( F(1,27) = 10.78, p = 0.003 \); Run 1 vs Run 3: \( F(1,27) = 13.62, p = 0.001 \)). Displayed in the RT interaction graph (see Figure 3) and results from the paired-samples t-tests, these effects reflect that incongruent conditions (compared to congruent) decreased RTs more in the first run (\( p = 0.001 \)) compared to run 2 (\( p = 0.018 \)), and not in run 3 (\( p = 0.69 \)). Paired-samples t-tests were then performed, showing that RTs significantly decreased during congruent conditions in run 2 (\( M = 825.64; SD = 219.72 \)) and run 3 (\( M = 809.87; SD = 235.40 \)) compared to run 1 (\( M = 940.24; SD = 256.02 \)), \( p = 0.001 \) and 0.002, respectively. While remaining constant for incongruent conditions across experimental runs (\( Ms \ (SD): \) Run 1 = 788.66 (155.92), Run 2 = 766.79(171.33), Run 3 = 801.93(229.73); \( ns \)). We did not find a significant interaction between emotion and video, which suggests that the congruency effect on RTs was consistent across the different valences.

Finally, we assessed the effect of congruency on accuracy across different emotional valences and across experimental runs. A three-way analysis of variance
did not produce any significant main effects of run, emotion or video, however, there was a significant interaction between experimental run and video, $F(2,54) = 7.42, p = 0.001$. The significant interaction was further analyzed using contrasts. There was an accuracy difference between run 1 and both run 2 and run 3, for congruent versus incongruent videos, (Run 1 vs Run 2: $F(1,27) = 8.10, p = 0.008$; Run 1 vs Run 3: $F(1,27) = 10.68, p = 0.003$). The accuracy interaction graph (see Figure 3) and paired-samples t-tests, indicate that incongruent conditions (compared to congruent) increased accuracy significantly in the first run ($p = 0.04$), but not in run 2 ($p = 0.30$) or run 3 ($p = 0.17$). Similar to the RT results, it was demonstrated by paired-samples t-tests that during congruent conditions accuracy increased in run 2 ($M = 99.31\%; SD = 1.51\%$) and run 3 ($M = 99.05\%; SD = 2.23\%$) compared to run 1 ($M = 97.32\%; SD = 3.90\%$), $p = 0.02$ (for both). While remaining constant during incongruent conditions across experimental runs ($Ms (SD)$: Run 1 = 99.36% (1.90%), Run 2 = 98.60% (3.77%), Run 3 = 98.31% (3.11%); ns). Again, we did not find a significant interaction between emotion and video, which suggests that the congruency effect on accuracy was consistent across the different valences.

Figure 3. Mean reaction times and accuracy for experimental runs (Run 1, Run 2, Run 3) and video (congruent and incongruent). Error bars show the standard error of the mean.

**fMRI-PLS Results**

Our focus was on spatiotemporal activity of brain regions that was differentially involved in congruent and incongruent dynamic emotion perception. The congruent conditions were happy videos preceded by the happy instruction cue and angry videos preceded by the angry instruction cue. The incongruent conditions were angry videos preceded by the happy instruction cue and happy videos preceded by the angry
instruction cue. For further analyses, involving baseline and neutral stimuli see the Supplementary Materials. Specifically, Supplementary Materials 1 show the analysis with baseline conditions, confirming that the novel DEP task successfully activates regions known to be involved in emotion perception; and Supplementary Materials 2 include the neutral conditions.

The significant LV accounted for 63.60% of covariance in the data and differentiated congruent and incongruent conditions (see Figure 4). In line with previous literature, the vmPFC and dorsal medial prefrontal (dmPFC) regions known to be involved in congruency with prior expectations (Barbalat et al., 2013; Summerfield and Koechlin, 2008) showed significantly greater activation for congruent compared to incongruent conditions. Additional areas active for dynamic emotions under congruent conditions were the, bilateral inferior frontal gyri, right amygdala, left putamen, and right insula.

Incongruent emotions recruited a wide network of regions, including the left middle occipital gyrus, cuneus, left precuneus, left superior parietal lobule, bilateral inferior parietal lobule, left middle frontal gyrus, medial frontal gyrus and left precentral gyrus, areas known to be involved in visual automatic change detection (Clery et al., 2013). Additional areas included the anterior mid-cingulate gyrus, left middle temporal gyrus, and bilateral insula, regions critical in prediction error (Barbalat et al., 2013; Summerfield and Koechlin, 2008). Finally, we identified the following regions during incongruent trials: the rTPJ, posterior mid-cingulate, right thalamus, and left claustrum (see Table 1).
Figure 4. Results of the mean-centered PLS analysis, differentiating congruent and incongruent conditions. The graph shows the brain scores from TR1, TR 4 and TR 5 (TR = 3 sec; error bars = 95% CIs from bootstrapping). All maxima have BSR ≥ 3.0 and cluster size ≥ 100 voxels (600 mm$^3$). Regions corresponding to areas activated during dynamic emotion under congruent conditions: vmPFC = ventromedial prefrontal cortex; rAMY = right amygdala; IPUT = left putamen; prefrontal cortex (bilateral inferior frontal gyri, bilateral superior frontal gyri); and incongruent: PC = posterior mid-cingulate; rTPJ = right temporoparietal junction; bilateral insula; occipital (left middle occipital gyrus, cuneus); left middle temporal gyrus; parietal (left superior
parietal lobule, left precuneus, bilateral inferior parietal lobule, bilateral postcentral); anterior mid-cingulate; premotor (left precentral, left middle frontal).
<table>
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<tr>
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<th>He m</th>
<th>BA</th>
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<th>Voxels</th>
<th>BSR</th>
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<td></td>
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Table 1

*Differences in activity during congruent vs. incongruent emotion perception*
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Abbreviations: Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; voxels = number of voxels (one voxel volume=6 mm$^3$). All reported activations are ≥100 voxels (600 mm$^3$). sgACC = subgenual anterior cingulate cortex; vmPFC = ventromedial prefrontal cortex; dmPFC = dorsomedial prefrontal cortex.

A critical advantage of PLS is that it provides temporal information for each of the activation peaks, enabling extraction of regional activity for each TR and plotting of time courses (McIntosh et al., 2004). To investigate the nature of changes in activity, the percent signal change was examined over time for specified regions of interest: vmPFC, right amygdala, rTPJ and posterior region of the mid-cingulate gyrus. We identified the time point of maximal difference for congruent conditions from incongruent conditions at TR 5, while incongruent conditions showed greatest divergence from congruent conditions at TR1 (see Figure 5).
Figure 5. For visualization purposes: time courses of activity from the whole-brain analysis, in the ventromedial prefrontal cortex (x = 46, y = 24, z = -16); right amygdala (x = 28, y = -2, z = -18); right temporoparietal junction (x = 56, y = -50, z = 32); posterior mid-cingulate (x = -2, y = -26, z = 30). Error bars show the standard error of the mean.

Discussion

The purpose of the current study was to develop and validate a novel fMRI paradigm with improved ecological validity, using dynamic, audio-visual video clips, instead of static pictures as stimuli. The DEP was designed to investigate the prior expectancy effect on response rate and spatiotemporal neural activity during naturalistic emotion perception. Our study using dynamic emotional stimuli, found some results consistent with previous literature, which used static stimuli (Summerfield and Koechlin, 2008; Barbalat et al., 2013; Clery et al., 2013); however, our study also revealed significant differences in brain activity and behaviour. Our findings show that:
(1) recognition of emotions congruent with prior expectations is initially slower than recognition of incongruent emotions, engaging the prefrontal cortices, which suggests greater executive functioning processes; (2) over time, recognition of emotions congruent with prior expectations becomes faster, engaging the amygdala and putamen, which suggests emotional learning (Hamann, 2001; Hooker et al., 2006) and conditioning (Brovelli et al., 2011; Tricomi et al., 2009); and (3) recognition of incongruent emotions is consistently fast, recruiting visual automatic change detection neural correlates and posterior mid-cingulate gyrus, which suggests quick adjustments of attention to change (Decety and Lamm, 2007; Vogt, 2014).

The newly developed DEP task provides convergent validity, high discriminability, suitability for use in fMRI, and increased ecological validity. The DEP task has convergent validity with the Perceiving Emotions and Understanding Emotions components of the MSCEIT, which is a reliable and valid measure of emotional intelligence (Mayer et al., 2012). In addition, the DEP task has increased ecological validity, as the presentation of emotion includes both biological movement and multimodal sensory information, which are critical in the investigation of emotion perception; previous work has relied on static pictures of emotional expressions, only representing a single facial pattern, resulting in decreased activity in key social-emotion regions (Arsalidou et al., 2011). In the current study, we replicated the findings of previous studies investigating prior expectation on emotion, such that the vmPFC was recruited for congruent emotion processing, whereas the anterior mid-cingulate gyrus, middle temporal gyrus, insula (Barbalat et al., 2013; Summerfield and Koechlin, 2008), occipital regions, posterior parietal regions and premotor gyri (Clery et al., 2013) were recruited during incongruent emotion processing. More importantly, however, the advantage of increased ecological validity enabled the recruitment of key regions of the social brain, namely amygdala and rTPJ (Kennedy and Adolphs, 2012; Van Overwalle, 2009). Thus, we were able to examine the contribution of these regions for conditions congruent and incongruent with prior expectations.

Congruency with prior expectation involved greater top-down processing of emotional content and emotion learning and conditioning, with regions peaking at later time points of hemodynamic response. Activations included the prefrontal cortex (superior, medial and inferior regions) and right amygdala, which are areas implicated in higher-order emotion processing, such as evaluation and decision-making during emotion processing, encoding of emotional experiences in memory and emotion-related learning (Baker and Kim, 2004; Hamann, 2001; Koenigs et al., 2007; Lindquist
et al., 2012). Activations were also found in the putamen, an area consistently active during conditioning and habituation induced by visual cues (Brovelli et al., 2011; Tricomi et al., 2009). The engaged network of activated regions was found to peak at later time points (i.e., TR 5), in addition we identified slower response times for congruent conditions, indicative of the need for greater cognitive engagement (Theeuwes et al., 2000). Slower response times for congruency with prior expectations have been reported previously in the literature (Dieguez-Risco et al., 2015), particularly for stimuli that carry greater uncertainty and require evaluation of congruency. It is argued that when asked to judge emotional congruency in dynamic displays of emotion there is initially higher uncertainty because the emotional expression may suddenly change, and therefore the evaluator takes longer than when judging emotional incongruency, which is immediately evident to differ from the emotional cue. With time, however, the response time to emotional congruent stimuli decreases as the nature of the emotional contingency is learned. This is demonstrated in our study as response times became faster over time (i.e., across the experimental runs), only during congruent conditions, giving support to temporal cue-related emotion learning. Thus, in contrast to previous prior expectancy studies (Barbalat et al., 2013), the results of the current study demonstrate that congruently cued emotions require greater cognitive engagement and processes such as evaluation and decision-making, and only after repeated cues and conditioning, prior expectations are reinforced to facilitate emotion perception. These findings are akin to real life where prior expectations are not instantaneous and develop through recurrent experience and learning.

In contrast to congruency with prior expectations, incongruent trials engaged regions previously associated with visual automatic change detection and rapid cognition. An area active during automatic change detection (Clery et al., 2013), which is also a key hub of the social brain (Kennedy and Adolphs, 2012; Van Overwalle, 2009), is the TPJ. This region was recruited during incongruent emotion conditions and it is known to be particularly involved in rapid processing of unexpected cues and enabling of immediate reactions to social situations (Ciaramelli et al., 2008; Decety and Lamm, 2007). Additionally, we found activations in the posterior parts of the midcingulate gyrus, an area critically important in rapid cognitive adjustments and cognitive efficiency (Leech and Sharp, 2014; Vogt, 2014). The activity of rTPJ and posterior mid-cingulate gyrus peaked early, demonstrating evidence for their roles in efficient and rapid detection of incongruent emotion. The suggested quick detection
for incongruent emotion is in line with the consistently fast response rates during incongruent conditions, relative to the initial slower responding during congruent conditions. Our findings for fast detection of incongruent emotion are novel and make sense from an evolutionary perspective, because in a dynamic and complex environment, advantageous or dangerous situations (critical to our survival) may arise unexpectedly, requiring automatic detection and attention directed towards a potential opportunity or threat (Bishop, 2008; Juth et al., 2005; Öhman, 2009).

Critically, we provide crucial new insights into the influence of prior expectations on perception of naturalistic emotion. In the case of dynamic audio-visual emotional stimuli, initial congruence detection may be slower because the participant is evaluating, with certainty, whether the emotional content (which is in constant motion, with changing audio-visual features) is congruent with previous information. The requirement to evaluate congruency is in line with our fMRI findings, as key brain regions in emotion evaluation and decision-making were associated with the congruent conditions. During incongruent trials, on the other hand, the participant reaches a decision sooner because once a different emotional valence is detected one can be certain that it is an incongruent emotion. We suggest that this quick detection of change in emotional valence during incongruent conditions may in part be driven by visual automatic change detection processes, as supported by the fMRI results. One limitation of this study is that the results pertain only to a male population. The male sample was recruited in order to reduce heterogeneity, as males and females may differ in their emotion perception (Lambrecht et al., 2014; Stevens and Hamann, 2012). The validity and reliability of this novel paradigm will be the focus of follow-up studies in which we will investigate the role of prior expectancy in healthy females and in patients suffering from disorders associated with dysfunctional emotion perception; specifically, in patients with schizophrenia who have been reported to have an overreliance on prior expectations (Chambon et al., 2011b).

**Conclusion**

In conclusion, we present a novel fMRI paradigm, which gauges the influence of prior expectations on dynamic emotion perception with increased ecological validity. The improved methodology enabled us to provide new insights into the behavioural and neural mechanisms of prior expectations and delineate the spatiotemporal activity of brain regions active during dynamic emotion perception. Specifically, prior expectations seem to be reinforced by top-down processes, which direct attention to emotional events congruent with expectations, and which, with repeated exposure,
facilitate perception. Conversely, emotional events incongruent with prior expectations gain attention via change detection processes, an adaptive behaviour, which allows one to automatically detect a change in one’s environment and identify a potential opportunity or threat.
Supporting Information 1

Results

fMRI-PLS Results

The significant LV accounted for 53.60% of the covariance, and differentiated emotion and baseline conditions (see Figure 1). Regions showing greater activation for emotion compared to baseline conditions, in line with previous emotion literature were the: amygdala, the prefrontal cortex, parahippocampal gyrus, insula and basal ganglia (see Table 1) (Adolphs, 2002; Murty et al., 2010; Phillips et al., 2003a; Vuilleumier and Pourtois, 2007).

![Figure 1](image.png)

**Figure 1.** Results of the Mean-Centered PLS analysis, the pattern identified by LV 1 separated emotion and baseline conditions. The graph shows the mean-centered brain scores from TR1 to TR 5 (error bar=95% CIs from bootstrapping). All maxima have BSR≥3.0 and cluster size ≥100 voxels (600 mm3).
### TABLE 1
Differences in activity during emotion conditions vs. baseline conditions

<table>
<thead>
<tr>
<th>Region</th>
<th>Peak MNI</th>
<th>MNI coordinates</th>
<th>Voxels</th>
<th>BSR</th>
</tr>
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<td></td>
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<td><strong>Baseline &gt; Emotion</strong></td>
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<tr>
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</tr>
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<td></td>
<td></td>
<td></td>
<td>10.66</td>
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</table>
R = right; L = left; B = bilateral; BSR = bootstrap ratio; voxels = number of voxels (one voxel volume=6 mm\(^3\)). All reported activations are ≥100 voxels (600 mm\(^3\)) and peak BSR between TR 1 and TR 5.
Supporting Information 2

Results

Behavioural Findings- Reaction Time

A factorial ANOVA 3 (experimental run) x 3 (emotion) x 2 (video) within-subjects design on RTs was conducted, with factors: experimental run (Run 1, Run 2, Run 3), emotion (angry, happy and neutral) and video (congruent and incongruent). We were interested in the effect of congruency on RTs across different emotional valences and across experimental runs. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity where the assumption of sphericity had been violated. A main effect of run was revealed, $F(1.52, 41.11) = 9.94, p = 0.001$, RTs in the first run ($M = 907.05; SD = 196.22$) were slower than Run 2 ($M = 825.39; SD = 197.56$) and Run 3 ($M = 834.72; SD = 221.21$), $p=0.002$ and 0.016, respectively. No significant difference was found between the second and third run. There was a main effect of emotion, $F(2, 54) = 37.19, p < 0.0001$, the angry video ($M = 802.34; SD = 189.47$) was faster than happy video ($M = 842.05; SD = 201.47$) and neutral video ($M = 922.78; SD = 210.70$), $p = 0.007$ and $p < 0.0001$, respectively, and happy video was faster than neutral video, $p < 0.0001$. There was a main effect of video, $F(1, 27) = 32.86, p < 0.0001$, the incongruently cued videos ($M = 814.03; SD = 175.37$) were faster than the congruent cued videos ($M = 897.41; SD = 221.39$), $p < 0.0001$.

There was a significant interaction between experimental run and video, $F(1.43, 38.58) = 12.86, p < 0.0001$. To test the significant interaction simple contrast were conducted. There was a RT difference between Run 1 and Run 2 for congruent versus incongruent videos, $F(1,27) = 14.36, p = 0.001$. Additionally, there was an RT difference between Run 1 and Run 3 for congruent versus incongruent videos, $F(1,27) = 15.04, p = 0.001$. Looking at the RT interaction graph (see Figure 1) these effects reflect that incongruent preceding cue (compared to congruent) decreased RTs more in the first run compared to run 2 and run 3.
Behavioural Findings - Accuracy

A factorial ANOVA 3 (experimental run) x 3 (emotion) x 2 (video) within-subjects design on accuracy was conducted, with factors: experimental run (Run 1, Run 2, Run 3), emotion (angry, happy and neutral) and video (congruent and incongruent). We were interested in the effect of congruency on accuracy across different emotional valences and across experimental runs. There were no significant main effects of run, emotion or video. There was a significant interaction between experimental run and video, $F(2, 54) = 6.34, p = 0.003$. To test the significant interaction simple contrast were conducted. There was an accuracy difference between Run 1 and Run 2 for congruent versus incongruent videos, $F(1,27) = 7.04, p = 0.013$. Additionally, there was an accuracy difference between Run 1 and Run 3 for congruent versus incongruent videos, $F(1,27) = 10.36, p = 0.003$. Looking at the accuracy interaction graph (see Figure 2) these effects reflect that incongruent preceding cue (compared to congruent) increased accuracy only in the first run and not in run 2 and run 3.

Figure 1. Mean reaction times for experimental runs (Run 1, Run 2, Run 3) and video (congruent and incongruent). Error bars show the standard error of the mean.
Figure 2. Mean accuracy for experimental runs (Run 1, Run 2, Run 3) and video (congruent and incongruent). Error bars show the standard error of the mean.

fMRI- PLS Results

The significant LV accounted for 27.40% of the covariance, and differentiated congruent (angry video + angry cue; happy video + happy cue; neutral video + neutral cue) and incongruent (happy video + angry cue; happy video + neutral cue; angry video + happy cue; neutral video + angry cue) conditions. Note that incongruent conditions: neutral video + happy cue and angry video + neutral cue did not significantly differentiate from congruent conditions. A region showing greater activation for congruent compared to incongruent conditions, in line with previous literature was the ventromedial prefrontal cortex (vmPFC) (Barbalat et al., 2013; Summerfield and Koechlin, 2008). Additional areas active for dynamic emotions under congruent conditions were the bilateral inferior frontal, right middle frontal, right precentral, left superior frontal, right parahippocampal, left amygdala, left caudate, bilateral claustrum, left insula, right globus pallidus, left putamen, left middle temporal, bilateral superior temporal, right transverse temporal and inferior temporal.

Incongruent emotions recruited a wide network of regions involved in incongruence detection: occipital cortex- cuneus and lingual, posterior parietal cortex- bilateral inferior parietal, and the bilateral postcentral (Clery et al., 2013). As well as
regions involved in prediction error: right anterior cingulate, right insula and bilateral superior frontal (Barbalat et al., 2013; Rahnev et al., 2011; Summerfield and Koechlin, 2008). Additional areas for incongruent conditions were: posterior cingulate, right hippocampus, left parahippocampal, right temporoparietal junction (TPJ) and bilateral thalamus (see Table 1).
TABLE 1

Differences in activity during congruent vs. incongruent emotion perception

<table>
<thead>
<tr>
<th>Region</th>
<th>Peak MNI</th>
<th>MNI coordinates</th>
<th>Voxels</th>
<th>BS R</th>
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<td></td>
<td></td>
<td>x    y    z</td>
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<td><strong>Congruent &gt; Incongruent</strong></td>
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<td>-64 -16 2</td>
<td>933 8.02</td>
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<td>62     -28  4</td>
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<td>1736 7.70</td>
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<tr>
<td>R. Inferior temporal</td>
<td>46     -72  0</td>
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## Incongruent > Congruent

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<th>R. Medial frontal (BA6)</th>
<th>B. Middle frontal (BA9, 8, 10)</th>
<th>Paracentral (BA31)</th>
<th>R. Postcentral (BA4)</th>
<th>B. Precentral (BA6, 4, 9, 43)</th>
<th>R. Sub-Gyral (BA6)</th>
<th>B. Superior frontal (BA9, 6)</th>
<th>R. Posterior Cingulate (BA30)</th>
<th>R. Anterior Cingulate (BA24)</th>
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</table>
B. Thalamus

<p>| | | | | |</p>
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</table>

R = right; L = left; B = bilateral; BSR = bootstrap ratio; voxels = number of voxels (one voxel volume=6 mm³). All reported activations are ≥100 voxels (600 mm³) and peak BSR between TR 1 and TR 5.
CHAPTER 3
Prior expectations influence functional networks underlying efficient threat processing
Preface

Chapter 2 describes a study in which I investigated the prior expectancy effect on dynamic emotion perception and the related neural activation. I was able to demonstrate that during dynamic emotion perception prior expectations develop gradually over the course of the experiment and are related to activity in ventromedial prefrontal cortex (vmPFC) and right amygdala (rAMY). In comparison, emotion perception that is incongruent with prior expectations may rely on fast change detection processes, associated with activity in the right temporoparietal junction (rTPJ). However, there is still a lack of understanding about the underlying neural networks associated with fast responding to emotions congruent and incongruent with prior expectations. Therefore, the following chapter describes a study that examined the functional brain networks connected to the vmPFC, rAMY and rTPJ (key regions active during congruency vs. incongruency with prior expectations), in order to identify the importance of these networks in fast detection of emotion. Using the same paradigm that I have used in my previous publication (Dzafic et al., 2016), the DEP task, I specifically focused on the fast detection of threatening emotion (i.e., anger). Examining threat processing and the underlying neural networks in healthy individuals provides implications for SCZ, as impaired threat processing is consistently reported in SCZ.

Note. The paper incorporated in this chapter is now under review in Journal of Cognitive Neuroscience.

Abstract

Speed of threat processing represents an ecologically essential ability for survival in an ever-changing social environment in which threatening emotions might be congruent or incongruent with one’s expectations. Research shows that the ability to quickly respond to threat is subserved by different cognitive processes, depending on its congruency with one’s expectations; however, the underlying functional circuitry is still poorly understood. The objective of this study was to delineate functional networks that subserve rapid recognition of threat in situations either congruent or incongruent with prior expectations. We used the Dynamic Emotion Perception task to elicit prior expectations and manipulate the probability of a threatening stimulus, and functional connectivity analysis to delineate the underlying functional networks. Our results show that fast detection of threat congruent with prior expectations engages a right amygdala subcortical network, suggesting selective attention to threat. In addition, we found a trend for faster detection of threat incongruent with prior expectations to engage a ventral frontoparietal network connected to the right temporoparietal junction. These findings shed light on the functional circuitries that facilitate rapid recognition of threat in a healthy brain, with implications for pathologies, such as schizophrenia, which are characterized by impaired processing of threatening emotions.
Introduction

In a dynamic social environment, the ability to quickly recognize potential threat is essential for survival. In social situations in which threat is expected, allocation of attention towards aggressive cues in order to enable faster reaction to danger is paramount (Barbalat et al., 2012). Conversely, in seemingly innocuous social situations threat may appear unexpectedly, requiring a rapid shift of attention towards aggressive cues (Ohman et al., 2001; Schmidt-Daffy, 2011). In a previous functional magnetic resonance imaging (fMRI) study we investigated the neural correlates underlying perception of expected or unexpected emotions; however, because of its survival relevance, the focus of the current study was on perception of anger (i.e., social threat), and, specifically, on its rapid recognition. As such, we examined the functional networks that subserve rapid response to threat presented in a dynamic, audio-visual environment in which threat was either congruent or incongruent with prior expectations.

Identification of the neural circuitry facilitating rapid recognition of life-like, dynamic representations of threat in a healthy brain is essential for our understanding of aberrant functional connectivity in pathologies characterized by impaired threat processing. Previous studies have consistently used static displays of anger to display threatening emotion (Marstaller et al., 2016; Ohman et al., 2001); and converging results implicate an amygdala and thalamus functional network as core circuitry in rapid threat processing, by means of selective processing of threatening cues (Dolan and Vuilleumier, 2003; Vuilleumier et al., 2001, 2003). In a recent multimodal neuroimaging, brain-behaviour study, Marstaller et al. (2016) have shown that individuals who are faster at detecting 'direct' threat (i.e., anger), have greater engagement of a right amygdala functional network. Furthermore, Marstaller and colleagues identified that these individuals also have greater integrity in the inferior longitudinal fasciculus, a white matter pathway connecting the amygdala to visual regions. Despite the crucial findings provided by the abovementioned work, the threat stimuli were presented in a form of static images of faces. Evidence shows that the greater complexity of real-life dynamic emotion requires predictive and evaluative processes, which are generated by the greater uncertainty in the constantly moving and changing features (Kaufman and Johnston, 2014; Palumbo and Jellema, 2013). Utilization of static stimuli in experimental paradigms, therefore, may fail to uncover processes involved in real-life fast detection of dynamic threatening emotions, and
would confound our understanding of functional circuitries that underlie these processes, which facilitate rapid recognition of threat in a healthy brain.

Furthermore, studies to date have not delineated the *expectancy-driven* functional networks, which subserve rapid detection of threat. Specifically, the underlying circuitry that enables rapid detection of expected threat compared to threat that is not expected. What we do know is that there are regional activation differences in specific areas, such as ventromedial prefrontal cortex (vmPFC), right amygdala (rAMY) and parietal cortex - in particular right temporoparietal junction (rTPJ) - between emotions congruent and incongruent with prior expectations, respectively (Bermpohl et al., 2006; Browning and Harmer, 2012; Dzafic et al., 2016; Ueda et al., 2003), but the functional circuitry has been relatively unexplored. In regards to incongruency with expectations, seminal work by Corbetta et al. (2008), has established that ‘reorienting’ to relevant, unexpected events, such as potentially threatening humans, involves a right ventral frontoparietal network, dubbed the ventral attention network (Corbetta and Shulman, 2002). There is only one fMRI study (Barbalat et al., 2013), which has investigated functional connectivity differences influenced by prior expectations, showing that increased functional connectivity between vmPFC and thalamus is associated with detection of expected rather than unexpected threat. On the other hand, in terms of speed of response to threat, Doty and colleagues (2014) found vmPFC activity to be associated with fast response for unexpected threat, rather than expected threat. Thus, there are inconsistencies in the literature, as neither of the aforementioned studies investigated the underlying functional network and its relation to speed of threat processing.

The objective of the current study was to delineate the functional networks underlying fast response to naturalistic threat, in conditions where threat was congruent or incongruent with prior expectations. For this purpose, we used a previously validated Dynamic Emotion Perception task, in which instruction cues were used to elicit prior expectations during viewing of angry, audio-visual videos (Dzafic et al., 2016). Based on the results of previous studies we predicted that faster recognition of threat *congruent* with prior expectations would involve the recruitment of rAMY functionally connecting to thalamus, as these are known to be involved in efficient threat processing (Bishop, 2008; Dolan and Vuilleumier, 2003; Marstaller et al., 2016; Vuilleumier and Pourtois, 2007). Conversely, faster recognition of threat *incongruent* with prior expectations would involve the recruitment of rTPJ (a core region of the ventral attention network (Corbetta et al., 2008)) functionally connecting to inferior
frontal regions, such as vmPFC, previously implicated in rapid processing of unexpected threat (Corbetta et al., 2008; Decety and Lamm, 2007).

**Methods**

**Participants**

Twenty-eight healthy, right-handed males (mean (M) age = 31.79, standard deviation (SD) = 4.95) were recruited through on-line advertising to staff and students across the University of Queensland. A telephone interview was conducted prior to recruitment to ensure that participants had normal or corrected-to-normal vision, were not taking medication, and had no history of neurological disorders, or metal implants in their body. The sample, on average, displayed normal intelligence and emotional intelligence scores, estimated using 2 subsets (vocabulary and matrix reasoning) of the Wechsler abbreviated scale of intelligence (WASI; Wechsler (1999)); M = 107.33; SD = 11.79) and the perceiving emotions component of Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT; Mayer et al. (2003); M = 108.46; SD = 12.66).

Participants were provided with an information sheet that included a full description of the study and fMRI procedure. After reading and understanding the document, written informed consent was obtained from each participant. The study conforms to World Medical Association Declaration of Helsinki. This research was approved by the Medical Research Ethics Committee of the University of Queensland. Participants received $30 as reimbursement.

**Materials**

Participants completed the Dynamic Emotion Perception (DEP) Task (Dzafic et al., 2016) during fMRI. The DEP task involved viewing audio-visual video clips of an actor expressing emotions congruent or incongruent with prior expectations. Prior expectations were induced by displaying an emotional instruction cue before the video clips (Barbalat et al., 2013) and by increasing the occurrence likelihood of emotion videos congruent with the emotion in the instruction cue (Chambon et al., 2011a).

Experimental stimuli included emotion videos, instruction cues, and emotion cues, as described below. For an in-depth description of the development, editing, and piloting of these stimuli, and for a schematic figure of the DEP task, please see Dzafic et al. (2016). (1) Emotion videos: there was a total of forty-eight emotional videos, 16 of each emotion condition: angry, happy, and neutral. The videos presented a female actor speaking sixteen different sentences, which were emotionally ambiguous (i.e., the semantic content made sense for multiple emotions). By keeping the content
constant across the 3 emotions, we controlled for linguistic confounds. (2) Instruction cue: the instruction cue contained a still picture of the actor expressing an emotion (either an angry, happy, or neutral), with the expressed emotion written in white text underneath the picture. The picture and the writing were presented in the centre of the cue and overlaid on a black background. Above the picture, the instruction cue contained white text, instructing the participant to make an “index finger press” for the emotion in the picture. (3) Emotion cues: the emotion cues were identical to the instruction cues, except that they did not contain the text above the picture to make an “index finger press”.

**Design**

The experimental procedure consisted of three runs of the DEP task and nine experimental conditions: 3 cues (happy, angry, or neutral) x 3 emotional videos (happy, angry or neutral). When the emotion in the cues and video matched, this was a ‘congruent’ condition, whereas when the emotion in the cues and video did not match, this was an ‘incongruent’ condition. Please note that the task included angry, neutral, and happy video clips. However, for the purposes of this study and research question we conducted analyses only on the angry video clips, as the objective of the study was to investigate specifically threat (anger) perception in different expectancy contexts. The different expectancy conditions were: anger congruent (angry video preceded by angry cues) and anger incongruent (angry video preceded by happy cues) (see Fig. 1).

**Figure 1.** A graphical depiction of a congruent and an incongruent threat (anger) trial within the Dynamic emotion perception task. In an angry block participants...
were asked to press a button with their index finger for angry videos (congruent trial). However, in other emotion blocks, for example a happy block, participants were asked to press a button with their middle finger for angry videos (incongruent trial).

Within each imaging run there were nine experimental blocks. Each experimental block began with an instruction cue (3 sec), followed by six or nine trials consisting of an emotion cue presented for 1 sec, followed by an inter-stimulus interval (ISI; a black screen presented for a mean duration of 1 sec), which was followed by an emotion video presented for 3 sec. The ISI was jittered within a block, with a uniform distribution between 500 ms and 1500 ms, of either 6 x 200 ms intervals (during blocks of 6 videos) or 9 x 125 ms intervals (during blocks of 9 videos). The reason the blocks were of different lengths was to reduce the predictability of how many trials each block contained, and thus the ability of participants to predict the number of incongruent or congruent videos. Alternating the number of videos was also done to eliminate repetitiveness, as this may result in fluctuations of attention. Within a block of six videos, there were four congruent and two incongruent videos (66.67% expectancy bias); and within a block of nine videos, there were five congruent and four incongruent videos (55.55% expectancy bias).

The experiment was a mixed design; meaning that different emotion videos were presented in an event-related fashion within the blocks of a specific emotion cue. In other words, within each block, the cue always carried the same emotion (e.g., angry) but the videos within that block would alternate the emotion (e.g., four angry videos, one happy video, and one neutral video). The video clips within a block were randomized in Microsoft Excel, so that the appearance of congruent or incongruent videos could not be predicted. The emotion blocks were counterbalanced between runs, as were the runs between participants, using the Balanced Latin Squares method.

**Procedure**

The participants were asked to respond to the video clips to indicate if the emotion presented in the instruction cue matched the emotion expressed in the video. Specifically, participants were told to press the button with their index finger when the video clip was concordant with the instruction cue emotion or press with their middle finger when it was not. All responses occurred within 3 seconds during the video clips. Accuracy and reaction times (RTs) were recorded for each trial.
Prior to the fMRI experiment, participants were trained with a practice task outside the MRI scanner. Both the practice task and fMRI task were presented using E-Prime 2.0 software (https://www.pstnet.com/eprime.cfm, 2013; Schneider et al., (2012)) on a Windows computer screen. The practice task consisted of 9 blocks and feedback was given if the correct/incorrect button was pressed. The goal was to ensure that participants understood the aim of the task and that the finger response became automated outside the scanner. During the fMRI experiment, the DEP task was seen by participants through a tilted mirror attached to the head coil on the MRI scanner. Responses were made on a custom-built MRI-compatible response box. Participants were instructed to respond as quickly and as accurately as possible and no feedback was given in the actual experiment.

After the fMRI experiment participants completed two questionnaires: WASI (Wechsler, 1999) and MSCEIT (Mayer et al., 2003), in a testing room outside the MRI scanner. The practice task, fMRI task, and questionnaires were completed at the Centre for Advanced Imaging, University of Queensland’s 3T scanner facility.

**MRI Procedure and Preprocessing**

Structural and functional MRI images were acquired by a 3T Siemens Magnetom TrioTim system using a 12-channel head coil. The scans collected for each subject, in a session were as follows: localizer, T1-weighted anatomical image MP2RAGE sequence (repetition time (TR): 1900 ms, echo time (TE): 2.32 ms, resolution: 1mm\(^3\), FoV: 230 mm, 196 slices), T2* weighted echo-planar sequence (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm\(^3\), slices: 46, FoV: 192 mm), Diffusion Weighted Imaging (TR: 8400 ms, TE: 100 ms, resolution: 2.3 mm x 2.3 mm x 2.5 mm, slices: 60, FoV: 300 mm, b-value: 2000 s/mm\(^2\), directions: 64), and resting-state (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm\(^3\), slices: 46, FoV: 192 mm). The total scanning time per session was 45 minutes.

Standard preprocessing of the images was carried out using Statistical Parametric Mapping (SPM8) (http://www.fil.ion.ucl.ac.uk/spm/software/spm8). The preprocessing steps were as follows: slice timing on the functional images, to correct for differences in slice acquisition times within each volume using the middle slice as reference; realignment (estimate and reslice) on the functional images, to correct for inter-scan movement within each run (no participant was excluded for excessive movement (defined as >3 mm translation, >2 degrees rotation); co-registration of the functional and structural images; segmentation of the structural image, with heavy regularisation (0.1) recommended for MP2RAGE sequence; normalization of the
resliced images into a standardized, stereotaxic space (according to the Montreal Neurological Institute template); and smoothing of normalized images with 6mm full-width-at-half-maximum isotropic Gaussian kernel.

Data Analysis

**Behavioural Analysis**

We conducted a factorial ANOVA on mean RTs with a 3 Run (Run 1, Run 2 and Run 3) x 2 Congruency (angry video congruent and angry video incongruent) within-subjects design. This was done in order to assess the effect of congruency on RTs to detect angry emotion across experimental runs. Congruent video indicates the pairing of angry cues with angry video clips, whereas incongruent video indicates the pairing of happy cues with angry video clips. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity where the assumption of sphericity had been violated. Significant interactions were further analyzed using Bonferroni-corrected simple contrasts and paired-samples t-tests, after running 1000 permutations. These analyses were conducted using the SPSS package.

**Functional Connectivity and Brain-Behaviour Analysis**

We utilized a multivariate, partial least squares (PLS) approach for the functional connectivity analysis and assessment of the relation of behavioural performance and activity in the delineated functional networks. PLS examines the relationship between activity in a selected brain region and activity in the rest of the brain across task conditions, as well as the relation of these functional networks with behavioural performance in each experimental condition. The PLS technique allows identification of distributed patterns of neural activity, rather than the independent activity of a single brain region; thus, this analysis is optimally suited to investigating emotion perception, which engages a widespread and interactive brain network (Arsalidou et al., 2011; Vuilleumier and Pourtois, 2007).

Based on the findings of regional activations underlying congruence with prior expectations or incongruence with prior expectations (Barbalat et al., 2013; Dzafic et al., 2016), we conducted a seed voxel analysis in which three seed voxels were selected: the ventromedial prefrontal cortex [-4 34 -12], the right amygdala [18 -8 -18] and the right temporoparietal junction [60 -50 34]. In our study, the selection of the seed voxels was both data-driven (please see Dzafic et al., 2016), and literature driven (Bishop, 2008; Vuilleumier & Pourtois, 2007; Marstaller et al., 2016; Corbetta, Patel, & Shulman, 2008; Decety & Lamm, 2007; Doty et al., 2014). In three separate
analyses, we correlated activity in the vmPFC, rAMY, and rTPJ with activity in the rest of the brain and with accurate RTs of the participants in the congruent and incongruent conditions. One participant was removed from the final analysis due to being an outlier (z-score < 3.25).

The procedure for the functional connectivity analysis involves extracting the Blood-oxygen-level dependent (BOLD) values from the three seed regions from the onset of each angry video across 6 time points (TRs), as this time period captures the hemodynamic response function. The averaged activity for each seed region is then correlated with activity in all other brain regions, across all participants, and within each experimental condition, to form correlation maps. Next, the correlation maps are decomposed with singular value decomposition (SVD), resulting in a set of orthogonal variables (latent variables; LVs). Each LV consists of three components: singular values (significance for a given LV), voxel saliences (spatiotemporal activity for a given LV), and task saliences (degree to which each condition is related to the brain-seed correlations within the given LV). Finally, the significance for each LV is determined by conducting 500 permutations (McIntosh et al., 1996). Corrections for multiple comparisons are not necessary in PLS, as the voxel saliences are calculated in a single mathematical step on the whole brain. For each LV, “brain scores” are computed for each participant, which indicate the degree to which each participant shows the pattern of brain activity identified, across conditions. We calculated the correlation between the brain scores from each significant LV and the seed BOLD values to assess the relation between the whole-brain pattern and activity in the three seed regions. The robustness of the voxel saliences was assessed with bootstrap estimation of the standard errors (SE), which in the present study was carried out 100 times. Bootstrap ratios are obtained by dividing each voxel’s mean salience by its bootstrapped SE. Peak voxels with a bootstrap ratio > 3.0 and cluster size of 100 or more voxels were considered, as this approximates p < 0.0001.

Results

Behavioural Findings

We assessed the effect of congruency on RTs to detect angry emotion across experimental runs. A main effect of congruency was revealed, $F(1, 27) = 21.95, p < 0.001$; such that angry videos congruent with the instruction cue ($M = 848.09; SE = 40.59$) obtained significantly slower RTs compared to angry videos incongruent with the instruction cue ($M = 756.58; SE = 33.28$). There was also a significant interaction
between experimental run and congruency ($F(1.61, 43.56) = 4.26, p = 0.027$), indicating that the difference between RTs for emotion videos congruent and incongruent with the instruction cue was dependent on which experimental run was being performed. Simple contrasts were conducted comparing experimental runs and congruency, an RT difference was revealed between run 1 and run 3, for congruent versus incongruent angry videos, ($F(1, 27) = 5.68, p = 0.024$). Paired-samples t-tests showed that RTs significantly decreased during angry congruent conditions in run 3 ($M = 808.14; SE = 43.16$) compared to run 1 ($M = 905.94; SE = 49.57$), $p = 0.028$, while RTs did not change for the incongruent condition across experimental runs ($Ms (SE):$ Run 1 = 745.53 (30.78), Run 2 = 747.13(33.12), Run 3 = 777.07(46.99); $ns$). The results show faster response times over the course of the experiment only for the congruent conditions, this was taken to indicate that prior expectations had developed, facilitating reaction time for angry videos congruent with cues.

**Functional Connectivity and Brain-Behaviour Findings**

**Functional connections with vmPFC.** The functional connectivity analysis with vmPFC identified one statistically significant LV, accounting for 46.60% of covariance in the data. The LV demonstrated a pattern of vmPFC functional connectivity common for both congruent and incongruent conditions (see Fig. 2). The functional network connected to the vmPFC included right ventrolateral prefrontal cortex (vlPFC), right dorsomedial prefrontal cortex (dmPFC), left inferior frontal cortex, left anterior cingulate gyrus, right posterior cingulate gyrus, right thalamus pulvinars, left subthalamic nucleus, and right putamen (see Table 1). However, no reliable correlation was observed for RTs in either congruent or incongruent conditions, meaning that the vmPFC network was not related to speed of response to threat in either condition.
Figure 2. Functional connections with vmPFC /behaviour PLS results. (Left panel) A pattern of correlated whole-brain activity. (Right panel) Correlations between activity in vmPFC seed and scores representing activity in the regions displayed in the left panel, with no reliable correlation for reaction times in either congruent or incongruent conditions. Asterisk denote a significant correlation based on 95% confidence intervals calculated from the bootstrap procedure.
Table 1

*Functional connections with vmPFC*

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<td></td>
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<td>x   y   z</td>
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<td>Functional network reflecting positive correlation with vmPFC seed</td>
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<td>-88 -26 644</td>
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Abbreviations: Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; sgACC = subgenual anterior cingulate cortex; vmPFC = ventromedial prefrontal cortex; vlPFC = ventrolateral prefrontal cortex; dmPFC =
dorsomedial prefrontal cortex; pre-SMA = pre-supplementary motor area; voxels = number of voxels (one voxel volume=6 mm3). All reported activations are from LV 1, ≥100 voxels (600 mm3).

**Functional connections with right amygdala.** The functional connectivity analysis with right amygdala seed identified two statistically significant LVs, accounting for 47.92% and 27.43% of covariance in the data. The first LV delineated a functional network connected to right amygdala whose activity positively correlated with RTs during incongruent conditions, meaning that the slower the recognition of threat incongruent with prior expectations the stronger the activity in this network (see Fig. 3a). This network involved vmPFC, bilateral middle and right inferior frontal cortices, and right anterior cingulate cortex (see Table 2). Critically, the second LV delineated a functional network connected to right amygdala whose activity negatively correlated with RTs during congruent conditions, meaning that the faster the recognition of threat congruent with one’s expectations the stronger the activation of this network (see Fig. 3b). This network involved left amygdala, left hippocampus, left posterior cingulate gyrus, right parahippocampal gyrus, left lingual gyrus, and right thalamus (see Table 3). Note that, although the network was engaged in both congruent and incongruent conditions, and associated with faster RTs, in the incongruent condition this network was not functionally connected to right amygdala (i.e., CI in the incongruent condition crosses zero), which was the area of interest in this study.
Figure 3. Functional connections with rAMY /behaviour PLS results. 3a. (Left panel) A pattern of correlated whole-brain activity. (Right panel) Correlations between activity in rAMY seed and scores representing activity in the regions displayed in the bottom panel, with positive correlation for reaction times during incongruent conditions. 3b. (Left panel) A pattern of correlated whole-brain activity. (Right panel) Correlations between activity in rAMY seed and scores representing activity in the regions displayed in the bottom panel, with negative correlation for reaction times during congruent conditions. Asterisk denote a significant correlation based on 95% confidence intervals calculated from the bootstrap procedure.
### Table 2

**Functional connections with rAMY**

<table>
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<th>Brain region</th>
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<th>BA</th>
<th>MNI coordinates</th>
<th>Voxels</th>
<th>BSR</th>
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<td>m</td>
<td></td>
<td>X</td>
<td>Y</td>
<td>Z</td>
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<tr>
<td>with rAMY seed</td>
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</tr>
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<tr>
<td>with rAMY seed</td>
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</tr>
<tr>
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<tr>
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<td>L</td>
<td>19</td>
<td>-28</td>
<td>-70</td>
<td>6</td>
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</table>
Abbreviations: Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; rAMY = right amygdala; vmPFC = ventromedial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; voxels = number of voxels (one voxel volume=6 mm$^3$). All reported activations are from LV 1, ≥100 voxels (600 mm$^3$).

**Functional connections with right temporoparietal junction.** The functional connectivity analysis with rTPJ as seed identified a statistically significant LV, accounting for 37.19% of covariance in the data. The LV demonstrated a pattern of rTPJ functional connectivity during both congruent and incongruent conditions. We found a trend for a negative correlation between RT’s and the rTPJ functional network during incongruent conditions, meaning that the faster the recognition of threat incongruent with one’s expectations the stronger the activation of this network (see Fig. 4). This network included the vmPFC, bilateral precentral gyrus, left inferior frontal gyrus, right pre-supplementary motor area, right insula, right inferior parietal lobule, left precuneus, left lingual and right fusiform gyri (see Table 4).
Figure 4. Functional connections with rTPJ /behaviour PLS results. (Left panel) A pattern of correlated whole-brain activity. (Right panel) Correlations between activity in rTPJ seed and scores representing activity in the regions displayed in the left panel, with a trend for negative correlation for reaction times during incongruent conditions. Asterisk denote a significant correlation based on 95% confidence intervals calculated from the bootstrap procedure.
### Table 3

**Functional connections with rTPJ**

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<th>MNI coordinates</th>
<th>Voxels</th>
<th>BSR</th>
</tr>
</thead>
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<td></td>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
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<td>-12</td>
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**Abbreviations:** Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; rTPJ = right temporoparietal junction; vmPFC = ventromedial prefrontal cortex; pre-SMA = pre supplementary motor area; voxels = number of voxels (one voxel volume=6 mm³). All reported activations are from LV 1, ≥100 voxels (600 mm³).

**Discussion**

The purpose of the current study was to identify the functional networks that subserve rapid response to threat, in situations either congruent or incongruent with prior expectations. We investigated the functional connectivity of the vmPFC, rAMY and rTPJ, with strong evidence for involvement of these brain regions in emotion perception influenced by prior expectations (Barbalat et al., 2013; Doty et al., 2014; Dzafic et al., 2016). In line with our hypotheses, our findings show that facilitation of
response to threat *congruent* with prior expectations was associated with a subcortical network functionally connected to rAMY. Although it did not reach significance, there was also a trend for faster response to threat *incongruent* with prior expectations associated with a ventral frontoparietal network functionally connected to rTPJ. Furthermore, we found that the vmPFC functional network was also engaged during threat congruent and incongruent with prior expectations; however, connectivity from here was not associated with the facilitation of response to threat. Finally, we found that rAMY connectivity with prefrontal regions and right anterior cingulate cortex (ACC) was associated with slower response times for incongruent threat. In summary, our data suggest that fast response to congruent (expected) threat is supported by selective processing of threatening emotional cues, related to a rAMY subcortical network, and slower response to incongruent threat associated with error-monitoring and correction, related to a rAMY-ACC network.

**Rapid detection of congruent threat**

Responses to threat congruent with prior expectations were found to be faster in those participants who recruited a subcortical network, including left hippocampus and right thalamus, connecting to the rAMY. The rAMY is argued to be involved in allocating processing resources to prioritize cues relevant in a given situation (Pessoa & Adolphs, 2010), and as such would facilitate rapid detection of threat, relevant for survival (Bishop, 2008; Marstaller et al., 2016; Vuilleumier and Pourtois, 2007). Connectivity between the amygdala and hippocampus has been strongly associated with contextual fear conditioning, in which participants are exposed to a particular context paired with a threatening stimulus, such as a shock, resulting in an automatic fear response during that specific context (Alvarez et al., 2008; Marstaller et al. 2016). Thus, our data align with the idea that fear conditioning and rapid processing of emotion underlies fast responding to expected threat. Moreover, in fear conditioning, forward connectivity from the thalamus to the rAMY is known to be involved in rapid processing of threatening emotion (Morris et al., 1999; Vuilleumier et al., 2003). This pathway is considered to be involved in the ‘coarse’ processing of emotion, without the input of more regulatory cortical areas (Garvert et al., 2014; Williams et al., 2006). The functional connections with the rAMY, left hippocampus and right thalamus support the idea that expecting and quickly responding to threat engages a bottom-up system, involved in fear conditioning, which unconsciously and selectively processes angry cues for rapid alerting in a threatening environment.
**Incongruent threat processing**

In a non-threatening context, rapid reorienting of attention may underlie fast response to unexpected threat. We found a trend for faster responding to threat incongruent with prior expectations to be associated with a network comprised of bilateral precentral gyri, right ventral frontoparietal regions, insula, sensorimotor regions and fusiform gyrus connecting to the rTPJ, identified as the ventral attention network (Corbetta et al., 2000; Corbetta and Shulman, 2002; Kincade et al., 2005). This functional network specializes in bottom-up processing, interrupting top-down attention and shifting focus to unexpected, but relevant stimuli (Corbetta et al., 2008). Conversely, we found that the functional network comprising prefrontal regions, right ACC and the rAMY was associated with slower responding to threat incongruent with prior expectations. The functional connections with the amygdala and ACC have been implicated in “inhibitory” top-down resolution of error during emotional incongruence (Etkin et al., 2006). Therefore, perception of incongruent threat may involve a fast ‘bottom-up’ reorienting network, which allows rapid response to threat, while slow response to incongruent threat reflects a greater burden of top-down pathways involved in error resolution.

**The role of the vmPFC in threat processing**

Previous literature provides inconsistent results regarding the vmPFC and threat processing. For instance, a functional connectivity study found that threatening emotions congruent with prior expectations engage a vmPFC network (Barbalat et al., 2013), but a brain-behaviour study found that faster detection of threatening emotions incongruent with prior expectations was related to vmPFC activity (Doty et al., 2014). Ours is the first study, which examined the role that the vmPFC functional network plays in speed of threat processing, finding that a vmPFC network (without the rTPJ) does not facilitate response for either congruent or incongruent threat processing. The vmPFC is a cortical area involved in top-down processes, such as social decision-making, emotion evaluation, and guiding behaviour based on previous outcomes (Amodio and Frith, 2006; Grossman et al., 2010; Teasdale et al., 1999). Our findings suggest that top-down mechanisms of prior expectations, which engage the vmPFC, may not directly facilitate rapid response to threat, but rather that these mechanisms may be crucial for longer-term cognitive strategies, such as evaluating that the social situation is threatening, and the conscious focus and biasing of attention to threat (Grossman et al., 2010; Moretti et al., 2009). Therefore, our findings provide further
evidence for the role of the rAMY subcortical network in automatic prioritization of angry cues, and fast response in a threatening context.

The results from the current study provide important insights into our neuroscientific understanding of impaired processing of threatening emotions, and their role in the pathogenesis of psychiatric disorders. In psychosis, for example, there is a deficit in recognizing threatening emotions (Behere, 2015; Mandal et al., 1998; van't Wout et al., 2007) and also misattribution of threat (Premkumar et al., 2008), with strong links to neurobiological factors; specifically, abnormal activation in similar brain regions as those identified in the current study (i.e., amygdala, prefrontal regions, ACC, and hippocampus) (Underwood et al., 2015). However, psychosis has been conceptualised as a disorder of functional connectivity (Schmidt et al., 2015), and a network level approach, such as the one used in the current study, may provide greater insight into the neural underpinnings of psychosis. Furthermore, new therapeutic advancements for treatment-resistant psychosis, such as neuromodulation therapies, rely on isolating abnormal neuroanatomic networks (Tye et al., 2009). Thus, future studies should investigate the rAMY subcortical network, in order to characterise maladaptive threat appraisals in psychosis, and increase the focus and efficacy of neuromodulatory therapeutic interventions.

Conclusions

The results of the present study confirmed that rapid detection of threat congruent with prior expectations is associated with the rAMY subcortical network. In threatening environments, attention is rapidly directed to cues that signal aggression, inducing forward connections between subcortical structures and rAMY. The present study was able to elucidate the role of vmPFC network in threat processing, which has been inconsistent in previous literature. We found that the vmPFC network is important for both congruent and incongruent threat recognition; however, activity in this network does not directly facilitate fast response. In terms of rapid detection of threat incongruent with prior expectations, we found a trend association with ventral frontoparietal attention network activity with a primary node of the rTPJ, which is known to interrupt top-down attention and shift focus to unexpected, behaviourally-relevant stimuli. Our results have implications for understanding the neurobiological underpinnings of psychiatric disorders where there is an impaired processing of threat, and may inform the application of neuromodulation therapies for treatment-resistant psychiatric disorders, such as psychosis.
CHAPTER 4

Neural correlates of dynamic emotion perception in schizophrenia and the influence of prior expectations
Preface
In the previous chapters, the focus was on understanding the influence of prior expectations on emotion perception in the healthy population. I delineated the expectancy-related neural activity during naturalistic emotion perception, and then I focused on the functional networks subserving rapid response to threatening emotion, which is congruent or incongruent with prior expectations. Critically, in the next chapter I investigated aberrancy of prior expectations during dynamic emotion perception in a SCZ sample. Previous literature has shown that individuals with SCZ have deficits in perception, and it has been theorised that this is due to reduced precision in prior expectations. I wanted to examine this theory and whether it applies to impaired emotion perception in SCZ. Therefore, in the following chapter I use the DEP paradigm to investigate emotion perception in SCZ and if impairment is related to aberrant prior expectations. For this purpose, I examine accuracy, discriminability and response speed during emotion perception in individuals with SCZ. Furthermore, I investigate the rAMY functional network in the SCZ sample, as this network was found to be important in efficient perception of threatening emotion in a healthy sample in the previous chapter.

Note. This paper is ready to be submitted to Biological Psychiatry: Cognitive Neuroscience and Neuroimaging.

Dzafic, I., Burianová, H., Martin, A. K. & Mowry, B. Neural correlates of dynamic emotion perception in schizophrenia and the influence of prior expectations.
Abstract
Impaired emotion perception is a well-established and stable deficit in schizophrenia; however, there is limited knowledge about the underlying aberrant cognitive and brain processes that result in emotion perception deficits. Recent influential work, based on predictive coding, has shown that perceptual deficits in schizophrenia may result from decreased precision in prior expectations, associated with under-activity in higher hierarchical brain regions, such as the frontal cortex. Nevertheless, the link between emotion perception, prior expectations, and the underlying aberrant brain processes has yet to be examined. In the present study, we investigated the perception of dynamic, multisensory emotion and the influence of prior expectations in schizophrenia. During a functional Magnetic Resonance Imaging scan, 32 participants (16 with schizophrenia and 16 healthy controls) completed the Dynamic Emotion Perception task, which induces prior expectations with emotion instruction cues. We delineated neural responses and functional connectivity in whole-brain large-scale networks underlying emotion perception. Compared to healthy individuals, schizophrenia patients had lower accuracy and response speed over time, specifically for emotions that were congruent with prior expectations. At the neural level, schizophrenia patients had less engagement of right inferior frontal gyrus, insula, and inferior parietal lobule, and right amygdala-subcortical dysconnectivity during discrimination of emotions congruent with prior expectations. The results indicate that individuals with schizophrenia may have aberrant prior expectations about the emotional dispositions of others, associated with under-activity in inferior frontal and parietal regions and right amygdala dysconnectivity, which results in inefficient perception of emotion.

Keywords: schizophrenia; emotion; functional connectivity; inferior frontal; right amygdala.
Introduction

Emotion perception is impaired in schizophrenia (SCZ) with a significant impact on overall functional outcome (Irani et al., 2012). In particular, deficits in emotion perception are thought to lead to impairment in social and community functioning and the ability to form quality relationships (Couture et al., 2006; Hooker and Park, 2002). Currently, there is inadequate knowledge of the cognitive processes underlying emotion perception difficulties in SCZ. The ‘Predictive Coding’ theory of cognition proposes that prior expectations facilitate emotion perception by directing attentional focus onto relevant emotional information, based on our prior experience (Barbalat et al., 2013; Brown and Brune, 2012). For example, prior expectations have been found to improve speed and accuracy during emotion perception in healthy individuals (Barbalat et al., 2013; Dzafic et al., 2016). Within the Predictive Coding theory, SCZ has been conceptualized as a disorder of reduced precision (increase in uncertainty) in prior expectations (Adams et al., 2016; Adams et al., 2013), meaning that individuals with SCZ have less confidence in their expectations, leading to inefficient directing of attention and nosier incoming sensory information. The effect of prior expectations on emotion perception in SCZ has only been investigated using static emotion displays (Barbalat et al., 2012), despite the fact that sensory information in emotional expressions is dynamic in nature and often changes rapidly in social situations. Naturalistic emotion displays may better capture the complex neural processes associated with emotion perception (Arsalidou et al., 2011) and better identify the underlying aberrant processes during emotion perception in SCZ. In the current study, we investigated whether aberrancy in prior expectations leads to impaired efficiency in recognising emotion in patients with SCZ, using ecologically valid, dynamic contexts.

Processing of emotions that are congruent with prior expectations is associated with activity in frontal areas (Barbalat et al., 2013) and the amygdala (Dzafic et al., 2016). Reduced precision in prior expectations in SCZ has been proposed to reflect impaired activity in higher hierarchical regions (e.g., frontal regions), resulting in less inhibitory top-down influence over primary sensory regions (Adams et al., 2016). This is compatible with the considerable evidence for frontal dysfunction in SCZ (Fan et al., 2013; He et al., 2013; Huang et al., 2010) and hyper-connectivity in sensory regions (Anticevic et al., 2014). In
addition to frontal dysfunction in SCZ, several converging lines of evidence have found that deficits in emotion perception are associated with dysconnectivity in functional networks involving the amygdala (Bjorkquist et al., 2016; Das et al., 2007; Mukherjee et al., 2012). However, no study to date has directly explored the neural circuitry underlying aberrant prior expectations in SCZ during emotion perception. In the current study, we investigated if attenuated prefrontal activity and rAMY dysconnectivity in SCZ are associated with impaired perception of emotions congruent with prior expectations.

In summary, our aim was to investigate the influence of prior expectations on naturalistic emotion perception in SCZ, and the underlying distinct patterns of brain activity, and functional connectivity with the rAMY. In line with previous studies (Hargreaves et al., 2016; Johnston et al., 2010) we predicted that SCZ patients would have deficits in discriminating dynamic emotion perception in general, with greater deficits compared to healthy controls when emotion perception requires precise prior expectations (Adams et al., 2016); in other words, detecting emotions congruent with prior expectations. At the neural level, we predicted reduced activation in frontal regions in patients with SCZ during emotion perception that is congruent with prior expectations (Adams et al., 2016; Anticevic et al., 2014). Finally, we predicted that SCZ patients would have greater difficulty using prior expectation to facilitate emotion perception, as indexed by poorer accuracy and increased response times, and this would be associated with rAMY dysconnectivity.

**Methods and Materials**

**Participants**

Sixteen, right-handed patients with chronic SCZ (age range = 30-57; mean age = 46.40, SD = 9.43) were recruited from the Queensland Centre for Mental Health Research (QCMHR). Sixteen age and sex matched, right-handed healthy controls (HC; age range = 34-58; mean age = 45.19, SD = 7.92) were recruited from a National Health and Medical Research Council (NHMRC)-funded, population-based Australian sample of individuals as controls for the SCZ participants. Trained clinicians conducted comprehensive interviews with the SCZ patients using the Diagnostic Interview for Genetic Studies (DIGS) (Nurnberger et al., 1994) and the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992). In addition, information on the SCZ cohort was
extracted from all available medical records. Two independent research psychiatrists assigned the Best Estimate Final Diagnosis (BEFD) (Leckman et al., 1982) after reviewing all the available information, and then together determined the consensus diagnosis. Diagnostic inter-rater reliability was assessed using standard procedures (Suarez et al., 2006).

Prior screening confirmed that all participants were without eye disease, a history of neurological disorders, or metal implants in their body, and the HCs were not currently taking medication. The Mini International Neuropsychiatric Interview (M.I.N.I.) version 5.0.0 (Sheehan et al., 1997) was used to screen participants who had current alcohol dependence and a major depressive episode. Intelligence quotient (IQ) was estimated using 2 subsets (vocabulary and matrix reasoning) of the Wechsler abbreviated scale of intelligence (WASI; Wechsler, 1999); please see Table 1 for demographic information. Participants were provided with an information sheet, which included a full description of the study and an MRI information sheet, which included further details regarding the MRI procedure. After reading the document, written informed consent was obtained. This research was approved by the West Moreton Hospital and Health Service Human Research Ethics Committee, and the University of Queensland Human Research Ethics. Participants received $40 in department store vouchers for the neurocognitive testing and MRI.
### Table 1

**Demographic information**

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<th>HC (SD)</th>
<th>t/χ²</th>
<th>p-value</th>
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<td><strong>Full Scale IQ (N = 16)</strong></td>
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<td>116.63</td>
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<tr>
<td></td>
<td>(12.35)</td>
<td>(10.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean SAPS (N = 15)</strong></td>
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<td>-</td>
<td>-</td>
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<tr>
<td><strong>Mean SANS (N = 15)</strong></td>
<td>1.87 (1.51)</td>
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</table>

**Medication (N = 15)**

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<td>Total Typical antipsychotic</td>
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<td>Total Sedative/hypnotic</td>
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</table>

χ² value derived from Pearson’s chi-squared test with variables group and gender.

Abbreviations: SD = standard deviation; N = sample size; M = male; F = female; SCZ = schizophrenia; HC = healthy controls; SAPS = Scale for the Assessment of Positive Symptoms (Global rating); SANS = Scale for the Assessment of Negative Symptoms (Global rating).

**Materials and Procedure**

Participants completed the Dynamic Emotion Perception (DEP) Task (Dzafic et al., 2016) during an fMRI scan. The DEP task involved viewing audio-visual videos of an actor expressing emotions congruent or incongruent with prior expectations. Prior expectations were induced in each experimental block by displaying an emotion instruction cue at the start of each block and by increasing the occurrence likelihood of emotional videos congruent with the emotion in the instruction cue in each block.

The emotional videos (duration: 3 sec) presented a female actor speaking different sentences in either an angry, happy, or neutral tone with
corresponding facial expressions. The videos were preceded by an emotion instruction cue (duration: 3 sec) at the start of each block and emotion cues (duration: 1 sec) before each video. Both types of cues presented a still picture of the actor expressing an emotion (angry, happy, or neutral) with the expressed emotion written in white text underneath the picture. The instruction cue also contained white text above the picture, instructing participants to make an “index finger press” when the emotion in the video was concordant with the instruction cue emotion (‘congruent’ emotion), or to make a “middle finger press” when the emotion in the video was not concordant with the instruction cue emotion (‘incongruent’ emotion; for more details, see Dzafic et al., 2016).

Prior to the fMRI experiment, participants were trained with a practice task outside the MRI scanner. Both the practice task and fMRI task were presented using E-Prime 2.0 software (https://www.pstnet.com/eprime.cfm, 2013; Schneider et al., (2012)) on a Windows computer screen. Responses were made on a custom-built MR-compatible response box. Participants were instructed to respond as quickly and as accurately as possible. Following the fMRI experiment participants completed the WASI questionnaire (Wechsler, 1999). The practice task, fMRI task, and questionnaire were completed at the Centre for Advanced Imaging, University of Queensland’s 3T scanner facility.

**MRI Procedure and Preprocessing**

Structural and functional MRI images were acquired by a 3T Siemens Magnetom TrioTim system using a 12-channel head coil. The scans collected for each participant were as follows: localizer, T1-weighted anatomical image MP2-RAGE sequence (repetition time (TR): 1900 ms, echo time (TE): 2.32 ms, resolution: 1 mm$^3$, FoV: 230 mm, 192 slices, inversion time (TI): 900 ms, flip angle: 9 degrees), whole-head T2*-weighted echo-planar sequence (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm$^3$, slices: 46, FoV: 192 mm, flip angle: 90 degrees), DWI (TR: 8400 ms, TE: 100 ms, resolution: 2.3 mm x 2.3 mm x 2.5 mm, slices: 60, FoV: 300 mm, b-value: 2000 s/mm$^2$, directions: 64), and resting-state (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm$^3$, slices: 46, FoV: 192 mm). The total scanning time per session was 45min.

Standard preprocessing of the images was carried out using Statistical Parametric Mapping (SPM8; http://www.fil.ion.ucl.ac.uk/spm/software/spm8, 2013; Friston (2003)). The preprocessing steps were as follows: slice timing on the functional images, to correct for differences in slice acquisition times within
each volume using the middle slice as reference; realignment (estimate and
reslice) on the functional images, to correct for inter-scan movement within
each run (no participant was excluded for excessive movement (defined as >3
mm translation, >2 degrees rotation); co-registration of the functional and
structural images; segmentation of the structural image, with heavy
regularisation (0.1) recommended for MP2-RAGE sequence; normalization of
the resliced images into a standardized, stereotaxic space (according to the
Montreal Neurological Institute template); and smoothing of normalized images
with a 8mm full-width-at-half-maximum isotropic Gaussian kernel.

Data Analysis

Behavioural Analyses

Discriminability was calculated using $d'$ scores (Macmillan and
Creelman, 1990) to assess emotion discriminability for each participant. The
discriminability index $d'$ provides an indication of ability to distinguish each
emotion video when it is congruent, compared to incongruent. A larger $d'$ score
indicates a better ability to detect an emotion when it is congruent compared to
when it is not. The discriminability index $d'$ can take into account the possibility
that participants may be more inclined to indicate that every emotional video is
congruent with the preceding cue, this would lead to a higher hit rate (HR) at
the expense of greater false alarms (FA). For this calculation, we adjusted $d'$
according to Corwin (1994) where HR = 1 or FA = 0. We conducted a two
way factorial ANOVA to investigate differences between SCZ and HC in
discriminability for each emotional video (angry, happy, and neutral).

Mean reaction times (RTs) and accuracy percentage from all responses
acquired during scanning were calculated for each participant, across 18
conditions (3 emotion videos x 2 congruency x 3 runs). Only RTs for correct
responses were retained for the analysis. Trials from blocks in which accuracy
was less than 50% (chance performance) were removed from further analyses.
Two types of factorial mixed-subjects ANOVAs were conducted on RTs and
percentage accuracy. First, a three-way ANOVA was conducted to investigate
the effect of prior expectations on emotion videos in SCZ compared to HCs,
with factors: 3 emotion videos (angry, happy, neutral) x 2 congruency
(congruent and incongruent) x 2 group (SCZ and HC). Next a three-way
ANOVA was conducted to investigate the effect of prior expectations across
time (experimental runs) in SCZ compared to HCs, with factors: 3 experimental runs (run 1, run 2, run 3) x 2 congruency (congruent and incongruent) x 2 groups (SCZ and HC).

All behavioural analyses were conducted using the SPSS package (SPSS Inc., Armonk, NY). Significant interactions were further analysed using Bonferroni corrected repeated contrasts, independent samples t-tests, and paired samples t-tests. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity where the assumption of sphericity had been violated. Any outliers, as defined by z-score > 3, were removed from further analyses. This resulted in removing one SCZ and one HC from the discriminability analysis, and two SCZ and one HC from the accuracy analysis.

**fMRI Whole-Brain Analysis**

The goal of the analysis was to investigate the regionally specific responses in SCZ compared to HC during congruent and incongruent conditions. Functional data were analysed using a general linear model for event-related designs in SPM8. At the first level, the onsets for the videos were selected as the event onsets and the videos were separated into 6 different conditions: angry congruent video, angry incongruent video, happy congruent video, happy incongruent video, neutral congruent video, and neutral incongruent video. Head motion parameters were included as a regressor to account for participant motion during the course of the experiment. A 1/128 Hz high-pass filter was used to remove slow signal drifts, and a canonical HRF with no derivatives was selected. Contrast images were entered into a second-level analysis using a full factorial model, with factors: group (SCZ vs. HC) and congruency (congruent and incongruent). T-test models examined the brain activity differences in SCZ vs. HC during congruent and incongruent conditions, separately. We included response accuracy, participant age, and IQ as covariates in the analysis. Voxel-level threshold was set at $p < 0.05$ family-wise error (FWE) corrected and a cluster threshold of $k = 10$ voxels.

**Functional Connectivity and Brain-Behaviour Analysis**

Functional connectivity during the DEP task and its relation to response speed was assessed using a multivariate, partial least squares (PLS) approach (McIntosh et al., 1996). The activity in a selected brain region and activity in the rest of the brain was examined, as well as the relation of these functional networks with behavioural performance in the congruent and incongruent
conditions. The selection of the right amygdala (rAMY) seed was literature driven (Das et al., 2007; Dzafic et al., 2016; Marstaller et al., 2016; Mukherjee et al., 2012). We correlated activity in the rAMY [18 -8 -18] seed region (Dzafic et al., 2016) with activity in the rest of the brain, and with response time scores of the participants in incongruent and congruent conditions. Specifically, the procedure involved extracting the blood-oxygen-level dependent (BOLD) values in the rAMY from SPM8 analysis, from the onset of each video, and then in PLS correlating these scores with activity in all other brain regions, and response times. This was done for each condition across all participants to form correlation maps. Next, the correlation maps were decomposed with singular value decomposition (SVD), resulting in a set of orthogonal variables (latent variables; LVs). Each LV consists of three components: singular values (significance for a given LV, determined by conducting 500 permutations), voxel saliences (spatiotemporal activity for a given LV, reliability assessed by conducting bootstrapping 100 times), and task saliences (degree to which each condition is related to the brain-seed correlations within the given LV) (McIntosh et al., 1996). For each LV, “brain scores” are computed, which indicate the degree to which each participant shows the pattern of brain activity identified. We calculated the correlation between the brain scores and the rAMY BOLD values to assess the relation between the whole-brain pattern and activity in the rAMY. Peak voxels with a bootstrap ratio > 2 and cluster size of 50 or more voxels were considered to be reliable, as this approximates p < 0.0025.

Results

Behavioural Findings – Emotion Discriminability

A factorial ANOVA 3 (emotion video) x 2 (group) on discriminability d’ showed a significant main effect of group, $F(1, 28) = 11.30, p = 0.002$, indicating that SCZ have a lower discriminability compared with HC across different emotions. There was also a trend for an interaction between group and emotion, $F(2, 56) = 2.65, p = 0.079$. The interaction indicated that sensitivity differences between SCZ and HC were statistically significant for angry videos ($t(18.77) = -2.54, p = 0.02$) and neutral videos ($t(16.74) = -4.21, p = 0.001$), but not for happy videos ($t(28) = -1.61, p = 0.12$) (see Fig. 1a).
**Behavioural Findings – The Effect of Prior Expectations on Emotion Perception**

A factorial ANOVA 3 (emotion video) x 2 (congruency) x 2 (group) on percentage accuracy revealed a main effect of congruency, $F(1, 27) = 5.76, p = 0.02$. Accuracy for congruent emotions ($M = 94.9\%, S.E. = 0.01$) was lower than for incongruent emotions ($M = 96.9\%, S.E. = 0.01$). There was also a main effect of group, $F(1, 27) = 7.88, p = 0.009$, SCZ ($M = 93.9\%, S.E. = 0.01$) had lower overall accuracy compared with HC ($M = 97.9\%, S.E. = 0.01$). There was a significant interaction between group and congruency, $F(1,27) = 4.64, p = 0.04$; independent samples t-tests revealed that SCZ patients, compared to HC, were significantly worse at detecting congruent emotions ($M$($S.E.$): SCZ = 92%(1.5); HC = 97.8%(1.4)), $t(17.23) = -2.74, p = 0.014$; however, SCZ patients were not significantly different at detecting incongruent emotions ($M = 95.8\%, S.E. = 0.7$) compared to HC ($M = 98\%, S.E. = 0.7$), $t(16.03) = -1.95, p = 0.07$ (see Fig. 1b).

The factorial ANOVA on RTs revealed a main effect of group, $F(1, 29) = 19.48, p < 0.001$. Individuals with SCZ ($M = 1592.15, S.E. = 90.64$) were slower than HC ($M = 1035.39, S.E. = 87.76$) in the overall experiment. There was a main effect of emotion video, $F(1.55, 44.83) = 28.16, p < 0.001$. The angry videos ($M = 1225.09, S.E. = 62.74$) and happy videos ($M = 1287.23, S.E. = 63.76$) were faster to detect than the neutral videos ($M = 1428.98, S.E. = 68.63$), $p < 0.001$. There was a main effect of congruency, $F(1, 29) = 35.23, p < 0.001$, the incongruently cued videos ($M = 1236.69, S.E. = 59.56$) were faster to detect than the congruently cued videos ($M = 1390.84, S.E. = 68.90$). There was a significant interaction between group and emotion video, $F(1.55, 44.83) = 5.67, p = 0.01$. Independent samples t-tests revealed that SCZ patients compared to HC were significantly slower at detecting happy ($M$($S.E.$): SCZ = 1549.74(91.62); HC = 1024.72(88.71)), neutral ($M$($S.E.$): SCZ = 1760.13(98.62); HC = 1097.84(95.49)) and angry ($M$($S.E.$): SCZ = 1466.57(90.15); HC = 983.62(87.29)) videos compared to HCs, $p < 0.001$.

**Behavioural Findings – Prior Expectations Over Time**

A factorial ANOVA 3 (experimental run) x 2 (cue) x 2 (group), on percentage accuracy revealed a main effect of run, $F(2, 54) = 8.35, p = 0.001$, accuracy in the first ($M = 95.1\%, S.E. = 0.01$) and third runs ($M = 95.6\%, S.E. = 0.01$) were lower than in the second run ($M = 97.1\%, S.E. = 0.01$), $p < 0.017$.  


There was a significant interaction between group and run, $F(2,54) = 5.46, p = 0.007$. Paired samples t-tests revealed that while HC had a stable accuracy across runs, SCZ patients were significantly worse during the first and third runs ($Ms(S.E.)$: Run1 = 92.2%(0.01); Run3 = 94.1%(0.01)) compared to the second run ($M = 95.8\%$, S.E. = 0.01), $t(13) = -5.87, p < 0.001$ and $t(13) = 2.92, p = 0.012$, respectively (see Fig. 1c).

The factorial ANOVA on RTs revealed a main effect of run, $F(1.39, 41.76) = 9.07, p = 0.002$. Participants were faster in run 3 ($M = 1296.33$, S.E. = 65.31) and run 2 ($M = 1305.51$, S.E. = 66.78) compared to run 1 ($M = 1424.85$, S.E. = 77.20), $p = 0.02$ and 0.002, respectively. There was a significant interaction between group, run, and congruency, $F(1.77, 52.98) = 7.31, p = 0.002$. Paired samples t-tests revealed that SCZ patients improved in their detection speed for incongruent videos from run 1 ($M = 1713.36$, S.E. = 112.99) to run 3 ($M = 1499.86$, S.E. = 90.89), $t(15) = 2.37, p = 0.03$, whereas HCs improved in their speed for congruent videos from run 1 ($M = 1195.47$, S.E. = 109.85) to run 3 ($M = 1027.99$, S.E. = 98.92), $t(15) = 3.85, p = 0.002$ (see Fig. 1d).
Figure 1. Behavioural results from schizophrenia patients and healthy controls during dynamic emotion perception. 1a) Discriminability index (d’) for emotion videos: angry, happy and neutral. 1b) Significant interaction, mean accuracy percentage for condition (congruent and incongruent) and group (schizophrenia and healthy controls). 1c) Significant interaction, mean reaction times for experimental run (Run 1, Run 2, Run 3) and group (schizophrenia vs. healthy controls). 1d) Three-way significant interaction, mean reaction times for experimental run (Run 1, Run 2, Run 3), group (schizophrenia and healthy controls), and condition (congruent and incongruent). Error bars show the standard error of the mean.

fMRI Regional Activity Findings

Results from a full factorial analysis revealed a significant main effect of congruency with activations in the bilateral superior temporal gyri, left lingual gyrus, bilateral cuneus, right precuneus, right insula, bilateral inferior parietal lobule, left putamen, left precentral gyrus, right inferior frontal gyrus, and left primary somatosensory cortex. There was also a significant interaction between congruency and group with the activation in the right primary
somatosensory cortex. Next, t-tests were conducted to examine the regionally specific responses in SCZ compared to HC during congruent and incongruent conditions separately. During congruent conditions (see Fig. 2a) SCZ recruited visual regions, such as the left lingual gyrus and cuneus, whereas HC recruited right inferior frontal gyrus, bilateral insula and bilateral inferior parietal lobule. During incongruent conditions (see Fig. 2b) SCZ recruited right precuneus, left fusiform gyrus, right cuneus, and right superior parietal lobule, whereas HC recruited right claustrum and globus pallidus (see Table 2).

Figure 2. Brain activity differences between schizophrenia patients and healthy controls during dynamic emotion perception. The left panel shows activity differences during perception of emotion incongruent with prior expectations, and the right panel shows activity differences during perception of emotion congruent with prior expectations. Warm colours indicate schizophrenia patients > healthy controls, and cool colours indicate healthy controls > schizophrenia.
### Table 2

**Regional activity during emotion perception**

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<tr>
<th>Brain region</th>
<th>Hemisphere</th>
<th>BA</th>
<th>MNI coordinates</th>
<th>Cluster size</th>
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<tr>
<td><strong>Congruent HC &gt; SCZ</strong></td>
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Incongruent SCZ > HC

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</table>

Abbreviations: Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; IPL = inferior parietal lobule; SPL = superior parietal lobule; HC = healthy controls; SCZ = schizophrenia. All reported activations are p < 0.05 FWE corrected.

Functional Connectivity of Right Amygdala and Brain-Behaviour

Findings

The functional connectivity analysis with rAMY identified one statistically significant LV (p = 0.012), accounting for 25.38% of covariance in the data (see Fig. 3). The LV differentiated two distinct expectancy-driven patterns of rAMY functional connectivity for SCZ and HC.

The first pattern of rAMY functional connectivity was observed during incongruent conditions for both SCZ and HC. Activity in this network was negatively correlated with response time, meaning that faster recognition of emotions incongruent with one’s expectations was associated with stronger activation of this network in both HC and patients with SCZ. This large-scale network involved bilateral prefrontal regions (dorsal, ventral, and medial), bilateral anterior and posterior cingulate gyri, left hippocampus, midbrain, bilateral occipital regions, bilateral parietal regions, left temporoparietal junction, bilateral insula, and thalamus (see Table 3).

Critically, the second pattern of rAMY functional connectivity was observed during congruent conditions, but only for HC. Activity in this network was negatively correlated with response times, meaning that faster recognition of emotions congruent with the HC’s expectations was associated with stronger
activation of this network. This network comprised rAMY, left hippocampus, right anterior cingulate gyrus, bilateral posterior cingulate gyrus, left parahippocampal gyrus, and right thalamus (see Table 3).

Figure 3. Functional connections with rAMY /behaviour PLS results for schizophrenia patients and healthy controls during dynamic emotion perception. (Left panel) A pattern of correlated whole-brain activity. (Right panel) Correlations between activity in rAMY seed and scores representing activity in the regions displayed in the left panel. Results indicate that healthy controls have differentiated rAMY functional connectivity for congruent and incongruent conditions, associated with faster response speed. Conversely,
schizophrenia patients show rAMY functional connectivity only for incongruent conditions, associated with faster response speed. Asterisks denote significant correlations based on 95% confidence intervals calculated from the bootstrap procedure.
### Table 3

**Functional connections with rAMY during emotion perception**

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<th>Voxels</th>
<th>BSR</th>
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<tr>
<td><strong>rAMY network for HC during congruent conditions</strong></td>
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</table>

Abbreviations: Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; rAMY = right amygdala; dlPFC = dorsolateral prefrontal cortex; SPL = superior parietal lobule; vlPFC = ventrolateral prefrontal cortex; vmPFC = ventromedial prefrontal cortex; SMA = supplementary motor area; SN = Subthalamic Nucleus; voxels = number of voxels (one voxel volume=6 mm³). All reported activations are ≥50 voxels.
Discussion

In the current study, we investigated dynamic emotion perception in patients with SCZ and the influence of prior expectations at the behavioural and neural levels. We identified reduced ability in SCZ patients to identify emotions that were congruently cued (i.e., congruent with prior expectations), as evidenced by poorer accuracy and less improvement in response speed over time. At the neural level, we found reduced activity in right inferior frontal gyrus, insula, and inferior parietal lobule, as well as rAMY-subcortical dysconnectivity during congruent emotion trials in the SCZ group. The findings show that although there is a general impairment during perception of dynamic emotions in SCZ, there is a more specific deficit in detecting emotion congruent with prior expectations, which is subserved by aberrant neural circuitry comprising right inferior frontal gyrus, insula, inferior parietal lobule, and rAMY-subcortical regions. These findings suggest that there is an important link between emotion perception difficulties in SCZ and aberrancy in prior expectations; aberrant prior expectations may lead to difficulty directing attention to relevant emotion cues, resulting in reduced control over sensory input.

Dynamic, audio-visual emotion perception was found to be impaired in patients with SCZ compared to healthy individuals, as demonstrated by poorer overall performance and aberrant neural activity. Specifically, we found that patients had lower accuracy, response speed and ability to discriminate emotional videos, in line with previous research (Chan et al., 2010; Feingold et al., 2016; Hargreaves et al., 2016; Huang et al., 2013; Johnston et al., 2010). In addition, patients with SCZ had less activity in the superior temporal gyri, a region often implicated in audio-visual integration and representation of dynamic emotional expressions (Robins et al., 2009; Said et al., 2010). Attenuated superior temporal activity in SCZ has been found in previous studies (Shin et al., 2015; Sugranyes et al., 2011), and has been associated with a reduced ability in SCZ to integrate audio-visual information (Szycik et al., 2009), which may result in a general deficit in dynamic, audio-visual emotion perception. Interestingly, in addition to the general deficit in emotion perception, patients with SCZ had a more specific deficit in detecting emotions that were congruent with prior expectations, indicated by poorer accuracy and less improvement in response speed over time. In comparison to the patients, healthy individuals were facilitated in their accuracy and response times by prior expectations, over the course of the study for congruent emotions only (Dzafic et al., 2016). Our findings support those of Chambon et al. (2011b) who found that during social perception SCZ patients have less
facilitation of performance by prior expectations. Furthermore, our findings are in line with the theoretical conceptualization of SCZ as a disorder of reduced precision in prior expectations (Adams et al., 2016).

The findings from the current study show that during perception of emotions congruent with prior expectations, patients with SCZ had decreased activity in right inferior frontal gyrus, insula, and inferior parietal lobule, and decreased connectivity in rAMY, compared to healthy controls. In contrast, patients with SCZ had greater engagement of early visual regions. This is in line with this notion that reduced precision in prior expectations is a result of attenuation in higher hierarchical brain regions (Adams et al., 2016), such as inferior frontal regions (Barbalat et al., 2013; Dzafic et al., 2016; Summerfield and Koechlin, 2008). The right inferior frontal gyrus (IFG) and the insula are neighbouring regions, with roles in higher cognitive processing, such as inhibitory control (Aron et al., 2004; Forstmann et al., 2008), emotion regulation (Morawetz et al., 2016), and emotional awareness (Gu et al., 2013). Activity in the inferior parietal lobule has been associated with the evaluation of prior expectations (Corbetta et al., 2008; O'Connor et al., 2010). The attenuation in these regions in SCZ may result in poorer emotion regulation and difficulty evaluating emotion-related prior expectations, leading to reduced control (from priors) to inhibit irrelevant sensory cues during emotion perception (Adams et al., 2016; Anticevic et al., 2014). Finally, our results show that the rAMY network, functionally connecting to thalamus and hippocampus, was recruited only by HCs and activity in this network was correlated with faster response times. The rAMY is a region consistently implicated in inhibiting irrelevant sensory cues and directing attention towards context relevant cues (such as emotional cues) (Garvert et al., 2014; Pessoa and Adolphs, 2010). We speculate that aberrancy in prior expectations associated with dyconnectivity with rAMY, may result in a noisier perceptual system and less predictable environment, impairing the ability to efficiently perceive the emotional dispositions of others.

**Conclusion**

The current study provides novel insights to our understanding about the influence of prior expectations on dynamic emotion perception in SCZ. Our results indicate that in a dynamic environment, individuals with SCZ may have aberrant prior expectations, resulting in inefficient perception of dynamic emotion. The related aberrant neural circuitry in SCZ involves inferior frontal and parietal regions, insula and rAMY-subcortical network. The results from the current study further our
understanding of the underlying cognitive and neural processing differences during emotion perception in patients with SCZ, which has the potential to better inform novel treatments, such as neuromodulation or cognitive remediation, allowing for a more targeted intervention.
CHAPTER 5

Association between schizophrenia polygenic risk and neural correlates of emotion perception.
**Preface**

In the chapters thus far, I investigated naturalistic emotion perception, the influence of prior expectations and the underlying neural circuitry, first in a healthy sample and then in a sample with SCZ. I found that patients with SCZ have aberrant prior expectations during emotion perception, and, importantly, this may be related to abnormal activity in inferior frontal gyrus, inferior parietal lobule, insula and dysconnectivity in a subcortical network connecting to the rAMY. As emotion perception has received considerable attention as a possible endophenotype of schizophrenia, in the next chapter I investigate the association between the underlying neural activity and SCZ risk as indexed by polygenic risk scores in a group of healthy controls. Therefore, in the following chapter I investigate naturalistic emotion perception using the DEP paradigm, in a group of healthy individuals with varying levels of SCZ genetic risk, as identified in a large genomewide association study. I applied the polygenic risk model to determine genetic risk of SCZ and delineated the related neural activity.

Note. The paper incorporated in this chapter is now under review in Psychological Medicine.

**Dzafic, I., Burianová, H., Periyasamy, S. & Mowry, B. Association between schizophrenia polygenic risk and neural circuitry during emotion perception.**
Abstract

The neural correlates of emotion perception have been shown to be significantly altered in schizophrenia (SCZ) patients as well as their healthy relatives, possibly reflecting genetic susceptibility to the disease. The aim of the study was to investigate the association between SCZ polygenic risk and brain activity whilst testing perception of multisensory, dynamic emotional stimuli. We created SCZ polygenic risk scores (PRS) for a sample of twenty-eight healthy individuals. The PRS was based on data from the Psychiatric Genomics Consortium and was used as a regressor score in the neuroimaging analysis. The results of a multivariate brain-behaviour analysis show that higher SCZ PRS are related to increased activity in brain regions critical for emotion regulation during the perception of threatening (angry) emotions. These results suggest that individuals with higher SCZ PRS may over-activate the neural correlates underlying emotion regulation during perception of threat, perhaps due to neural inefficiency in emotion-regulation areas in individuals who have higher genetic susceptibility to SCZ. Moreover, over-recruitment of emotion regulatory regions might function as a compensation to maintain normal emotion regulation during threat perception. If replicated in larger studies, these findings may have important implications for understanding the neurophysiological biomarkers relevant in SCZ.

Keywords: schizophrenia polygenic risk; fMRI; emotion perception; emotion regulation.
Introduction

Schizophrenia (SCZ) is a highly debilitating and heritable mental illness, characterized by impairment in diverse abilities spanning perception, reasoning, and social cognition (de Jong et al., 2013; Green et al., 2004). Evidence suggests that the SCZ-related impairment in these abilities may be due to the dynamic interplay between genes and brain (Martin et al., 2014; Roffman et al., 2006). Current estimates from twin and family studies put the heritability of SCZ at 81% (Wahlstrom et al., 1986), which has led to large collaborative efforts to identify genes able to explain this heritability. However, to date, the identified genes explain only 7% of the liability to SCZ (Ripke et al., 2014b). As SCZ is considered an umbrella term, likely encompassing a large number of aetiologies, one approach to explaining the missing heritability is to search for intermediate traits that lie on a causal pathway between the genotype and clinical phenotype. An endophenotype is described as an intermediate trait that theoretically should be measurable, heritable, and state-independent, with neurophysiological and neurocognitive qualities (Cannon and Keller, 2006; Mowry and Gratten, 2013). One of these neurocognitive intermediate traits is emotion perception, as it has well-established evidence of impairment and aberrant underlying brain function in SCZ (Namiki et al., 2007; Romero-Ferreiro et al., 2016; Vai et al., 2015). Robust evidence has emerged identifying emotion perception as a viable endophenotype for SCZ, as deficits in emotion perception are heritable, associated with SCZ risk genes, and apparent before illness onset (Bediou et al., 2007; Germine et al., 2016b). It is thus of importance to investigate the specific processes and neurobiological underpinnings of emotion perception and their relationship with risk genes, as such research would lead to a clearer understanding of the pathway from genotype to clinical phenotype.

The endophenotypic role of emotion perception in schizophrenia has been highlighted with evidence of robust deficits in SCZ, which persist throughout the course of illness and which are also present in healthy individuals with high SCZ susceptibility (Behere, 2015). In a recent review, Behere (2015) posits that emotion recognition difficulties are trait markers for SCZ, particularly difficulties recognizing threatening emotions, such as anger, and misattribution of threat onto ambiguous facial expressions (Behere, 2015). Impaired threat processing was found to be a stable deficit, present in both acute and remission phases of SCZ; however, these deficits were particularly heightened in those with psychotic symptoms. Thus, aberrant processing of facial expressions could potentially be linked to development of
psychopathology. In support of this notion, a number of SCZ common genetic risk variants have been reported to be associated with emotion and facial processing ability and its underlying neural circuitry (Greenwood et al., 2011; Martin et al., 2014). Moreover, previous research investigating genetic susceptibility to SCZ has identified that healthy relatives of those with SCZ have impaired emotion perception, such as misattributing threat onto neutral expressions (Eack et al., 2010). In addition, research shows that healthy individuals with high SCZ risk have altered activity in emotion neural correlates, such as frontal regions, anterior cingulate gyrus, nucleus accumbens, amygdala, and hippocampus (Phillips and Seidman, 2008). Considering these abovementioned findings, in the current study we hypothesized that SCZ genetic risk in healthy individuals would be associated with aberrant brain processing during emotion perception, particularly for threatening emotions.

With large genome-wide association studies (GWAS) identifying over 100 common risk variants significantly associated with SCZ (Ripke et al., 2014a), a new direction in the field has been to consider the polygenic nature of SCZ by quantifying the combined effect of variants into a polygenic risk score (PRS). Recently, a large polygenic study has shown that deficits in emotion recognition are significantly associated with SCZ polygenic risk across development, supporting the notion that impaired processing of facial expressions may constitute a link between genetic risk for SCZ and development of psychosis (Germine et al., 2016b). Notwithstanding the important findings from this study, Germine and colleagues utilized an emotion tasks with static pictures as stimuli, consequently decreasing ecological validity and examining different underlying mechanisms compared to naturalistic, dynamic displays of emotion (Arsalidou et al., 2011; Palumbo and Jellema, 2013). As the field is moving towards more ecologically valid tasks, emotion perception should be assessed using dynamic, audio-visual displays in order to gauge real-life emotion processing. In addition, the emotion perception tests utilized by Germine and colleagues did not evaluate different contexts of emotion recognition; for example, when a certain emotion is expected or unexpected. Recent evidence shows that individuals with SCZ have reduced precision in their prior expectations, which impairs perception (Adams et al., 2016); thus, it seems critical to explore the influence of SCZ genes on the underlying processes driving emotion perception impairment.

The impact of SCZ polygenic risk on emotion recognition has, thus far, been examined only at the behavioral level, with no study to date investigating the influence on brain function underlying emotion perception. As robust evidence highlights that
SCZ is a disorder of aberrant brain function (Coyle et al., 2016; Liang et al., 2006), the objective of this study was to investigate the association between polygenic risk of SCZ in healthy individuals and the neural correlates of emotion perception. A recently new field of study, 'imaging genetics' has focused on the neurobiological intermediate traits of SCZ, informed by neuroimaging. Imaging genetics allows us to map the neural activity of abilities, such as emotion perception, as a function of genotype. Using the imaging genetics technique, a number of genome-wide significant risk variants have been investigated with respect to their association with the emotion brain network. The findings reveal that risk alleles of certain candidate genes are associated with increased and inefficient connectivity between frontal regions and limbic regions, which play a key role in emotion regulation and emotional learning (Curcic-Blake et al., 2012; Mothersill et al., 2013; Surguladze et al., 2012). As schizophrenia has a complex polygenetic architecture, recent imaging genetic studies have investigated the cumulative effects of risk variants (applying the polygenic risk model) on brain function (Birnbaum and Weinberger, 2013). Imaging polygenic studies have found associations between SCZ genetic risk and white matter volume (Terwisscha van Scheltinga et al., 2013), as well as neural inefficiency in frontal regions (Lancaster et al., 2016; Walton et al., 2013a). However, no imaging genetic study to date has explored the association between polygenic risk of SCZ and the brain regions underlying emotion perception.

The objective of the current study was to investigate the association between polygenic risk of SCZ in healthy individuals and neural correlates of emotion perception, in different expectancy contexts. For this purpose, we used a previously validated Dynamic Emotion Perception (DEP) task (Dzafic et al., 2016), with increased ecological validity, three emotional conditions (anger, happiness, and neutral) expressed by an actor in an audio-visual video and two levels of expectation (congruency and incongruency with prior expectations). Our focus was on healthy individuals for the following reasons: (1) robust evidence shows that genetic risk for SCZ in healthy individuals influences emotion processing (Eack et al., 2010; Germine et al., 2016b) and (2) healthy individuals present a cleaner sample, without the confounds of illness and medication, which affect brain function. For the polygenic risk scores (PRS) we used summary data from SCZ PGC2 (Ripke et al., 2014a) to assess differences in brain activation during a functional magnetic resonance imaging (fMRI) scan. We conducted a multivariate analysis to characterize activity in brain regions that covaries with individual SCZ PRS during the DEP task. Based on the results of
previous studies, we predicted that SCZ PRS would be associated with aberrant activity in neural correlates of emotion regulation and emotional learning (Curcic-Blake et al., 2012; Mothersill et al., 2014), such as the inferior frontal cortex, right amygdala, cingulate gyrus, and parahippocampal gyrus. Additionally, we predicted that schizophrenia PRS would have the strongest association with aberrant brain activity during the viewing of threatening emotions, as perception of these types of emotions is most impaired in schizophrenia (Behere, 2015) and healthy relatives of those with SCZ (Eack et al., 2010).

Materials and Methods

Participants

Twenty-eight Caucasian right-handed healthy controls (HC; mean age = 49.61, SD = 9.09, 18 male) were recruited from a population-based Australian sample. Screening was conducted over the phone prior to the recruitment, to confirm that participants had no history of eye disease, neurological disorders, metal implants, or current medication. The Mini International Neuropsychiatric Interview (M.I.N.I.) version 5.0.0 (Sheehan et al., 1997), was used to ensure that participants did not have current alcohol dependence and were not experiencing a major depressive episode. Intelligence quotient (IQ) was estimated using 2 subsets (vocabulary and matrix reasoning) of the Wechsler abbreviated scale of intelligence (WASI; Wechsler (1999). There were no significant associations between age, IQ, performance on the DEP task and SCZ PRS, $p > 0.09$, in all cases (see Table 1). Participants were provided with an information sheet, which included a full description of the study and an MRI information sheet. Written informed consent was obtained. This research was approved by the West Moreton Hospital and Health Service, and The University of Queensland Human Research Ethics Committees.
Table 1

Pearson Correlation Matrix among Age, IQ, performance during the DEP task and SCZ PRS

<table>
<thead>
<tr>
<th></th>
<th>Age (N = 28)</th>
<th>IQ (N = 26)</th>
<th>DEP accuracy (N = 27)</th>
<th>DEP reaction time (N = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCZ PRS</td>
<td>0.14</td>
<td>-0.34</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>Age</td>
<td>-0.10</td>
<td>-0.10</td>
<td>-0.25</td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td></td>
<td>0.02</td>
<td></td>
<td>-0.39*</td>
</tr>
</tbody>
</table>

Abbreviations: DEP = Dynamic Emotion Perception; SCZ = schizophrenia; PRS = polygenic risk score; N = sample size. *p = 0.049.

Genotyping and Quality Control

Genotyping was conducted using Illumina OmniExpress-12 arrays containing > 712,000 markers for 12 controls and PsychChip arrays containing > 570,000 markers for 17 controls respectively. Standard quality control procedures for samples and markers were conducted using established protocols (Anderson et al., 2010). These two datasets were merged and single nucleotide polymorphisms (SNPs) were excluded if the minor allele frequency was < 5%, if the call rate was < 97%, or if the $\chi^2$-test for Hardy–Weinberg Equilibrium had a $p$-value < 1e-06. After filtering, 220,707 variants were used for PRS analysis.

Generation of Polygenic Risk Scores

SCZ genetic risk was estimated using publicly available results from an international GWAS of 34,241 SCZ cases and 45,604 controls (Schizophrenia Workgroup of the Psychiatric Genomics Consortium). SCZ PRS was calculated using the method described by the International Schizophrenia Consortium (Wray et al., 2014), implemented in the software PRSice v1.25: Polygenic Risk Score (Euesden et al., 2014). A list of SNPs in common between discovery and target samples was determined and subsequently clumped for linkage disequilibrium ($r^2 < 0.2$) across
500kb regions. This ensured that all SNPs included in each SCZ PRS model were independent. The SNP list was limited to those with association \( p \)-values less than the standard GWAS threshold for genome-wide significance \( (5 \times 10^{-8}) \). Individual PRS were calculated using this SNP list. The sample did not contain any outliers and the PRS was normally distributed (Kolmogorov-Smirnov: \( p = 0.20 \)). A power analysis was performed (R software: “pwr.r.test” function (1988)), yielding 80% power to observe a moderate effect \( (r = 0.5) \) of PRS on Blood-oxygen-level dependent (BOLD) during emotion perception \( (n = 28, \alpha = 0.05, \text{two-sided}) \).

**Dynamic Emotion Perception Task**

The Dynamic emotion perception (DEP) task involved viewing emotional audio-visual videos that were either congruent or incongruent with the emotional prior expectations. In the task, prior expectations were manipulated by displaying an emotion instruction cue before the videos and by increasing the occurrence likelihood of emotional videos congruent with the emotion in the instruction. The DEP task consisted of forty-eight 3-second videos of a Caucasian female actor speaking different sentences in an emotional manner (either in an angry, happy, or neutral tone). The emotion instruction cues presented a still picture of the actor expressing an emotion, with the expressed emotion written in white text underneath the picture. There were 3 still pictures of the actor and, in each one, the actor expressed either an angry, happy, or neutral emotion. At the start of each block, the cue contained white text above the picture, instructing to make an “index finger press” for the target emotion (for further details, see Dzafic et al. (2016)).

**Design**

The experimental procedure consisted of nine experimental conditions: 3 emotion instruction cues (happy, angry or neutral) \( \times \) 3 emotional videos (happy, angry or neutral). Each experimental block began with an instruction emotion cue (3 sec), followed by six or nine sequences of trials consisting of an emotion cue (1 sec), a black screen as the inter-stimulus interval (ISI; mean duration of 1sec) and an emotional video clip (3 sec). The ISI was jittered within a block, with a uniform distribution between 500ms and 1500ms, of either 6 x 200ms intervals (during blocks of 6 video clips) or 9 x 125ms intervals (during blocks of 9 video clips). Within a block of six video clips, there were four congruent and two incongruent video clips; and within a block of nine video clips, there were five congruent and four incongruent video clips.

The experiment was a mixed design, with the same emotion cues presented several times within a block (block design), but with varying emotional videos.
(alternating in emotional content) presented within a block (event-related design). ‘Congruent’ videos matched the emotional content of the cues, whereas ‘incongruent’ videos did not. The behavioral and fMRI analyses involved the conditions ‘angry congruent’ (angry cue + angry video), ‘angry incongruent’ (happy/neutral cue + angry video), ‘happy congruent’ (happy cue + happy video), ‘happy incongruent’ (angry/neutral cue + happy video), ‘neutral congruent’ (neutral cue + neutral video), ‘neutral incongruent’ (happy/angry cue + neutral video). The videos within a block were randomized so that the appearance of congruent or incongruent video clips could not be predicted. The emotion blocks were counterbalanced between runs, as were the runs between participants, using the Balanced Latin Squares method.

**Procedure**

The participants were asked to respond to the videos to indicate if the emotion presented in the instruction cue matched the emotion expressed in the video. Specifically, participants were told to press the button with their index finger when the video was congruent with the instruction cue and press with their middle finger when it was not. All responses occurred within 3 seconds during the videos, giving sufficient time for the participant to respond. Accuracy and reaction times (RTs) were recorded for each trial.

Prior to the fMRI experiment, participants were trained with a practice task outside the MRI scanner. Both the practice task and fMRI task were presented using E-Prime 2.0 software (https://www.pstnet.com/eprime.cfm, 2013; Schneider et al., (2012)) on a Windows computer screen. The practice task consisted of 9 blocks and feedback was given if the correct/incorrect button was pressed. The goal was to ensure that participants understood the aim of the task and that the finger response became automated outside the scanner. During the fMRI experiment the DEP task was seen by participants through a tilted mirror attached to the head coil on the MRI scanner. Responses were made on a custom-built MRI-compatible response box. Participants were instructed to respond as quickly and as accurately as possible and no feedback was given in the actual experiment. After the fMRI experiment, participants completed the two questionnaires: WASI (Wechsler, 1999) and Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT; Mayer et al. (2003)), in a testing room outside the MRI scanner. The practice task, fMRI task, and questionnaires were completed at the Centre for Advanced Imaging, University of Queensland 3T scanner facility.
**MRI Procedure and Preprocessing**

Structural and functional MRI images were acquired by a 3T Siemens Magnetom TrioTim system using a 12-channel head coil. The scans collected for each subject, in a session were as follows: localizer, T1-weighted anatomical image MP2RAGE sequence (repetition time (TR): 1900 ms, echo time (TE): 2.32 ms, resolution: 1 mm$^3$, FoV: 230 mm, 196 slices), T2* weighted echo-planar sequence (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm$^3$, slices: 46, FoV: 192 mm), DWI (TR: 8400 ms, TE: 100 ms, resolution: 2.3 mm x 2.3 mm x 2.5 mm, slices: 60, FoV: 300 mm, b-value: 2000 s/mm$^2$, directions: 64), and resting-state (TR: 3000 ms, TE: 30 ms, resolution: 2.5 mm$^3$, slices: 46, FoV: 192 mm). The total scanning time per session was 45 minutes.

Standard preprocessing of the images was carried out using SPM8 (Welcome Department of Imaging Neuroscience, UCL, UK, http://www.fil.ion.ucl.ac.uk/spm/spm5.html). The preprocessing steps were as follows: slice timing on the functional images to correct for differences in slice acquisition times within each volume using the middle slice as reference; realignment (estimate and reslice) on the functional images to correct for inter-scan movement within each run (no participant was excluded for excessive movement (defined as >3 mm translation, >2 degrees rotation); co-registration of the functional and structural images; segmentation of the structural image with heavy regularisation (0.1) recommended for MP2RAGE sequence; normalization of the resliced images into a standardized, stereotaxic space (according to the Montreal Neurological Institute template); and smoothing of normalized images with 6mm full-width-at-half-maximum isotropic Gaussian kernel.

**Data Analysis**

**PRS-fMRI Analysis**

We conducted the fMRI analyses using a multivariate approach, Partial least-squares (PLS), to examine the direct association between brain activity and PRS. PLS investigates the distributed patterns of neural activity and is optimally suited for complex cognitive functions (McIntosh and Lobaugh, 2004), such as naturalistic emotion perception, which engages a widespread and interactive brain network (Arsalidou et al., 2011; Vuilleumier and Pourtois, 2007). PLS identifies the fundamental relations (latent variables: LVs) between brain activity, experimental conditions, and individual differences variables (such as PRS) that account for maximum covariance in the data. Similar to principal component analysis, PLS
decomposes the data into orthogonal LVs by conducting singular value decomposition (SVD) (McIntosh et al., 2004). For each LV, “brain scores” are computed for each participant, which indicate the degree to which each participant shows the pattern of brain activity identified. Each LV consists of three components: singular values (significance for a given LV), voxel saliences (spatiotemporal activity for a given LV), and task saliences (degree to which each condition is related to the brain-PRS correlations within the given LV).

In order to examine the relationship between brain and PRS as a function of experimental condition, we included fMRI data during each video condition in a data matrix cross-correlated with the PRS. We isolated activity during the videos by conducting the analysis across five TRs (TR 0 – TR 5) starting at the onset of the video clips. Activity at each time point was normalized to the first TR (labelled TR 0 in the figures). For all analyses, we ran 1000 permutations (Le Floch et al., 2012; McIntosh et al., 1996) to determine significant LVs at p < 0.001. In addition, we ran 100 bootstraps, estimating the standard errors of the salience for each voxel in order to assess the reliability and robustness of each voxel’s contribution to a pattern of brain activity. We used the mean-centering approach in PLS, which involves subtracting the grand mean of the data matrix from the task means. We restricted the bootstrap ratio threshold to ± 3 (p < 0.001) and reported areas with a cluster size of 100 or more voxels. Confidence intervals (95%) were calculated from the bootstrap procedure; for the mean brain scores in each condition across the five TRs, significant differences between conditions were determined by a lack of overlap in the confidence intervals.

Results

Schizophrenia PRS and Neural Correlates of Dynamic Emotion Perception

The first LV (p < 0.001) accounted for 30.98% of covariance in the data. The LV differentiated brain activity during congruent angry conditions and congruent/incongruent happy conditions, based on PRS. Specifically, PRS correlated positively with brain activity during perception of angry videos congruent with prior expectations, and PRS correlated negatively with brain activity during perception of happy videos congruent and incongruent with prior expectations (see Fig. 1). The brain regions activated include the bilateral dorsolateral prefrontal cortex (dlPFC), dorsomedial prefrontal cortex (dmPFC), bilateral inferior frontal gyri, bilateral supplementary motor area, bilateral amygdala, right anterior cingulate gyrus, right anterior insula (rAI), right temporoparietal junction (TPJ), bilateral precuneus, superior
temporal gyrus, left hippocampus and right thalamus (see Table 2). Activation in these areas is critical for emotional regulation (Etkin et al., 2015; Frank et al., 2014; Kohn et al., 2014).
Figure 1. Results of the behavioural PLS analysis, association between brain activity during emotion perception and schizophrenia polygenic risk scores. (Left panel) Whole-brain activity in emotion regulation regions, such as the prefrontal cortex, inferior frontal gyri, bilateral amygdala and right anterior insula. (Right Panel) Correlations between brain scores representing activity in the regions displayed in the left panel and polygenic risk scores, with positive correlations during the angry congruent conditions and negative correlations during the happy congruent and incongruent conditions. Asterisk denote a significant correlation based on 95% confidence intervals calculated from the bootstrap procedure, and effect size with 80% power calculated from the power analysis.
Table 2

*Brain activity during emotion perception associated with SCZ PRS*

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Hem</th>
<th>BA</th>
<th>MNI coordinates</th>
<th>Voxels</th>
<th>BSR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td>dlPFC</td>
<td>B</td>
<td>6, 8</td>
<td>16</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-8</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Precentral</td>
<td>R</td>
<td>6</td>
<td>34</td>
<td>-6</td>
<td>54</td>
</tr>
<tr>
<td>dmPFC</td>
<td>L</td>
<td>6, 11</td>
<td>-4</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>Inferior frontal</td>
<td>B</td>
<td>13, 47</td>
<td>32</td>
<td>14</td>
<td>-18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-24</td>
<td>20</td>
<td>-22</td>
</tr>
<tr>
<td>SMA</td>
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<td>-12</td>
<td>48</td>
<td>2205</td>
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<tr>
<td>Amygdala</td>
<td>B</td>
<td>-20</td>
<td>-10</td>
<td>-24</td>
<td>360</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>-12</td>
<td>-24</td>
</tr>
<tr>
<td>Posterior mid-cingulate</td>
<td>B</td>
<td>23, 24</td>
<td>8</td>
<td>-14</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-20</td>
<td>-14</td>
<td>44</td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td>L</td>
<td>30</td>
<td>-12</td>
<td>-66</td>
<td>16</td>
</tr>
<tr>
<td>Anterior Cingulate</td>
<td>R</td>
<td>24</td>
<td>8</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
<td>32</td>
<td>-16</td>
<td>-20</td>
<td>464</td>
</tr>
<tr>
<td>Middle occipital</td>
<td>L</td>
<td>19</td>
<td>-56</td>
<td>-68</td>
<td>-2</td>
</tr>
<tr>
<td>Cuneus</td>
<td>R</td>
<td>19</td>
<td>32</td>
<td>-80</td>
<td>30</td>
</tr>
<tr>
<td>Precuneus</td>
<td>B</td>
<td>7, 19</td>
<td>20</td>
<td>-44</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-28</td>
<td>-74</td>
<td>48</td>
</tr>
<tr>
<td>Postcentral</td>
<td>B</td>
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<td>32</td>
<td>-26</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-48</td>
<td>-14</td>
<td>46</td>
</tr>
<tr>
<td>Temporoparietal junction</td>
<td>R</td>
<td>40</td>
<td>68</td>
<td>-26</td>
<td>34</td>
</tr>
<tr>
<td>Superior parietal lobule</td>
<td>R</td>
<td>7</td>
<td>26</td>
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<td>72</td>
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<tr>
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<td>10</td>
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<td>-8</td>
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<tr>
<td>Caudate</td>
<td>R</td>
<td>4</td>
<td>24</td>
<td>-4</td>
<td>218</td>
</tr>
</tbody>
</table>
### Abbreviations:
- Hem = hemisphere; BA = Brodmann area; R = right; L = left; B = bilateral; BSR = bootstrap ratio; dlPFC = dorsolateral prefrontal cortex; dmPFC = dorsomedial prefrontal cortex; SMA = supplementary motor area; vlPFC = ventrolateral prefrontal cortex; voxels = number of voxels (one voxel volume=6 mm³).
- All reported activations are ≥100 voxels (600 mm³).

### Discussion

The aim of the current study was to investigate the association between SCZ polygenic risk and brain function in healthy participants during emotion perception. To our knowledge, this is the first imaging genetics study to investigate the association of SCZ polygenic risk on emotion perception. The results show a positive correlation between SCZ PRS and increased brain activity during anger (threat) perception, and a negative correlation between SCZ PRS and decreased brain activity during perception of happiness. The pattern of the activated brain regions involved emotion regulation neural correlates, such as the dlPFC, parietal cortex, anterior cingulate, amygdala and right anterior insula (rAI). These results suggest that individuals with higher SCZ PRS may over-activate emotion-regulation areas during perception of threat, particularly when threat is congruent with prior expectations. The over-activation in emotion regulatory brain regions may reflect neural inefficiency in those with a greater genetic risk for SCZ, resulting in more effort to regulate the experience of threat.

Emotion dysregulation is a core feature of SCZ (Ellgring and Smith, 1998; Kring and Werner, 2004). A recent review and meta-analysis shows that individuals with SCZ have global emotion regulation difficulties, engaging in maladaptive strategies to regulate their emotions (O'Driscoll et al., 2014). There is also behavioural and neurophysiological evidence that individuals with SCZ particularly struggle with regulating their experience of negative emotions, such as anger (Cohen and Minor, 2010; Strauss et al., 2013). Emotion regulation is an integral component during...
emotion perception (Phillips et al., 2003b) and those with maladaptive emotion regulation have impaired recognition of emotional expressions (Swart et al., 2009). Underlying the emotion regulation difficulties in SCZ is aberrant brain activity in regions such as the left vIPFC, inferior frontal gyrus, and anterior insula (van der Meer et al., 2014) and disconnection between frontal and limbic regions during regulation of negative emotion (Morris et al., 2012). A recent real-time fMRI study revealed that individuals with SCZ are able to improve their recognition of negative emotions by learning to regulate activation of the rAI (Ruiz et al., 2013), a region with a central role in emotion regulation (Gu et al., 2013). Therefore, aberrant neural activity during emotion regulation may provide an explanation for poorer emotion perception performance in SCZ.

Our findings provide support for a link between SCZ common variant genetic risk profiles and aberrant activity in neural correlates of emotion regulation (Curcic-Blake et al., 2012; Mothersill et al., 2014). In our study, the BOLD response in neural correlates of emotion regulation was increased in healthy individuals with greater SCZ genetic risk when viewing expected threatening emotions. Regions with greater activity included the rAI and bilateral amygdala, both implicated in emotional reactivity and the signaling of salience and, consequently, the need to regulate (Phillips et al., 2003a); the dorsal prefrontal cortex (dPFC), which is critically involved in driving regulatory processes, such as attention, reasoning, and the representation of emotional associations (Kohn et al., 2014; Ochsner and Gross, 2005); the superior temporal gyrus, angular gyrus, and SMA, regions implicated in executing the regulatory processes initiated by dPFC (Kohn et al., 2014); the right anterior cingulate gyrus, a region critical in effortful error-related regulation (Bush et al., 2000); and finally, the left hippocampus, a region with a role in inhibiting negative emotions and response to threat (Gray and McNaughton, 2003). Although these emotion-regulation areas were recruited to a greater extent in those individuals with higher SCZ PRS, we did not find that their performance was better in discriminating emotions during the DEP task. Increased activation in these brain regions in the presence of no improvement in behavioural performance may indicate inefficient emotion regulation activity in those with higher SCZ PRS, particularly when viewing threatening emotions. In comparison, viewing happy emotions was not related to increased activation in the emotion-regulation areas in those with higher PRS, furthermore individuals with lower PRS were found to have decreased activity in the emotion regulation regions during happiness perception. This may be because regulation is found to be easier for happy
emotions compared to threatening emotions (Kim and Hamann, 2007), and thus lesser engagement of emotion regulation neural correlates is required.

Our results seem to reflect that healthy individuals with higher genetic risk over-recruit the emotion-regulation areas in an attempt to maintain normal emotion regulation during threat perception. Previous research supports this notion, with studies finding that healthy individuals with a greater risk of developing SCZ over-activate regulatory regions in order to effectively regulate their experience of negative emotions (Modinos et al., 2010; Mohanty et al., 2005). Interestingly, we found over-activity in emotion regulation regions particularly during perception of threatening emotions that were congruent with prior expectations; in other words, threat that was expected. This finding may reflect that individuals with greater genetic risk for SCZ, similarly to individuals with SCZ, have reduced precision in prior expectations (Adams et al., 2016), and, therefore, require more regulation to processes expected threat. Further examination and replication of these findings in larger studies may link emotion regulation as a potential biomarker of SCZ pathophysiology, with important implications for risk identification, early intervention, and improving psychosocial therapy for SCZ.

Limitations

A major limitation of the current study is the sample size compared to other imaging genetic studies. Although we had sufficient power (80%) and observed robust effects, larger replication studies are needed to investigate the effects of SCZ PRS on neural activity underlying emotion perception. Nonetheless, we have incorporated methods to increase the power of this study by: (1) utilising the cumulative genetic risk approach, which increases the effect size of liability for SCZ compared to the candidate gene approach; (2) calculating and using the SCZ PRS as a regressor variable rather than a predictor score, and (3) basing our PRS on summary statistics from the most recent, large schizophrenia PGC2 (Ripke et al., 2014a) dataset (n = 34,241 SCZ cases; 45,604 controls), with resultant increased power and predictive capacity (Dudbridge, 2013).

Conclusions

Our results suggest that the alterations in neural activity during emotion perception identified previously in SCZ may be explained to a significant extent by common genetic variants. We observed associations between SCZ PRS and increased activity in emotion regulation neural correlates during processing of threatening emotions. These results provide evidence that genetic risk for SCZ may
be associated with widespread neural inefficiency in the emotion-regulation areas. When combined with other pathological factors, this neural inefficiency during emotion perception may increase risk for psychosis. In this study, we build on the work in imaging genetics by assessing how cumulative genetic susceptibility for SCZ influences emotion perception and related neural activity. We provide support for the PRS approach in delineating potential biomarkers for SCZ, and the potential to contribute to translational neuroscience efforts to improve risk identification, early intervention, and psychological treatments.
CHAPTER 6
General Discussion
Aim

The goal of this thesis was to apply an integrative approach to investigate naturalistic emotion perception and the underlying impaired processing in schizophrenia (SCZ). To this end, I examined the role of prior expectancy, aberrancy in neural circuitry and SCZ risk genes in explaining variability on dynamic emotion perception. This thesis provides novel insights into the influence of prior expectancy on naturalistic emotion perception in healthy adults and delineates the nature of aberrant prior expectations in patients with SCZ. Specifically, I found that, in healthy adults naturalistic emotion perception is facilitated by prior expectations with reduced reaction time over time. This was associated with increased prefrontal and right amygdala (rAMY) activity. In contrast, reaction times to unexpected emotions remained stable over time. These trials engaged the right temporoparietal junction (rTPJ) and occipital regions and may represent automatic change detection processes. In patients with SCZ there may be impairment in prior expectations, as our results show reduced ability in SCZ patients to identify emotions that were congruent with prior expectations. The impaired perception of congruent emotions was related to reduced activity in inferior frontal, parietal, and insula cortex, as well as aberrant activity in a rAMY-subcortical network. Thus, the present thesis provides primary evidence for the expectancy-driven neural circuitry underlying emotion perception, and how this circuitry may be impaired in SCZ. Furthermore, I provide preliminary evidence for the association between SCZ polygenic risk in healthy adults and altered activity in emotion neural networks, paving the way for larger studies into the association between genetic risk profiles and emotion perception in SCZ. In the following chapter, I summarise how the findings from the current thesis contribute to the literature investigating dysfunctional emotion perception in SCZ and conclude with clinical implications and future directions.

Contributions to the Literature

The current thesis has contributed to the body of knowledge pertaining to naturalistic emotion perception and the underlying processes and neural correlates. Previous studies have found that prior expectations facilitate perception of emotions, presented in static images, by improving speed and accuracy to discriminate emotions congruent with prior expectations (Barbalat et al., 2013; Barbalat et al., 2012). In the current work, I explored the influence of prior expectations during naturalistic emotion perception, using dynamic, audio-visual videos displaying anger, happy and neutral
expressions. In the first study (presented in Chapter 2), I developed and validated a novel fMRI paradigm The Dynamic Emotion Perception (DEP) task to investigate the prior expectancy effect at the behavioural and neural levels. In contrast to previous findings, the results in the current study demonstrate that congruently cued dynamic emotions initially require greater effort to discriminate, as indicated by slower reaction times and lower accuracy. Only after repeated cues over the course of the experiment, prior expectations were reinforced to facilitate emotion perception. Conversely, emotional information incongruently cued (incongruent with prior expectations) acquired consistently fast responses relative to the initial slower responding for congruently cued emotions. These findings are novel and they are akin to real life, where prior expectations are not instantaneous and develop through recurrent experience and learning. In regards to the finding of fast detection of incongruent emotion; in a dynamic world, advantageous or dangerous situations may arise unexpectedly, thus it is an adaptive ability to quickly detect incongruency.

Previous studies investigating the neural basis of prior expectation on emotion, have consistently shown that the vmPFC is engaged during congruent emotion processing, whereas the anterior mid-cingulate gyrus, middle temporal gyrus, insula (Barbalat et al., 2013; Summerfield and Koechlin, 2008), occipital regions, posterior parietal regions and premotor gyri (Clery et al., 2013) are engaged during incongruent emotion processing. The findings in this thesis show that during dynamic emotion perception key regions of the social brain, namely amygdala and rTPJ (Kennedy and Adolphs, 2012; Van Overwalle, 2009) were engaged for conditions congruent and incongruent with prior expectations. I found that recognition of emotions congruent with prior expectations engaged prefrontal regions, amygdala and putamen, previously associated with greater top-down monitoring (Mitchell, 2011), emotional learning (Hamann, 2001; Hooker et al., 2006) and conditioning (Brovelli et al., 2011; Tricomi et al., 2009), respectively. In comparison, recognition of incongruent emotions engaged visual automatic change detection neural correlates (Clery et al., 2013), such as the rTPJ, associated with quick re-orienting of attention to change (Decety and Lamm, 2007; Vogt, 2014). Thus, Chapter 2 provided new insights into the behavioural and neural mechanisms of prior expectations during naturalistic emotion perception.

The current thesis has also extended the threat processing literature, providing new insights into efficient expectancy-related threat processing and the underlying functional networks. In the second study (presented in Chapter 3), I investigated the functional connectivity of key emotion brain regions and their association with faster
responding to threatening (angry) emotion, either congruent or incongruent with prior expectations. Functional networks were mapped for the three seed regions identified in Chapter 1, the vmPFC, rAMY and rTPJ (Dzafic et al., 2016). Previous literature has implicated these regions in threat processing (Doty et al., 2014; Marstaller et al., 2016) and rapid re-orienting of attention to relevant stimuli (Corbetta et al., 2008). I found that faster recognition of congruent threat was associated with a subcortical network functionally connected to rAMY, implicated in fear conditioning and rapid processing of coarse emotional cues. In comparison, faster recognition of incongruent threat showed a trend association with a ventral frontoparietal network functionally connected to rTPJ, implicated in rapid reorienting of attention to relevant change. Conversely, the functional network connected to vmPFC was not associated with the facilitation of response to threat, in either congruent or incongruent contexts. Therefore, in Chapter 3, the expectancy-related functional networks, important in efficient processing of threatening emotion, were delineated. Specifically, the rAMY-subcortical network, which may be critical in environments where one is conditioned to expect threat, in directing attention to cues that signal aggression.

The current thesis has provided novel insights regarding the underlying aberrant processes and neural circuitry contributing to impaired emotion perception in SCZ. In the third study (presented in Chapter 4), I identified reduced ability to identify emotions that were congruent with prior expectations, in SCZ patients compared to healthy individuals. In addition, the patients failed to show the same pattern of learning and facilitation by prior expectations as indexed by reduced response time over the course of the experiment. Therefore, the nature of SCZ-related aberrancy in prior expectations was elucidated, which in previous literature has been inconsistently reported (Barbalat et al., 2012; Chambon et al., 2011b). Specifically, the results from study 3 indicate that SCZ patients have decreased ability to either form or use priors to facilitate emotion perception. At the neural level, I found that patients had reduced engagement of inferior frontal, parietal and insula regions, and rAMY-subcortical dysconnectivity when viewing emotions congruent with prior expectations. Aberrancy in the higher hierarchical frontal, parietal and insula regions may explain the impaired ability to form prior expectations (Adams et al., 2016), whereas rAMY dysconnectivity with subcortical regions may reduce efficiency in discriminating context-congruent emotions (Marstaller et al., 2016). The results from chapter 4 delineate the underlying aberrant processes during naturalistic emotion perception in SCZ by indicating that there might be a specific deficit in the prior expectancy effect, this results in a failure
to direct attention to context-relevant emotional information and reduces the facilitatory influence prior expectations provide in healthy perception of emotion. Thus, in a dynamic environment, individuals with SCZ may have aberrant prior expectations leading to inefficient perception of emotion.

Finally, in the fourth study (presented in Chapter 5), I provide preliminary evidence for an association between SCZ genetic risk scores in healthy adults and variability in the neural processing of emotion. To our knowledge this is the first imaging genetics study to investigate SCZ polygenic risk in the context of naturalistic emotion perception and prior expectancy. The results show a positive correlation between increasing SCZ polygenic risk scores (PRS) in a group of healthy individuals and increased activity in emotion regulation neural correlates, during threat perception. These results suggest that in individuals with higher genetic risk for SCZ, greater activity in brain regions involved in emotion regulation may reflect neural inefficiency in this circuitry. Moreover, greater recruitment of emotion regulation circuitry may function as a compensatory mechanism to normalise performance during threat perception. The results from Chapter 5 provide new knowledge in the field of imaging genetics by assessing how cumulative genetic susceptibility for SCZ influences the neural correlates of emotion perception. Furthermore, these results provide preliminary data that should inform larger replication studies able to examine whether the underlying neural networks of naturalistic emotion perception may be potent neurophysiological biomarkers of SCZ.

Implications of Findings

Current treatment for psychiatric disorders, such as SCZ, are ineffective at treating the (social) cognitive effects (Herbener et al., 2005), leading to an extensive search for new treatments that are able to target the underlying aberrant processes that cause and maintain impairment. In combination, the four empirical studies presented in the current thesis provide methodological, theoretical, clinical and translational implications for the development of new treatments for emotion perception deficits. The findings from the current thesis shed light on the functional circuitries that facilitate emotion perception and rapid recognition of threat in a healthy brain. These neurophysiological findings have the potential to increase the focus and efficacy of neurofeedback therapeutic interventions for those with SCZ, or with a greater risk to develop SCZ and other disorders with pathological emotion processing. Recent advances in neuroimaging have made neurofeedback of localized brain areas a
feasible, reliable technique that could potentially revolutionize treatment for psychiatric disorders (Thibault et al., 2016). Neurofeedback is a process conducted using devices such as electroencephalography (EEG) or fMRI; the process involves providing the participant with real-time feedback about their brain activity and training the participant to consciously correct aberrant brain activity (Arns et al., 2016). Real-time fMRI neurofeedback is already gaining ground in its application to improve emotion regulation in SCZ by targeting insular activity (Ruiz et al., 2013).

The research from the current thesis also provides essential data for future targeting of networks involved in emotion perception difficulties by transcranial direct current stimulation (tDCS). Non-invasive brain stimulation techniques, such as tDCS, have a direct effect on brain activity in comparison to neurofeedback techniques (Luft et al., 2014). Techniques such as tDCS are able to affect social cognition (Martin et al., 2017) and could have significant potential to improve cognitive outcomes in patients with serious mental health issues (Baeken et al., 2016; Tortella et al., 2015). For example, based on the evidence in studies 1 and 2, the rTPJ may provide an excellent first candidate stimulation site in order to improve recognition of unexpected but relevant emotion. Research into tDCS and its influence on emotion perception is still in its infancy; thus, future studies should examine the effect of stimulating the rTPJ on response times during emotion perception in a healthy population. The results from the healthy sample may provide evidence of the causal role of rTPJ in efficient processing of emotion, and also provide the baseline for future work investigating the applicability of tDCS in improving emotion processing in clinical groups.

In regards to the theoretical and clinical implications of the current thesis, the research studies within provide a better understanding of the cognitive and underlying neural processing differences during naturalistic emotion perception in patients with SCZ. The results from study 3 support the theoretical conceptualisation of SCZ as a disorder of reduced precision in prior expectations, as impairment in emotion perception was specific to conditions in which emotions were congruent with prior expectations. In contrast participants with SCZ did not have a significant impairment to discriminate emotions incongruent with (and not reliant on) prior expectations. Therefore, our results highlight the need for future interventions to target and improve formation of valid prior expectations in dynamic contexts. For instance, novel treatments, such as real-time fMRI coupled with cognitive remediation may target the activity of the rAMY whilst the individual is instructed to form cue-emotion associations. If in SCZ there is a faulty pattern of activation in this target area, resulting in reduced
precision in prior expectations, real-time fMRI may assist in overriding the aberrant connectivity. With repeated practice, a healthier pattern of brain activity may emerge that can support formation of valid prior expectations to facilitate emotion perception in SCZ.

In regards to the methodological implications, I developed a novel fMRI paradigm, which can be used in future studies to examine naturalistic emotion perception and the effect of prior expectancy in a range of pathologies with emotion processing deficits, for example: autism spectrum disorders (Kita and Inagaki, 2012; Sigman et al., 2004), bipolar disorder (Temmerman et al., 2015) and eating disorders (Oldershaw et al., 2011). In addition, future studies may administer the DEP task to specifically study impaired processing of threat in SCZ (Henry et al., 2010; Pinkham et al., 2014b) and anxiety disorders (Booth et al., 2016; Chen et al., 2016). A major benefit of the DEP task is that it allows the exploration of functional networks underlying prior expectancy in a dynamic emotional environment. As a consequence, the results of this study and the results of future studies applying the DEP paradigm may inform the application of neuromodulatory techniques, which rely on isolating and modifying abnormal functional networks in psychopathology (Luft et al., 2014; Schlapfer and Bewernick, 2009).

Finally, the imaging genetics findings in the current thesis provide initial evidence that SCZ polygenic risk is associated with altered activity in the healthy emotion regulation neural circuitry. With abundant research showing emotional dysregulation among individuals with schizophrenia (Davidson et al., 2000; Khoury et al., 2015), this study supports the benefit of therapy, which targets emotion regulation, consequently improving the emotion regulation brain network. In addition, the finding that healthy individuals with higher genetic risk have increased neural activity during emotion perception may indicate that this circuitry is a potential biomarker for SCZ. Our findings give support to using the polygenic risk model in delineating neurophysiological biomarkers for SCZ and other neuropsychiatric diseases, with the potential to contribute to translational efforts aimed at preventing the onset of psychosis.

Future Directions and Limitations

Suggestions for future directions, particularly relating to the translation of the findings presented in the thesis have been mentioned in the implications section of the discussion. Here, I will therefore focus on further consideration for future work and the limitations that should be addressed in future research.
Future studies will benefit by employing naturalistic presentations of emotion in the study of emotion perception. By using dynamic and multisensory emotional stimuli in the new DEP task, I was able to demonstrate how prior expectations may emerge in a more dynamic real-life scenario and therefore disambiguate the SCZ-related abnormality in prior expectations during the processing of dynamic social stimuli. It is fundamental that future studies utilise ecologically valid stimuli when investigating underlying mechanisms, as non-canonical stimuli may rely on different mechanisms.

In the current thesis, I focused on functional connectivity analyses to investigate healthy and aberrant neural networks. Functional connectivity provides correlational evidence for a relationship between regions, and their effect on behaviour. However, further studies using effective connectivity techniques, such as dynamic causal modelling may be required to understand the causal relationships between areas of interest. Future studies may wish to model how inferior frontal regions causally influence the functioning of the rAMY-subcortical network and, consequently, how prior expectations influence or direct attentional resources to certain aspects of the perceptual field.

A limitation of the current thesis is that the results are based on a heterogeneous sample of chronic, medicated schizophrenia patients. I did not assess the relationship between medication and aberrant emotion neural circuitry in SCZ; this may provide interesting avenues for future research. Future studies may wish to investigate performance on the DEP task before medication or include medication history as a covariate in the analyses. The heterogeneous medication history of the SCZ cohort in the present thesis made this inappropriate and likely misleading. Furthermore, the SCZ and the imaging genetics HC sample sizes were small to moderate; although there was sufficient statistical power to report robust effects. As such, larger replication studies are needed to investigate the prior expectancy effect in SCZ and the effects of schizophrenia PRS on emotion perception neural circuitry.

**Overall Summary**

The research presented in this thesis highlights the processing and neurophysiological mechanisms that underlie naturalistic emotion perception and how these may be impaired in SCZ. Prior expectations were found to facilitate healthy emotion perception ability. At the neural level, viewing emotions congruent with prior expectations was associated with prefrontal and rAMY activity; with rAMY-subcortical connectivity particularly important in rapid congruent threat processing. In contrast,
viewing emotion incongruent with prior expectations was associated with activity in neural correlates of automatic change detection, such as the rTPJ-ventral attentional network. In SCZ, there was a specific impairment in perceiving emotion congruent with prior expectations, which was associated with aberrant inferior frontal, parietal and insula activity, and rAMY-subcortical dysconnectivity. Finally, I found that SCZ risk genes were associated with greater activity in the neural correlates of emotion regulation. These findings demonstrate that activity in emotion brain regions is associated with polygenic risk for SCZ. Taken together, the findings in this thesis provide new insights into the behavioural, neural, and genetic correlates of naturalistic emotion perception impairment in SCZ.
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Appendix A

Here is the link for the published version of Study 1: