2015

Clarifying the functional process represented by reduced P50 suppression in schizophrenia

Anna Dalecki
University of Wollongong, adalecki@uow.edu.au

School of Psychology

Faculty of Social Sciences

Clarifying the Functional Process Represented by Reduced P50 Suppression in Schizophrenia

Anna Dalecki

This thesis is presented as part of the requirement for the

Award of the Degree of

Doctor of Philosophy

of the

University of Wollongong

August 2015

Certification

I, Anna Dalecki, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Psychology, Faculty of Social Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other institution.

Anna Dalecki

August 2015

Acknowledgements

I am fortunate enough to owe my sincere thanks to some very dear people, who in so many ways contributed to this thesis:

Firstly, to my primary supervisor Professor Rodney Croft, for his intellectual, emotional and financial support, for all the academic opportunities he provided me with, for his genuine love of science, and for all the meetings where we got to talk about ideas. I also extend my thanks to Associate Professor Stuart Johnstone, for his guidance, advice and support along the way.

To all of the participants in the sometimes onerous studies within, and in particular to all the patients for generously contributing their time and effort to science, as well as to Amity and Lisa-marie, for their help in data collection.

To the School of Psychology administration staff: Julie, Michelle, Len, Monica and Jill, for going above and beyond on many occasions and making things easier wherever they could.

To my fellow PhD students, especially Nat, Lisa-marie, Lisa L, Gen, Frances, April and Louise, for the distractions, laughs, coffee breaks, advice, encouragement, and company on late nights and weekends spent at the office.

To my friends, particularly Jess, Gunjan, Uwana and Sarah, for their motivation and encouragement, for knowing when to ask about 'the thesis', and when to spend time talking about and doing anything else.

To Paul, for his hand and heart, for his rational, reassuring words, for his encouragement whenever I needed it, for making me laugh often, and for reminding me of all the things there are to look forward to post-submission.

To my family: my grandparents, and in particular to Dziadzio who passed away earlier this year; to Ciocia Renia, Wujek Janusz, Emilia, Kamil and Bella for their support; and most of all, to my mum Maria, dad Dariusz, Kat, Evelina, and Michal, for their love, care, belief in me and most generous support for all these years. I dedicate this thesis to them.

Abstract

P50 suppression is a measure of the brain's ability to modulate its response to incoming repetitive auditory stimuli that is derived from the electroencephalogram. This measure is widely used in psychiatric research and is argued to be a schizophrenia endophenotype. However, inconsistent results within this literature suggest that P50 suppression, even in healthy populations, is poorly understood and limits the 'endophenotype' claim. An understanding of methodological issues relevant to P50 suppression measurement, variables influencing P50 suppression, and the neural function it indexes in healthy subjects will inform psychiatric research as to the nature of impairments observed in schizophrenia.

The overall aim of this doctoral thesis is to clarify the neural function that P50 suppression represents in schizophrenia. In order to achieve this, Study 1 investigated how various methodological factors influence the magnitude and reliability of P50 suppression and the P50 amplitudes from which it is derived. The results show, firstly, that P50 suppression is not invariant in magnitude, but reduces over time within-session and is dependent on the duration of the interval between auditory stimulus pairs within the P50 paired-stimulus paradigm; and secondly, that P50 suppression is more reliable when: 1) quantified using the P50 Difference as opposed to the P50 Ratio metric; 2) P50 amplitudes are defined relative to the pre-stimulus baseline rather than the negative trough preceding the P50 event-related potential (ERP) component; and 3) more (70-100) versus less (10-30) event-related responses contribute to averaged P50 responses.

Study 2 aimed to determine whether the refractory period (RP) or inhibitory input (II) hypothesis best explained P50 suppression. Starting from the premise that more neural generator recovery results in larger P50 amplitudes to the second stimulus in a pair (S2P50)

and more inhibition results in smaller S2P50 amplitudes, Study 2 manipulated the functionality of the hypothesised process(es) underpinning P50 suppression via manipulations of: 1) The duration of the interval separating consecutive stimulus pairs (inter-pair interval; IPI; 2 s versus 8 s); and 2) the direction of selective attention; Attention (directed toward) versus Non-Attention (directed away from) auditory stimuli. The rationale was that the 'relative' reduction of attention (Non-Attention, compared to Attention) and IPI (2 s compared to 8 s) would produce a relative reduction of the functionality of the process(es) underpinning P50 suppression, with reduced function of II *increasing* S2P50 amplitude and reduced function of RP *decreasing* S2P50 amplitude. Results supported the II, but not RP, hypothesis of P50 suppression. Reducing IPI from 8 to 2 s (in the Non-Attention protocol) tended to *increase* S2P50 amplitude (consistent with having reduced the recovery/functionality of inhibitory inputs; thus resulting in larger S2P50 amplitude). In the 2 s IPI paradigm, enhancing attention *reduced* S2P50 amplitude (consistent with Attention enhancing the functionality of inhibitory inputs; thus resulting in smaller S2P50 amplitude). Thus Study 2 demonstrated that scalp-recorded P50 suppression in healthy participants is consistent with the operation of underlying inhibitory inputs, and not a simple refractory period. Further it demonstrated that attention is relevant to P50 suppression, which means that contrary to speculation, it is not an entirely pre-attentive process.

Study 3 extended upon the second by determining whether the effect of attention on P50 suppression differs between schizophrenia patients and healthy controls, and thus whether the well-characterised attention deficit in schizophrenia is a confound in P50 suppression research. Replicating the Study 2 results, attention increased P50 suppression, which confirms that P50 suppression is not entirely pre-attentive. Further, the effect of attention differed between groups: enhancing attention increased P50 suppression (i.e., *reduced* S2P50

amplitude, *increased* P50 Difference) in healthy subjects but did not affect P50 measures in schizophrenia patients. No group differences in P50 suppression were found. Attention is thus a confound in schizophrenia P50 research, and needs to be carefully controlled. That group differences in P50 suppression were not found when attention was controlled, suggests the possibility that the schizophrenia-control difference in P50 suppression reported in the literature does not reflect P50 suppression but rather attentional differences between groups.

Taken together, the findings from this doctoral thesis support the view that P50 suppression reflects the operation of inhibitory input mechanisms. It demonstrates that the operation of these mechanisms is enhanced by attention, and that this attention-related enhancement is present in healthy controls but not schizophrenia patients. It follows that attention confounds P50 suppression measurement when comparing schizophrenia patients to healthy controls, and suggests the need to carefully control the direction of attention in schizophrenia P50 research in order to be able to more appropriately interpret findings of P50 suppression impairments (or lack thereof).

Publications Constituting this Thesis

Published Manuscripts

Dalecki, A., Croft, R. J., & Johnstone, S.J. (2011). An evaluation of P50 paired-click methodologies. *Psychophysiology. 48,* 1692-1700. (Chapter 2)

Dalecki, A., Johnstone, S.J., & Croft, R. J. (2015). Clarifying the functional process represented by P50 suppression. *International Journal of Psychophysiology.* (Chapter 3)

Manuscript Submitted for Peer Review

Dalecki, A., Green, A. E., Johnstone, S.J., & Croft, R. J. (*Under Review*). The relevance of attention in schizophrenia P50 paired-stimulus studies. *Clinical Neurophysiology.* (Chapter 4)

Statement of Verification

This statement verifies that the greater part of the work in the above-named manuscripts is attributed to the candidate. Anna Dalecki, under the guidance and supervision of her supervisors, took primary responsibility for the design of each study, all data collection and analysis, prepared the first draft of each manuscript, and prepared the papers for submission to relevant journals. Co-authors, who were also supervisors to the candidate, contributed to the thesis by providing guidance on the design, analysis and general structure of each study, and providing editorial suggestions for each paper.

Anna Dalecki (PhD Candidate)

Professor Rodney Croft (Primary supervisor)

August 2015

Table of Contents

Certification i

Acknowledgements ii

Abstract iii

Publications Constituting this Thesis vi

Statement of Verification vii

Table of Contents viii

List of Tables xi

List of Figures xii

Abbreviations xiv

1. CHAPTER 1: INTRODUCTION 1

1.1. Background 1

1.2. P50 Suppression 3

1.3. P50 Suppression: Neuroanatomical Substrates 3

1.4. P50 Suppression in Schizophrenia 6

1.5. P50 Suppression: Methodological Considerations 8

1.6. Clarifying the Functional Process Represented by P50 Suppression 12

1.7. Can the P50 Suppression Deficit Reported in Schizophrenia be More Appropriately Attributed to Attentional Impairments? 16

1.8. Data Analysis Variation and Strategy for the Present Thesis 19

1.9. Research Aims 20

1.10. Significance and Originality 21

1.11. References 23

2. CHAPTER 2: AN EVALUATION OF P50 PAIRED-CLICK METHODOLOGIES 37

2.1. Abstract....37
2.2. Introduction....38
2.3. Method....42
2.4. Results....47
2.5. Discussion....56
2.6. References....62
3. CHAPTER 3: CLARIFYING THE FUNCTIONAL PROCESS REPRESENTED BY P50 SUPPRESSION....67
3.1. Abstract....67
3.2. Introduction....68
3.3. Methods....72
3.4. Results....78
3.5. Discussion....81
3.6. References....84
4. CHAPTER 4: THE RELEVANCE OF ATTENTION IN SCHIZOPHRENIA P50 PAIRED STIMULUS STUDIES....89
4.1. Abstract....89
4.2. Introduction....91
4.3. Methods....94
4.4. Results....101
4.5. Discussion....105
4.6. References....109
5. CHAPTER 5: DISCUSSION....117
5.1. Summary of Thesis Findings....117
5.2. Contribution of the Thesis Findings to the Literature....118
5.3. Limitations....137

5.4. Future Directions 139
5.5. Conclusion 142
5.6. References 144

List of Tables

Table 2.1. Intraclass Correlation Coefficients for P50 Paired-Click Metrics Calculated from 100 Trials and Defined from Peak-to-Peak And Baseline-to-Peak.

Table 4.1. Demographic Characteristics of the Sample are Shown.

Table 4.2. S1P50 and S2P50 Amplitudes, Latencies and P50 Difference and Ratio Values for Non-Attention and Attention Conditions, in Schizophrenia Patients and Healthy Controls.

List of Figures

Figure 1.1. Reliability coefficients reported in the literature for S1 and S2P50 amplitudes, P50 Difference and P50 Ratio.

Figure 2.1. P50 ERPs to S1 and S2 at 19 electrode sites.

Figure 2.2. Intraclass Correlation Coefficient values for the best difference and ratio method shown as a function of electrode site.

Figure 2.3. Average standard deviations (with standard error), and Intraclass Correlation Coefficients (and 95% confidence intervals) shown for the: (a) S1 – $S2_{BP}$ difference; (b) $S2/S1_{PP}$ ratio; (c) $S1_{PP}$ P50 amplitude; and (d) $S2_{PP}$ P50 amplitude calculated from averages comprised of between 10 and 100 trials.

Figure 2.4. The S1 – $S2_{BP}$ difference and $S2/S1_{PP}$ ratio in each of five Time blocks.

Figure 2.5. Grand average ERP waveforms elicited by S1 and S2 are shown for Time 1 and Time 2 separately, at Cz.

Figure 2.6. P50 amplitudes (with standard error) to S1 and S2 in each of five Time blocks.

Figure 2.7. The S1 – $S2_{BP}$ difference and $S2/S1_{PP}$ ratio measured in each of five Inter-Pair Interval conditions.

Figure 2.8. Grand average Event Related Potential waveforms elicited by S1 and S2 are shown for the IPI1, IPI3, and IPI9 conditions, at Cz.

Figure 3.1. Event Related Potentials elicited in the Non-Attention and Attention conditions at Inter-Pair Intervals of 8 and 2 s.

Figure 4.1. S1P50 and S2P50 ERPs under Attention and Non-Attention conditions for healthy controls and schizophrenia patients.

Abbreviations

ACx	Auditory cortex
BP	Baseline-to-peak P50 amplitude definition method
EEG	Electroencephalogram
EOG	Electrooculogram
ERP	Event related potential
II	Inhibitory input hypothesis of P50 suppression
IPI	Inter-pair interval (between two consecutive stimulus pairs)
ISI	Inter-stimulus interval (between S1 and S2)
P50 Difference	S1P50–S2P50
P50 Ratio	S2P50/S1P50
PP	Peak-to-peak P50 amplitude definition method
RP	Refractory period hypothesis of P50 suppression
S1	Stimulus 1 in the auditory paired-stimulus paradigm
S2	Stimulus 2 in the auditory paired-stimulus paradigm
S1P50	P50 ERP component elicited in response to S1
S2P50	P50 ERP component elicited in response to S2

1. CHAPTER 1: INTRODUCTION

This doctoral research investigated the neural function theorised to underpin P50 suppression in two healthy samples and a sample of schizophrenia patients (with age and gender-matched healthy controls). The empirical studies comprising this thesis specifically sought to clarify the factors influencing P50 suppression and the reliability of its measurement (*Study 1; Chapter 2*), the functional process represented by P50 suppression in healthy subjects (*Study 2; Chapter 3*), and whether the knowledge gained from Studies 2 and 3 have bearing on our interpretation of the functional process represented by 'impaired' P50 suppression in schizophrenia patients (*Study 3; Chapter 4*). The following section outlines the general background and rationale for this research, the research aims, and the significance and originality of this research.

1.1. Background

Schizophrenia is a chronic psychiatric disorder comprising disparate symptoms (categorised as positive, negative and cognitive), with a lifetime prevalence of 1% (Mueser & McGurk, 2004). Although many genetic and environmental risk factors have been characterised, the cause of schizophrenia remains elusive. Further, although first and second generation antipsychotics ameliorate some (particularly positive) symptoms (van Os & Kapur, 2009), they are generally less efficacious at improving the negative symptoms and cognitive impairments (Lindenmayer, Nasrallah, Pucci, James, & Citrome, 2013) which substantially impact on schizophrenia patients' daily functioning and quality of life (Chue & Lalonde, 2014; Murphy, Chung, Park, & McGorry, 2006). The combination of a highly complex disease phenotype (schizophrenia) and a paucity of satisfactory treatments have led to a large

increase in research focused on schizophrenia endophenotypes (Braff, 2015; Thibaut et al., 2015). Endophenotypes are simple, readily measurable, 'intermediate phenotypes' lying in between genes and the ultimate disease phenotype (Gottesman & Gould, 2003). To be classified as such, endophenotypes should be associated with the disorder in the population, heritable, state-independent (present regardless of whether the disorder is 'active'), co-segregate with illness within families, and be present in unaffected family members at a higher rate than seen in the general population (Gottesman & Gould, 2003; Thibaut et al., 2015). The rationale is that endophenotypes will, being more proximal to genes than the disorder itself, be underpinned by fewer genes than the schizophrenia phenotype, and thus reveal more about the genetic and neural underpinnings of schizophrenia (Braff, 2015). Further, it may be the case that these biomarkers will be useful in treatment development, with endophenotypes (rather than manifest symptoms) the treatment target (Smucny, Stevens, Olincy, & Trellegas, 2015; Thaker, 2007; van der Stelt & Belger, 2007). One such (putative) schizophrenia endophenotype is P50 suppression (Allen, Griss, Folley, Hawkins, & Pearlson, 2009; Earls, Curran, & Mittal, 2016; Smucny et al., 2015; Ross & Freedman, 2015; Thaker, 2007).

Despite a considerable amount of research conducted using P50 suppression over the past 30 years (Adler et al., 1982; Earls, Curran, & Mittal, 2016), the functional process that it represents has not been fully elucidated. This limits the ability to interpret both the process P50 suppression represents in healthy subjects as well as in psychiatric populations that demonstrate alterations in these responses (such as schizophrenia; Heinrichs, 2004). Further, a paucity of research addressing the appropriate methodology for measuring P50 suppression means that a wide range of methods are adopted in the literature, further complicating the interpretation of results (de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007b; Patterson

et al., 2008). This Introduction will describe P50 suppression, the underlying neuronal process(es) it is thought to represent, and review some of the methodological considerations relevant to its measurement both in healthy subjects and schizophrenia samples.

1.2. P50 Suppression

The P50 event-related potential (ERP) component is a positive-going deflection in the electroencephalogram (EEG) occurring approximately 50 ms after, and elicited in response to, the presentation of a brief auditory stimulus (Freedman, Adler, Waldo, Pachtman, & Franks, 1983). P50 suppression is measured in an auditory paired-stimulus task, in which a pair of identical, brief auditory stimuli (S1, S2) are presented 500 ms apart with subsequent measurement of the amplitude of the P50 elicited in response to each stimulus. P50 suppression itself refers to the amplitude-reduction of the P50 to the second (S2) relative to the first (S1) of these auditory stimulus pairs (Siegel, Waldo, Mizner, Adler, & Freedman, 1984). The magnitude of P50 suppression is quantified using the P50 Difference (S1P50-S2P50; where a large Difference indexes a large reduction in P50 amplitude from S1 to S2) and the P50 Ratio (S2P50/S1P50; where a small Ratio indexes a large reduction in P50 amplitude from S1 to S2).

1.3. P50 Suppression: Neuroanatomical Substrates

Research into the neural underpinnings of P50 generation and suppression suggests the involvement of several neuroanatomical regions. Specifically, the presentation of any brief auditory stimulus (in the context of the P50 auditory paired-stimulus paradigm: S1 and S2)

elicits a P50 ERP component as recorded at the scalp. Evidence from magnetoencephalographic source localisation (Godey, Schwartz, de Graaf, Chauvel, & Liégeois-Chauvel, 2001; Huotilainen et al., 1998; Yoshiura, Ueno, Iramina, & Masuda, 1995), neuroimaging (Ehlis et al., 2009), and intracranial electrode studies (Korzyukov et al., 2007; Liégeois-Chauvel, Musolino, Badier, Marquis, & Chauvel, 1994) has identified auditory cortex (ACx) as a P50 generator. In addition to replicating findings of a temporal P50 source, a number of studies have also found evidence of frontal cortex involvement in P50 generation (Kurthen et al., 2007; Weisser et al., 2001) or of frontal cortex being the primary P50 generator (Jensen, Oranje, Wienberg, & Glenthøj, 2008).

With regard to the source of brain activity related to P50 *suppression*, there are a number of candidate regions: Initially the primary source of suppression-related activity was thought to be hippocampus (Bak, Glenthoj, Rostrup, Larsson, & Oranje, 2011; Bak, Rostrup, Lasson, Glenthoj, & Oranje, 2014; Grunwald et al., 2003; Leonard et al., 1996; Moxon, Gerhardt, Gulinello, & Adler, 2003; Thoma et al., 2008; Williams, Nuechterlein, Subotnik, & Yee, 2011). However, there is also evidence for the involvement of frontal cortex (Ehlis et al., 2009; Knight, Richard, Swick, & Chao, 1999; Korzyukov et al., 2007; Kurthen et al., 2007; Weisser et al., 2001; Williams et al., 2011), as well as other structures including thalamus (Williams et al., 2011) and reticular activating system (Erwin & Buchwald, 1986) in suppressing P50.

1.3.1. P50 Suppression: Hypothesised Neuronal Mechanisms

With respect to the neuronal mechanism(s) hypothesised to underlie P50 suppression, two primary hypotheses have been proposed: the refractory period (RP) and inhibitory input (II)

hypotheses of P50 suppression. The RP hypothesis posits the refractory period of the neuronal population generating the P50 (herein referred to as the 'P50 generator') as the process explaining the reduction in P50 amplitude to S2 (relative to S1). According to this hypothesis, the P50 generator fires in response to the arrival/presentation of an auditory stimulus (thus generating the P50). Following this, the P50 generator is temporarily exhausted and recovers gradually, with the magnitude of its subsequent response dependant on its recovery status. In terms of the auditory paired-stimulus paradigm: at the arrival of S1 (≈ 8 – 12 s after the previous stimulus pair), the P50 generator is fully recovered/functional (Zouridakis & Boutros, 1992) and generates the S1P50. By comparison, at the arrival of S2 (only 500 ms after S1), the P50 generator has not yet recovered fully and thus generates an S2P50 of smaller amplitude than to S1.

ERP evidence supporting the RP hypothesis comes from P50 paired-stimulus studies demonstrating that reducing the inter-stimulus interval (ISI) between stimuli within a pair (thus reducing the time available for the P50 generator to recover before S2 is presented) reduces S2 (but not S1) P50 amplitude, while increasing ISI increases S2 (but not S1) P50 amplitude (Cardenas, McCallin, Rachel, & Fein, 1997; Dolu, Suer, & Ozesmi, 2001). Further, studies using trains of more than two stimuli find P50 amplitude reduction from the first to the second stimulus, but no further reductions to later stimuli in the train (Rosburg et al., 2006; Rosburg et al., 2004). That P50 amplitude depends solely on the duration of the ISI of the immediately preceding stimulus suggests that the magnitude of P50 paired-click measures depends on the recovery status of the P50 generator.

The II hypothesis of P50 suppression posits the involvement of inhibitory inputs, activated by the P50 generator and, in a feedback loop, acting on it in order to reduce (or suppress) the

P50 generators' response to the arrival of an identical auditory stimulus (Adler et al., 1998; Javitt & Freedman, 2015; Miwa, Freedman & Lester, 2011). Specifically, the presentation of S1 is thought to simultaneously excite two neuronal populations: 1) the P50 generator; and 2) inhibitory inputs that serve to dampen the P50 generator's response in the event of the arrival of an identical auditory stimulus (Freedman et al., 1996). Once activated by S1/the P50 generator, these inhibitory inputs are thought to remain active for > 500 ms (Miller & Freedman, 1995). Thus, in the context of the P50 paired-stimulus paradigm, the inhibitory inputs are still active when S2 is presented (500 ms after S1) and so suppress the P50 generators' response to S2 resulting in a lower amplitude S2P50 compared to S1P50 where inhibitory inputs are not active. Evidence in support of II comes from intracranial recordings in humans, which show hippocampal activation approximately 250 ms after the presentation of S1 (Grunwald et al., 2003), possibly reflecting the activation of inhibitory inputs that then suppress the response to S2. Evidence from invasive animal experiments also supports the hypothesis that long lasting inhibitory circuits are involved in P50 suppression. For example, recordings from intracranial electrodes in rats showed that following the generation of the P20-N40 (the rat analogue of the human P50), interneurons fire in bursts for over 500 ms (with this activity related to P20-N40 suppression), which suggests that hippocampal interneurons mediate P20-N40 suppression (Miller & Freedman, 1995).

1.4. P50 Suppression in Schizophrenia

Compared to healthy participants, who typically exhibit robust P50 suppression (Patterson et al., 2008), P50 suppression is reduced in schizophrenia (e.g., Mao et al., 2016; Oranje & Glenthoj, 2014), with this reduction argued to be one of strongest findings in the

schizophrenia neurobiological literature (Adler et al., 1982; Bramon, Rabe-Hesketh, Sham, Murray, & Frangou, 2004; Chang, Arfken, Sangal, & Boutros, 2011; de Wilde et al., 2007b; Earls, Curran, & Mittal, 2016; Freedman et al., 1983; Heinrichs, 2004). Reduced P50 suppression in schizophrenia is also argued to be a biomarker for the disorder (Thaker, 2008), in that it is present in schizophrenia patients' unaffected first-degree relatives (Allen, Griss, Folley, Hawkins, & Pearlson, 2009; Clementz, Geyer, & Braff, 1998b; Earls, Curran, & Mittal, 2016; Hall, Taylor, Salisbury, & Levy, 2011; Myles-Worsley, 2002; Olincy et al., 2010), is heritable (Anokhin, Vedeniapin, Heath, Korzyukov, & Boutros, 2007; Greenwood et al., 2007; Greenwood et al., 2016; Hall et al., 2006; Young, Waldo, Rutledge III, & Freedman, 1996) and state-independent. Also, it is present in at-risk (Brockhaus-Dumke et al., 2008), first-episode (Brockhaus-Dumke et al., 2008; Chen, Li, Smith, Xiao, & Wang, 2011; Hong et al., 2009) as well as in chronically ill schizophrenia patients (Brockhaus-Dumke et al., 2008), which shows that impaired P50 gating is not just an artefact of chronic antipsychotic treatment or of the symptoms of the acute phase of the illness.

However this view has been called into question by failures to find impaired P50 suppression in schizophrenia (Arnfred, Chen, Glenthoj, & Hemmingsen, 2003; Bertelsen et al., 2015; de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007a; Domjan, Csifsak, Drotos, Janka, & Szendi, 2012; Gjini, Burroughs, & Boutros, 2011; Kathmann & Engel, 1990; Rentzsch et al., 2007), including in recent studies with large patient samples (Light et al., 2012). There have also been failures to replicate P50 suppression heritability (Aukes et al., 2008; Greenwood et al., 2007) and a report of potential publication bias against negative P50 suppression (P50 Ratio) findings (Chang et al., 2011). The reasons behind failures to find reduced P50 suppression in schizophrenia, or of robust P50 suppression in healthy participants (Kathmann & Engel, 1990), merit consideration. It may be that some of the discrepant results reported in

the literature may be reconciled and/or better understood by taking into account methodological differences across studies.

1.5. P50 Suppression: Methodological Considerations

A review of the literature has shown that P50 studies vary substantially with respect to methodologies employed (Patterson et al., 2008). These methodological differences may be important, with one meta-analysis showing a larger P50 effect from one research group compared with others combined (de Wilde et al., 2007b). However, a dearth of research determining how methodological variables affect P50 suppression means that it is difficult to determine how such methodological differences impact on our interpretation of schizophrenia P50 suppression research, and further that we do not have an evidence base with which to guide research in the selection of the most appropriate methods.

One approach that could be taken in providing such an evidence base is a demonstration of how methodological factors affect P50 suppression reliability, particularly as its low (or absent) reliability (see Figure 1.1; Clementz, Geyer, & Braff, 1997; Fuerst, Gallinat, & Boutros, 2007; Kathmann & Engel, 1990; Lamberti, Schwarzkopf, Boutros, Crilly, & Martin, 1993; Lu et al., 2007; Naber, Kathmann, & Engel, 1992; Schwarzkopf, Lamberti, & Smith, 1993) shows that the methods that allow for adequate P50 suppression measurement have not yet been delineated. Such knowledge would be useful for the design and analysis of P50 suppression studies as well as for the interpretation of the extant literature where, for example, method-related variations in reliability may explain some of the inconsistent results in the literature - it may be that positive findings have come from studies using methodologies that produce reliable P50 estimates and negative findings have come from

those utilising unreliable methods (or vice versa).

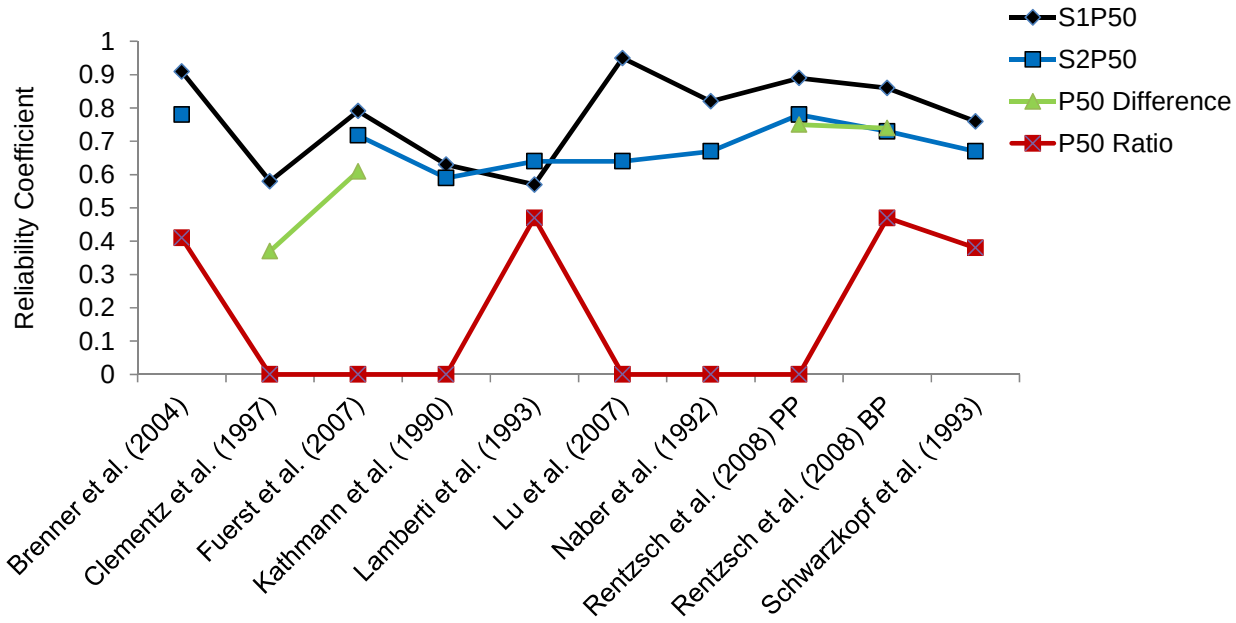

Figure 1.1. Reliability coefficients reported in the literature for S1 and S2P50 amplitudes, P50 Difference and P50 Ratio[1].

With respect to P50 suppression reliability, there are numerous methodological questions that require clarification, including the determination of the most appropriate P50 suppression metric (Difference versus Ratio), P50 amplitude definition method, and number of trials to present in an experiment. There have been suggestions that the P50 Difference is more

[1] Note that the correlation coefficients provided in Figure 1.1 are not all directly comparable, with some being calculated within-, and others between-session, and with the reliability coefficients used also differing between studies. These are provided simply to illustrate the numerous findings, including poor (or zero) reliability for the P50 Ratio; the most commonly reported P50 metric in the P50 suppression literature. Note also that not all studies calculated reliability coefficients for S2P50 amplitude or P50 Difference.

reliable than the P50 Ratio (Clementz et al., 1997; Fuerst et al., 2007; Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008), and thus that it may potentially be a more useful P50 suppression metric. However, reports of superior reliability for P50 Difference (compared to P50 Ratio) come mainly from studies which have failed to achieve non-zero reliability for P50 Ratio (Clementz et al., 1997; Fuerst et al., 2007; Rentzsch, Jockers-Scherubl, et al., 2008), (see Figure 1). This could be due to the true reliability of P50 Ratio being zero, or alternatively, it could suggest that the methods used in those studies were inadequate to obtain reliable P50 Ratio measures. Thus, whether P50 Difference reliability exceeds that of P50 Ratio reliability when P50 Ratio *is* reliably measured remains to be determined.

Importantly, and as shown in Figure 1.1., P50 Ratio reliability may be dependent on the method used to define P50 amplitudes; with Rentzsch, Jockers-Scherubl, et al. (2008) showing reliable P50 Ratio when P50 amplitudes were defined relative to the pre-stimulus baseline (baseline-to-peak method; BP) but not relative to the negative trough preceding the P50 (peak-to-peak method; PP). This finding requires replication and may be important, particularly as the P50 amplitude definition method used varies between studies. For example, some define P50 amplitudes from PP (e.g., Louchart-de la Chapelle et al., 2005; Mao et al., 2016; Oranje & Glenthoj, 2014), others from BP (e.g., Clementz & Blumenfeld, 2001) and others utilise a combination of these methods (e.g., defining peaks using the PP method, but where the preceding trough is ambiguous using the BP method; Lamberti et al., 1993).

Finally, the number of trials required to be averaged in order to get adequate measures of P50 and suppression requires clarification. This too varies between studies with some studies averaging relatively large numbers of trials (e.g., n = 110, Johannesen et al., 2005; n = 120,

Oranje & Glenthoj, 2014) and others considerably fewer (e.g., n = 32; Hong et al., 2009). Averaging fewer trials has the advantage of shortening the duration of the P50 paired stimulus paradigm, which is particularly desirable when working with a psychiatric sample. However, it is important to know the number of trials required in order obtain reliable and stable measures within-subjects.

Without reducing the number of trials presented, the duration of the P50 paired-stimulus paradigm could (theoretically) be reduced by reducing the duration of the inter-pair interval (IPI) separating one stimulus pair from the next. As the IPI duration typically employed in P50 suppression studies is relatively long (≈ 8-12 s), this parameter is the primary contributor to experiment duration. Long IPIs are thought to be necessary in order to allow the process(es) involved in P50 suppression sufficient time to recover prior to the presentation of a new stimulus pair (Zouridakis & Boutros, 1992). However, if a shorter period was sufficient for the recovery of these process(es), shorter IPIs could be employed; either shortening the duration of P50 studies or allowing for the presentation of more stimulus pairs within a given amount of time (potentially producing a more reliable measure):

Given that S2P50 amplitude is reduced, or 'suppressed' in healthy subjects when S2 follows S1 by 500 ms (Adler et al., 1982), whatever mechanism(s) is responsible for P50 suppression would appear to be active for at least 500 ms. However, when S2 follows S1 by 750-2000 ms (Cardenas et al., 1997; Dolu et al., 2001) P50 suppression no longer occurs; that is, S2P50 amplitude equals that of S1P50 at these intervals. This suggests that P50 suppression mechanism(s) may cease being active at some point between 500 and 2000 ms. If this mechanism recovers quickly, waiting 8-12 s before presenting the next stimulus pair (Adler et al., 1982; Zouridakis & Boutros, 1992) may be unnecessary, which suggests that the IPI

separating successive stimulus pairs could be substantially reduced. One study (Rentzsch, Gomez-Carrillo de Castro, Neuhaus, Jockers-Scherubl, & Gallinat, 2008) has suggested that reducing IPI from 8 to 2.8 s does not affect P50 Ratio or S2P50 amplitudes, however the attentional tasks given in the two IPI conditions of that study were different and thus the two IPI protocols were not directly comparable. Thus the results of the Rentzsch, Gomez-Carrillo de Castro, et al. (2008) study require replication before it can be concluded that IPI can be shortened without affecting P50 suppression.

Related to this is the question of the temporal stability of P50 suppression within-session; that is, whether P50 suppression is invariant or changes over time within an experiment. This too has not been determined, with findings in the literature inconsistent (Lamberti et al., 1993; Naber et al., 1992; Waldo et al., 1992; White & Yee, 2006). However, knowledge of the temporal course of P50 suppression within-session in healthy participants may identify where suppression is most robust and thus potentially different from schizophrenia patients. Further, such knowledge is relevant to the design of P50 studies, particularly with respect to the importance of carefully counterbalancing experimental conditions.

The above issues are dealt with in the first experiment of the Thesis (Study 1; Chapter 2).

1.6. Clarifying the Functional Process Represented by P50 Suppression

As described in Section 1.3.1, inhibitory (Freedman et al., 1983; Javitt & Freedman, 2015) or refractory (Jerger, Biggins, & Fein, 1992) neuronal processes (the II and RP hypotheses, respectively) are thought to underpin P50 suppression. Clarifying which of these best explains “normal” P50 suppression (such as in healthy participants) has relevance for

understanding the nature of alterations in P50 suppression such as those seen in schizophrenia. Further, such knowledge could suggest more sensitive paradigms with which to test for P50 suppression impairments. For instance, if RP was found to account for P50 suppression, a paradigm incorporating several ISIs (and thus mapping the recovery cycle at several time points) may be more useful for differentiating groups than one capturing recovery at the 500 ms time point following S1 only. This issue is dealt with in the second experiment of the Thesis (Study 2; Chapter 3), with logical considerations important to that experiment dealt with here.

The standard (8 s IPI, 500 ms ISI) P50 paradigm cannot clarify whether inhibitory or refractory processes are responsible for the reduction in S2P50 amplitude. It may be that the P50 generator is not fully recovered at the presentation of S2, or that S1-activated inhibitory inputs are still active and thus acting to suppress the brains' response to S2. In order for EEG research to determine which of these competing hypotheses best account for P50 suppression, alternative P50 paradigms measuring the activity of the hypothesised processes are necessary. For instance, where refractory cycles are thought to be involved, P50 protocols utilising smaller/larger intervals (thus allowing for less/more recovery) should engage the P50 neuronal generator population less/more and result in smaller/larger P50 amplitudes respectively. In turn, where this neuronal population is hypothesised to be involved in engaging some subsequent process (e.g., inhibition), it should be less/better able to do this where it is less/more engaged itself. The present thesis proposes such a novel P50 paradigm as a way of clarifying which of the II and RP hypotheses best explain P50 suppression. This paradigm seeks to test the contrasting predictions that II and RP hypotheses make about the effects of manipulating the functionality of the P50 suppression mechanism(s) (i.e., relatively enhancing versus relatively reducing) on S2P50 amplitude. Specifically, *reducing*

functionality of P50 suppression mechanisms should:

1. According to II, *increase* S2P50 amplitude: If inhibitory inputs underlie P50 suppression, reducing their functionality should result in less inhibition and therefore larger S2P50 amplitude (and vice versa for enhancing functionality of II).
2. According to RP, *decrease* S2P50 amplitude: If refractory periods underlie P50 suppression, reducing recovery should result in smaller S2P50 amplitudes (and vice versa for increasing recovery).

The thesis proposes two strategies for manipulating the function of the processes underlying P50 suppression in order to determine the functional process(es) underlying P50 suppression, which are discussed in turn.

1.6.1. Functional Process: Relevance of Inter-Pair Intervals.

Manipulating the duration of the IPI parameter in the P50 paired-stimulus paradigm is one strategy for manipulating the functionality of P50 suppression mechanisms. After all, the rationale for using long IPIs is to allow the mechanism(s) underlying P50 suppression mechanisms to recover (Zouridakis & Boutros, 1992). Following this logic, reducing IPI to the point where P50 suppression mechanisms are *not* fully recovered will relatively *reduce* their functionality. As described in Section 1.6, the II and RP hypotheses of P50 suppression make different predictions with respect to the effect a reduction of IPI will have on P50 amplitudes:

According to the RP hypothesis of P50 suppression, which posits that P50 suppression is explained by the recovery status of the P50 generator, reducing IPI from the standard 8 s to <

3 s will mean that S1 is presented while the P50 generator is relatively less recovered (< 3 s) compared to IPIs of 8 s or more, where the P50 generator is fully recovered (Zouridakis & Boutros, 1992). Thus, RP predicts that short (compared to long) IPIs will reduce S1P50 amplitude due to less recovery at short IPIs. Further, as S2 is always presented 500 ms after S1 (Earls, Curran, & Mittal, 2016), reducing IPI should either not affect S2P50 (if S1 completely exhausts the P50 generator and S2P50 is thus generated from a minimal neural reserve regardless of the IPI preceding S1), or reduce S2P50 amplitude (if S2P50 is generated from a proportion of the reduced neural reserve).

According to the II hypothesis of P50 suppression, which posits that P50 generator activation of inhibitory inputs accounts for P50 suppression, reducing IPI would result in either the P50 generator being insufficiently activated (due to less recovery, and as indexed by reduced S1P50) to adequately engage inhibitory inputs, or reduction of the functionality of the inhibitory mechanism (due to less recovery of the inhibitory inputs). Both of these possibilities would result in *increased* S2P50 (in the short relative to the long IPI condition).

1.6.2. Functional Process: Relevance of Attention

Manipulations of attention may represent another strategy for manipulating P50 suppression mechanism function. This is because enhanced attention triggers an amplified and more selective neuronal response to the attended stimuli (Hillyard, Vogel, & Luck, 1998; Kauramäki, Jääskeläinen, & Sams, 2007; Murray & Wojciulik, 2004). Attention can thus be used to differentiate between the RP and II hypotheses because:

According to RP, which posits only the P50 generator involvement in P50 suppression,

enhancing attention will enhance the P50 generator response. Thus directing attention toward (versus away from) stimulus pairs should increase both S1 and S2P50 amplitudes (both of which are subserved by the P50 generator).

According to II, which posits the involvement of both the P50 generator *and* the inhibitory inputs it activates to suppress the P50, enhancing attention could theoretically enhance the P50 generator, the inhibitory inputs it engages, or both of these. If attention enhances:

- The function of the *P50 generator only* – directing attention toward auditory stimuli would *increase* both S1 and S2P50 amplitudes (noting that if the enhanced S1 response subsequently engaged more inhibitory inputs then the S2P50 increase may by countered by greater inhibition and thus result in no change in S2P50).
- The function of *inhibitory inputs only* – directing attention toward auditory stimuli would not affect S1P50 (as inhibitory inputs are not active at the time of S1 presentation), but would *reduce* S2P50 (due to attentional enhancement of inhibitory action on S2).
- The function of both the P50 generator response *and* inhibitory inputs, directing attention to auditory stimuli should increase S1P50 (due to attentional enhancement of the P50 generator) and *reduce* S2P50 (due to greater P50 generator engagement of inhibitory inputs and/or attention enhancing inhibitory inputs directly).

The above considerations are used in the second experiment (Study 2; Chapter 3) to help delineate the functional process(es) underlying P50 suppression.

1.7. Can the P50 Suppression Deficit Reported in Schizophrenia be More

Appropriately Attributed to Attentional Impairments?

The attentional strategy of manipulating P50 suppression is also relevant because the degree of attention (or the degree to which attention is controlled) varies substantially across the P50 suppression literature. For example, some studies direct attention toward auditory stimulus pairs by asking participants to pay attention to the stimuli (e.g., Clementz, Geyer, & Braff, 1998a) or respond to 'oddball' trials embedded within the task (e.g., Johannesen et al., 2005). Conversely, others ask participants to ignore the stimuli (e.g., Mazhari, Price, Waters, Dragović, & Jablensky, 2011). In yet other studies the direction of participants' attention is unclear as they are sometimes asked to focus on a fixation point (e.g., Brockhaus-Dumke et al., 2008; Cadenhead, Light, Shafer, & Braff, 2005; Olincy et al., 2010; Turetsky, Bilker, Siegel, Kohler, & Gur, 2009), relax with their eyes closed (e.g., Jin et al., 1998; Winterer et al., 2013), given no instructions relating to the stimuli (e.g., Tregellas et al., 2007) or instructions given to participants are not reported. If, as suggested above in relation to Study 2, attention may relatively enhance the engagement of mechanism(s) involved in/underlying P50 suppression, this suggests that, to the degree that studies differ on this aspect, they may be measuring different neural processes. Specifically, studies directing attention away from auditory stimuli may be measuring P50 suppression in the absence of any attentional effects on it, whereas those directing attention toward auditory stimuli may be capturing attention-related enhancement of stimulus processing, *plus* an attention-related enhancement of processes involved in the suppression of the P50 response. Where attention is not controlled or not described/reported, it is possible that some participants attend to the auditory stimuli and others do not, and the neuronal processes measured in those studies could not be determined.

Furthermore, attention is directly relevant to schizophrenia research more generally. This is because schizophrenia patients have significant attentional impairments, with impaired attention itself argued to be a schizophrenia endophenotype (Chen & Faraone, 2000; Cornblatt & Malhotra, 2001), and so if P50 suppression is not pre-attentive, such differences in the attentional capabilities of the groups may affect P50 suppression results. For example, where participants are not given any instructions with respect to the auditory stimuli, it could be that healthy subjects, in the absence of anything else to focus their attention on, will direct their attention toward the auditory stimuli regardless of not being asked to do so. Due to their impaired attentional capabilities, schizophrenia patients may on the other hand *not* direct attention toward the auditory stimuli (which, in the context of a 'passive' paradigm, may be more consistent with the instructions/or lack thereof). In such a scenario, and assuming attention affects P50 suppression, healthy subjects would obtain the attention-related effect on P50 suppression whereas schizophrenia patients would not. It would be difficult to disentangle any resulting P50 suppression group differences to correctly apportion the degree to which they arose from differences in P50 suppression rather than differential allocation of attention. It could also be argued that the same could occur in studies where attentional instructions *are* provided to participants. Healthy subjects, whose better attentional capabilities may allow for more appropriate adherence to such instructions (and thus allocation of attention), may obtain the attention-related effect on P50 suppression not seen in schizophrenia patients, whose attentional impairments may not allow for appropriate allocation of attention to the auditory stimuli. Thus again, schizophrenia patients would not obtain the attention-related effect on P50 suppression that healthy controls might be expected to.

This suggests that it may be important not only to provide attentional instructions, but to

ensure that these are able to be adequately adhered to by schizophrenia patients. Indeed there is evidence from the schizophrenia memory literature that when patients are encouraged to utilise attentional/encoding strategies that they perform similarly to controls on memory tasks (e.g., Bonner-Jackson, Haut, Csernansky, & Barch, 2005; Weiss et al., 2003). Thus the degree to which schizophrenia patients will engage attentional processes is not only variable, but also dependent on task instruction. As the relation between attention and P50 suppression is still unclear, and as a variety of attentional instructions are provided in the literature, the extant literature is neither able to answer the question of the extent to which attention confounds are a problem in the schizophrenia research nor of the most appropriate attentional instructions to adopt. However, in the first instance, it is necessary to determine whether attention affects P50 suppression in the healthy samples against which schizophrenia patients are compared. Secondly, it is important to determine whether attention similarly affects P50 suppression in schizophrenia.

1.8. Data Analysis Variation and Strategy for the Present Thesis

Although the schizophrenia P50 suppression literature is substantial, a number of issues remain to be resolved. These include a delineation of the most appropriate methods for quantifying P50 suppression, clarification of the neuronal process(es) represented by P50 suppression in both healthy and schizophrenia patients, as well as the identification of relevant confounds in this literature. The present thesis is an attempt to address these issues in a manner relevant to the schizophrenia P50 suppression literature. That is, in any individual study, there are a number of ways in which P50 suppression is quantified, for instance using principal components analysis (Clementz & Blumenfeld, 2001), spectral analysis

(Johannesen et al., 2005), single trials analysis (Jin et al., 1997), or by measuring averaged ERP peak amplitudes (Adler et al., 1982). Also, the EEG data may have been filtered in various ways, for example, 1-20 or 20-50 (Clementz & Blumenfeld, 2001), 0.5-100 Hz (Kathmann & Engel, 1990), or with high-pass values anywhere between 0-10 Hz and low pass values between 30-10,000 Hz (Patterson et al., 2008). The relative merits of these variations have not been resolved, and are beyond the scope of the present thesis. However, given that, and in order for the results of the present thesis to be directly relevant to the schizophrenia P50 suppression literature, the present thesis will adopt methods (namely peak detection and utilisation of a 10 Hz high-pass filter) that match those of recent, large sample, multi-centre studies schizophrenia P50 suppression studies (e.g., Hall et al., 2014; Light et al., 2012; Olincy et al., 2010).

1.9. Research Aims

The overall aim of this doctoral research was to better understand the functional process represented by P50 suppression and thus the nature of its reported reduction in schizophrenia. Specifically, this research sought to evaluate P50 methodologies with respect to how these affect P50 suppression reliability and magnitude. Further it aimed to apply this knowledge to an investigation of the mechanism (RP or II) underlying P50 suppression in, firstly, a healthy sample, and secondly, a sample of schizophrenia patients (compared to age and gender-matched healthy controls).

This research addressed three specific research aims:

1. To determine whether: a) the number of trials and P50 peak amplitude definition method

influence P50 suppression reliability (for both P50 Ratio and P50 Difference); b) P50 suppression is stable, or changes across time within an experiment; and c) reducing the interval between stimulus pairs in the P50 paired-stimulus paradigm affects the magnitude of P50 and P50 suppression (Study 1, Chapter 2);

2. To determine which hypothesised mechanism of P50 suppression (II or RP) best predicts the effect on S2P50 amplitude of manipulating the functionality of the mechanism(s) underlying P50 suppression, and thus which best explains P50 suppression in healthy participants (Study 2, Chapter 3); and
3. To determine the relevance of attention in schizophrenia P50 suppression research by testing whether attention affects P50 measures, and (if present) whether this attentional effect differs between schizophrenia patients and healthy controls (Study 3, Chapter 4).

This doctoral thesis is presented as a collection of manuscripts prepared for publication (Style 2), in accord with the requirements of University of Wollongong. Each chapter represents a manuscript written for a specific journal with a defined audience. The structure of the abstract and headings within each paper is consistent with the style used by the journal for which it is written. Chapter 2 has been published in *Psychophysiology*, Chapter 3 has been published in the *International Journal of Psychophysiology*, and Chapter 4 is under review in *Clinical Neurophysiology*. While each journal requires a specific referencing style, for consistency all chapters in this thesis are referenced in the style of the American Psychological Association (6th edition).

1.10. Significance and Originality

This doctoral research compares P50 methodologies within a study in order to determine how

these variations affect P50 suppression and the reliability of its measurement, with a goal to providing empirical evidence to guide methodological choices and interpret the extant P50 literature. While across the literature the methods used in P50 studies vary, this between-study variability is uninformative with respect to guiding appropriate methodological choices in current P50 research and interpreting the results of P50 studies where they have used disparate methods. The present doctoral research aims to fill this gap.

P50 suppression is most often discussed in terms of it reflecting the operation of inhibitory inputs. By extension, reductions in P50 suppression, such as those reported in schizophrenia, are assumed to reflect impairment in the operation of these inhibitory circuits. This doctoral research will clarify the neuronal process (II or RP) represented by P50 suppression in a sample of healthy participants, thus demonstrating what process is referred to by "P50 suppression". Further, in demonstrating whether attention has a role in P50 suppression and whether any possible effect of attention differs between schizophrenia patients and healthy controls, this research will determine whether the P50 suppression deficit reported in the schizophrenia literature is due to impairment in P50 suppression mechanisms, or can be more appropriately attributed to the known attentional impairment in these patients.

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2. CHAPTER 2: AN EVALUATION OF P50 PAIRED-CLICK METHODOLOGIES

Dalecki, A., Croft, R. J., & Johnstone, S. J. (2011). An evaluation of P50 paired-click methodologies. *Psychophysiology, 48*, 1692-1700.

2.1. Abstract

The utility of P50 paired-click measures is limited by their unestablished reliability, unknown effects of time, and long protocol. This study measured within-session reliability, temporal course, effect of varying inter-pair interval (IPI), and peak definition and ratio calculation methods on P50 paired-click measures in healthy participants. Results indicate higher reliability for difference (ICC = .72) than ratio (ICC = .44) method; when P50 peaks are defined as baseline-to peak than peak-to-peak; time-related changes; and comparable P50 paired-click measures at long (9 s) and short (3–7 s) IPIs. After controlling for time effects, P50 paired-click measures are relatively reliable within-session and are best measured using the difference method and defined as baseline-to-peak amplitude; time effects must be taken into account when measuring P50 paired-click measures in a long paradigm; and IPI can be shortened in studies with healthy samples.

2.2. Introduction

The magnitude of P50 paired-click measures are widely reported to differ between schizophrenia populations and healthy controls (Adler et al., 1982; Becker et al., 2004; Clementz, Geyer, & Braff, 1997; Judd, McAdams, Budnick, & Braff, 1992; Yee, Nuechterlein, Morris, & White, 1998). The reduced P50 ratio in schizophrenia has been confirmed meta-analytically (Bramon, Rabe-Hesketh, Sham, Murray, & Frangou, 2004; de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007), and is argued to be the most robust case-control difference in the schizophrenia neurobiological literature (Heinrichs, 2004) and an endophenotypic trait marker for the disorder (Braff & Freedman, 2002; but also see Greenwood et al., 2007, who failed to find heritability of the P50 ratio). However, research using P50 paired-click measures is limited because of the unestablished reliability of these measures, the unknown effect of time on P50 paired-click measures, and the long recording sessions required to obtain P50 paired-click measures (due in part to the assumption that long intervals are required between trials; Zouridakis & Boutros, 1992). The present study addresses these issues in a sample of healthy participants.

Given that P50 paired-click measures are regarded to be automatic, preattentive trait markers of auditory information processing (Braff & Light, 2004), one might expect the measures to be reliable. However, numerous studies of P50 paired-click reliability, over different test-retest time periods, have failed to find adequate reliability for the P50 ratio: within-session (Clementz et al., 1997; Lamberti, Schwarzkopf, Boutros, Crilly, & Martin, 1993; Schwarzkopf, Lamberti, & Smith, 1993), within-day (Fuerst, Gallinat, & Boutros, 2007), and between days (Kathmann & Engel, 1990; Lu et al., 2007; Naber, Kathmann, & Engel, 1992). There have been only few exceptions to this (Lamberti et al., 1993: intraclass correlation

coefficient [ICC] = .47). Thus it is important to clarify which methods might improve P50 paired-click reliability.

Prior studies quantifying P50 paired-click reliability within-session have averaged between 30 and 60 trials (e.g., Clementz et al., 1997). Given the poor signal-to-noise ratio of P50 amplitudes and the failure to demonstrate P50 paired-click reliability, these numbers of trials may be insufficient. However, increasing the number of trials aggregated may improve reliability. For example, Boutros, Overall, and Zouridakis (1991) found that increasing the number of trials averaged from one block of 60 trials to three blocks of 120 trials improved the P50 ratio one week test-retest reliability from r = .03 to r = .29. However, even with a large number of trials presented, reliability remained low suggesting other sources of variability remain to be accounted for. Further, numerous studies have found no significant reliability despite presenting large numbers of trials (Naber et al., 1992; Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008; Smith, Boutros, & Schwarzkopf, 1994). Thus the relation between the number of trials averaged and P50 paired-click reliability remains to be clarified.

The method used to measure P50 peak amplitudes may also affect P50 paired-click reliability. For example, Rentzsch, Jockers-Scherubl, et al. (2008) found the P50 ratio was reliably measured from the pre-stimulus baseline to P50 peak (baseline-to-peak; BP), but not from the P50 peak relative to the preceding trough (peak-to-peak; PP), the amplitude measurement method typically used in P50 paired-click studies. However, this finding remains to be replicated.

Alternatively, the reduced P50 amplitude to the second (S2) of paired clicks (S1, S2) might be more reliably measured as the difference between P50 amplitudes (S1–S2) rather than

their ratio (S2/S1). A number of studies have found the S1–S2 difference to be reliable in the absence of S2/S1 ratio reliability (Fuerst et al., 2007; Lu et al., 2007; Smith et al., 1994). The current literature thus suggests that the S1–S2 difference is more reliable than the S2/S1 ratio, but further research is required to confirm this. Notably, the S1 P50 amplitude and S1–S2 difference are highly correlated (e.g., Fuerst et al., 2007; Lu et al., 2007). This suggests that the S1–S2 difference is not a P50 suppression metric, but rather reflects S1.

As the P50 paired-click paradigm is long (circa 20–60 min), it is important to determine the effects of time on P50 paired-click measures. However, how time affects P50 paired-click indices is unclear as studies of the temporal course of P50 paired-click indices report conflicting results, describing invariant (Naber et al., 1992; White & Yee, 2006), increasing (Lamberti et al., 1993), and decreasing (Waldo et al., 1992) P50 ratio scores in healthy participants over time within a session.

Another issue not adequately addressed in the literature concerns the optimal duration of the interval between successive click-pairs in the P50 paired-click paradigm (the inter-pair interval; IPI). Accumulating evidence supports the view that P50 paired-click measures equivalent to that measured at IPIs of 8 s can be obtained at shorter IPIs (Dolu, Suer, & Ozesmi, 2001; Rentzsch, Jockers-Scherubl et al., 2008). For example, Rentzsch, Gomez-Carrillo de Castro, Neuhaus, Jockers-Scherubl, and Gallinat (2008) failed to find a difference between P50 ratios obtained in P50 paired-click paradigms with short (2.8 s) and long (8 s) IPIs, suggesting that P50 paired-click measures could be obtained at an IPI of 2.8 s. However, Rentzsch, Gomez-Carrillo de Castro, et al. (2008) also failed to find a significant correlation between P50 ratios obtained in the short and long IPI paradigms, suggesting that the lack of difference may be due to an inability to obtain stable P50 ratio measures. In addition,

Rentzsch, Gomez-Carrillo de Castro, et al. (2008) asked participants to respond with a button press to an oddball stimulus in the short but not long IPI condition, and so the two paradigms were not directly comparable. However, if identical P50 paired-click measures were demonstrated at shorter IPIs, this could be a great advantage in that it could reduce the recording time of the P50 paired-click paradigm.

The present study thus addressed four issues. The first was the hypothesis that P50 paired-click metrics (S2/S1 and S1–S2) are reliable within-session in healthy participants, and that reliability depends on P50 peak-amplitude measurement method (PP vs. BP). Second, it tested the hypothesis that P50 paired-click measure reliability varies as a function of the number of trials contributing to the averaged P50 event-related potentials (ERPs). Third, it assessed the effect of time on P50 paired-click measures in healthy participants, determining whether P50 paired-click measures obtained in the first block of 150 trials would differ from that of four subsequent blocks. Fourth, it tested whether P50 paired-click measures can be adequately obtained at IPIs of less than 9 s (1, 3, 5, 7, and 9 s). As the effect of time is unclear, reliability and IPI effects were quantified for trials equally distributed across time within the session. This ensured that any temporal effects would not affect measurements. To ensure reliability was not underestimated by averaging too few trials, the present study quantified P50 paired-click reliability from averages of 100 click-pairs.

2.3. Method

2.3.1. Participants

Twenty undergraduate psychology students (13 females, mean age 20.95, *SD* = 3.26 years) from the University of Wollongong volunteered to participate in the study in return for course credit. All provided written informed consent. Exclusion criteria were self-reported hearing difficulties.

2.3.2. Procedure

Participants were fitted with electroencephalogram (EEG) recording apparatus and completed the Schizotypal Personality Questionnaire (SPQ) (Raine, 1991) and demographic and smoking history questionnaires.[2] Participants were asked to abstain from smoking and drinking coffee for 60 min prior to the start of the recording session and were not permitted to smoke or consume caffeine during the experiment.

Participants were seated in a dimly illuminated, sound attenuated room, facing a computer screen at a distance of 80 cm. Participants were presented with a total of 750 click pairs over five experimental blocks. The order of block presentation was counterbalanced between participants: order 1 (150, 135, 150, 165, and 150 click pairs) and order 2 (150, 165, 150, 135, and 150 click pairs). Blocks had a mean duration of 14 min, and participants were given a 2-min break following the completion of each block, thus the paradigm lasted a total of 78 min.

[2] This is not relevant to the present study and will not be discussed further.

2.3.3. P50 Task

To begin each block, a fixation cross appeared in the center of the computer monitor for 2000 ms. No other visual stimuli were presented. In order to minimize attentional variability, participants were orally instructed to silently count the click pairs as they were presented, responding with a button-press after every 25th click pair. Participants were asked to keep their eyes open, and to look at the computer monitor in front of them in order to minimize head and eye movements.

2.3.4. Auditory Stimulation

Auditory clicks were presented through two speakers placed on either side of the computer monitor, 1 m in front of participants. Click intensity, as measured with a Bruel and Kjaer Precision Sound Level Meter (Type 2235), was 80 dB.

Stimuli were pairs (S1 and S2) of identical auditory clicks (< 1 ms). Click duration at the speakers was approximately 20 ms. The inter-stimulus interval (ISI) between S1 and S2 was constant (500 ms). The IPI was the interval between the second click in a click pair (S2) and the first click of a subsequent click pair (S1), with click pairs presented at one of 5 mean IPIs (1, 3, 5, 7, and 9 s), referred to as IPI1, IPI3, IPI5, IPI7, and IPI9. To provide the appearance of randomness in the IPIs, each mean IPI was achieved by combining 3 randomly chosen IPIs whose mean equalled the target IPI, but which individually did not equal the target IPI: 1 s (930, 990, 1080 ms), 3 s (2946, 3016, 3038 ms), 5 s (4952, 4992, 5056 ms), 7 s (6954, 6984, 7062 ms) and 9 s (8930, 8990, 9080 ms). An equal number of trials with each IPI were

presented during each time block, such that IPIs were distributed equally in time across the total 750 trials.

2.3.5. EEG Data Collection

EEG data were recorded continuously using Ag-AgCl electrodes from 19 scalp sites (FP1, FP2, Fz, F3, F4, F7, F8, Cz, C3, C4, Pz, P3, P4, T3, T4, T5, T6, O1, and O2) placed according to the 10/20 International System. EEG data were grounded midway between Fz and FPz and referenced to the left ear. Electrooculogram (EOG) was recorded using Ag-AgCl electrodes placed above (E1) and below (E3) the left eye, and at the outer canthi of left (E5) and right (E6) eyes. Vertical EOG was derived as E1-E3. All electrode impedances were below 20 kΩ at the start of the recording.[3] The EEG/EOG data were amplified with a gain of 1338, digitized at 500 Hz, and digitally filtered using a 0.1 to 100 Hz (24 dB/octave roll-off) bandpass filter (NuAmps, Neuroscan). Continuous EEG/EOG data were stored for subsequent off-line analysis.

2.3.6. EEG Data Analysis

Data were analyzed offline using Neuroscan software (Scan 4.4). The data were EOG corrected (Semlitsch, Anderer, Schuster, & Presslich, 1986), bandpass filtered from 10 to 45 Hz (12 dB/octave roll-off), re-referenced to digitally linked ears (Miller, Lutzenberger, & Elbert, 1991), epoched from 100 ms pre- to 300 ms post-stimulus, and baseline corrected using the 100 ms pre-stimulus interval. Before averaging, an automatic artefact rejection procedure identified and rejected trials with activity exceeding ±50 μV in any EEG channel.

[3] Note that although some studies report electrode impedances lower than this (e.g., <5 kOhms is often employed as a cut-off), signal-to-noise ratio is dependent on the *combination* of electrode and amplifier impedances, with 20 kOhms electrode impedance providing adequate signal-to-noise given the NuAmps system impedance of 80 MOhms.

For reliability analyses, ERP averages were calculated from trials in IPI7 and IPI9 conditions combined (i.e., n = 300 click pairs) to obtain a mean IPI of 8 s (for consistency with the literature). ICCs estimated S2/S1 ratio, S1–S2 difference, S1, and S2 reliability, for ERP averages computed from three sets of 100 trials and P50 peaks defined from PP and BP. For the most reliable P50 paired-click metric (i.e., $S1–S2_{BP}$), ICCs additionally estimated $S1–S2_{BP}$ difference stability as a function of number of trials; that is, for ERP averages computed from three sets each of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 trials (referred to as ICC_{10}, ICC_{20}, etc.). The trials comprising each set were pseudorandomly chosen, such that sets contained an equal number of trials from each time block. Within-subject standard deviations (WSD) were then calculated for the $S1–S2_{BP}$ difference generated from each number of trials (referred to as WSD_{10}, WSD_{20}, etc.). For analysis of time effects, ERP averages were calculated from trials in IPI7 and IPI9 conditions combined (n = 300 click pairs, mean IPI = 8 s). ERP averages were calculated for each block of time (Time 1, 2, 3, 4, and 5), with 60 click-pairs contributing to each average. For analysis of IPI effects, ERP averages were calculated for each IPI condition (IPI1, IPI3, IPI5, IPI7, and IPI9). Each IPI average comprised 150 click-pairs. For all analyses, ERP averages were calculated for S1 and S2 separately.

2.3.7. P50 Peak Selection

Data were analyzed from Cz only, as per Clementz, Geyer, and Braff (1998). P50 peaks were identified manually by one rater. The P50 peak was identified as the largest positive peak ±15 ms of the grand mean average P50 peak latency (62 ms; i.e., 47–77 ms post-stimulus) following the Pa wave (48 ± 15 ms), and was required to exhibit frontocentral topography to be accepted as a true P50 peak (no minimum amplitude was required for a peak to be

classified as such). If no peak was present within the required latency range, or if a peak within the latency range did not have a frontocentral topography, the P50 amplitude was scored as '0.' P50 peak amplitudes were measured relative to the negative trough preceding the P50 peak (PP) and relative to the average amplitude of the 100 ms pre-stimulus baseline interval (BP). The P50 ratio was defined as the ratio of the P50 amplitudes to clicks 2 and 1 (i.e., S2/S1), and the P50 difference was the difference between P50 amplitudes to clicks 1 and 2 (i.e., S1–S2).

2.3.8. Statistical Analyses

The one-way random-effects (single measure) ICC model (1, 1) was used to measure within-session reliability for P50 paired-click metrics (S2/S1 and S1–S2), measured from each of PP and BP, and calculated from three sets of 100 randomly chosen trials (mean IPI = 8 s). The most reliable of these was the $S1–S2_{BP}$ difference; therefore, this metric was used for all remaining analyses. Following criteria provided by Fleiss (1986), we took ICCs ≤ .40 to be poor, .40–.75 to be fair to good, and > .75 to be excellent. To determine whether stability of the $S1–S2_{BP}$ difference improves as a function of the number of trials aggregated in generating P50 ERPs, planned contrasts compared the WSD_{100} with each of WSD_{10}, WSD_{20}, WSD_{30}, WSD_{40}, WSD_{50}, WSD_{60}, WSD_{70}, WSD_{80}, and WSD_{90}. To determine whether the P50 difference is affected by time, planned contrasts compared the $S1–S2_{BP}$ difference, at Time 1, with each of those from Times 2, 3, 4, and 5. To test for an effect of IPI on the P50 difference, planned contrasts compared the $S1–S2_{BP}$ difference measured at IPI9, against each of those from the IPI1, IPI3, IPI5, and IPI7 conditions.

2.4. Results

An S1 P50 peak was absent for one subject in the IPI1 condition. An S2 P50 peak was absent for one subject in Times 2–5, IPI5, and IPI9 conditions; two subjects in the IPI3 condition, and five subjects in the IPI1 condition (see Figure 1 for grand average P50 ERPs to S1 and S2).

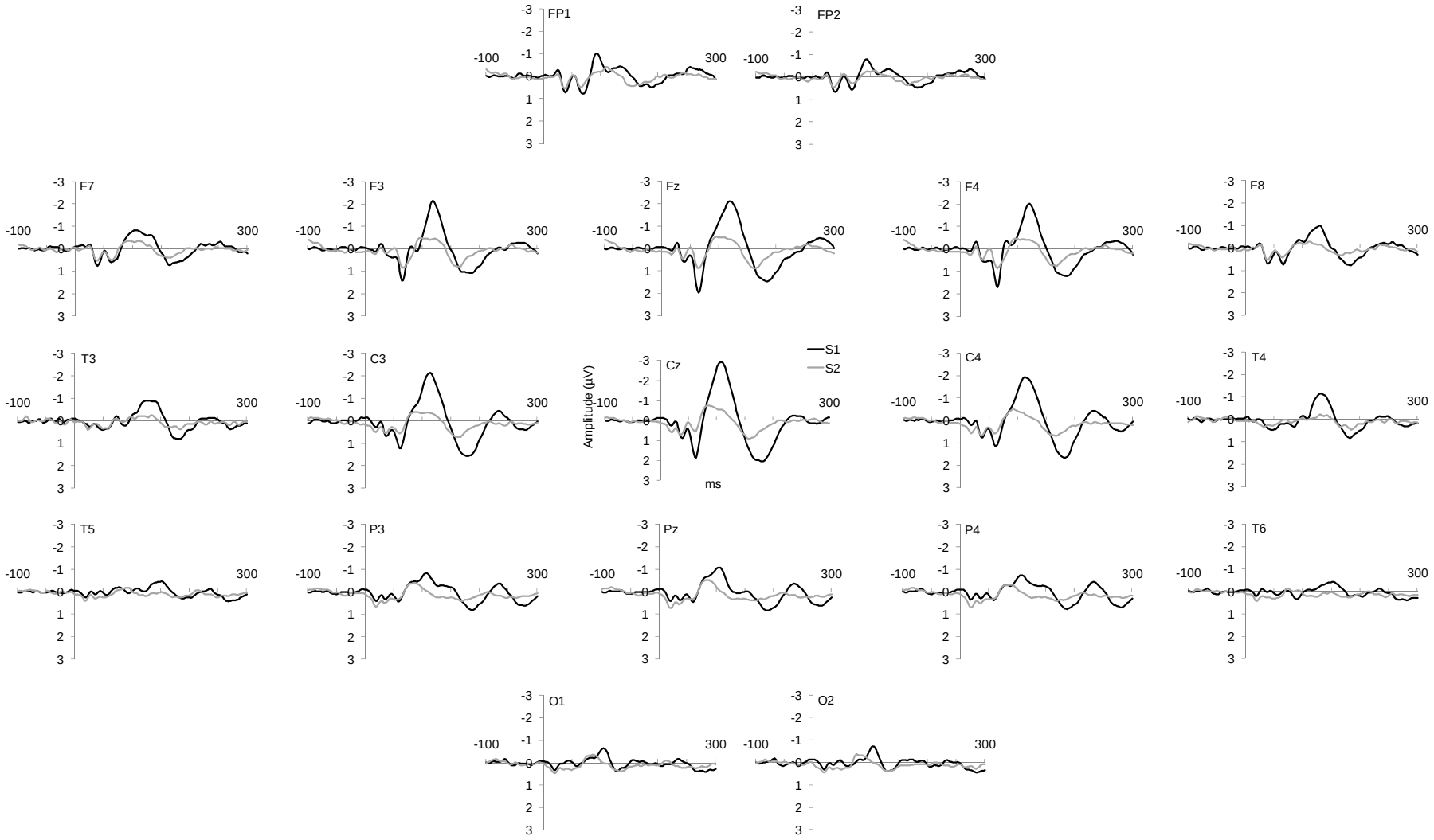

Figure 2.1. P50 ERPs to S1 and S2 at 19 electrode sites.

2.4.1. Reliability

Statistically significant reliability was found for all P50 paired-click metrics, for PP and BP peaks, and calculated from 100 trials (see Table 2.1 and Figure 2.2).

Table 2.1

ICCs for P50 Paired-Click Metrics Calculated from 100 Trials and Defined from PP and BP

	Peak-to-peak	*Baseline-to-peak*
P50 Peak Metrics		
S1	*.89 [.78; .95]****	*.89 [.78; .95]****
S2	*.66 [.43; .83]****	*.49 [.22; .73]****
P50 Paired-Click Metrics		
S2/S1 Ratio	*.44 [.16; .71]***	*.30 [.02; .61]**
S1 – S2 Difference	*.64 [.40; .82]****	*.72 [.50; .87]****

*Note. 95% confidence intervals are presented in parentheses; * < .05; ** < .01; *** < .001.*

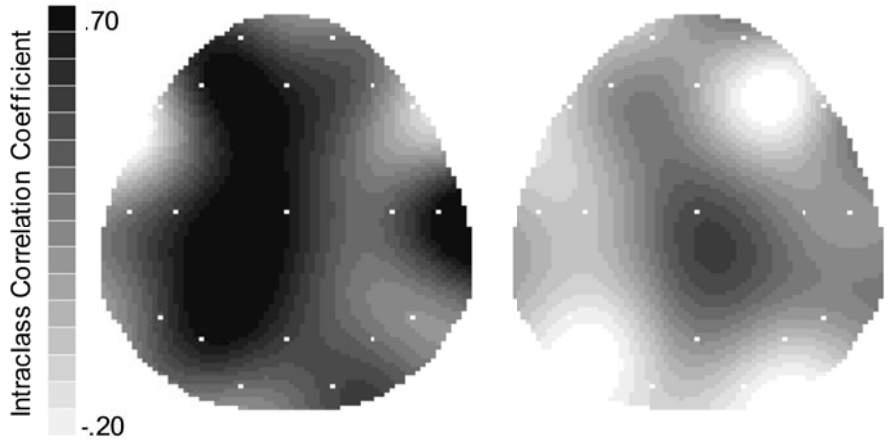

Figure 2.2. ICC values for the best difference (left; peak-to-peak) and ratio method (right; baseline-to-peak) shown as a function of electrode site.

S1–$S2_{BP}$ reliability was statistically significant when calculated from 20–100 trials (all $p < .01$), but not from 10 trials ($p > .05$). However, when calculated from 30 trials S1–$S2_{BP}$ reliability was poor. Only once 40 or more trials were averaged was S1–$S2_{BP}$ reliability consistently in the 'fair-to-good' range. Further, planned comparisons ($df = 1,19$) indicated that for S1–$S2_{BP}$, WSD_{100} was statistically significantly lower than WSD_{10}, WSD_{20}, WSD_{30}, WSD_{40}, WSD_{60}, WSD_{80}, and WSD_{90} (all $p < .05$); see Figure 3a.

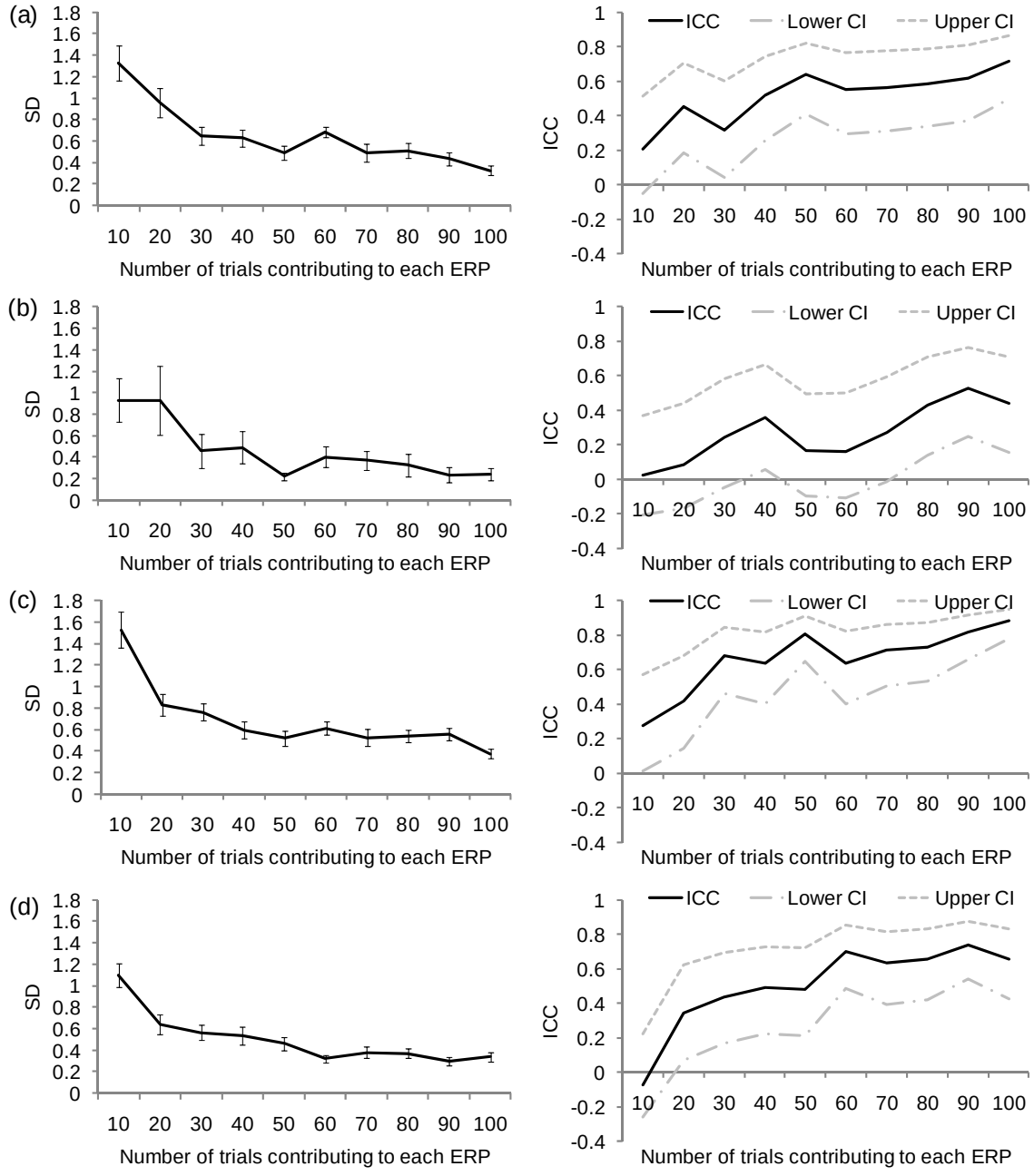

Figure 2.3. Average standard deviations (with standard error) [Left], and ICCs (and 95% confidence intervals) [Right] are shown for the: (a) S1 – $S2_{BP}$ difference; (b) $S2/S1_{PP}$ ratio; (c) $S1_{PP}$ P50 amplitude; and (d) $S2_{PP}$ P50 amplitude calculated from averages comprised of between 10 and 100 trials.

Although the most reliable P50 paired-click measure was the S1–S2 difference, reliability coefficients are also presented for the S2/S1 ratio (as this is the most commonly reported P50 paired-click measure), and S1 and S2 P50 peaks (in order to provide information on what affects the reliability of the derived P50 paired-click measures). $S2/S1_{PP}$ ratio reliability first reached significance when calculated from 40 trials ($p < .05$). However, significant nonzero reliability was not *consistently* observed until $S2/S1_{PP}$ ratios were calculated from 70 trials or more (all $p < .05$). Further, only once 80 or more trials were averaged was $S2/S1_{PP}$ ratio reliability consistently in the 'fair-to-good' range. Planned comparisons ($df = 1,17$) revealed that for $S2/S1_{PP}$, the WSD_{100} was significantly lower than the WSD_{10}, WSD_{20}, and WSD_{40} (all $p < .05$); see Figure 2.3b.

$S1_{PP}$ reliability was statistically significant when calculated from 10 ($p < .05$), 20 ($p < .01$), and 30–100 trials (all $p < .001$). $S1_{PP}$ reliability was 'excellent' when 90–100 trials were averaged. Planned comparisons ($df = 1,19$) indicated that for $S1_{PP}$, WSD_{100} was statistically significantly lower than WSD_{10}, WSD_{20}, WSD_{30}, WSD_{40}, WSD_{50}, WSD_{60}, WSD_{70}, and WSD_{80}, (all $p < .05$); see Figure 2.3c.

$S2_{PP}$ reliability was statistically significant when calculated from 30 ($p < .01$) and 40–100 (all $p < .001$), but not 10–20 trials ($p > .05$). $S2_{PP}$ reliability was 'fair-to-good' when 30–100 trials were averaged. Planned comparisons ($df = 1,19$) indicated that for $S2_{PP}$, WSD_{100} was statistically significantly lower than WSD_{10}, WSD_{20}, and WSD_{30} ($p < .05$); see Figure 2.3d.

2.4.1. Time

The S1–$S2_{BP}$ difference at Time 1 was significantly higher than at each of Times 2, 3, 4, and 5; $p < .05$, $df = 1,19$.

To allow for more direct comparison with the literature, the above analyses were repeated with the $S2/S1_{PP}$ ratio. Planned contrasts ($df = 1,17$) revealed that the $S2/S1_{PP}$ ratio at Time 1 was significantly lower than at Time 2, $F = 11.22$, $p < .01$. No differences were found between the $S2/S1_{PP}$ ratio at Time 1, and Times 3, 4, or 5 ($p > .10$); see Figures 2.4 and 2.5.

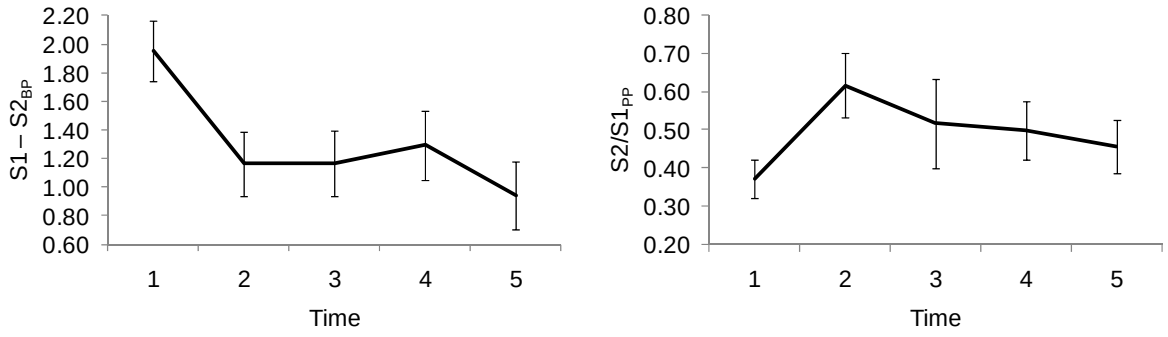

Figure 2.4. The S1 – $S2_{BP}$ difference (left) and $S2/S1_{PP}$ ratio (right) in each of five Time blocks. Error bars represent standard error.

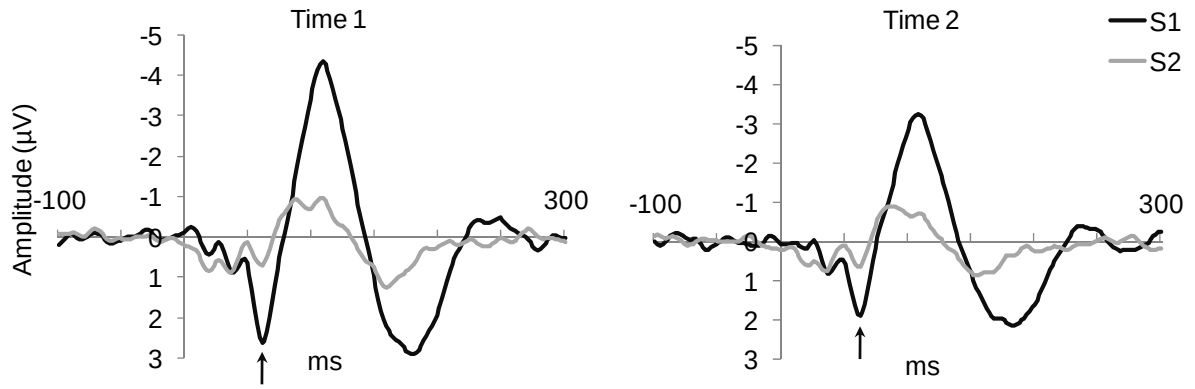

Figure 2.5. Grand average ERP waveforms elicited by S1 and S2 are shown for Time 1 (left) and Time 2 (right) separately, at Cz. Stimulus onset occurred at 0 ms. Arrows indicate P50 peaks.

In order to identify the source of changes in the S2/S1 ratio and S1–S2 difference over time, the effect of Time on S1 and S2 amplitudes was also tested. Planned contrasts (*df = 1*,19) revealed that S1 P50 amplitude at Time 1 was significantly larger than at Times 2 (F = 7.16, p < .05), 3 (F = 9.25, p < .01), 4 (F = 4.96, p < .05), and 5 (F = 9.65, p < .01). In contrast, planned contrasts (*df = 1*,19) revealed that S2 P50 amplitude at Time 1 did not differ from that at Times 2, 3, 4, or 5 (p > .10); see Figure 2.6.

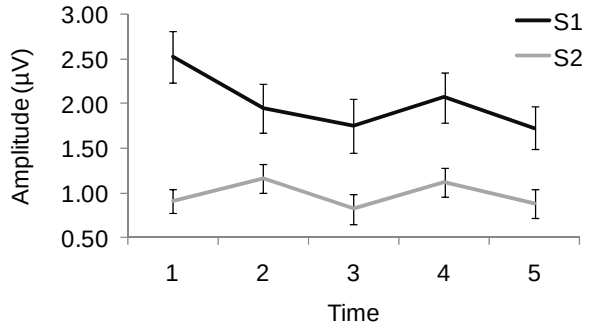

Figure 2.6. P50 amplitudes (with standard error) to S1 and S2 in each of five Time blocks.

2.4.1. Interpair Interval

There were no statistically significant differences between the S1–$S2_{BP}$ difference at IPI9, and either IPI3, IPI5, or IPI7 ($p > .10$). However, the S1–$S2_{BP}$ difference was significantly larger at IPI9 than at IPI1; $F = 7.13$, $p < .05$; $df = 1,19$.

To allow for more direct comparison with the literature, the above analyses were repeated with the $S2/S1_{PP}$ ratio. Planned contrasts ($df = 1,15$) indicated no statistically significant differences between the mean $S2/S1_{PP}$ ratio at IPI9, and IPI1, IPI3, IPI5, or IPI7 ($p > .05$); see Figure 2.7.

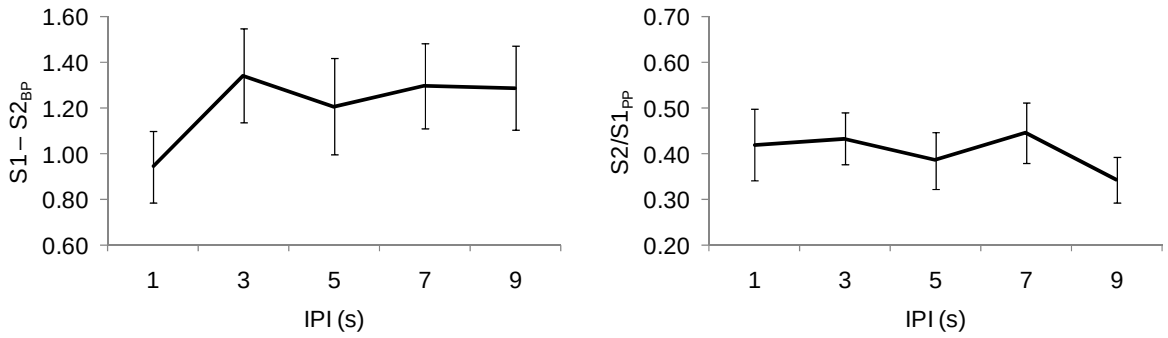

Figure 2.7. The S1 – $S2_{BP}$ difference (left) and $S2/S1_{PP}$ ratio (right) measured in each of five IPI conditions. Error bars represent standard error.

There was an apparent difference between the IPI1 and IPI9 S1 peaks (Figure 2.8). To determine whether this was statistically significant, paired samples *t* tests compared IPI1 to IPI9, for each of the S1 and S2 P50 amplitudes. No differences were found for S1 (IPI1, $M = 1.59$, SE = .21; IPI9, $M = 1.80$, $SE = .26$), $p > .10$, or S2 (IPI1, $M = .78$, $SE = .18$; IPI9, $M = .74$, $SE = .14$), $p > .10$.

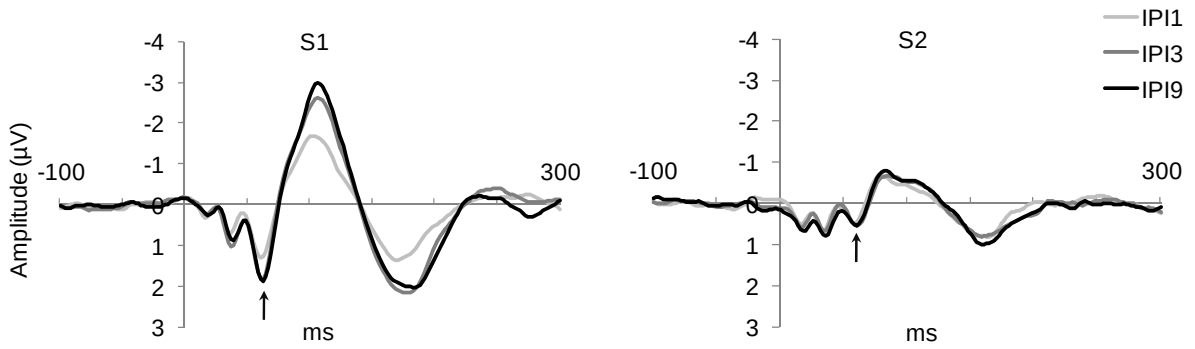

Figure 2.8. Grand average ERP waveforms elicited by S1 (left) and S2 (right) are shown for the IPI1, IPI3, and IPI9 conditions, at Cz. Stimulus onset occurred at 0 ms. Arrows indicate P50 peaks.

2.5. Discussion

2.5.1. Reliability

The present study found statistically significant reliability for both the S2/S1 ratio ($S2/S1_{PP}$ = .44; $S2/S1_{BP}$ = .30) and S1–S2 difference ($S1–S2_{PP}$ = .64; $S1–S2_{BP}$ = .72) when calculated from 100 trials. Following the classification criteria provided by Fleiss (1986), the reliability of the S2/S1 ratio falls within the 'poor' to 'fair-to-good' range, while the reliability of the S1–S2 difference falls within the 'fair-to-good' range. That the S1–S2 difference was more reliable than the S2/S1 ratio is consistent with earlier reports of greater reliability for the difference metric (Fuerst et al., 2007; Lu et al., 2007; Smith et al., 1994). Furthermore, S1–S2 difference reliability was greater when P50 peaks were measured from BP (rather than PP), whereas S2/S1 ratio reliability was greater when PP (rather than BP, which was barely significant) method was employed.

Although the reliability of the S2/S1 ratio was only 'poor' to 'fair-to-good' according to Fleiss (1986), the reliability of the ratio was statistically significant, and this does not correspond to reports in the literature of nonsignificant reliability. Methodological improvements in the present study may account for this difference. For example, we quantified reliability from subsets of the 300 trials distributed equally across time within the session—a method that should theoretically yield the maximum possible reliability as it negates variance due to time and participant state effects (Walhovd & Fjell, 2002). In contrast, others have measured within-session reliability between successive blocks of trials (e.g., Clementz et al., 1997; Lamberti et al., 1993; Schwarzkopf et al., 1993). In doing so, those studies may have introduced variance from time-related changes. Indeed, Lamberti et al. (1993) reported effects of time on P50 paired-click ratios, as did the present study, and Clementz et al. (1997) and Schwarzkopf et al. (1993) reported effects of time on P50 amplitudes.

To the extent that individuals vary in the temporal course of P50 paired-click measures, this will serve to undermine P50 paired-click measure stability quantified over time. This appears to be the case, as Lamberti et al. (1993) reported considerable individual variation in P50 paired-click indices within-session. Furthermore, Croft, Lee, Bertolot, and Gruzelier (2001) demonstrated differential temporal changes in P50 paired-click indices in different subsets of healthy participants, indicating that identical time-related changes cannot be assumed for healthy participants. This suggests that prior studies may have underestimated P50 paired-click measure reliability coefficients due to a failure to account for effects of time. In support of this interpretation, we calculated the S1–S2 difference reliability from Times 1–5 (thus not accounting for the effect of time). This reduced S1–$S2_{BP}$ difference reliability to ICC (1, 1) (single measure) = .39, $p < .001$. Therefore, we suggest that future studies take

effects of time into account in order to avoid underestimating the reliability of P50 paired-click indices. However, even when taking into account the effects of time, and averaging 100 trials, the reliability of the $S2/S1_{PP}$ ratio was only .44. This shows that additional sources of variance contributing to P50 ratio variability remain unaccounted for. The much lower S2/S1 ratio reliability suggests that studies using the P50 ratio metric would have less power, and thus require larger sample sizes in order to detect effects when compared with the P50 difference measure.

The reliability of the S1–S2 difference increased with a greater number of trials averaged, and consistently resulted in ICCs in the 'fair-to-good' range when 40 or more trials contributed to ERP averages. However, the within-subject variability of the S1–S2 difference continued to decrease as the number of trials increased from 10 to 100. Indeed, a statistically significant decrease in within-subject variability was found as the number of trials averaged was increased from 90 to 100, suggesting that averaging at least 100 trials is necessary to maximize within-subject stability.

2.5.2. Time

The present data indicate that P50 paired-click measures vary over time such that healthy participants exhibit smallest P50 ratio and largest P50 difference at the beginning of an experiment. The direction of the change is in accordance with the findings of Lamberti et al. (1993), but not Waldo et al. (1992) or Naber et al. (1992), who reported decreasing and invariant P50 ratio scores over time, respectively. However, direct comparison between these studies and the present study is problematic due to the different durations of their respective recording sessions. In an experiment of comparable duration, White and Yee (2006) reported

invariant P50 ratio scores over time. The present study differed methodologically from that of White and Yee (2006) in that we directed attention toward the stimuli, while White and Yee only required that subjects sit and listen quietly for the duration of their study. A study with both directed and uncontrolled attention conditions is necessary to determine whether this methodological difference explains the differential temporal pattern of P50 ratio scores within-session between these studies.

The mean P50 ratio in healthy participants of .37 (*SE* = .05) observed in the first block of the present study increased to .62 (*SE* = .08) in the second block (although $S2/S1_{PP}$ in subsequent blocks did not differ from that in the first). However, measured with the more reliable $S1–S2_{BP}$ difference, a significantly greater P50 difference occurred in the first block relative to *all* subsequent blocks. To put this increase in context, a meta-analysis of 46 P50 paired-click studies reported a mean P50 ratio of .39 (*SD* = .15) in healthy participants as compared to .80 (*SD* = .24) in schizophrenia patients (Patterson et al., 2008). This demonstrates that healthy participants' P50 ratios can be robust, or alternatively can approach levels reported in schizophrenia patients, depending on the point in the recording session at which P50 paired-click indices are quantified. To further assist the design of P50 paired-click studies, it would be useful for future research to determine whether P50 paired-click measures are also affected by tasks preceding the P50 paired-click paradigm.

2.5.3. Interpair Interval

The data presented here indicate that a P50 paradigm with an IPI of 9 s yields $S2/S1_{PP}$ ratio and $S1–S2_{BP}$ difference measures not different from those obtained with IPIs of 3, 5, or 7 s. Furthermore, the present study showed that reducing the IPI from 9 to 1 s had no statistically significant effect on either S1 or S2 P50 amplitudes. However, in the grand mean ERP

waveform, the S1 P50 amplitude appeared smaller in the IPI1 relative to the IPI9 condition. This was not the case for the grand mean IPI3 and IPI9 waveforms, which appeared practically identical. Thus, we would have reservations in recommending the use of 1-s IPIs, and would rather suggest that IPIs in the P50 paradigm could be reduced to 3 s and still generate S2/S1 ratio and S1–S2 difference scores equivalent to those obtained using a long (9-s) IPI, supporting the findings of Rentzsch, Gomez-Carrillo de Castro, et al. (2008). In practice, this would reduce the time needed to collect 100 trials from 16 to 6 min. Given the variability introduced into P50 paired-click measurement by the effect of time, reducing IPI duration could potentially improve P50 paired-click reliability by allowing more trials to be presented in a given time period. However, future studies manipulating the IPI parameter are needed to verify whether this finding generalizes to schizophrenia spectrum populations, and thus whether the duration of the P50 recording session can be similarly shortened within schizophrenia samples.

2.5.4. Limitations

The within-session P50 paired-click reliability observed in clinical samples and reliability observed over longer test-retest intervals may be lower than that observed in the healthy undergraduates in the present study. An important consideration in interpreting the reliability of a measure is the relative heterogeneity of the sample within which it was estimated. That is, as sample heterogeneity increases (thus increasing between-subject variance), the ICC magnitude will concurrently increase (assuming within-subject variance is unchanged) (see, e.g., Bland & Altman, 1990). Thus, the relatively homogenous sample in the current study (healthy undergraduates) is likely to under- rather than overestimate ICC values.

2.5.5. Implications

The present study demonstrated that P50 paired-click measures can be more reliably measured within-session with both the ratio and difference metrics, where adequate numbers of trials are acquired and effects of time are accounted for. The reliability of the difference metric was higher than that of the ratio, suggesting that studies would have more power to detect effects using the difference metric. The reliability of the S1–S2 difference was increased when P50 peaks were measured from BP (rather than PP), suggesting that the BP amplitude measurement method is optimal for P50 paired-click measurement. It may be useful for future research to determine what factors affect between-session reliability. Further, it was demonstrated that effects of time need to be accounted for in P50 paired-click research, as the presence of temporal effects is large enough to create apparent group differences and/or obscure real differences. Finally, the present results suggest that future studies using healthy participant samples may reduce the IPI parameter in the P50 paradigm from 9 to 3 s, substantially reducing the duration of the P50 recording session.

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3. CHAPTER 3: CLARIFYING THE FUNCTIONAL PROCESS REPRESENTED BY P50 SUPPRESSION

Dalecki, A., Johnstone, S. J., & Croft, J. R. (2015). Clarifying the functional process represented by P50 suppression. *International Journal of Psychophysiology, 96*, 149-154.

3.1. Abstract

P50 suppression refers to the amplitude-reduction of the P50 event related potential (ERP) to the second (S2) relative to the first (S1) of identical auditory stimuli presented 500 ms apart. Theory suggests that refractory periods (RP) and/or inhibitory inputs (II) underlie P50 suppression. The present study manipulated interval between stimulus pairs (IPI: 2, 8 s) and direction of participants' attention (Attention, Non-Attention) in order to determine which theory best explains P50 suppression. The rationale is that: 1) Reducing IPI will reduce the effect of the mechanism responsible for S2P50 change due to reduced recovery of the relevant mechanism at shorter (vs. longer) IPIs; 2) Increasing attention will increase the effect of the mechanism responsible for S2P50 change due to enhanced processing, particularly at the short IPI where the mechanisms are challenged; and 3) The two theories predict that the same direction of effect on the mechanism will result in opposing effects on the S2P50 (more recovery resulting in larger S2P50 and more inhibition resulting in smaller S2P50 amplitudes). In the Non-Attention paradigm, reducing IPI from 8 to 2 s reduced S1P50 (p = .001) and tended to *increase* S2P50 (p = .051), and in the 2 s IPI paradigm, directing attention towards stimuli had no effect on S1P50 (p = .146) but reduced S2P50 (p = .008), with both effects supporting the II hypothesis only.

3.2. Introduction

Presenting two identical auditory stimuli (S1 and S2) separated by 500 ms elicits a P50 event-related potential (ERP)—a positive deflection in the electroencephalogram (EEG)—approximately 50 ms after each stimulus. The P50 amplitude elicited by S2 is usually smaller relative to the S1 P50 amplitude. This reduction is termed "P50 suppression" (Siegel, Waldo, Mizner, Adler, & Freedman, 1984) and is quantified primarily using two derived ERP measures: the P50 Difference (S1—S2) and the P50 Ratio (S2/S1).

Theory suggests that two processes may account for P50 suppression: a refractory period (RP) (Jerger, Biggins, & Fein, 1992) and/or an inhibitory input (II) process (Freedman et al., 1996). The RP hypothesis posits (Siegel et al., 1984) that after responding to an auditory stimulus, the neuronal population generating the P50 response (thought to be in the auditory cortex; ACx) (Godey, Schwartz, de Graaf, Chauvel, & Liégeois-Chauvel, 2001; Korzyukov et al., 2007; Liégeois-Chauvel, Musolino, Badier, Marquis, & Chauvel, 1994; Weisser et al., 2001; Yvert, Crouzeix, Bertrand, Seither-Preisler, & Pantev, 2001) is temporarily exhausted. Following this, it gradually recovers, and P50 amplitudes depend on the recovery status of this neuronal population. Thus, under the RP hypothesis, S2P50 is reduced relative to S1P50 because at the arrival of S2 (500 ms after S1), the ACx neuronal population has not recovered fully, whereas at the arrival of S1 (≈ 8 s after the previous S2), it has recovered to a greater extent, perhaps fully (Zouridakis & Boutros, 1992).

According to the II hypothesis, the ACx neuronal population generating the P50 response also engages inhibitory inputs which, in a feedback loop with these ACx neurons, act to inhibit the responding of ACx to the subsequent arrival of an identical auditory stimulus. These inhibitory inputs were originally thought to originate in the CA3

hippocampal region (Bickford-Wimer et al., 1990; Freedman et al., 1996; Hershman, Freedman, & Bickford, 1995) however there is also evidence implicating frontal lobe (Knight, Richard, Swick, & Chao, 1999) and reticular activating system (Erwin & Buchwald, 1986) involvement in this purported inhibitory process. The II process is thought to be long lasting (> 500 ms; Miller & Freedman, 1995). Thus, since they are still active when S2 is presented (500 ms after S1) these inhibitory inputs serve to reduce the ACx P50 response to S2.

P50 suppression is important in schizophrenia (Bramon, Rabe-Hesketh, Sham, Murray, & Frangou, 2004; Chang, Arfken, Sangal, & Boutros, 2011; de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007; Heinrichs, 2004; Patterson et al., 2008) and across the schizophrenia spectrum (Croft, Dimoska, Gonsalvez, & Clarke, 2004; Croft, Lee, Bertolot, & Gruzelier, 2001). It has been argued to be an endophenotype for schizophrenia (Thaker, 2008) however failures to find P50 suppression impairments in schizophrenia (Greenwood, Light, Swerdlow, Radant, & Braff, 2012; Light et al., 2012) have called this assertion into question. The lack of clarity as to the functional relevance of P50 suppression may contribute to this uncertainly, as it is difficult to control for confounds in schizophrenia research without such knowledge. The logic underpinning these hypotheses can be used to help determine their adequacy, and thus their relation to schizophrenia:

If, as per the RP hypothesis, the ACx is temporarily exhausted after responding to an auditory stimulus and gradually recovers from this, then presenting auditory stimuli at incrementally greater intervals should produce a commensurate increase in P50 amplitudes as recovery increases. The results of such studies suggest that P50 amplitude (and thus the ACx response generating it) fully recovers 3000 ms after responding to a prior stimulus (Dalecki,

Croft, & Johnstone, 2011), but shorter intervals (250–1000 ms) are insufficient for full recovery (Dalecki et al., 2011; Dolu, Suer, & Ozesmi, 2001). So if recovery of the ACx *alone* accounts for P50 suppression, then reducing the time in between stimulus pairs from the standard 8 s to < 3 s should reduce S1P50 (due to less recovery), but as S2 always occurs 500 ms after S1, S2P50 should be either unaffected (if S1 exhausts the P50 generator and S2P50 is thus generated from a minimal neural reserve regardless of the IPI preceding S1) or reduced (if it is generated from a proportion of the reduced neural reserve). On the other hand, if P50 suppression is due to ACx activating inhibitory inputs, then reducing the time in between stimulus pairs from the standard 8 s to < 3 s would result in either the ACx itself being insufficiently activated (as indexed by reduced S1P50) to adequately engage inhibitory inputs, or reduction of the inhibitory resource (due to insufficient time for full recovery of the inhibitory inputs). Both of these possibilities would result in *increased* S2P50 (in the 2 s relative to 8 s IPI condition). Thus the present study will manipulate the interval between stimulus pairs in order to determine which theory best explains P50 suppression, with similar or smaller S2P50s in the 2 s (relative to 8 s) condition supporting the refractory period hypothesis, and larger S2P50s in the 2 s condition supporting the inhibition hypothesis.

Another way to address this issue is via the manipulation of attention. As set out above, the RP hypothesis postulates that ACx recovery status underlies P50 suppression. Thus, if RP accounts for P50 suppression and attention enhances the ACx response, then directing attention towards stimuli should enhance both S1 and S2 P50s. On the other hand, as II posits inhibitory input involvement (and ACx as the trigger for this inhibitory input) in P50 suppression, attention could enhance neither, one, or both of these. If attention enhances the *ACx response only*, then directing attention towards stimuli should enhance both S1 and S2P50s (although, if the enhanced S1 response subsequently engaged more inhibitory inputs

then the S2P50 ACx increase may by countered by greater inhibition and thus result in no change in S2P50). If attention enhances the *inhibitory inputs only,* then directing attention towards auditory stimuli should not affect S1P50 (as inhibitory inputs are not active at the time of S1 presentation), and should reduce S2P50 (due to attentional enhancement of inhibitory action on S2). Finally if attention enhances both the ACx response *and* inhibitory inputs, directing attention to auditory stimuli should enhance S1P50 (due to attentional enhancement of the ACx response) and reduce S2P50 (due to greater ACx engagement of inhibitory inputs and/or attention enhancing inhibitory inputs directly). Thus the present study also manipulated the direction of attention in the 2 s IPI condition (where the mechanisms for each hypothesis are challenged) in order to determine which theory best explains P50 suppression, with larger S2P50s in the Attention condition (relative to Non-Attention) supporting the recovery hypothesis, and similar or smaller S2P50s supporting the inhibition hypothesis.

The question of attention effects on P50 is particularly relevant to schizophrenia, as patients are known to have attentional impairments. That is, if attention modulates either the ACx response or inhibitory inputs, this raises the possibility that group differences may arise due merely to the well-described attentional differences between the groups (Braff, 1993), rather than because of specific differences in ACx response and/or inhibitory processing.

Few studies have examined the effects of directing attention *towards* and *away from* auditory stimuli on P50 measures. Some studies have compared the standard 'passive' P50 paradigm with one where attention is directed toward auditory stimuli (Rosburg, Trautner, Elger, & Kurthen, 2009; Yee et al., 2010). However, this comparison is problematic as it is difficult to determine where attention is directed under 'passive' conditions. That is, despite

not being given specific instructions to attend to the stimuli, it is possible that participants may do so regardless, thus removing the difference between this and an 'attention' condition. Only two studies have compared P50 across conditions where attention is directed toward and away from auditory stimuli (Gjini, Burroughs, & Boutros, 2011; Kho et al., 2003). Neither of these studies found differences in P50 across these conditions. However, as they used only a long IPI (≈ 8 s), they do not answer the question of whether attention effects are present at shorter IPIs where refractory and/or inhibitory mechanisms are challenged. The present study will address this.

The present study aims to clarify which theory (RP or II) best explains P50 suppression by manipulating the inter-pair interval (IPI; interval between successive auditory stimulus pairs) and attention, within the P50 auditory paired-stimulus paradigm.

3.3. Methods

3.3.1. Participants

24 undergraduate psychology students from the University of Wollongong volunteered to participate in the study in return for course credit. Three were excluded due to technical difficulties with the data recording. Thus data from 21 participants (16 females) aged 18 to 49 (M = 21.26, SD = 6.59 years) were analyzed. All provided written informed consent. Exclusion criteria were self-reported hearing difficulties.

3.3.2. Procedure

Participants were fitted with EEG recording apparatus and completed the Schizotypal Personality Questionnaire (SPQ; Raine, 1991) and demographic and smoking history questionnaires[4]. Participants were asked to abstain from smoking and drinking coffee for 60 minutes prior to the start of the recording session and were not permitted to smoke or consume caffeine during the experiment.

Participants were then seated in a chair in a dimly illuminated room with a computer screen 80 cm in front of them. The experiment contained 20 experimental blocks, with each block 6.6 minutes in duration. Participants were given a brief (approximately 30 s) break in between each block, thus the experiment lasted a total of 132 minutes. This design allowed for the presentation of a large number of tone pairs (n = 1,440; thus 360 tone pairs in each attention/IPI combination).

3.3.3. P50 Paired-Stimulus Paradigm

Tones were presented as pairs (S1, S2) with inter-stimulus interval (ISI) constant (500 ms). The inter-pair interval (IPI) was the interval between the second tone in a tone pair (S2) and the first tone of the subsequent tone pair (S1). Tone pairs were presented at one of two mean IPIs (2 and 8 s), referred to as IPI2 and IPI8. To provide the appearance of randomness in the IPIs, each mean IPI was achieved by combining 3 IPIs whose mean equalled the target IPI, but which individually did not equal the target IPI: 2 s (1950, 2000, 2050 ms) and 8 s (7500, 8000, 8050 ms).

[4] These were administered as P50 gating is also thought to be related to the schizotypal personality trait and modulated by smoking status (Croft et al., 2004; Croft et al., 2001). However, this is not relevant to the present paper and will not be discussed further.

Half of the blocks (n = 10) were 'Attention' blocks and half were 'Non-Attention' blocks. 'Attention' and 'Non-Attention' blocks were pseudo-randomly distributed across the experiment such that they were equally spread over time and no more than two of the same block type occurred in a row.

At the beginning of the experiment, participants were orally instructed that during half of the blocks the tones they would hear were unimportant and they should ignore them and watch the silent movie playing on the computer screen in front of them ('Non-Attention' condition). For the other half of the blocks, participants were instructed to pay attention to the tone pairs ('Attention' condition), and respond with a button press when tones within a pair differed in pitch, but not when tones within a pair were identical in pitch. This instruction served to direct participants' attention towards both tones in the pair. To begin each block, instructions appeared on the screen (these were repeated orally by the experimenter). Participants were also instructed to keep their eyes open and watch a silent movie ("Shaun the Sheep") which was played throughout the entire experiment.

The 1,440 paired-stimuli were presented over the 20 experimental blocks (72 paired-stimulus trials per block), with an equal number of IPI2 and IPI8 trials, distributed equally across time within the experiment. 'Decision' trials were those where tones within a pair differed in pitch from one another. These made up 10% of the trials and were not analyzed but served only to direct participants' attention towards the tones in the 'Attention' condition.

Auditory stimuli were presented through headphones. Tone intensity, as measured with a Bruel and Kjaer Precision Sound Level Meter (Type 2235), was 80 dB. Auditory stimuli were pairs (S1 and S2) of brief (1 ms duration) tones. Individual tones were either low (L; 1000 Hz) or high (H; 2000 Hz) in pitch such that tones could be paired in one of four

combinations: HH (45%), LL (45%), HL (5%), or LH (5%).

3.3.4. EEG Data Collection

EEG data were recorded continuously using Ag-AgCl electrodes from 19 scalp sites (FP1, FP2, Fz, F3, F4, F7, F8, Cz, C3, C4, Pz, P3, P4, T3, T4, T5, T6, O1, and O2) placed according to the 10/20 International System, and from both mastoids (M1 and M2). EEG data were grounded midway between Fz and FPz and referenced midway between Cz and CPz. Electrooculogram (EOG) was recorded using Ag-AgCl electrodes placed above (E1) and below (E3) the left eye, and at the outer canthi of left (E5) and right (E6) eyes. Vertical EOG was derived as E1−E3. All electrode impedances were below 20 kΩ at the start of the recording. The EEG/EOG data were amplified with a gain of 1338, digitized at 2000 Hz, and digitally filtered using a 0.1 to 100 Hz (24 dB/octave roll-off) bandpass filter (NuAmps, Neuroscan). Continuous EEG/EOG data were stored for subsequent off-line analysis.

3.3.5. EEG Data Analysis

Data were analyzed offline using Neuroscan software (Scan 4.5). The data were EOG corrected (Semlitsch, Anderer, Schuster, & Presslich, 1986), and bandpass filtered using a 10-45 Hz zero-phase shift filter (12 dB/octave roll-off). Note that although there are a number of possible filters that could be applied, as the most appropriate remains to be determined we used that which has been used for schizophrenia P50 suppression research, in order to ensure that the present results are relevant to the schizophrenia P50 suppression literature (Anokhin, Vedeniapin, Heath, Korzyukov, & Boutros, 2007; Hall, Taylor, Salisbury, & Levy, 2011; Patterson et al., 2008; Schulze et al., 2007). Data were then re-referenced to digitally linked

mastoids (Miller, Lutzenberger, & Elbert, 1991), epoched around S1 (from 100 ms pre- to 900 ms post-stimulus), and baseline corrected using the 100 ms pre-stimulus interval[5]. The average amplitude of the 100 ms activity preceding S2 (400 to 500 ms) was also calculated to enable baseline correction of S2 to its immediately preceding baseline. Before averaging, an automatic artefact rejection procedure identified and rejected trials with activity exceeding ± 50 μV in any EEG channel.

3.3.6. P50 Peak Selection

Data were analyzed from Cz only, as per Clementz, Geyer, and Braff (1998). Although some P50 studies use principal components analysis (e.g., Clementz & Blumenfeld, 2001), simple peak detection was used here to allow comparison with the majority of schizophrenia P50 studies, including recent studies with large patient samples (Light et al., 2012). P50 peaks were identified manually by one rater. Additional constraints were imposed on putative P50 peaks to discern these from non-P50 deflections. To be accepted as a true P50 peak they were required to exhibit a fronto-central topography (determined by visual inspection of topographic maps). No minimum amplitude was required for a P50 peak to be classified as such. If no peak was present within the required latency range, or if a peak within the latency range did not have a fronto-central topography, the P50 amplitude was scored as '0'.

P50 peak amplitudes were defined relative to the average amplitude of the S1 pre-

[5] To evaluate whether an alternative choice of pre-stimulus baseline (i.e., the 100ms period preceding S1, which is also occasionally reported in the literature: e.g., (Knott, Millar, & Fisher, 2009)) had any appreciable effect on the results, ERP peaks were also defined relative to this baseline.

stimulus baseline for S1P50 and S2 pre-stimulus baseline for S2P50. The P50 Ratio was defined as the ratio of the P50 amplitudes to tones 2 and 1 (i.e., S2/S1), and the P50 Difference was the difference between P50 amplitudes to tones 1 and 2 (i.e., S1–S2). ERP averages were generated for IPI2, IPI8, Attention and Non-Attention conditions separately (n = 360 tone pairs in each combination) and a grand mean across IPI and attention conditions combined.

The Group grand mean P50 was defined as the largest positive deflection 40–80 ms post-stimulus (following Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008). Each subject's Individual grand mean P50 was then defined as the largest positive peak ± 15 ms of the Group grand mean P50 latency. Each subject's P50 peak (for each individual Attention and IPI condition) was defined as the positive deflection ± 5 ms of their Individual grand mean P50 latency.

3.3.7. Statistical Analyses

Paired t-tests compared S1P50 and S2P50 in the Non-Attention IPI8, Non-Attention IPI2, Attention IPI8 and Attention IPI2 conditions separately to determine whether P50 suppression was evident in each. To determine whether reducing IPI from 8 s to 2 s affected P50, paired samples t-tests compared S1P50 and S2P50 across IPI8 and IPI2 in the Non-Attention condition. To determine whether attention manipulations affected P50, paired samples t-tests compared S1P50 and S2P50 across Non-Attention and Attention conditions in the IPI2 condition (where the mechanism underlying P50 suppression is challenged due to reduced recovery) and the IPI8 condition typically used in the P50 suppression literature. To evaluate the effect of IPI and attention on P50 suppression, the above paired samples t-tests

were also conducted on the P50 Difference[6].

3.4. Results

3.4.1. Behavioral Results

A one-sample t-test revealed that the sensitivity (d') score of the sample (M = 2.78, SE = .21) differed significantly from '0', ($p < .001$) indicating that the group performed the Attention task at above chance levels over the experiment. The mean correct hit rate was .70 (SE = .05) and the mean false alarm rate was .01 (SE < .01).

3.4.2. P50 Suppression

P50 suppression was evident in each attention and IPI condition, with paired *t*-tests revealing that S2P50 was significantly smaller than S1P50 in the Non-Attention IPI8 ($p < .001$), Non-Attention IPI2 ($p = .003$), Attention IPI8 ($p < .001$) and Attention IPI2 conditions ($p < .001$) (Figure 3.1).

[6] The P50 Difference was chosen as it has been shown to be more reliable than the P50 Ratio (Dalecki et al., 2011; Fuerst et al., 2007; Smith et al., 1994). However, for completeness, and to enable comparisons with studies who also report the P50 Ratio, statistical tests were also performed on this metric and are reported here.

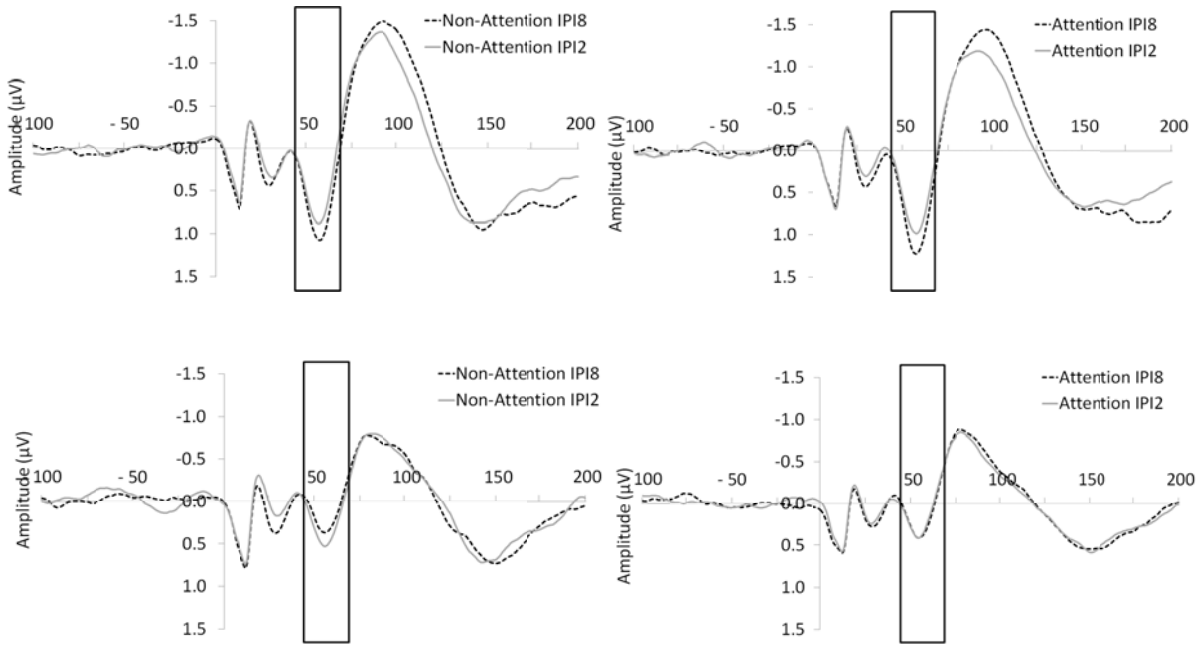

Figure 3.1. ERPs elicited in the Non-Attention (left) and Attention (right) conditions at IPI8 (dotted black line) and IPI2 (solid grey line). P50 ERPs to S1 (top) and S2 (bottom) are boxed. Stimulus onset is at 0 ms. Positive is plotted downwards.

3.4.3. RP vs. II

3.4.3.1. Effects of Reducing IPI from 8 s to 2 s in the standard Non-Attention condition.

Under Non-Attention conditions, paired samples t-tests revealed that reducing IPI from 8 s to 2 s reduced S1P50 ($p = .001$) and tended to increase S2P50 ($p = .051$) (see Figure 3.1), which is consistent with II, but inconsistent with the RP hypothesis. It also reduced P50 suppression as indexed by a smaller P50 Difference ($p < .001$) and larger P50 Ratio ($p = .001$).

3.4.3.2. Effects of Directing Attention towards Paired Stimuli in the IPI2 condition.

In the IPI2 condition, compared to Non-Attention, directing attention towards auditory stimuli did not affect S1P50 (p = .146), but reduced S2P50 (p = .008), which is consistent with attention enhancing inhibitory inputs, but not ACx. It also increased P50 suppression as indexed by a larger P50 Difference (p = .004) and smaller P50 Ratio (p = .002).

3.4.3.3.Effects of Directing Attention towards Paired Stimuli in the standard IPI8 condition.

In the standard IPI8 condition, compared to Non-Attention, directing attention towards auditory stimuli increased S1P50 (p = .040), but did not affect S2P50 (p = .799), which is consistent with attention enhancing ACx and II (as there was no commensurate increase in S2P50). It had no effect on P50 suppression as indexed by either the P50 Difference (p = .248) or P50 Ratio (p = .846).

3.4.4. Follow-up Analyses:

For completeness, we also examined the effect of IPI under Attention conditions. In the Attention condition, paired samples t-tests revealed that reducing IPI from 8 to 2 s reduced S1P50 (p = .001) but did not affect S2P50 (p = .248), tended to reduce P50 suppression as indexed by a (trend-level) smaller P50 Difference (p = .054), and did not affect the P50 Ratio (p = .803).

3.5. Discussion

In the standard Non-Attention protocol, reducing IPI from 8 s to 2 s reduced S1P50 and tended to *increase* S2P50, which is consistent with less inhibitory input engagement and thus supports the II hypothesis as the best explanation for P50 suppression. This is further supported by the observation that directing attention towards auditory stimuli (in the IPI2 protocol) *reduced* S2P50, as the RP hypothesis would predict an increase to S2P50 as more attention was allocated to S2, whereas the II hypothesis would predict reduced S2P50 due to enhanced suppression. Taken together this suggests that P50 suppression is underpinned by inhibitory inputs, with attention enhancing this inhibition (either via greater engagement of inhibitory inputs by the ACx when it itself is enhanced by attention, or by attention directly enhancing the inhibitory inputs), and reduced IPI consequently reducing inhibition (due to the IPI not being sufficient to overcome the refractory period of the inhibitory processes).

At IPI8, given that Attention increased S1P50 and had no effect on S2P50, this suggests that attentional differences between schizophrenia patients and controls will affect group differences on P50 suppression metrics (through attention's influence on S1P50 amplitudes). Thus we did not replicate earlier failures to find attention effects on S1P50 when comparing conditions where attention is directed toward, and away from stimulus pairs (Gjini et al., 2011; Kho et al., 2003). However, it should be noted that this effect was not strong enough to significantly alter P50 ratio or difference scores, which questions the relevance of this finding to the schizophrenia P50 suppression research. This issue cannot be resolved from the current data, but as S1P50 contributes to P50 suppression measures, and as the attention effect was dependent on refractory period (with significant P50 suppression effects at IPI2), and as the effect of refractory period is currently unknown in schizophrenia, the

present results highlight the possibility that P50 suppression reductions in schizophrenia reported in the literature may have been confounded by attention. Taken together, our results thus show that differences between schizophrenia patients and healthy controls P50 suppression measures could be due to either:

(a) Impaired II (failing to attenuate S2P50); or
(b) Impaired attention (failing to enhance S1P50 and/or subsequent reduction in II activation).

Our attention results thus demonstrate that when P50 measures of ACx and/or inhibitory function (and *not* attention) are of interest, the most appropriate paradigm is one where attention is directed away from the auditory stimuli. If attention is directed toward the stimuli (e.g., by instructing participants to count the stimuli), group differences related to ACx and/or inhibitory processes will be confounded by any attentional differences between the groups. For example, part of the group difference would be due to the attentional enhancement of S1P50 in healthy subjects and a lack thereof in schizophrenia patients (to the extent that attentional impairments are present in this sample).

A limitation of the present study is that we are unable to conclusively demonstrate that participants' attention was indeed directed away from the auditory stimuli in the Non-Attention blocks of the experiment. However, we were able to show that participants performed the Attention (auditory discrimination) task successfully; suggesting that during this task participants' attention was directed *toward* the auditory stimuli. Given this, and that we found an attention-related difference in S1P50 amplitudes, this suggests that in the Non-Attention condition participants directed their attention away from the auditory stimuli.

The present results demonstrate that both inter-pair interval and attention can

modulate P50 amplitude and resultant P50 suppression measures, and that the particular pattern of effects provides support for the II hypothesis of P50 suppression (Freedman et al., 1996). The attention effects also provide caution for the interpretation of the P50 suppression deficits in schizophrenia, as they show that P50 suppression changes can be due to either attention or independent inhibitory processes.

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4. CHAPTER 4: THE RELEVANCE OF ATTENTION IN SCHIZOPHRENIA P50 PAIRED STIMULUS STUDIES

Dalecki, A., Green, A. E., Croft, R. J., & Johnstone, S. J. (*Under Review*). The relevance of attention in schizophrenia P50 paired stimulus studies. *Clinical Neurophysiology*

4.1. Abstract

Objective

P50 suppression refers to the P50 ERP amplitude-reduction to the second (S2) relative to the first (S1) of identical brief auditory stimuli (SOA = 500 ms). Its reduction in schizophrenia is argued to represent impaired inhibitory input (II) mechanisms. Enhancing attention enhances II functionality (*reducing* S2P50 amplitude and increasing P50 Difference) in healthy subjects. We determined whether the effect of attention on P50 suppression differs between schizophrenia patients (SCZ) and controls (CON) and thus is a confound in P50 schizophrenia research.

Methods

We manipulated the direction of attention (Attention, Non-Attention) in 21 SCZ and 18 CON in the P50 suppression task.

Results

Directing attention toward stimulus pairs (versus Non-Attention) increased P50 suppression (P50 Difference). This effect tended to differ between groups, with Attention increasing

S1P50, *reducing* S2P50 and increasing P50 suppression (P50 Difference and reducing P50 Ratio) in CON only. No group differences were found for P50 Difference or Ratio.

Conclusions

Attention is a confound in schizophrenia P50 research and thus should be carefully controlled. When attention was controlled, P50 group differences were not found.

Significance

The SCZ–CON P50 difference reported in the literature may be related to uncontrolled attention (and not impaired P50 suppression *per se*).

4.2. Introduction

Presenting two identical auditory stimuli (S1 and S2) separated by 500 ms elicits a P50 event-related potential (ERP) — a positive deflection in the electroencephalogram (EEG) — approximately 50 ms after each stimulus. The P50 amplitude elicited by S2 is usually smaller relative to the S1 P50 amplitude. This reduction is termed "P50 suppression" (Siegel, Waldo, Mizner, Adler, & Freedman, 1984) and is quantified using the P50 Difference (S1—S2) and P50 Ratio (S2/S1) metrics.

P50 suppression is thought to reflect the operation of a neuronal circuit consisting of the P50 generator (thought to be in auditory cortex: ACx; Godey, Schwartz, de Graaf, Chauvel, & Liégeois-Chauvel, 2001; Korzyukov et al., 2007; Liégeois-Chauvel, Musolino, Badier, Marquis, & Chauvel, 1994; Weisser et al., 2001; Yvert, Crouzeix, Bertrand, Seither-Preisler, & Pantev, 2001) which, once activated by S1, in turn activates inhibitory inputs that suppress the ACx response to the subsequent arrival of an identical stimulus, S2 (Freedman et al., 1996). These inhibitory inputs are thought to be active for > 500 ms (Miller & Freedman, 1995), and so as they are still active when S2 is presented (500 ms after S1), the inhibitory inputs reduce the magnitude of the ACx response to S2. The inhibitory inputs were originally thought to be hippocampal (Freedman et al., 1996; Hershman, Freedman, & Bickford, 1995), however subsequent evidence also suggests the involvement of other areas, including frontal cortex (Ehlis et al., 2009; Knight, Richard, Swick, & Chao, 1999; Korzyukov et al., 2007) and reticular activating system (Erwin & Buchwald, 1986).

The P50 suppression impairment reported in schizophrenia (Bramon, Rabe-Hesketh, Sham, Murray, & Frangou, 2004; Chang, Arfken, Sangal, & Boutros, 2011; Earls, Curran & Mittal, 2016) is thus assumed to reflect an inhibitory input impairment and argued to be an

endophenotype for schizophrenia (Thaker, 2008). However, failures to find impaired P50 suppression in schizophrenia (Greenwood, Light, Swerdlow, Radant, & Braff, 2012; Light et al., 2012) have called this view into question.

Importantly, the methodology employed across P50 studies varies substantially (de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007b). These variations explain some of the variability in results (Patterson et al., 2008) and suggest the possibility that different neural functions are being assessed across studies. One of these methodological differences relates to how attention is controlled; most commonly, a participant's attention is not controlled (e.g., participants are not given instructions pertaining to the stimuli; Tregellas et al., 2007), or simply instructed to fixate a cross and relax during stimulus presentation; (Hazlett et al., 2015), however it may also be directed away (Mazhari, Price, Waters, Dragović, & Jablensky, 2011), or toward the task stimuli (Johannesen et al., 2005). Thus it is important to know whether attention is relevant to P50 suppression, as it may be that the variable P50 suppression findings in schizophrenia are due to a combination of the well-characterised attention deficit in the disorder, and the task dependent variability of attentional demands.

Indeed, we have previously demonstrated (Dalecki, Johnstone, & Croft, 2015) that attention is important for P50 suppression. In that study we challenged the mechanisms underlying P50 suppression by reducing the interval between stimulus pairs (IPI; inter-pair interval) from the standard 8 s to 2 s (thus not allowing the mechanism underlying P50 suppression to recover fully (Dalecki, Croft, & Johnstone, 2011; Dolu, Suer, & Ozesmi, 2001; Zouridakis & Boutros, 1992). Under these conditions, enhancing attention *reduced* S2P50 amplitude and increased P50 suppression (and also increased the P50 Difference). Thus attention can enhance P50 suppression in healthy subjects, and it is not only pre-

attentive inhibitory inputs that are captured in the P50 paired-stimulus task. This attentional effect may be relevant to schizophrenia, a population in which attentional impairments are well established (Braff, 1993). Specifically, schizophrenia patients and healthy controls may differently apply attention to paired stimuli in the P50 paradigm. Where attention is relatively enhanced (such as in healthy controls relative to schizophrenia patients), this may result in enhanced P50 suppression in controls and apparent P50 suppression failure in schizophrenia patients. Thus, since schizophrenia patients have impaired attention, the attention-related enhancement of P50 suppression in controls (Dalecki et al., 2015) may explain the "P50 suppression" difference often reported in schizophrenia (Adler et al., 1982).

Consistent with this, evidence within the schizophrenia memory literature has shown that while schizophrenia patients have impaired recognition in memory tasks relative to controls (Paul, Elvevåg, Bokat, Weinberger, & Goldberg, 2005), these group differences may reduce or disappear when patients are given a strategy for directing their attention (Ragland et al., 2003). This suggests the possibility that under 'normal' circumstances (i.e., in the absence of any task instructions) groups may be differentially applying attention and that this may in part underlie group differences in outcome measures. Further, given that attention is relevant to P50, it is possible that patients and controls allocate attention differently during 'normal' P50 tasks: for example, in tasks where participants are simply asked to 'listen to' the stimuli (de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007a; Grunwald et al., 2003). Although ostensibly controlling for attention, these commonly used instructions do not require that attention is actually directed toward stimuli for successful completion of the task, nor do they allow for measurement of where attention has been directed. Nevertheless, if controls are better at following these attentional instructions, then they (but not patients) will have an

attention-related improvement of P50 suppression whereas patients, who are not as good at allocating attention, do not get this improvement of P50 suppression.

The present study will compare the effect of attention on P50 suppression between schizophrenia patients and healthy controls in order to determine whether attention is a confound in schizophrenia P50 research. The present P50 paradigm, in the Non-Attention condition, instructs participants to ignore the auditory stimuli while they watch a concurrently playing silent movie. In the Attention condition, it instructs participants to attend to the auditory stimuli and to respond to infrequently occurring target pairs (where one stimulus in the pair is louder than the other). In order to adequately perform this Attention task, attention must be directed to the auditory stimuli and thus is different from 'Attention' conditions of studies where participants are simply asked to 'listen to' auditory stimuli. Further, as it provides strategies for directing attention to the participants, through the combination of an attentional task and simple instructions (i.e., 'press the button to the loud stimuli'), the present paradigm may act to remove or reduce attentional differences between SCZ and CON (as has been shown to occur in the schizophrenia memory literature, e.g., Ragland et al., 2003).

4.3. Methods

4.3.1. Participants

Twenty-one patients and 18 healthy controls participated in the study (Table 1). To be eligible for inclusion in the study, patients had to be aged between 18 and 55, have a diagnosis of schizophrenia (n = 16) or schizoaffective disorder (n = 5) (SCZ) (American Psychiatric

Association, 2000), be on a stable dose and type of antipsychotic medication (no change over the 4 weeks prior to inclusion in the study), not taking clozapine and not pregnant or breastfeeding. Patients were recruited through the Alfred Hospital outpatient clinic, the Monash Alfred Psychiatry Research Centre participant database, advertisements placed at community mental health organisations and via referral through psychiatrists (with whom the researchers made contact to inform about the study). The Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein, & Opler, 1987) was conducted to assess patients' current clinical symptoms. The MINI International Neuropsychiatric Inventory (MINI) (Sheehan et al., 1998) was administered in order to confirm the diagnosis in the SCZ sample. Healthy controls (CON) were recruited via word of mouth and advertisements placed around community noticeboards. The MINI screen was conducted with CON to screen for the presence of any Axis I psychiatric disorders. All participants gave written informed consent to participate and the study was approved by the University of Wollongong, the Alfred Hospital and Monash University Human Research Ethics Committees.

Table 4.1. Demographic characteristics of the sample are shown.

	SCZ	CON
Age (years)	M = 37.90; SD = 8.26	M = 37.17; SD = 8.82
Gender	9 male; 12 female	9 male; 9 female
Education (years)	13.86 (SD = 3.10)	18.11 (SD = 3.51)
Handedness	20 right; 1 left	17 right; 1 left
PANSS Scores		
Total (range)	73 (52 – 93)	*n/a*
Positive (range)	17 (9 – 28)	*n/a*
Negative (range)	17 (9 – 28)	*n/a*
General (range)	38 (28 – 51)	*n/a*

4.3.2. Procedure

The P50 paradigm was recorded as part of a 3-hour battery of cognitive tasks administered in a fixed order. The P50 paradigm (20 mins duration) was the third task in this battery, and followed a logical memory and word encoding task which together lasted approximately 15 minutes.

Auditory click stimuli were square waves (1 ms duration) generated by Stim2 software, Neuroscan. Stimuli within a pair (S1 and S2) could either be identical in loudness (80 dB), or differ from one another such that one click within a pair was louder (100 dB) than the other.

A total of 200 stimulus pairs were presented over four experimental blocks (two Attention and two Non-Attention blocks) containing 50 stimulus pairs each. The order of experimental blocks was counterbalanced between participants. Half of the participants received the Attention bock first, and half the Non-Attention block first. In the Non-Attention blocks, participants watched a silent movie (Shaun the Sheep), and were told that they would hear auditory clicks through the headphones but that these were unimportant and that they should ignore them and focus on the movie instead. In the Attention blocks, participants were instructed to attend to the auditory clicks and respond with a button press whenever the clicks within a pair differed from one another in loudness. The silent movie was also played during the Attention condition.

The inter-stimulus interval (ISI) between S1 and S2 was constant (500 ms). The inter-pair interval (IPI) between the S2 of one stimulus pair and the S1 of the subsequent stimulus pair was either 2 or 8 s (referred to as IPI2 or IPI8). To provide the appearance of randomness in the IPIs, each mean IPI was achieved by combining 3 randomly chosen IPIs whose mean equalled the target IPI, but which individually did not equal the target IPI: IPI2 (1879, 2035, 2086 ms), and IPI8 (7927, 7964, 8109 ms). In order to be relevant to schizophrenia P50 studies (where 8-10 s IPIs are used: e.g., Boutros, Korzyukov, Jansen, Feingold, & Bell, 2004; Olincy et al., 2010), only the IPI8 trials are analysed here.

Participants sat in a chair 80 cm away from a computer screen, and were fitted with headphones (Sennheiser HD 280 Pro 64Ω). At the beginning of each block, on-screen instructions appeared identifying the block as an Attention or Non-Attention block and the experimenter verbally repeated these instructions. Participants received a short break in between each block, and the P50 paired-stimulus paradigm lasted 20 minutes.

4.3.3. EEG Acquisition

EEG data were recorded continuously using Ag-AgCl electrodes from 19 scalp sites (FP1, FP2, Fz, F3, F4, F7, F8, Cz, C3, C4, T7, T8, Pz, P3, P4, P7, P8, O1, O2) placed according to the international 10-20 system, and from both mastoids (M1, M2). EEG data were grounded midway between Fz and FPz and referenced to the nose. Electrooculogram (EOG) was recorded using Ag-AgCl electrodes placed above (E1) and below (E3) the left eye, and at the outer canthi of the left (E5) and right (E6) eyes. Vertical EOG was derived as E1−E3. All electrode impedances were below 20 kΩ at the start of the recording. The EEG/EOG data were digitised at 500 Hz, and digitally filtered using a .1–100 Hz bandpass filter (SynAmps2). Continuous EEG/EOG data were stored for subsequent offline analysis.

4.3.4. EEG Data Analysis

Data were analysed offline using Neuroscan software (Scan 4.5). The data were EOG corrected (Semlitsch, Anderer, Schuster, & Presslich, 1986), and bandpass filtered from 10–45 Hz (zero-phase shift, 12dB/octave roll-off). Note that although there are a number of possible filters that could be applied, as the most appropriate remains to be determined we used that which has been used for schizophrenia P50 suppression research, in order to ensure that the present results are relevant to the schizophrenia P50 suppression literature (Anokhin, Vedeniapin, Heath, Korzyukov, & Boutros, 2007; Hall, Taylor, Salisbury, & Levy, 2011; Patterson et al., 2008; Schulze et al., 2007). Data were then re-referenced to digitally linked mastoids (Miller, Lutzenberger, & Elbert, 1991), epoched around S1 (from 100 ms pre- to 900 ms post-stimulus), and baseline corrected using the 100 ms pre-stimulus interval. The average amplitude of the 100 ms activity preceding S2 (400 to 500 ms) was calculated in order to baseline correct S2 to its pre-stimulus baseline. Before averaging, an automatic

artefact rejection procedure identified and rejected trials with activity exceeding ± 50 μV in any EEG channel.

4.3.5. P50 Peak Selection

Data were analysed from Cz only (Clementz, Geyer, & Braff, 1998; Mao et al., 2016; Oranje & Glenthoj, 2014), however topographic constraints were imposed on putative P50 peaks to discern these from non-P50 positive-going deflections occurring within the P50 latency range: To be accepted as a true P50 peak they were required to exhibit a fronto-central topography (determined by visually inspecting 2D topographic maps constructed from data obtained from all 19 EEG sites; as per Croft, Dimoska, Gonsalvez, & Clarke, 2004). This resulted in the exclusion of n = 1 CON and no SCZ. Three subjects (CON: n = 2; SCZ: n = 1) were also excluded as their average ERPs contained Pa deflections (15-40 ms post-stimulus; Knott, Millar, Fisher, & Albert, 2010) whose amplitudes were extreme statistical outliers (± 3 times the inter-quartile range) indicating the presence of movement artefacts. Although some P50 studies use principal components analysis (e.g., Clementz & Blumenfeld, 2001), simple peak detection was used here to allow comparison with the majority of schizophrenia P50 studies, including recent studies with large patient samples (Light et al., 2012). P50 peaks were identified manually by one rater. No minimum amplitude was required for a P50 peak to be classified as such. If no peak was present within the required latency range, or if a peak within the latency range did not have a fronto-central topography, the P50 amplitude was scored as '0'. These requirements, however, did not result in any participant's ERPs being assigned a '0' voltage.

P50 peak amplitudes were defined relative to the average amplitude of the S1 pre-stimulus baseline for S1P50 and S2 pre-stimulus baseline for S2P50. The P50 Difference was

defined as the difference between P50 amplitudes to clicks 1 and 2 (i.e., S1P50–S2P50) and the P50 Ratio as the ratio of the P50 amplitudes to clicks 2 and 1 (i.e., S2P50/S1P50). ERP averages were generated for the Attention and Non-Attention conditions separately (n = 50 click pairs in each combination).

The Group grand mean P50 was first defined as the largest positive deflection 40–80 ms post-stimulus (following Rentzsch, Gomez-Carrillo de Castro, Neuhaus, Jockers-Scherubl, & Gallinat, 2008) in the Group grand mean ERP (collapsed across all participants and experimental conditions). Next, we used the Group grand mean P50 latency (± 15 ms) to define each individual subject's grand mean P50 (collapsed across all experimental conditions). Finally, we used the Individual grand mean P50 latency (± 5 ms) to define each subjects' P50 for each attention condition.

4.3.6. Statistical Analyses

To verify task compliance, d', a measure of response sensitivity, was calculated using signal detection analysis (Stanislaw & Todorov, 1999) and compared against '0' for the sample as a whole to determine whether participants performed at greater than chance level (1-sample t-test). To determine whether sensitivity differed between the groups, d' was compared using a between subjects t-test.

For the remainder of the research questions, a repeated measures ANOVA was conducted for P50 amplitudes, with Stimulus (S1P50 versus S2P50 amplitude) and Attention (Non-Attention versus Attention) the within-subjects factors and Group (SCZ versus CON)

the between-subjects factor. The relationships between research questions and aspects of this ANOVA are described below:

1) The main effect of Stimulus was used to determine whether there was a difference between S1P50 and S2P50 amplitudes (i.e., verifying that P50 suppression had occurred);

2) The Attention by Stimulus interaction was used to determine whether, consistent with Dalecki et al. (2015), enhancing attention increased P50 suppression;

3) The Attention by Stimulus by Group interaction was used to determine whether the effect of Attention on P50 suppression differed between SCZ and CON;

4) The Stimulus by Group interaction was used to determine whether P50 suppression differed between the groups overall, in a 'guided attention' P50 suppression task.

Further, as P50 Ratio measures have also been heavily used in the schizophrenia P50 suppression literature, the above P50 suppression analyses were re-run for P50 Ratio for completeness.

4.4. Results

4.4.1. Task Compliance Verification

In the Attention condition, the sensitivity (d') index of the whole sample (M = 2.20, SE = .18) differed significantly from '0' ($p < .001$), and a between-subjects t-test found that d' did not differ between the groups (CON: M = 2.31, SE = .28; SCZ: M = 2.13, SE = .24) ($p = .633$), indicating that the sample performed the attention task appropriately and equivalently.

4.4.2. P50 Suppression Verification

The Stimulus × Attention × Group repeated measures ANOVA revealed a main effect of Stimulus ($p < .001$), with S1P50 amplitude (M = 1.82, SE = .15) larger than S2P50 amplitude (M = .94, SE = .10), indicating that P50 suppression was evident.

4.4.3. Testing for an Attention-Related P50 Suppression Increase

There was an Attention × Stimulus interaction ($p = .001$), with P50 Difference larger under Attention (M = 1.24, SE = .19) than Non-Attention (M = .54, SE = .18) conditions, consistent with Attention enhancing P50 suppression.

4.4.4. Testing for Confounding Effects of Attention on P50 Suppression

There was a trend-level Stimulus × Attention × Group interaction ($p = .100$). Exploratory post hoc analyses are described in section 4.4.7.

4.4.5. Testing for Group Differences in P50 Suppression

There was no significant Stimulus × Group interaction ($p = .823$), indicating that in a 'guided attention' P50 task SCZ did not differ from CON in terms of P50 suppression.

4.4.6. Parallel Analyses (P50 Ratio)

There were no main effects of Attention (p = .255) or Group (p = .200), however there was a trend-level Attention × Group interaction for P50 Ratio (p = .079).

4.4.7. Exploratory Analyses

Although there was no significant Stimulus × Attention × Group interaction for P50 Difference or Attention × Group interaction for P50 Ratio, as both of these interactions reached trend level, to explore whether the effects of attention on P50 metrics were the same across groups paired-samples t-tests compared each of P50 Difference and P50 Ratio, between Non-Attention and Attention conditions, for CON and SCZ separately:

In CON, Attention (versus Non-Attention) increased S1P50 amplitude (p = .006), decreased S2P50 amplitude (p = .037), increased P50 Difference (p = .001) and decreased P50 Ratio (p = .008) (see Table 4.2 and Figure 4.1), consistent with attention enhancing II in healthy participants. In SCZ, Attention (versus Non-Attention) did not affect S1P50 amplitude (p = .119), S2P50 amplitude (p = .867), P50 Difference (p = .215) or P50 Ratio (p = .684) (see Table 4.2 and Figure 4.1).

Table 4.2. S1P50 and S2P50 amplitudes, latencies and P50 Difference and Ratio values for Non-Attention and Attention conditions, in SCZ and CON, are shown.

	Non-Attention	Attention
Controls		
S1P50 amplitude (μV)	1.41 (.13)	2.11 (.22)
S2P50 amplitude (μV)	1.07 (.16)	.74 (.08)
S1P50 latency (ms)	70.67 (1.90)	70.40 (1.40)
S2P50 latency (ms)	71.20 (1.88)	68.27 (1.81)
P50 Difference	.33 (.22)	1.37 (.27)
P50 Ratio	.87 (.15)	.43 (.07)
Schizophrenia Patients		
S1P50 amplitude (μV)	1.72 (.25)	2.05 (.28)
S2P50 amplitude (μV)	.98 (.16)	.95 (.18)
S1P50 latency (ms)	70.60 (1.70)	70.40 (1.21)
S2P50 latency (ms)	67.60 (1.83)	66.60 (1.31)
P50 Difference	.74 (.27)	1.10 (.26)
P50 Ratio	.35 (.21)	.45 (.17)

Note: Standard error is shown in parentheses.

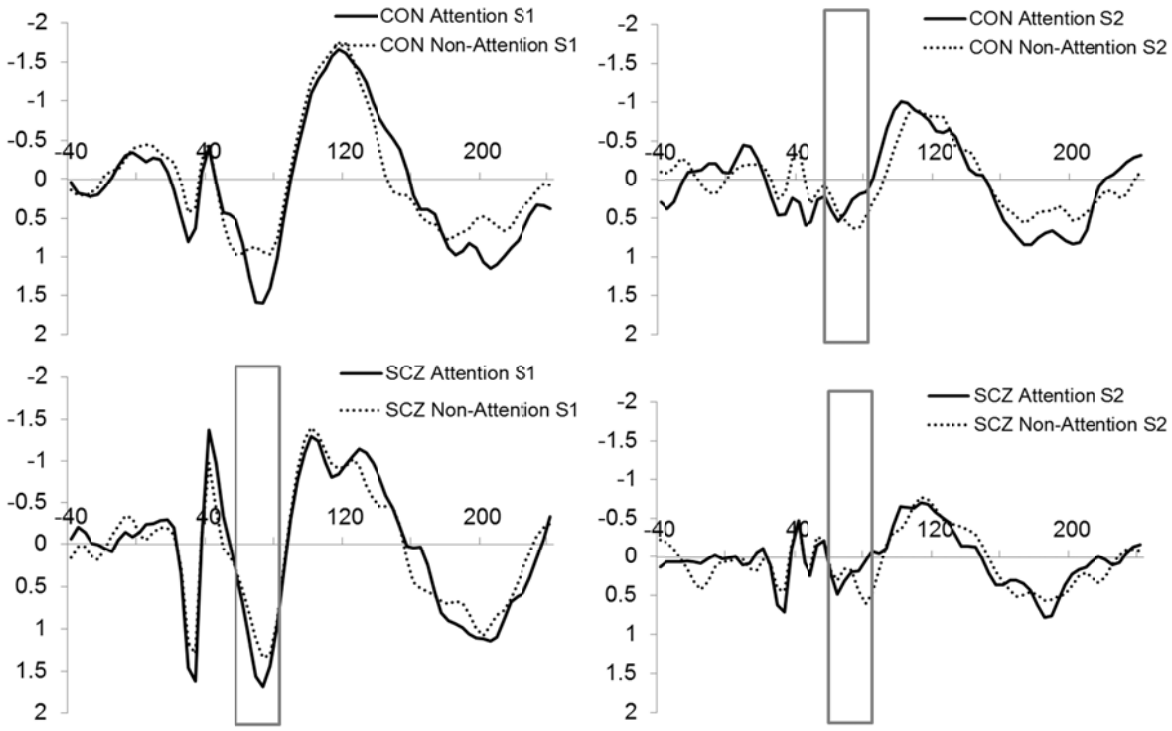

Figure 4.1. S1P50 (left) and S2P50 (right) ERPs under Attention (solid line) and Non-Attention (dotted line) conditions for healthy controls (top) and schizophrenia patients (bottom). P50 ERPs are boxed, and positive is plotted downward.

4.5. Discussion

The present study replicated the finding of Dalecki et al. (2015) in that enhancing attention (Attention versus Non-Attention) increased P50 suppression (P50 Difference), using an 8 s IPI, which is typical in the schizophrenia P50 suppression literature (e.g., Hall et al., 2012; Olincy et al., 2010; Oranje, Aggernaes, Rasmussen, Ebdrup, & Glenthøj, 2013). This demonstrates that attention is relevant to P50 suppression, and that it is not just pre-attentive mechanisms that are captured in the P50 paired-stimulus paradigm.

Although in the present study the effect of attention on P50 Difference did not interact significantly with Group ($p = 0.10$, failing to demonstrate that the effect of attention was reliably different between the groups), when we explored the effect of attention in each group separately we found that attention affected P50 in healthy controls only, and did not affect any P50 measure in schizophrenia patients. Specifically, the present study found that Attention (compared with Non-Attention) increased S1P50, *decreased* S2P50 amplitude and consequently increased P50 suppression (as indexed by a larger P50 Difference) in healthy controls. This pattern of effects is consistent with previous research showing that enhancing attention enhances the functionality of inhibitory inputs; that is, it increases P50 suppression (i.e., *reduces* S2P50 amplitude) (Dalecki et al., 2015).

The results of the present study demonstrate a role for attention in P50 suppression. Taken together with the fact that attention is often poorly controlled in P50 suppression studies, and that schizophrenia patients are known to have attentional impairments (Cornblatt & Malhotra, 2001; Gold, Fuller, Robinson, Braun, & Luck, 2007; Hong et al., 2011), these findings provide strong evidence that attention may be an important confound in the schizophrenia P50 suppression literature. That is, in studies that utilise 'passive' P50 suppression paradigms, in which participants are not provided with an overt task with respect to the auditory stimuli (Tregellas et al., 2007), it is unclear how participants are directing their attention. It is therefore possible that healthy participants may direct their attention toward auditory stimuli while schizophrenia patients do not, resulting in an attention-related enhancement of P50 suppression in healthy subjects but not schizophrenia patients. This may also occur in studies where participants are simply instructed to 'listen to' the auditory stimuli (de Wilde et al., 2007a). Since no measure of adherence to these instructions (and thus of correct, and similar, orientation of attention across groups) is made in these cases, again it is

possible that it is controls who, better able to follow attentional instructions, will demonstrate an attention-related enhancement of S1P50 and reduction of S2P50 and thus a larger P50 Difference, and again, that this difference in attention will produce apparent group differences in P50 suppression. The strength of this conclusion is limited by the fact that we did not include an "uncontrolled attention" condition (for example a condition with either no instructions, fixating a cross, or sitting with eyes closed) in the present study. Consequently, we cannot demonstrate that, in the absence of task instructions, controls selectively attend to the auditory stimuli more than schizophrenia patients do, thus obtaining an attention-related enhancement of P50 suppression that is not seen in patients. Future studies will be necessary in order to evaluate this possibility.

Nevertheless, the effect of attention on P50 suppression found in the present study suggests that controlling attention in schizophrenia studies is important in order to exclude the possibility that group differences in P50 suppression arise due to attentional differences between the groups. In the present study, attention was carefully controlled by assigning participants an easy task thus: 1) not challenging the participants (particularly the schizophrenia patients), as stressful or difficult tasks have been shown to impair P50 suppression (White & Yee, 1997) and; 2) giving simple instructions that schizophrenia patients could easily follow. Indeed, an analysis of the behavioural data showed that both schizophrenia patients and healthy controls performed the task appropriately and similarly. Under these conditions, where differential allocation of attention (and thus differential attention-related effects on P50 suppression mechanisms) across the groups is reduced we did not find impaired P50 suppression in schizophrenia patients (as indexed by either P50 Difference or P50 Ratio) (Adler et al., 1986; Olincy et al., 2010). Thus it may be that the

effect seen in the literature is due not to impaired P50 suppression in schizophrenia per se, but related to uncontrolled attention.

The present study has shown that attention affects P50 suppression such that enhancing attention increases P50 suppression. The presence of attentional impairments in SCZ suggests the potential for differences in the direction of selective attention between SCZ and CON, particularly in cases where attentional instructions are not given. Thus where the direction of attention is not controlled, differences between SCZ and CON attributed to P50 suppression, may instead reflect attentional differences.

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5. CHAPTER 5: DISCUSSION

5.1. Summary of Thesis Findings

Despite 30 years of schizophrenia P50 suppression research, whether schizophrenia patients have reduced P50 suppression has, to date, remained unclear due to failures to replicate this finding, reports of low (or absent) P50 suppression reliability, and the unclear relation between methodology and both P50 suppression magnitude and reliability. Further, what a possible P50 suppression reduction in schizophrenia may represent is unclear as the functional process represented by P50 suppression in healthy samples is in itself poorly understood. Finally, whether attention is a confound in schizophrenia P50 suppression remains to be determined, with the answer to this question having implications for the interpretation of the extant schizophrenia P50 suppression literature as well as for P50 suppression study design (e.g., the possible need for careful control of attention in these studies).

The present doctoral research demonstrated (in Study 1; Dalecki, Croft, & Johnstone, 2011) that P50 suppression can be reliably measured using both the P50 Ratio and P50 Difference metrics, and that P50 Difference reliability is greater than that of P50 Ratio, showing that this is the more robust P50 suppression metric. P50 suppression (Difference) was also more reliable for P50 amplitudes defined from BP (compared to PP) and when more (n = 40-100) than less (n = 10-30) trials were averaged in obtaining P50 measures. That, with adequate methodology, P50 suppression can be reliably measured suggests that studies with adequate methodology should be able to detect P50 suppression impairments in schizophrenia (where they exist). In terms of what functional process this represents, this doctoral research has provided support for the hypothesis that inhibitory inputs (and not simple refractory periods)

underlie P50 suppression in healthy participants and that attention enhances this inhibition (Study 2; Dalecki et al., 2011). Finally, the present research replicated the attention-related enhancement of P50 suppression in healthy participants, and, failing to find the same in schizophrenia patients, showed that attention is a confound in P50 suppression schizophrenia research (Study 3). This shows that where P50 *suppression* is of interest, attention should be carefully controlled in order to ensure that potential group differences do not arise purely due to attentional differences between the groups (in the absence of true P50 suppression differences between them). As the direction of attention was carefully controlled in Study 3, and no schizophrenia-healthy control differences were found for P50 suppression, the results of the present doctoral research suggest the possibility that the schizophrenia-control P50 difference reported in the literature may indeed reflect attentional, and not P50 suppression differences between these groups.

5.2. Contribution of the Thesis Findings to the Literature

5.2.1. Methodology

With respect to methodological questions, the present research found that under conditions where participants (a) were provided with attentional instructions (and thus attentional variability within-session was minimised), (b) 100 stimulus-pairs were presented and averaged to obtain P50 ERP components, and (c) the effect of time on P50 was taken into account when estimating reliability, P50 suppression was reliable as measured by both P50 Ratio (ICC = .44) and P50 Difference methods (ICC = .72). Further, that P50 Difference reliability was greater than that for Ratio supports the view that P50 Difference is the more reliable P50 paired-stimulus metric (Rentzsch, Jockers-Scherubl, Boutros, & Gallinat, 2008).

In support of this, P50 Difference was more sensitive in detecting time-related changes in P50 suppression within-session (finding a difference between P50 Difference in the first 14 minutes versus each of four subsequent 14-minute blocks of the experiment), whereas P50 Ratio detected the time-related change in P50 suppression only where it was largest (i.e., first 14 minutes compared to the second 14-minute block only, not compared to the third, fourth or fifth 14-minute blocks).

However, in itself the P50 metric utilised (i.e., P50 Difference versus Ratio) does not account for the difference between positive and negative schizophrenia P50 suppression studies. For example, while some negative schizophrenia P50 suppression studies (i.e., those failing to find a P50 suppression deficit in schizophrenia), analysed only P50 Ratio (and not P50 Difference; Arnfred, Chen, Glenthoj, & Hemmingsen, 2003; Gjini, Burroughs, & Boutros, 2011; Kathmann & Engel, 1990; Turetsky, Bilker, Siegel, Kohler, & Gur, 2009), which may suggest that the failure to detect a difference could be due to poorer P50 Ratio reliability, some negative studies analysed both P50 Ratio and Difference and failed to find a group difference for either measure (de Wilde, Bour, Dingemans, Koelman, & Linszen, 2007; Domjan, Csifsak, Drotos, Janka, & Szendi, 2012; Light et al., 2012). Indeed, among recent positive studies, schizophrenia patient-healthy control differences have been reported for P50 Difference but not P50 Ratio (Hazlett et al., 2015), for P50 Ratio but not P50 Difference (Sanchez-Morla et al., 2013), for P50 Ratio (where Ratio was the only P50 measure analysed) (Wonodi et al., 2014), and for both P50 Difference and P50 Ratio (Smith et al., 2013).

The present doctoral research also showed that P50 amplitude definition affects P50 suppression reliability, with BP (versus PP) producing more reliable P50 Difference measurements (Study 1; Dalecki et al., 2011). This finding is in line with the results of

Rentzsch, Jockers-Scherubl, et al. (2008) who similarly found superior P50 suppression reliability for BP P50 amplitude derivations. However, Rentzsch, Jockers-Scherubl, et al. (2008) achieved superior reliability for P50 *Ratio* using BP P50 amplitude derivations while Study 1 achieved superior reliability for P50 Difference. This difference in results may stem from the fact that Rentzsch, Jockers-Scherubl, et al. (2008) used the pre-stimulus baseline preceding S1 to derive both S1 and S2P50 amplitudes, whereas the present study used the S1 pre-stimulus baseline to derive S1P50 amplitude and the S2 pre-stimulus baseline to derive S2P50 amplitude, which provides a more relevant baseline for the S2 P50 peak. A future study would be necessary to determine whether this methodological difference accounts for the difference in results. Nevertheless, the present demonstration of superior P50 suppression reliability for BP P50 amplitude derivations also fits with observations reported in the literature (Clementz & Blumenfeld, 2001; Smith et al., 2013) regarding the difficulty in unambiguously identifying a trough preceding the P50 relative to which to measure its PP amplitude. If this trough is difficult to identify reliably, the variability introduced into the resultant measurement may account for the superior reliabilities achieved with BP amplitude derivations reported here (Study 1; Dalecki et al., 2011) and by Rentzsch, Jockers-Scherubl, et al. (2008). Yet, despite the potential problems with measuring P50 amplitudes relative to the preceding negative trough (i.e., PP), this is the more commonly used measure within the P50 suppression literature. It may thus be useful for future studies to report PP alongside BP values as was done here, and by Rentzsch, Jockers-Scherubl, et al. (2008) and Arnfred et al. (2003) in order to accumulate evidence as to whether the more reliable BP measurements result in more statistically robust results.

Again however, P50 amplitude definition method is not, on its own, sufficient to account for the difference between positive and negative studies. While the majority of negative P50

suppression schizophrenia studies defined P50 amplitudes from PP (de Wilde et al., 2007; Domjan et al., 2012; Gjini et al., 2011; Light et al., 2012; Turetsky et al., 2009), there are also reports of negative findings for P50 amplitudes defined from BP (Arnfred et al., 2003). Similarly, positive findings come from studies using both PP (Hazlett et al., 2015; Sanchez-Morla et al., 2013; Wonodi et al., 2014) and BP (Clementz & Blumenfeld, 2001; Smith et al., 2013) defined P50 amplitudes.

Study 1 (Dalecki et al., 2011) also demonstrated that P50 reliability is dependent on the number of trials contributing to averaged P50 ERP components, suggesting that P50 paired-stimulus paradigms with too few trials may yield P50 metrics with inadequate reliability and thus have limited statistical power to detect relationships between P50 and other variables. This may be a particular issue for studies where the P50 Ratio is the primary suppression metric as P50 Difference showed reliability values consistently in the 'fair-to-good' range (defined as .40 - .75 by Fleiss, 1986) with 40 or more trials averaged, whereas 80 or more trials were needed to achieve this with P50 Ratio. It is possible that insufficient numbers of trials averaged may have contributed to some negative findings in the literature.

For example, among negative P50 suppression schizophrenia studies, the number of trials contributing to averaged P50 ERP components ranged from 64 (Kathmann & Engel, 1990) to 120 (Arnfred et al., 2003; Light et al., 2012; Turetsky et al., 2009). One hundred and twenty trials should be sufficient to obtain adequate measures of both P50 Ratio and Difference according to Study 1 (where reliable P50 measures were obtained with 100 trials averaged). However, as 80 or more trials were necessary in order to consistently obtain fair-to-good reliability for P50 Ratio (Study 1; Dalecki et al., 2011), the 64 trials recorded in the Kathmann and Engel (1990) study (where P50 Ratio was the only suppression metric

analysed) may not have been sufficient to obtain a reliable measure of P50 suppression and thus may have contributed to that study's failure to find impaired P50 suppression in schizophrenia. However, among positive studies, the number of trials contributing to averaged P50 ERP components did not always exceed 80 but can range from 32 to 150 (e.g., Hazlett et al., 2015; Sanchez-Morla et al., 2013; Wonodi et al., 2014). Thus again, the number of trials averaged does not, alone, differentiate between positive and negative studies and thus does not explain the different results in the schizophrenia P50 suppression literature.

Given the number of methodological parameters on which individual studies differ from one another, it is difficult, from a review of the literature, to conclude whether the methodology of any one individual study is adequate to achieve reliable P50 suppression. For example, while some studies averaged together a sufficiently large number of trials (as per Study 1), they may have measured only P50 Ratio (whereas Study 1 suggested Difference was more reliable) (e.g., Turetsky et al., 2009), or where they measured P50 Difference with amplitudes defined from BP (more reliable than PP as per Study 1), they then averaged together an *in*sufficient number of trials (as per Study 1) (e.g., Smith et al., 2013) or failed to provide participants with attentional instructions (as per Study 2, described below; e.g., Hazlett et al., 2015; Kathmann & Engel, 1990), thus possibly introducing variability due to attentional fluctuations within those experiments. Therefore, the approach taken in Study 1 (Dalecki et al., 2011), namely, an evaluation of P50 methods *within* a study, is likely to be more useful than an overview of the variable literature in discerning the most appropriate methodologies for P50 suppression measurement.

With respect to methodological effects on P50 *magnitude*, the present research has also demonstrated that time-related effects on P50 have important implications for study design.

Study 1 (Dalecki et al., 2011) demonstrated that P50 suppression is not invariant across time within an experiment, but reduces within-session such that it is strongest at the start (e.g., first 14-minute block) than all later times (e.g., four subsequent 14-minute blocks) of an experiment. Firstly, this justifies our quantification of P50 suppression reliability for trials equally distributed across time within-session (thus ensuring measurements are each equally affected by time related P50 changes) and suggests that where this was not done before, studies may have underestimated 'true' P50 reliability (e.g., Lamberti, Schwarzkopf, Boutros, Crilly, & Martin, 1993). Further, the reduction in P50 suppression over time within-session highlights the importance of carefully counterbalancing experimental conditions within an experiment and of experimental condition order across groups (e.g., schizophrenia versus control). This is necessary as the within-session time effect in Study 1 (Dalecki et al., 2011) was large enough to reduce P50 suppression in healthy participants to the point where P50 Ratio in these participants, initially robust, approached values reported in schizophrenia (Patterson et al., 2008). Thus careful counterbalancing is required in order to avoid obtaining P50 suppression measurements for different conditions from different time points across the session, and thus creating apparent differences where none may exist.

Further, the finding that P50 suppression reduces across time within-session also suggests that shortening the duration of P50 suppression studies may be useful, in that such experiments would obtain P50 measurements at the point where P50 suppression is most robust in healthy participants and thus may be more sensitive in capturing alterations in P50 suppression among psychiatric comparison groups. Although this could be achieved by reducing the number of trials presented, the results of the present thesis do not support this approach as P50 suppression reliability did not differ from '0' when small (i.e., $n = 10$ for P50 Difference; $n < 40$ for P50 Ratio) numbers of trials were averaged together. Further, P50

suppression reliability only fell within the 'fair-to-good' range (.40 - .75, as defined by Fleiss, 1986) when more than 40 (P50 Difference) or 80 (P50 Ratio) trials contributed to averaged P50 ERP components.

However, Study 1 demonstrated that the duration of the P50 paradigm duration could also be reduced by reducing the duration of the IPI separating consecutive stimulus pairs. We demonstrated this by showing that the IPI can be reduced from 9 s to 3 s without affecting P50 suppression (P50 Difference or Ratio), S1P50 or S2P50 amplitudes, thus replicating the findings of Rentzsch, Gomez-Carrillo de Castro, Neuhaus, Jockers-Scherubl, and Gallinat (2008), who similarly, failed to find a difference between P50 Ratios obtained at IPIs of 8 and 2.8 s and extending this conclusion to IPIs of 7, 5, and 3 s using more rigorous methodology; Rentzsch, Gomez-Carrillo de Castro, et al. (2008) provided different attentional instructions to participants across the two IPI conditions in their study, meaning that these were not exactly comparable, whereas participants in Study 1 performed only one (the same) attentional task, meaning that differing attentional tasks could not have been a confound with respect to IPI in the present research (Study 1; Dalecki et al., 2011). Thus these results demonstrate that P50 measures equivalent to those obtained using an 8 s IPI can be obtained when the duration of this parameter is reduced to 3 s. Employing a shorter IPI in the P50 paired-stimulus paradigm would allow for a larger number of trials to be presented in a shorter time period. For example, using a 3 s IPI would mean that 240 trials could be presented in 14 minutes (the time period where P50 suppression was strongest in Study 1), whereas a 9 s IPI would only allow for the presentation of 90 trials over the same time period. However the use of short IPIs is not yet common practice in the P50 suppression literature, where the vast majority of studies employ IPIs of at least 8 s. Indeed to the best of our knowledge, there are only two P50 suppression studies (with the exception of the two studies

comparing long and short IPIs: Dalecki et al., 2011; Rentzsch, Gomez-Carrillo de Castro, et al., 2008) that have reported data using shorter IPIs: Crawford, McClain-Furmanski, Castagnoli, and Castagnoli (2002) who used an IPI of 5 s, and Rentzsch, Jockers-Scherubl, et al. (2008) who used an average IPI of 2.8 s (achieved by averaging trials with IPIs of 1.5, 3.0, 3.8 and 4.6 s).

That reducing IPI from 9 s to 3 s did not affect any P50 metric is also revealing about the behaviour of the neuronal mechanism(s) underpinning P50 suppression. That is, these results suggest that whatever neuronal process underlies P50 suppression, it is as functional (recovered) 3 s after the presentation of the previous stimulus pair as it is after 9 s. While the earliest point at which these mechanisms recover is yet to be firmly established, accumulating evidence (Dolu, Suer, & Ozesmi, 2001), including Study 1, which showed that a 1 s IPI was insufficient for full recovery, suggests that it takes up to 3 s for the processes involved in P50 suppression to fully recover and thus be fully functional in response to the next pair of auditory stimuli. Conversely, this suggests that at IPIs of less than 3 s the processes involved in P50 suppression are *not* fully recovered. This suggests novel paradigms for understanding P50 suppression, such as comparisons of P50 amplitudes in the context of the P50 paired-stimulus paradigm across conditions where P50 suppression mechanisms are fully functional (e.g., IPI of 8 s) against those where they are not (e.g., IPI of 2 s)[7].

The results of the evaluation of P50 methodology conducted in Study 1 have relevance for the design of P50 suppression studies and were applied in the design of Studies 2 and 3 of the present thesis. P50 amplitudes in Studies 2 and 3 were defined relative to the pre-stimulus baseline and P50 Difference was used as the primary suppression metric, with P50 Ratio

[7] Discussed in Sections 5.2.2 and 5.2.3.

results provided alongside P50 Difference where appropriate (in order to allow for comparison with much of the P50 suppression literature, where P50 Ratio is often reported). However, noting concerns about the validity of this approach (given that P50 Difference is correlated highly with S1P50 amplitude and thus may reflect stimulus registration more closely than it does P50 suppression; Fuerst, Gallinat, & Boutros, 2007; Lu et al., 2007), the present thesis also reported S1P50 and S2P50 amplitudes- with effects on S2P50 amplitude (upon which putative suppression mechanisms are theorised to act) taken to be strong evidence of changes in P50 suppression. To ensure the time-related effects on P50 did not disproportionately affect certain experimental manipulations (e.g., attention direction and IPI) these were equally distributed across time within-session.

The reliability of P50 suppression in any given study is thus dependent on the adequacy of its methodology (with the parameters evaluated here discussed above and other parameters remaining to be addressed in future research). Notwithstanding unresolved methodological questions, following the demonstration that P50 suppression can be reliably measured, it is important to better clarify what process P50 suppression (and its impairment) represents in both healthy and schizophrenia samples. The resolution of this question was the aim of Studies 2 and 3 and is discussed below.

5.2.2. Functional Significance of P50 Suppression

Study 2 (Dalecki, Johnstone, & Croft, 2015) utilised a P50 paradigm incorporating both long (8 s) and short (2 s) IPIs that, respectively, do and do not (as per Study 1; Dalecki et al., 2015) allow for the full recovery of the mechanism(s) involved in P50 suppression, with the aim of determining which of the current hypotheses of P50 suppression (II or RP) best

account for the effects of this manipulation on P50 suppression. It also addressed this question by manipulating attention as another method by which mechanisms underlying P50 suppression could be manipulated (for example, enhanced, by increasing attention toward auditory stimuli). This was done in order to characterise the process represented by "normal" P50 suppression (i.e., intact P50 suppression in healthy subjects). These manipulations of the functionality of mechanisms underlying P50 suppression were consistent with the II, but not RP hypothesis of P50 suppression. The effects of reducing P50 suppression mechanism functionality (by reducing IPI) and enhancing it (by enhancing attention) are discussed in turn.

Reducing the functionality of P50 suppression mechanisms (by reducing IPI from 8 to 2 s, in the Non-Attention protocol: thereby challenging P50 suppression due to incomplete recovery at the short IPI) tended to both reduce S1P50 amplitude (reflecting less engagement of the neuronal population proposed to engage inhibitory inputs; Freedman et al., 1996) and *increase* S2P50 amplitude (consistent with less inhibitory input engagement, and thus less inhibition occurring at the arrival of S2). *Enhancing the functionality of P50 suppression mechanisms* by directing attention toward (versus away from) auditory stimuli (in the IPI2 protocol where P50 suppression mechanisms were challenged) *reduced* S2 P50 amplitude, consistent with Attention enhancing inhibitory inputs, and inconsistent with RP, which predicts increased S2P50 amplitude with enhanced Attention (as, if Attention enhanced the P50 generator response, this would have produced an increase in both the S1 and S2P50 amplitudes). That the effects of two methods of manipulating the functionality of P50 suppression mechanisms (via IPI and Attention manipulations) were both consistent with II but not RP (Dalecki et al., 2015), this provides strong support to the view that inhibitory inputs subserve P50 suppression in healthy participants (Freedman et al., 1996).

This demonstration (of the functional process underlying 'normal' P50 suppression in a healthy sample) is important as it has relevance for the conceptualisation of P50 suppression 'impairment', such as that reported in schizophrenia. Specifically, as the results of Study 2 suggest that P50 suppression in healthy participants reflects the *attentionally mediated* function of *inhibitory inputs* operating on, and triggered/engaged by P50 generator neurons, this suggests that impaired P50 suppression in schizophrenia could reflect either: 1) Impaired inhibitory input functionality; or 2) Impaired attention (manifesting in either less direct attentional enhancement of inhibitory inputs or in smaller S1P50 amplitudes, reflecting less P50 generator activation, and the subsequent reduction/failure to engage inhibitory inputs).

That Study 2 (Dalecki et al., 2015) found support for inhibitory inputs subserving P50 suppression is consistent with the schizophrenia neurobiological literature showing that schizophrenia patients have reduced expression of inhibitory (GABAergic) receptors in brain regions presumed to be involved in P50 suppression, such as hippocampus (Mizukami et al., 2000), and pre-frontal (Fatemi, Folsom, & Thuras, 2011) and temporal cortices (Mizukami et al., 2002), as well as reduced expression of nicotinic acetylcholine receptors which activate GABAergic interneurons (Freedman, Hall, Adler, & Leonard, 1995; Miwa, Freedman, & Lester, 2011). Further, these results are consistent with the results of intervention studies showing that enhancing nicotinic receptor functioning (e.g., via smoking; Adler, Hoffer, Wiser, & Freedman, 1993) or through rapid eye movement sleep (which allows desensitised nicotinic receptors to re-sensitise) (Griffith, Waldo, Adler, & Freedman, 1993) improves P50 suppression. Further, given the role of frontal cortex in attention (Herrmann & Knight, 2001; Kane & Engle, 2002), the present findings of attentional enhancement of P50 suppression are also consistent with neuroimaging (Ehlis et al., 2009) and lesion (Knight, Richard, Swick, & Chao, 1999) studies showing more haemodynamic activation of pre-frontal cortex in

participants with robust P50 suppression, and impaired P50 suppression in subjects with pre-frontal lesions. However given the role of attention in P50 suppression, the results of Study 2 (Dalecki et al., 2015) suggest the need for careful consideration of attention in P50 suppression research generally, its potentially confounding effects in schizophrenia P50 suppression research, as well as novel avenues by which future research could probe the nature of inhibitory input function (and thereby dysfunction in schizophrenia). These will be discussed in turn.

Despite it being commonly assumed within the P50 suppression literature that P50 suppression is a pre-attentive process (Braff & Light, 2004; Cadenhead, Light, Shafer, & Braff, 2005; Martin et al., 2007), only few studies have empirically tested this assumption. Further, only two prior studies have specifically compared the effects on P50 of manipulating the direction of attention *toward* versus *away from* auditory stimuli (Gjini et al., 2011; Kho et al., 2003), with neither study finding an effect between those conditions. As Study 2 (Dalecki et al., 2015) found that attention modulated P50 suppression (P50 Difference) at both the 8 s (in contrast with Gjini et al., 2011; Kho et al., 2003) and 2 s intervals (which had not been looked at previously), possible reasons for the difference in results between the studies merit consideration.

In Study 2, the auditory (paired tone) and visual (silent movie) stimuli presented to participants were identical across attention conditions and only the direction of attention was presumed to change. That is, in the Attention task participants were instructed to attend to the auditory stimuli in order to perform an auditory discrimination task while a silent movie played on screen. In the Non-Attention task, participants were instructed to ignore the auditory stimuli and instead watch the silent movie which was playing on screen. On the

other hand, in the Gjini et al. (2011) and Kho et al. (2003) studies, the 'attention toward' and 'attention away from auditory stimuli' conditions differed not only in the direction of attention; the stimuli participants were presented with also differed across conditions. For example, Kho et al. (2003) directed attention *toward* auditory stimuli by asking participants to silently count the stimulus pairs but directed attention *away* from stimuli by asking them to complete a reverse digit span task. This may have been more stressful/difficult than counting stimuli in the 'attention' task (with stress known to affect P50 suppression; White & Yee, 1997). In addition, the timing of stimulus presentation differed across the two tasks; in the 'attention away' (reverse digit span) task, the digits for the task were presented first, and then 3 stimulus pairs were presented while the participant rehearsed for later recall. Further, visual stimuli (i.e., the visually presented digits) were shown only in the 'attention away' but not 'attention toward' task. Similarly, Gjini et al. (2011) directed attention *toward* stimuli by asking participants to respond to infrequent deviant pairs, but directed attention *away* from stimuli using a visual continuous performance task. Thus again, participants were shown visual stimuli in one, but not the other attention condition. Variability in the task difficulty, timing of stimulus presentation (Kho et al., 2003) and modality of stimuli presented to participants across attention conditions (Gjini et al., 2011; Kho et al., 2003) may have introduced additional variability into P50 measurements, which limits the power of these studies to detect any possible attention effects. Finally, the wide age range of participants (≈ 24-70 years) in the Gjini et al. (2011) pilot study may have introduced variability into the measurements and obscured any potential effects.

The finding (Study 2; Dalecki et al., 2015) that attention enhances P50 suppression in healthy participants also has relevance for P50 suppression research more generally. Firstly, it suggests that where P50 suppression *per se* (as opposed to the attentional enhancement

thereof) is the neuronal process of research interest, P50 paired-stimulus protocols with attention directed toward the auditory stimuli may not be appropriate. This is because such protocols will capture P50 suppression combined with the attention-related enhancement of the P50 generator (S1P50) and/or of inhibitory inputs (acting on S2P50). Secondly, this finding also calls into question the appropriateness of P50 protocols which do not provide participants with any instructions pertaining to the auditory stimuli. Such protocols cannot discern where participants are orienting their attention and thus cannot discern the neuronal process they are measuring (i.e., whether it is P50 suppression, or the attentional enhancement of P50 suppression). The same argument applies to protocols which nominally provide attention instructions (e.g., 'listen to' the auditory stimuli), but whose compliance is not measurable. Finally, this finding raises questions about the appropriateness of 'uncontrolled attention' P50 suppression protocols in schizophrenia research, due to the known attention impairments present in this population (Chen & Faraone, 2000; Cornblatt & Malhotra, 2001). This issue will be dealt with further in Section 5.2.3.

The Study 2 findings (Dalecki et al., 2015) also suggest novel strategies to further P50 suppression research both in terms of probing the nature of P50 suppression in healthy samples, as well as the nature of the P50 suppression deficit in schizophrenia patients and identifying interventions for ameliorating it. For example, given the demonstration (Study 2; Dalecki et al., 2015) that scalp-recorded P50 suppression is consistent with the operation of underlying inhibitory inputs, it may be useful for future research to map the temporal course of this inhibition in both healthy and schizophrenia populations. Currently, a constant (500 ms) ISI is ubiquitously used in P50 suppression research, based predominantly on an early finding (Freedman, Adler, Waldo, Pachtman, & Franks, 1983) that this interval provides the greatest separation between schizophrenia patients and healthy controls. However, the

information provided using this one, constant ISI cannot resolve interesting questions about the nature of inhibitory differences between patients and controls. For example, it cannot determine whether it is the *magnitude* of inhibition/suppression that differs between groups or the *timing* of it (i.e., it is possible that controls demonstrate robust suppression at 500 ms, whereas schizophrenia patients demonstrate equally robust suppression at an earlier or later point, but not at 500 ms). Research incrementally manipulating the ISI separating S1 and S2 would be needed to resolve this question. Such basic research delineating the time course of P50 suppression in both groups would also inform the design of P50 protocols more sensitively-able to capture relevant differences.

The results of the present doctoral research are also applicable to research aimed at identifying interventions capable of ameliorating P50 suppression deficits. Much of this research is conducted on healthy subjects. Such studies often split their samples into 'good/high' and 'poor/low P50 suppressors', and probe the efficacy of, for example, pharmacological (e.g., nicotine administration; Knott et al., 2013) interventions in ameliorating these relative P50 suppression reductions. The P50 protocol used in Study 2 may complement (or present a novel alternative) to such research strategies given that P50 suppression was relatively reduced (i.e., impaired/challenged) in healthy participants by reducing IPI from 8 to 2 s in the standard Non-Attention P50 paired-stimulus paradigm or by reducing attention (Non-Attention versus Attention) (Dalecki et al., 2015). Future studies might profitably investigate whether these experimentally induced reductions of P50 suppression (perhaps in combination with stratifying a participant sample into 'good' and 'poor' suppressors) impair P50 suppression in poor suppressors further, and would thus enhance the sensitivity of studies seeking to remediate this impairment via, for example, pharmacological (Knott et al., 2013) strategies.

Overall, and consistent with the II hypothesis of P50 suppression (Freedman et al., 1996; Javitt & Freedman, 2015) as well as a large body of literature including genetic (Freedman et al., 2003; Leonard & Freedman, 2006) and binding (Freedman et al., 1995) studies, Study 2 (Dalecki et al., 2015) demonstrated effects on S2P50 amplitude (after manipulation of the strength of P50 suppression functionality) consistent with inhibitory input action and inconsistent with simple refractory periods. Further, Study 2 found that P50 suppression was not pre-attentive, but that attention increased P50 suppression. This suggests that attention needs to be carefully controlled in P50 suppression studies generally (something that is not often done) and, due to known attentional impairments in schizophrenia, suggests a strong possibility that attention may be an important confound in this research. Study 3 addressed this issue.

5.2.3. Attention Confounds P50 Suppression in Schizophrenia

Consistent with Study 2 (Dalecki et al., 2015), Study 3 demonstrated that enhanced attention increased P50 suppression (in the long IPI protocol typical of P50 paired-stimulus schizophrenia research), providing further support for the conclusion that P50 suppression is not pre-attentive. Furthermore, and also consistent with the results of Study 2, enhancing attention increased S1P50 amplitude and *decreased* S2P50 amplitude in healthy controls, consistent with the pattern of effects predicted by the II, but not the RP hypothesis of P50 suppression. That these attentional effects were seen in controls only (attention did not affect any P50 metric in schizophrenia patients) demonstrates that attention is a confound in schizophrenia P50 suppression research. Finally, when attention was controlled, no difference in P50 suppression was seen between schizophrenia patients and healthy controls suggesting

that the schizophrenia-control P50 suppression difference reported in the literature may reflect attentional differences rather than differences in P50 suppression between the groups. However, it should be qualified that although statistically significant effects of attention were found in the controls but not patients, statistical differences between the groups in terms of the effect of attention were not observed. This finding highlights the importance of attention in P50 schizophrenia research, but also the need for future research to clarify it further.

With respect to the functional nature of P50 suppression, in exploring the trend-level interactions between Attention and Group for P50 suppression (Difference and Ratio), Study 3 found that in healthy controls, enhancing Attention increased S1P50 amplitude, *decreased* S2P50 amplitude and increased P50 suppression (as indexed by a larger P50 Difference and smaller P50 Ratio). In schizophrenia patients on the other hand, enhancing Attention (versus Non-Attention) failed to affect any P50 measure. This pattern of effects is consistent with the II hypothesis of P50 suppression and the results of Study 2 (Dalecki et al., 2015). Specifically, the attention-related increase in S1P50 amplitude is consistent with attention enhancing P50 generator firing/functionality. The attention-related *decrease* in S2P50 amplitude is consistent with either the enhanced firing of the P50 generator enhancing inhibitory input engagement, or with attention directly enhancing inhibitory input firing/functionality. In Study 3, as in Study 2, the effect of attention on P50 amplitudes was not consistent with the RP hypothesis of P50 suppression, which would predict attentional enhancement of the P50 generator population, and thus an increase in both S1 and S2P50 amplitudes. Thus, taken together, the results of Studies 2 and 3 provide strong support for the II hypothesis of P50 suppression (Freedman et al., 1996; Javitt & Freedman, 2015).

That Study 3 replicated the attention-enhancing effect of P50 suppression found in Study 2

suggests that the direction of participants' attention must be taken into consideration in interpreting the results of any P50 suppression study (irrespective of the study sample). This is because the neuronal process(es) measured depends on the direction of attention. That is, without attention directed to the auditory stimuli, the neuronal process measured is P50 suppression itself (i.e., the action of inhibitory inputs on the P50 generator neuronal population). However, with attention directed *toward* auditory stimuli (i.e., enhanced), the neuronal process measured is P50 suppression combined with the attentional enhancement of P50 suppression. It follows that P50 suppression studies which fail to provide any attentional instructions to participants will be limited in terms of the interpretation of the neuronal process(es) that are reflected in their studies.

The attention effect on P50 suppression is particularly relevant in schizophrenia P50 suppression research, where attentional impairments are argued to be biomarkers for schizophrenia, independent of P50 suppression itself (Chen & Faraone, 2000; Cornblatt & Malhotra, 2001). This difference in attentional capabilities between the groups suggests the strong possibility that in P50 suppression protocols where attention is not controlled (for example, where no instructions pertaining to the auditory stimuli are provided to participants; e.g., Oranje & Glenthoj, 2014), that controls, with superior attentional capabilities, may (even in the absence of any instruction to do so) attend to the stimuli, and thereby obtain an attentional enhancement of P50 suppression. Conversely, schizophrenia patients may not direct their attention toward stimuli and thereby not obtain the same attentional enhancement of P50 suppression. In such a scenario, and with the knowledge that attention enhances P50 suppression, it is possible that any resultant differences in P50 suppression between the groups may be more properly attributed (at least in part) to differences in attention between the groups.

It is possible that the provision of attentional instructions to the groups may not (in all cases) resolve this difficulty. Due to the differences in attentional capabilities, the attention instructions provided must be clear and simple enough for the psychiatric sample to adequately follow. If the attention instructions provided are too difficult, the controls will again (being better able to follow them) be the only group who obtains the attention-related enhancement of P50 suppression. Indeed, simple instructions have been used in the schizophrenia memory and encoding literature, where schizophrenia patients' performance (under conditions where they are provided with appropriate attentional strategies) is similar to that of healthy controls (Bonner-Jackson, Haut, Csernansky, & Barch, 2005; Weiss et al., 2003). Similarly, in Study 3 where attention instructions were simple (i.e., press the button to the loud stimulus), schizophrenia patients performed the task similarly to healthy control subjects. The careful control of attention in P50 suppression studies (via the provision of similar attentional instructions) would increase the strength of conclusions that group differences in P50 suppression reflect differences in inhibitory input function as opposed to differences in attention between groups.

Without such control, it is impossible to unambiguously interpret whether P50 suppression differences between schizophrenia patients and controls reflect a difference in P50 suppression, or merely in attention. Yet many (both positive and negative) schizophrenia P50 suppression studies do not provide participants with explicit instructions pertaining to the auditory stimuli (e.g., Hazlett et al., 2015; Kathmann & Engel, 1990; Light et al., 2012; Sanchez-Morla et al., 2013; Turetsky et al., 2009). And where they do, the extent to which participants follow instructions such as "listen to" (de Wilde et al., 2007; Wonodi et al., 2014) or 'ignore" (Smith et al., 2013) the stimuli is not measureable. In light of the attention-enhancing effects of P50 suppression in healthy participants (Studies 2 and 3; Dalecki et al.,

2015), and given that attention is impaired in schizophrenia (Braff, 1993) yet poorly controlled (or not controlled at all) in many P50 studies, there is a strong possibility that attention is an important confound in this P50 research. However, as the direction of attention is not measured in most of these studies, it is impossible to determine from the extant literature whether this is the case. Study 3 controlled attention (and verified that both controls and schizophrenia patients performed the attentional task appropriately and similarly) and failed to find a group difference in P50 suppression (as indexed by either the P50 Difference or Ratio metrics) or P50 amplitudes. Given that the schizophrenia-healthy control P50 suppression difference was not found after removing (or at least reducing) attentional differences between the groups suggests that the group difference reported in the literature may reflect attentional differences between schizophrenia patients and controls, rather than differences in P50 suppression between them.

5.3. Limitations

The attentional manipulations used in Studies 2 and 3 were limited in that we were not certain that participants' attention was in fact directed away from paired stimuli in the Non-Attention task. We were, however, confident that participants' selective attention was directed *toward* paired stimuli in the Attention condition; behavioural analyses showed that both groups performed the task appropriately. Further, that in Studies 2 and 3 we found an effect of attention on P50 provides strong support that attention did in fact differ between the Attention and Non-Attention conditions. Nevertheless, it may be beneficial for future studies to provide attentional instructions to participants which allow for unambiguous verification that attention is directed away from stimuli under Non-Attention conditions.

Secondly, the strength of the conclusion that 'P50 suppression differences reported in the literature may be due to attentional differences between schizophrenia patients and controls (due to uncontrolled attention in P50 studies)' is limited by the fact that the present thesis (whilst controlling attention) did not include a condition where attention was not controlled (such as a passive condition where no instructions pertaining to the stimuli are provided to the participants). Therefore the present research cannot determine whether P50 suppression differences between the groups would have arisen under 'uncontrolled attention' conditions and future research would be required in order to determine this.

Finally, although Study 1 (Dalecki et al., 2011) demonstrated optimal P50 metric reliability was achieved when large (n = 80-100) numbers of trials were averaged together in generating P50 ERP components, the necessity to minimise experiment duration when working with a schizophrenia sample meant that in Study 3 only 50 trials were presented for each experimental condition (i.e., Attention and IPI) combination. This may have meant that reliability, and thus the statistical power to detect group differences may not have been as high as it otherwise would have been had larger numbers of trials been presented. However, we carefully controlled attention in order to reduce variance due to attentional variability, and reported the P50 Difference as the primary P50 suppression metric (for P50 amplitudes derived from BP) in order to maximise reliability. Further, we recorded data from 19 channels in order to be able to use topographic information to help identify 'true' P50 peaks (a methodological improvement on some schizophrenia P50 studies which record EEG data from the Cz channel only, thus limiting the ability to verify that positive-going EEG deflections in the P50 latency range are indeed true P50 ERP components; e.g., Kathmann & Engel, 1990; Mao et al., 2016; Olincy et al., 2010).

It should be noted that although first investigated in relation to the P50 ERP, later auditory mid-latency ERPs are increasingly being studied in the context of the paired-stimulus paradigm. These include the N1 (Boutros et al., 2009; Boutros, Korzyukov, Jansen, Feingold, & Bell, 2004; Brockhaus-Dumke et al., 2008) and P2 (Anokhin, Vedeniapin, Heath, Korzyukov, & Boutros, 2007; Boutros et al., 2004). As these later ERPs differ from the P50 with respect to their reliability (Fuerst, Gallinat, & Boutros, 2007), heritability (Anokhin et al., 2007), as well as how they are affected by sleep (Kisley et al., 2003), the results of the present thesis, which has not evaluated these later auditory components, apply solely to the P50 and without further research cannot be generalised to other mid-latency auditory ERPs.

5.4. Differences in Methodologies Employed Across Studies 1-3

Studies 1, 2 and 3 of the present thesis differed from one another in several methodological respects including: duration, task instructions, number and characteristics (clicks vs. tones) of auditory stimulus pairs and the duration of the IPIs between successive stimulus pairs. In order to characterise P50 paired click measures over time and in order to present enough stimuli in order to be able to characterise P50 paired click measures under different IPIs (1, 3, 5, 7 and 9 seconds), Study 1 was long in duration (≈ 78 minutes; broken up into 5**,** 14-minute blocks). In order to avoid imposing unreasonable time demands on participants, Study 1 did not additionally address the issue of attention and was carried out with only one task instruction (namely that participants attend to and silently count the click pairs, responding with a button press after every 25th click pair). Study 2 was likewise long in duration (≈ 132 minutes; broken up into 20**,** 6.6-minute blocks) and extended on Study 1 in that it involved a manipulation of attention (with attention alternately directed toward the stimulus pairs or

toward a concurrently playing silent movie). As stimuli were being presented under one of two attention conditions (vs. one in Study 1), only two IPI conditions (2 and 8 seconds) were included in Study 2. Finally the need to minimise demands on the schizophrenia sample in Study 3 meant that the duration of that study was limited to 20 minutes (broken up into 4, 5-minute blocks). In order to replicate the attention and IPI results from Study 2 and determine their relevance to schizophrenia, the same Attention and IPI conditions were incorporated in Study 3, however the numbers of trials presented was greatly reduced (1,440 in Study 2 vs. 200 in Study 3). The studies also differed from one another in respect of the auditory stimulus characteristics, with the stimuli in Studies 1 and 3 being 'clicks' (square waves of 1ms duration) and Study 2 being 1000 and 2000 Hz tones (also 1ms in duration).

It follows that differences between the results of the different studies are not unexpected, making it particularly important to focus on the main hypotheses in each study, rather than trying to extrapolate all features of one study to another.

5.5. Future Directions

Future research might profitably pursue further clarification of methodological issues likely to be relevant to P50 suppression measurement and about which little is currently known. One avenue which might usefully be pursued is the clarification of how digital filter parameters, and specifically the direction of digital filter application (i.e., analogue simulation versus zero-phase shift) and digital filter high- and low-pass values affect P50 suppression measurement. The 10-45 Hz digital filter used in the studies that form the basis of the present thesis was chosen as these filter values are most commonly applied in the P50 literature (see Patterson et al., 2008 for a review) as well as in recent (e.g., Mao et al., 2016;

Winterer et al., 2013) and large P50 schizophrenia studies (e.g., Hall, Taylor, Salisbury, & Levy, 2011) and thus render the present results comparable to the extant literature, and in particular other P50 schizophrenia studies. However, for a number of theoretical reasons, a better understanding of several characteristics of this filter may be useful.

Firstly, the 'filter direction' is important because the application of a zero-phase shift filter may in principle confound the P50 due to activity from later latencies (containing the larger amplitude N1 and P2 ERP components) being artificially 'moved' to the latency of the P50, whereas an analogue simulation digital filter (applied in the left-to-right direction only) would not have this effect. As the vast majority of P50 studies do not report the direction in which their filters are applied, whether this is a problem in the P50 suppression literature cannot currently be discerned and further, if it is a problem, it is impossible to determine which studies in the literature may be affected by this potential confound. Secondly, a better understanding of how digital filter high pass values affect P50 may be useful. The commonly used 10 Hz high pass (Patterson et al., 2008) separates the P50 from the lower-frequency, attentionally-mediated N1 (Jerger, Biggins, & Fein, 1992). However, numerous studies employ lower high-pass values (ranging from .1 to 8 Hz; Patterson et al., 2008). If zero-phase shift filters indeed confound earlier (e.g., P50) ERP components with activity from later ones (e.g., N1, P2), the use of lower (e.g., 2 Hz) high-pass values may produce artefactual effects on the P50 which should be properly attributed to later (lower-frequency) ERP components. Further, and precisely because of the extent of methodological variability between studies, this approach could usefully be extended to an evaluation of other methodological parameters. One such useful extension may be an examination of the instructions provided to participants. Although this was not experimentally manipulated in Study 1, and in the absence of empirical evidence regarding the best instructions to provide to participants, in

Study 1 participants were instructed to silently count the number of trials presented. The rationale behind this being that a) it may reduce attentional variability, as attention would, if instructions were followed appropriately, be directed toward the stimuli for the duration of the experiment; and b) provide a measurable index (i.e., number of trials counted) that would allow for the determination of how appropriately the attentional task was performed. By contrast, the majority of both the positive and negative studies discussed above neither provided explicit instructions pertaining to the stimuli, or where they did, the extent to which participants followed these were not measurable. Two studies (one positive, one negative) did not report what instructions were provided to participants; two (one positive, one negative) instructed participants to 'listen to' the stimuli, three (two negative, one positive) instructed participants to fixate a distant target, one (negative) study instructed participants to read a newspaper, one (positive) instructed participants to ignore the stimuli, and finally in one (negative) study participants were seated upright with their eyes closed (but no further instructions were reported).

5.6. Conclusion

The present research demonstrated that the manner in which attention and IPI manipulations affect P50 amplitudes is consistent with inhibitory inputs, and not simple refractory periods of the P50 generator, subserving P50 suppression. This is significant because it demonstrates what process is represented by 'normal' P50 suppression; that is, in healthy individuals. Further, this research was the first of those manipulating attention *toward* versus *away from* auditory stimuli to demonstrate that enhancing attention toward auditory stimuli in the P50 paradigm increases P50 suppression in healthy participants (Dalecki et al., 2015). It subsequently replicated this result in an independent sample (Study 3). This is significant

because it shows that P50 suppression is not entirely pre-attentive, but rather, that attention is relevant to P50 suppression (Studies 2 and 3). Finally, this research demonstrated (in Study 3) that attention is a confound in schizophrenia P50 suppression research, increasing P50 suppression in healthy participants but not in schizophrenia patients. This highlights a clear need for rigorous control of attention in future schizophrenia P50 suppression studies in order that the direction of participants' selective attention is measurable/known and in order to avoid attributing group differences to P50 suppression 'impairment' where they may be occurring due to attentional differences between the groups. Further, to the extent that P50 suppression itself (versus attentional *enhancement of* P50 suppression) is of interest to researchers, providing participants with an attentional task with respect to the auditory stimuli may obscure this aim by conflating inhibition and attention. Finally, in the present research, where attention was controlled and schizophrenia patients and healthy controls performed the attentional task provided to them appropriately (and equally well), group differences in P50 suppression (as measured by both the P50 Difference and P50 Ratio) were not found, suggesting that the schizophrenia patient–healthy control difference in P50 suppression reported in the literature may reflect impaired attention in patients, and not suppression *per se*.

5.7. References

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