Multisensory Integration Impairment in Rodent Models of Schizophrenia: Converging Evidence for Remediation by Nicotinic Receptor Stimulation of the GABAergic System

by

Jacob Milton Cloke

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Doctor of Philosophy
in
Psychology + Neuroscience

Guelph, Ontario, Canada
© Jacob Milton Cloke, 2016
ABSTRACT

MULTISENSORY INTEGRATION IMPAIRMENT IN RODENT MODELS OF SCHIZOPHRENIA: CONVERGING EVIDENCE FOR REMEDIATION BY NICOTINIC STIMULATION OF THE GABAERGIC SYSTEM

Jacob Milton Cloke  
University of Guelph, 2016

This thesis investigated the selective multisensory integration impairment in ketamine-treated rats, a rodent model of schizophrenia. Previous findings have shown that ketamine-treated rats are impaired on a crossmodal object recognition (CMOR) task, but not on similar tests that do not require multisensory binding. In the current thesis, this effect was replicated and extended using a novel multisensory object oddity (MSO) task, which directly assesses multisensory binding in the absence of significant memory demands; ketamine-treated rats were impaired on tactile-visual and olfactory-visual MSO, but not on similar unimodal oddity tasks. Systemic administration of nicotine reversed the CMOR and MSO impairments in ketamine-treated rats. This effect appears to be mediated by α4β2 nicotinic acetylcholine receptors (nAChR), as the selective α4β2 agonist ABT-418, but not the selective α7 nAChR agonist GTS-21, restored CMOR and MSO performance in ketamine-treated rats. The involvement of nAChRs in remediating this cognitive impairment was explored in the orbitofrontal (OFC) and medial prefrontal (mPFC) cortices. Intra-OFC, but not intra-mPFC, ABT-418 restored multisensory cognition in ketamine-treated rats. Prompted by past research indicating dysfunctional GABAergic transmission in schizophrenia and ketamine-treated rats, we hypothesized that decreased OFC GABAergic function disrupts multisensory cognition and that OFC nAChRs restore performance in ketamine-treated rats by enhancing GABAergic release. Accordingly, the
GABA\textsubscript{A} antagonist bicuculline blocked the remediating effect of intra-OFC ABT-418 on CMOR and MSO performance. Additionally, whole-cell electrophysiology revealed disrupted GABAergic transmission in the OFC of ketamine-treated rats, and activation of $\alpha_4\beta_2$ nAChRs restored GABAergic function. Moreover, immunohistochemical analyses indicated that GABAergic parvalbumin (PV)-interneuron (PV-IN) expression was decreased in the OFC of ketamine-treated rats. Thus, DREADDS (Designer Receptors Exclusively Activated by Designer Drugs) were used to silence OFC PV-INs in PV-Cre mice, revealing a selective MSO deficit that could be reversed by ABT-418. This thesis therefore proposes that dysfunction of OFC PV-INs in ketamine-treated rats causes their multisensory impairment and that activation of $\alpha_4\beta_2$ nAChR on PV-INs restores essential inhibitory transmission reversing the multisensory deficit. Given the parallels between schizophrenia and the current rodent model, pharmacological therapies targeting the GABAergic system and/or GABAergic-nicotinic interactions hold promise for treating specific cognitive symptoms in schizophrenia.
Acknowledgements

Thank you to my advisor, Dr. Boyer Winters, for his support and guidance throughout my PhD. You ‘spur’-red my interest in behavioural neuroscience and contributed greatly to my ‘arsenal’ of skills. Completing my PhD under your supervision was a fantastic experience and I will never forget it.

Thank you to my committee members, Dr. Craig Bailey and Dr. Francesco Leri. Craig, you have helped me immensely throughout my PhD and I learned what dedication and hard work are by working in your lab. Francesco, you’ve supported the development of my career since my undergraduate degree, and I’m glad to have had that continue into my graduate career.

Thank you to the many undergraduate students that contributed to my PhD research. In particular, I would like to give a special thanks to Stephanie De Lisio who helped me develop the multisensory oddity task. As well, I would like to thank Warren Bignell who helped me learn whole-cell electrophysiology.

Thank you to the Winters Lab and NACS group for their help and support throughout my PhD. I am lucky to have shared these past couple years with a great group of people and I wish the best to everyone. To Stephen, Dave, and Tom: thanks for giving me an excuse to get out of the house.

Thank you to my parents, Lisa and Norm Koeth, for their continuous support throughout my PhD. You always encourage me to work harder and I wouldn’t be here without either of you.

Lastly, I would like to thank Mikaela Stiver. You have supported my dreams and I have supported yours. You have been my motivation and I have been yours. Through all the early mornings, late nights, and long days, you have always been there. I am so unbelievably lucky to have you in my life. Together we can accomplish anything, and I cannot wait to see what is next for us.
Table of Contents

Abstract ................................................................................................................................. i
Acknowledgments ................................................................................................................ iv
List of Tables .......................................................................................................................... ix
List of Figures ....................................................................................................................... x
List of Abbreviations ............................................................................................................. xi

Chapter 1: Literature Review .............................................................................................. 1

Schizophrenia ......................................................................................................................... 2
Cognition in Schizophrenia .................................................................................................... 2
  Abnormal Multisensory Integration ...................................................................................... 3
Neurobiological Bases of Schizophrenia .............................................................................. 7
  Glutamate Hypothesis ......................................................................................................... 7
    NMDA Receptor Hypofunction Rodent Model of Schizophrenia ...................................... 8
GABA Hypothesis .................................................................................................................. 9
Integration of Glutamate and GABA Hypotheses ................................................................. 12
GABA and Multisensory Integration .................................................................................... 13
Cognition in Animal Models of Schizophrenia ................................................................. 15
  Object Recognition .......................................................................................................... 15
  Crossmodal Object Recognition ....................................................................................... 17
  Oddity Task ....................................................................................................................... 19
The Cholinergic System ......................................................................................................... 21
  Nicotinic Acetylcholine Receptors and Schizophrenia ....................................................... 22
  Nicotinic Acetylcholine Receptor Interaction with GABA ................................................ 23
Orbitofrontal Cortex ............................................................................................................. 24
Current Study ....................................................................................................................... 26

Chapter 2: α4β2 Nicotinic Receptor Stimulation of the GABAergic System
Within the Orbitofrontal Cortex Ameliorates the Severe Crossmodal Object
Recognition Impairment in Ketamine-Treated Rats: Implications for
Cognitive Dysfunction in Schizophrenia. Neuropharmacology, 90, 42-52 ........... 28

Abstract ............................................................................................................................... 29
Introduction ............................................................................................................................ 30
Methods .................................................................................................................................. 33
  Subjects .............................................................................................................................. 33
  Sub-Chronic Drug Administration ...................................................................................... 33
Chapter 3: Rat Multisensory Oddity Task Development

Abstract.................................................................................62
Introduction...........................................................................63
Methods..................................................................................65
   Subjects..............................................................................65
   Oddity Tasks......................................................................65
   Object Recognition..........................................................68
   Experiments......................................................................69
      Experiments 1.1 & 1.2.....................................................69
      Experiments 2.1 & 2.2.....................................................69
      Experiment 3..................................................................69
Data Analysis..........................................................................70
Results...................................................................................70
   Experiments 1.1 & 1.2.........................................................70
   Experiments 2.1 & 2.2.........................................................71
Chapter 4: Generalized Multisensory Oddity Task Impairment in Ketamine-Treated Rats is Reversed by $\alpha_4\beta_2$ Nicotinic Receptor Stimulation of the GABAergic System in the Orbitofrontal Cortex

Abstract .......................................................................................................................... 76
Introduction ....................................................................................................................... 77
Methods ............................................................................................................................. 79
  Subjects ............................................................................................................................ 79
  Sub-Chronic Drug Administration .................................................................................. 80
  Surgery ........................................................................................................................... 80
  Infusion Procedure ........................................................................................................ 81
  Histology ....................................................................................................................... 81
  Immunohistochemistry ................................................................................................. 81
  Brain Slice Preparation and Electrophysiology ............................................................ 82
  Oddity Tasks ................................................................................................................. 84
Experiments ...................................................................................................................... 84
  Experiments 1.1 & 1.2 .................................................................................................. 84
  Experiments 2.1 & 2.2 .................................................................................................. 84
  Experiments 3.1 & 3.2 ................................................................................................. 85
  Experiments 4.1 & 4.2 ................................................................................................. 85
  Experiments 5.1 & 5.2 ................................................................................................. 86
  Experiments 6.1 & 6.2 ................................................................................................. 86
  Experiments 7.1 & 7.2 ................................................................................................. 86
  Experiments 8.1 & 8.2 ................................................................................................. 87
  Experiment 9 ................................................................................................................. 87
  Experiment 10 ............................................................................................................... 88
Data Analysis .................................................................................................................... 88
Results ............................................................................................................................... 89
  Experiments 1.1 & 1.2 ................................................................................................. 89
  Experiments 2.1 & 2.2 ................................................................................................. 90
  Experiments 3.1 & 3.2 ................................................................................................. 91
  Experiments 4.1 & 4.2 ................................................................................................. 92
  Experiments 5.1 & 5.2 ................................................................................................. 92
  Experiments 6.1 & 6.2 ................................................................................................. 93
  Experiments 7.1 & 7.2 ................................................................................................. 96
  Experiments 8.1 & 8.2 ................................................................................................. 96
  Experiments 9 ................................................................................................................. 98
  Experiments 10 ............................................................................................................. 101
Discussion ....................................................................................................................... 101
Chapter 5: Pharmacogenetic Inhibition of Orbitofrontal GABAergic Parvalbumin-Interneurons Impairs Multisensory Oddity Task Performance in Mice: Remediation by $\alpha_4\beta_2$ Nicotinic Receptor Activation ......................... 108

Abstract ................................................................................................................. 109

Introduction ............................................................................................................. 110

Methods .................................................................................................................... 111
  Subjects .................................................................................................................. 111
  AAV Vector Construction ....................................................................................... 111
  Surgery .................................................................................................................... 112
  Drugs ...................................................................................................................... 112
  Immunohistochemistry .......................................................................................... 112
  Oddity Tasks .......................................................................................................... 113
  Experiments ........................................................................................................... 113
    Experiment 1 ......................................................................................................... 113
    Experiment 2 ......................................................................................................... 113
    Experiment 3 ......................................................................................................... 114
    Experiment 4 ......................................................................................................... 114
    Experiment 5 ......................................................................................................... 114
  Data Analysis ......................................................................................................... 115

Results ...................................................................................................................... 115
  Experiment 1 .......................................................................................................... 115
  Experiment 2 .......................................................................................................... 116
  Experiment 3 .......................................................................................................... 116
  Experiment 4 .......................................................................................................... 119
  Experiment 5 .......................................................................................................... 119

Discussion .............................................................................................................. 120

Chapter 6: General Discussion .............................................................................. 124

Viable Tasks to Assess Multisensory Cognition in Rodents ................................... 127

Generalized Multisensory Impairment in a Rodent Model of Schizophrenia ........... 129

Prefrontal $\alpha_4\beta_2$ nAChRs Restore Multisensory Cognition in Ketamine-Treated Rats 132

GABAergic-nAChR Mechanism Restores Multisensory Cognition in Ketamine-Treated Rats. 136

Future Directions ................................................................................................... 142

Conclusions ............................................................................................................. 144

References .............................................................................................................. 147
### List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>Total sample exploration for CMOR task experiments</td>
<td>59</td>
</tr>
<tr>
<td>Table 2</td>
<td>Total choice exploration for CMOR task experiments</td>
<td>60</td>
</tr>
<tr>
<td>Table 3</td>
<td>Electrophysiological properties of neurons recorded from ketamine- and saline-treated rats</td>
<td>105</td>
</tr>
<tr>
<td>Table 4</td>
<td>Total exploration for rats oddity task experiments</td>
<td>106</td>
</tr>
<tr>
<td>Table 5</td>
<td>Total exploration for mice oddity task experiments</td>
<td>123</td>
</tr>
</tbody>
</table>
**List of Figures**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oddity task design.</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>CMOR task design.</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Effect of systemic co-administration of nicotine and bicuculline on CMOR</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Effect of systemic co-administration of nicotine and MK-801 on CMOR</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Effect of ABT-418 and GTS-21 systemic administration on CMOR</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Effect of systemic co-administration of ABT-418 and bicuculline on CMOR</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Cannulations of the orbitofrontal cortex.</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Effect of orbitofrontal cortex nicotine administration on CMOR performance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Effect of orbitofrontal ABT-418 administration and co-administration with</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>bicuculline on CMOR performance in ketamine-treated rats.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>MSO task design.</td>
<td>67</td>
</tr>
<tr>
<td>10</td>
<td>MSO task (tactile-visual &amp; olfactory-visual) and unimodal oddity task (tactile-, visual-, olfactory-only) performance in rats.</td>
<td>70</td>
</tr>
<tr>
<td>11</td>
<td>Culmination of MSO task performance in rats across 5 min.</td>
<td>71</td>
</tr>
<tr>
<td>12</td>
<td>MSO object control experiment.</td>
<td>72</td>
</tr>
<tr>
<td>13</td>
<td>Selective MSO task impairment in ketamine-treated rats.</td>
<td>89</td>
</tr>
<tr>
<td>14</td>
<td>Effect of systemic nicotine on MSO task performance in rats</td>
<td>90</td>
</tr>
<tr>
<td>15</td>
<td>Effect of systemic ABT-418 on MSO task performance in rats</td>
<td>91</td>
</tr>
<tr>
<td>16</td>
<td>Effect of systemic GTS-21 on MSO task performance in rats</td>
<td>92</td>
</tr>
<tr>
<td>17</td>
<td>Cannulations of the orbitofrontal cortex.</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>Effect of orbitofrontal cortex nicotine administration on MSO task</td>
<td></td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Cannulations of the medial prefrontal cortex.</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Effect of medial prefrontal cortex nicotine administration on MSO task</td>
<td></td>
</tr>
<tr>
<td></td>
<td>performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Effect of orbitofrontal cortex ABT-418 administration and co-administration</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>with bicuculline on MSO task performance in ketamine-treated rats</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Whole-cell electrophysiology recordings of GABAergic postsynaptic currents</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>in ketamine- and saline-treated rat orbitofrontal cortex.</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Expression of parvalbumin in ketamine- and saline-treated rat orbitofrontal</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>cortex.</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>MSO task (tactile-visual &amp; olfactory-visual) and unimodal oddity task (tactile-, visual-, olfactory-only) performance in mice.</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>Effect of systemic ketamine on MSO task and unimodal oddity performance in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mice. Effect of systemic CNO on MSO task performance in mice.</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Expression of hM4D-mCherry in the orbitofrontal cortex of PV-Cre mice.</td>
<td>118</td>
</tr>
<tr>
<td>24</td>
<td>Effect of pharmacogenetic inhibition of orbitofrontal cortex PV-INs in PV-</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Cre on MSO and unimodal oddity task performance. Effect of ABT-418 on CNO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>induced MSO impairment.</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Overview of GABAergic-nAChR mechanism.</td>
<td>145</td>
</tr>
</tbody>
</table>
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>CMOR</td>
<td>Crossmodal object recognition</td>
</tr>
<tr>
<td>CNO</td>
<td>Clozapine-N-oxide</td>
</tr>
<tr>
<td>CNTRICS</td>
<td>Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia</td>
</tr>
<tr>
<td>DHβE</td>
<td>Dihydro-β-erythroidine hydrobromide</td>
</tr>
<tr>
<td>DNMS</td>
<td>Delayed non-match-to-sample</td>
</tr>
<tr>
<td>DREADDS</td>
<td>Designer Receptors Exclusively Activated by Designer Drugs</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GAD67</td>
<td>Glutamic acid decarboxylase 67</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>MATRICS</td>
<td>Measurement and Treatment Research to Improve Cognition in Schizophrenia</td>
</tr>
<tr>
<td>MLA</td>
<td>Methyllecaconitine</td>
</tr>
<tr>
<td>mPFC</td>
<td>Medial prefrontal cortex</td>
</tr>
<tr>
<td>MSO</td>
<td>Multisensory oddity</td>
</tr>
<tr>
<td>nAChR</td>
<td>Nicotinic acetylcholine receptor</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-d-aspartate</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbitofrontal cortex2</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>PolyI:C</td>
<td>Polyinosinic-polycytidylic acid</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PV</td>
<td>Parvalbumin</td>
</tr>
<tr>
<td>PV-IN</td>
<td>Parvalbumin interneuron</td>
</tr>
<tr>
<td>PV-ir</td>
<td>Parvalbumin immunoreactivity</td>
</tr>
<tr>
<td>SOM</td>
<td>Somatostatin</td>
</tr>
<tr>
<td>sPSCs</td>
<td>Spontaneous postsynaptic currents</td>
</tr>
<tr>
<td>VIP</td>
<td>Vasoactive-intestinal peptide</td>
</tr>
</tbody>
</table>
Chapter 1

Literature Review
Schizophrenia

Schizophrenia is a debilitating mental disorder with a lifetime prevalence rate of 1%. The Diagnostic and Statistical Manual (DSM)-IV characterizes the disorder by three types of symptoms: positive symptoms – hallucinations, delusions, and loss of contact with reality; negative symptoms – negative affect, anhedonia, apathy, and alogia; and cognitive symptoms – impairment in attention, working memory, learning/memory, and social cognition (Mueser & McGurk, 2004). The wide range of symptoms in schizophrenia produces complications in areas such as work, relationships, social interactions, and personal life (Mueser & McGurk, 2004); this affects everyday functioning in patients. Both genetic and environmental factors, such as poverty and social economic status, appear to increase likelihood of the disorder. Furthermore, schizophrenia is associated with increased risk for drug use, disease, and other mental illnesses, which escalates the mortality rate by 5% (Mueser & McGurk, 2004). Schizophrenia is one of the top 10 causes of disability, as people with the disorder make up 50% of all hospital admissions weighing substantial economic and social costs (Mueser & McGurk, 2004). Thus, the aetiology and possible treatments for symptoms related to schizophrenia require further investigation to help ease these personal and societal burdens.

Cognition in Schizophrenia

Cognitive dysfunction in schizophrenia is thought to be a core component of the disorder with cognitive symptoms often present prior to full diagnosis (Nuechterlein et al., 2004). Moreover, cognitive impairment seems to be a stronger predictor of the outcome of the disorder than the positive or negative symptoms (Green, Kern, & Heaton, 2004). Interest in studying the cognitive deficits associated with schizophrenia has arisen from their association with quality of everyday function, and novel treatment approaches are targeting this component of the disorder. Two recent initiatives, Measurement and Treatment Research to Improve Cognition in
Schizophrenia (MATRICS) and Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS), are assessing cognitive dysfunction in schizophrenia in order to advance treatment strategies. MATRICS has identified eight cognitive domains that are impaired in schizophrenia and replicate across the disorder: speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, verbal comprehension, and social cognition (Young, Powell, Risbrough, Martson, & Mark, 2009). Thus, the cognitive symptoms in schizophrenia appear to be a core component of the disorder and continuing research is required to reduce the severity of these impairments. Accordingly, CNTRICS has suggested, among other things, the establishment of a battery of tests to assess multiple facets of cognition in animal models of schizophrenia. Such research, in combination with clinical studies, should yield a more complete understanding of the underlying neurobiology and potential therapies for schizophrenia-related cognitive deficits.

**Abnormal multisensory integration in schizophrenia**

Incoming sensory information from multiple modalities (vision, touch, hearing, etc.) generates our everyday experience in the world. Single sensory modalities are experienced simultaneously, and the brain facilitates the integration of sensory information into multisensory representations; this process is called multisensory integration. Recently, the literature has indicated that there appears to be abnormal multisensory integration in schizophrenia (Tseng et al., 2015). Indeed, CNTRICS has suggested the use of a ‘cross-modal integration’ task to be included in their recommended battery of tests for use with animal models of schizophrenia (Butler, Silverstein, & Dakin, 2008). Despite this recommendation, few viable options currently exist for the study of crossmodal or multisensory integration in rats or mice, the species most
commonly used in such studies. One of the major aims of the current thesis was to address this problem.

Multisensory integration is facilitated by congruent presentation of task-relevant stimuli; this is known as multisensory facilitation. For example, our natural ability to perceive speech is enhanced by the simultaneous presentation of auditory and visual information or both hearing speech and seeing the appropriate lip movements. The benefit of providing visual articulation for perceiving speech is deficient in patients with schizophrenia (Ross et al., 2007). At a more basic level, congruent presentation of visual and auditory targets typically reduces reaction time to produce simple responses in comparison to reaction times for unimodal targets. Evidence suggests that schizophrenia patients show increased (Williams, Light, Braff, & Ramachandran, 2010), reduced (Stone et al., 2011), or normal (Wynn, Jahshan, & Green, 2014) reaction times to the presentation of bimodal targets compared to controls, depending on testing conditions. Furthermore, susceptibility to the rubber hand illusion, which requires the integration of congruent proprioceptive, visual, and tactile information, has been shown to be increased (Peled, Pressman, Geva, & Modai, 2003; Peled, Ritsner, Hirschmann, Geva, & Modai, 2000) or decreased in schizophrenia patients (Ferri et al., 2013). In further support of basic multisensory processing deficits, a dampened N100 (a negative evoked potential 100 milliseconds following stimulus presentation) evoked potential response, which is associated with multisensory integration in normal participants, is not apparent in schizophrenia patients during audio-visual integration (Stekelenburg, Maes, Van Gool, Sitskoorn, & Vroomen, 2013) (Stekelenburg & Vroomen, 2007). Thus, while it is apparent that multisensory integration is abnormal in schizophrenia, the specific nature of this abnormality appears to vary with task requirements.
Conversely, incongruent presentation of stimuli is known to decrease facilitation effects; this is known as multisensory interference. Incongruent presentation of audio-visual information can produce a fusion effect known as the McGurk effect (McGurk & MacDonald, 1976). For example, the presentation of visual “ga” while hearing “ba” can produce the perception of “da” (McGurk & MacDonald, 1976). This is a prime illustration of how the brain integrates incoming sensory information into a unified percept. Multiple studies have shown that patients with schizophrenia are less susceptible to the McGurk Effect (de Gelder, Vroomen, Annen, Masthof, & Hodiamont, 2002; Pearl et al., 2009; White et al., 2014). Furthermore, patients with schizophrenia appear to have intact speech recognition without the support of visual information, as well as intact lip reading (de Gelder et al., 2002; Ross et al., 2007; White et al., 2014). These results suggest a deficit in multisensory integration in schizophrenia patients independent of unisensory impairments. Although there is inconsistency in the literature, with studies demonstrating that patients and control subjects do not differ in their susceptibility to the McGurk Effect (Martin, Giersch, Huron, & van Wassenhove, 2013; Surguladze et al., 2001), the weight of evidence suggests atypical multisensory integration in schizophrenia patients.

Social cognition is also compromised in patients with schizophrenia (Green, Olivier, Crawley, Penn, & Silverstein, 2005), and the link between social cognition and multisensory integration has previously been examined. The ability to use emotional vocalizations to aid in categorizing emotional faces appears to be decreased in schizophrenia patients (de Gelder et al., 2004; de Jong, Hodiamont, & de Gelder, 2010; de Jong, Hodiamont, Van den Stock, & de Gelder, 2009; Van den Stock, de Jong, Hodiamont, & de Gelder, 2011). Furthermore, Seubert et al. (2010) examined the effect of odour priming on facial recognition and demonstrated decreased
priming in schizophrenia patients, specifically within the disgust category. Clearly there is a connection between social cognition and multisensory integration in schizophrenia patients.

Schizophrenia patients display a longer window of simultaneity, such that they require a longer time between presentation of stimuli to determine whether one is independent of the other (Foucher, Lacambre, Pham, Giersch, & Elliott, 2007; Martin et al., 2013), and this may be linked to atypical multisensory integration in the disorder. Furthermore, alterations in either perception or attention may also be associated with abnormal multisensory integration, since this form of cognition is thought to require attentional processes (Talsma, Senkowski, Soto-Faraco, & Woldorff, 2010). Impairments in attention are apparent in the disorder (Laurent et al., 1999; Luck & Gold, 2008) and attention has been connected to multisensory impairments in schizophrenia patients (de Jong et al., 2010; Zvyagintsev, Parisi, Chechko, Nikolaev, & Mathiak, 2013). However, at a neural level the P50 component (a positive evoked potential 50 milliseconds following stimulus presentation) is decreased in schizophrenia patients, and this is associated with cross-sensory suppression, suggesting a deficit in low-level perception (Magnée, Oranje, van Engeland, Kahn, & Kemner, 2009; Roa Romero et al., 2016). Additional research is required to investigate whether abnormal multisensory integration is reflective of a perceptual or attentional impairment in patients.

Atypical multisensory integration in schizophrenia has only recently drawn greater attention, but this symptom has potential implications for other aspects of the disorder, such as social cognition (Green et al., 2005) and multisensory hallucinations (Jardri et al., 2009), which are likely linked to dysconnectivity aspects of the schizophrenia brain (Stephan, Baldeweg, & Friston, 2006). Indeed, early impairments in multisensory integration may be a potential predictor of vulnerability to the disorder in children with parents with schizophrenia (Gamma et
Research in this area is therefore warranted to specify the nature of abnormal multisensory processing and possible neural bases for this symptomatology.

**Neurobiological Bases of Schizophrenia**

Considerable research has investigated how specific neurotransmitter systems and their interactions are affected in schizophrenia. Alterations in specific neurotransmitter systems, such as dopamine, glutamate, and gamma-aminobutyric acid (GABA), have been implicated in regulating the positive, negative, and cognitive symptomology of schizophrenia. For example, first- and second-generation antipsychotics, which block dopaminergic activity (as well as other neurotransmitter systems) (Kane, 1996) can reduce positive symptoms, but are not as effective in treating the cognitive or negative symptoms (Johnstone, Crow, Frith, Carney, & Price, 1978; Lieberman et al., 2003). This thesis explored the interaction between the glutamatergic and GABAergic systems in regulating cognitive symptomology related to schizophrenia.

**Glutamate hypothesis**

A leading theory on the neuropathology of schizophrenia proposes that dysfunction or blockade of the N-methyl-d-aspartate (NMDA) glutamate receptor is an underlying factor in the disorder (Jentsch & Roth, 1999; Olney, Newcomer, & Farber, 1999). This theory was developed from early findings showing that ketamine and phencyclidine (PCP), non-competitive NMDA receptor antagonists, administered in humans produce schizophrenia-like symptoms (Krystal et al., 1994). These antagonists appear to mimic the positive, negative, and cognitive symptoms seen in the disorder. Furthermore, administration of such drugs exacerbates these symptoms in schizophrenia patients (Itil, Keskiner, Kiremitci, & Holden, 1967; Malhotra et al., 1997). In support of potential dysfunction of these receptors, post-mortem studies of schizophrenia patient brains show altered expression of NMDA receptors affecting glutamatergic pathways (Akbarian...
et al., 1996; Kristiansen, Huerta, Beneyto, & Meador-Woodruff, 2007). Furthermore, genetic knockout of forebrain pyramidal neuron NMDA receptors in mice induces a schizophrenia-like phenotype (Tatard-Leitman et al., 2015). These findings implicate potential alterations of the NMDA receptor in schizophrenia, which may be linked to the symptomatology in the disorder.

Administration of ketamine or PCP in humans severely affects cognitive functions, such as recognition memory, attention, and perception (Krystal et al., 1994; Malhotra et al., 1997; Malhotra et al., 1996). In particular, the effects of ketamine are specific to disrupting encoding, but not retrieval of spatial and verbal working memory (Rowland et al., 2005). Chronic ketamine users also exhibit an altered rubber hand illusion, which requires the integration of visual, tactile, and proprioperception information (Tang et al., 2015). This finding suggests that ketamine administration induces atypical multisensory integration similar to what is seen in schizophrenia patients. Evidence therefore supports the hypothesis that dysfunction of the NMDA receptor is connected to cognitive dysfunction in schizophrenia.

**NMDA receptor hypofunction rodent model of schizophrenia**

Evidence from the human literature shows that acute administration of PCP and ketamine induces schizophrenia-like symptoms, suggesting that hypofunction of the NMDA receptor contributes to the symptomatology of schizophrenia. Acute injection of NMDA receptor antagonists in rodents appears to mimic symptoms of the disorder, particularly the negative (social withdrawal/deficits and anhedonia) and cognitive symptoms (deficits in prepulse inhibition, working memory, and object recognition etc.; Gilmour et al., 2012; Neill et al., 2010). However, acute injections limit the ability to model schizophrenia in rodents due to their short-lasting effects. In addition, rodents are tested under the influence of the drug, which may result in potentially confounding effects, including hyperlocomotion (Caixeta, Cornelio, Scheffer-
Teixeira, Ribeiro, & Tort, 2013; Chan, Chiu, Sou, & Chen, 2008; Hou et al., 2013). Treating rodents sub-chronically with these drugs appears to model schizophrenia more appropriately, as these animals can be tested in a drug-free state. Treated rodents exhibit symptoms and cellular changes similar to those seen in schizophrenia (Gilmour et al., 2012; Neill et al., 2010). For example, sub-chronic administration of NMDA receptor antagonists induces a decrease in parvalbumin within the prefrontal cortex and hippocampus of rodents, similar to post-mortem findings from schizophrenia patients (Abdul-Monim, Neill, & Reynolds, 2007; Behrens et al., 2007; Cochran et al., 2003; Pratt, Winchester, Egerton, Cochran, & Morris, 2008). Impaired cognitive abilities, such as working memory, attention, learning and memory, and social cognition, are also seen in rodents sub-chronically treated with NMDA receptor antagonists (Gilmour et al., 2012; Neill et al., 2010). Thus, the NMDA receptor hypofunction rodent model of schizophrenia has established validity within this area, but further examination is required to develop an understanding of the underlying causes of the various impairments reported in this model.

**GABA hypothesis**

The aetiology of schizophrenia has also been associated with dysfunction of the GABAergic system, and this may be linked to the glutamatergic changes discussed above (Lewis, Curley, Glausier, & Volk, 2012; Lewis, Hashimoto, & Volk, 2005). Decreased markers of the calcium-binding protein parvalbumin (PV), a constituent of a specific sub-type of GABAergic interneurons, found post-mortem in the brains of schizophrenia patients, specifically within the prefrontal cortex, hippocampus, and entorhinal cortex, is a well-documented finding in the literature (Beasley & Reynolds, 1997; Beasley, Zhang, Patten, & Reynolds, 2002b; Hashimoto et al., 2003; Hoftman et al., 2015; Reynolds, Beasley, & Zhang, 2002; Volk et al., 2012; Zhang &
Moreover, the changes in PV expression appear to be specific to this calcium-binding protein, as alterations in other calcium-binding proteins calreitinin and calbindin are generally unaffected post-mortem in schizophrenia patients (Lewis et al., 2005). The importance of changes to PV-containing interneurons (PV-INs) is their crucial role in the production of gamma oscillatory activity (Cardin et al., 2009; Sohal, Zhang, Yizhar, & Deisseroth, 2009). Gamma oscillations, the synchronous firing of neurons in the 30-90 Hz range, are essential for the integration of cortical network activity and likely contribute to cognitive functions (Bartos, Vida, & Jonas, 2007). PV-INs synchronize the activity of cortical pyramidal neurons through inhibitory feedback (Cobb, Buhl, Halasy, Paulsen, & Somogyl, 1995) leading to gamma oscillatory activity. Reductions in PV are suggested to affect gamma oscillatory activity in schizophrenia. Supporting this, abnormal gamma oscillatory activity is another common finding in patients with schizophrenia (Cho, Konecky, & Carter, 2006; McNally, McCarley, & Brown, 2013; Uhlhaas & Singer, 2010). Furthermore, in schizophrenia patients, alterations in GABA activity in vivo is positively correlated with changes in gamma oscillatory activity (Frankle 2015). Thus, PV-INs activity may be an important factor in schizophrenia patients with severely affected cognition (Gonzalez-Burgos, Cho, & Lewis, 2015).

Additionally, altered expression of the primary GABA synthesizing enzyme glutamic acid decarboxylase 67 (GAD67; Duncan et al., 2010; Hashimoto et al., 2003; Hofman et al., 2015; Volk, Austin, Pierri, Sampson, & Lewis, 2000), the vesicular GABA transporter (vGAT; Hofman et al., 2015), the GABA membrane transporter (GAT1; Volk, Austin, Pierri, Sampson, & Lewis, 2001), and GABA receptors (Akbarian et al., 1995; Beneyto, Abbott, Hashimoto, & Lewis, 2011; Duncan et al., 2010; Hofman et al., 2015; Lewis, Hashimoto, & Morris, 2010; Volk et al., 2002) have all been reported post-mortem in the brains of schizophrenia patients.
Binding of $[^{11}\text{C}]$flumazenil (a benzodiazepine PET radiotracer) in the presence of a GAT1 inhibitor is significantly increased in all cortical regions in controls, but not schizophrenia patients (Frankle et al., 2015) and cortical GABA is decreased in patients (Thakkar et al., 2016), consistent with altered GABAergic system function. Indeed, pharmacological decrease of GABA with the GABA$_A$ antagonist bicuculline within the medial prefrontal cortex (mPFC) of rodents induces similar behavioural and cognitive deficits, and changes in dopaminergic tone to that of schizophrenia patients (Auger & Floresco, 2014; Enomoto, Tse, & Floresco, 2011; Piantadosi & Floresco, 2014).

Recent evidence strengthens the hypothesis that deficient PV-IN activity induces a schizophrenia behavioural phenotype. First, inhibition of PV-INs within the ventral hippocampus using DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) produces deficits in prepulse inhibition and startle reactivity in mice, as well as altering oscillatory network activity in vivo (Nguyen et al., 2014). Second, knock down of the GAD1 transcript (encodes GAD67) within PV-INs in transgenic mice induces impairments in object recognition and fear extinction and reduces GABA release in the prefrontal cortex (Brown et al., 2015; Lazarus, Krishnan, & Huang, 2015). Thirdly, knockout of GAD67 within PV-INs impairs prepulse inhibition, social memory, and alters GABAergic transmission (Fujihara et al., 2015). Lastly, an early deficit in cognitive flexibility induced by decreased PV development apparent in Dlx5/6$^{+/\sim}$ mice, which is associated with task related decreases in gamma oscillatory activity, is restored through optogenetic stimulation of PV-INs at gamma frequency within the prefrontal cortex (Cho et al., 2015). PV-INs therefore appear to contribute significantly to a schizophrenia related cognitive phenotype; however, the role of this interneuron sub-type – like GABA itself - in the disorder requires further study.
Integration of glutamate and GABA hypotheses

Dysfunction of the GABAergic system in schizophrenia has been linked to NMDA receptor hypofunction (Gonzalez-Burgos & Lewis, 2012; Lisman et al., 2008). In rodents, sub-chronic administration of NMDA receptor antagonists’ ketamine or PCP induces a decrease in markers of PV and GAD67 in the prefrontal cortex and hippocampus (Abdul-Monim et al., 2007; Behrens et al., 2007; Cochran et al., 2003; Pratt et al., 2008). Furthermore, GABAergic currents are decreased in the prefrontal cortex of rodents sub-chronically treated with NMDA receptor antagonists in comparison to controls (Jeevakumar & Kroener, 2014; Kjaerby, Broberg, Kristiansen, & Dalby, 2014; Zhang, Behrens, & Lisman, 2008). In culture, exposure to sublethal doses of ketamine decreases expression of PV and GAD67 within PV-INs (Kinney et al., 2006). These findings mimic the cellular changes seen in schizophrenia, suggesting a link between GABA alterations and NMDA receptor hypofunction. Recent genetic evidence suggests that both GABAergic and glutamatergic signalling is disrupted in schizophrenia, although they may be independent processes (Pocklington et al., 2015).

PV-INs are clearly affected in schizophrenia, and these interneurons are important for the production of gamma oscillations (Cardin et al., 2009; Sohal et al., 2009). Administration of NMDA receptor antagonists in rodents and humans alters gamma activity (Anderson, Pinault, O'Brien, & Jones, 2014; Hakami et al., 2009; Hiyoshi, Kambe, Karasawa, & Chaki, 2014; Pinault, 2008; Sullivan, Timi, Hong, & O'Donnell, 2015; Young, Stubbendorff, Valencia, & Gerdjikov, 2015). Specifically, deletion of NMDA receptors on PV-INs in PV-Cre/NR1 mutant mice interferes with gamma oscillatory activity (Carlén et al., 2012; Korotkova, Fuchs, Ponomarenko, von Engelhardt, & Monyer, 2010). Optogenetic activation of PV-INs is impaired in PV-Cre/NR1 mutant mice, and these animals display decreased sensitivity to NMDA receptor
antagonist disruption of gamma activity (Carlén et al., 2012). Aberrant gamma oscillatory activity is a common feature of schizophrenia and has been linked to cognitive deficits (Senkowski & Gallinat, 2015). In support of this, PV-Cre/NR1 mutant mice display impairments in cognitive functions, such as habituation, working memory, and associative learning (Carlén et al., 2012; Korotkova et al., 2010).

**GABA and Multisensory Integration**

The GABAergic system, specifically PV-IN activity, has been implicated in multisensory integration. The integration of tactile-auditory information is impaired in mouse models of autism, which appears linked to dysfunction of GABA (Gogolla, Takesian, Feng, Fagiolini, & Hensch, 2014). Consequently, decreased inhibitory transmission, parvalbumin, and GAD65 markers were found in these autistic mouse models, and the multisensory impairment was rescued by administration of the benzodiazepine diazepam (alosteric GABAA agonist; Gogolla et al., 2014). Postnatal enhancement of inhibitory transmission through sub-chronic administration of diazepam rescued the decreases in PV and GAD65, as well as the impairment in multisensory integration (Gogolla et al., 2014). This effect was not found in older mice (Gogolla et al., 2014), suggestive of reorganization of the GABAergic system in early development leading to the impairment in multisensory integration. Gogolla et al. (2014) suggest that an optimal level of excitatory-inhibitory activity may be linked to proper multisensory integration and an increase or decrease in the balance impairs multisensory integration. Excitatory-inhibitory balance has also been hypothesized to be dysfunctional in schizophrenia (Lewis et al., 2012), paralleling the idea of Gogolla et al. (2014). In support of this, PV-containing interneurons show little multisensory enhancement, unlike surrounding pyramidal cells; however, optogenetic activation of PV-INs disrupts multisensory enhancement in
pyramidal cells (Olcese, Iurilli, & Medini, 2013). Decreased markers of PV are also found in schizophrenia and animal models (Abdul-Monim et al., 2007; Beasley & Reynolds, 1997; Beasley et al., 2002b; Behrens et al., 2007; Cochran et al., 2003; Duncan et al., 2010; Hashimoto et al., 2003; Hofman et al., 2015; Pratt et al., 2008; Reynolds et al., 2002; Volk et al., 2000; Volk et al., 2012; Zhang & Reynolds, 2002), providing a potential connection between abnormal multisensory integration in schizophrenia and impaired GABAergic activity.

PV-INs synchronize neuronal activity, and this is crucial for the production of gamma oscillatory activity (Cardin et al., 2009; Sohal et al., 2009). Gamma oscillatory activity is postulated to be important for the synchronous activity across and within cortical regions, and this appears important for multisensory integration. Indeed, gamma-band activity has previously been connected to the binding of sensory information in humans (Schneider, Lorenz, Senkowski, & Engel, 2011; Senkowski, Schneider, Foxe, & Engel, 2008; Senkowski, Schneider, Tandler, & Engel, 2009; Siegle, Pritchett, & Moore, 2014). Therefore, altered gamma-band activity in schizophrenia (Cho et al., 2006; McNally et al., 2013; Uhlhaas & Singer, 2010), which is linked to decreases in PV, may be associated with abnormal multisensory integration. Stone, Coffman, Bustillo, Aine, and Stephen (2014) assessed this possibility and showed that increased gamma oscillatory activity was associated with abnormal multisensory integration in schizophrenia patients. Furthermore, increased gamma oscillatory activity was negatively correlated with MATRICS scores, such that increased gamma power was associated with decreased scores, suggesting a more general role of altered gamma activity in cognitive impairments in schizophrenia (Stone et al., 2014). Balz et al. (2016) also provide support for this hypothesis, demonstrating that individual differences in GABAergic transmission modulate audio-visual perception by controlling gamma oscillatory activity. Further work is required to examine the
role of altered gamma and GABAergic activity in schizophrenia and its link to abnormal multisensory integration.

**Cognition in Animal Models of Schizophrenia**

CNTRICS have proposed the testing of animal models of schizophrenia to further understand cognition in schizophrenia (Carter & Barch, 2007). Multiple animal models of schizophrenia have been developed, each encompassing a different aspect of the disorder, including: pharmacological, genetic, and developmental models. Importantly, in terms of studying cognition, these rodent models display similar cognitive deficits, such as prepulse inhibition, sensory gating, working memory, cognitive flexibility, and social memory (Amitai & Markou, 2010; Auger & Floresco, 2014; Enomoto et al., 2011; Hlinak & Krejci, 1994; Jentsch, Tran, Le, Youngren, & Roth, 1997; Mansbach & Geyer, 1989; Neill et al., 2010; Nguyen et al., 2014). Further systematic assessment of these models in related, but understudied facets of cognition, will add to the growing understanding of the neurobiological bases of cognition in schizophrenia and potential therapeutic strategies. The current thesis is primarily concerned with the processing of multisensory object features in such models; accordingly, I will focus in the following sections on object-based cognitive tasks that form the basis for the novel tests used in the present work.

**Object recognition**

Ennaceur and Delacour (1988) developed the object recognition task to study the neurobiology of memory in rodents. The task is based on rodents’ natural tendency to prefer novel, rather than familiar objects. The standard object recognition task consists of two phases: a sample and choice phase. These two phases are separated by a short or long delay depending on the form of memory that is to be examined. Rodents are typically presented with two identical
objects in a sample phase and allowed to explore them. Following a delay, rodents are presented with a triplicate copy of the object from the same phase (familiar object) and a novel object. Rodents with intact memory will preferentially explore the novel object more than the familiar object demonstrating recognition of the sample object. Performance of this task can be investigated in either an open-field or Y-shaped apparatus depending on the experiment. The open-field apparatus appears to recruit the hippocampus for spatial identification and the perirhinal cortex for object identification. However, object recognition in the Y-shaped apparatus, which restricts spatial cues, does not require the hippocampus (Winters, Forwood, Cowell, Saksida, & Bussey, 2004).

The object recognition task has commonly been used to examine declarative memory in rodent models of schizophrenia (Lyon, Saksida, & Bussey, 2012), as the MATRICS initiative recognizes this task as a preclinical measure of the visual and learning memory domain (Young et al., 2009). This task has been used to investigate the cognitive enhancing effects of different pharmacological manipulations in rodent models. Severe impairments in object recognition performance are commonly found in pharmacological, genetic, developmental, and lesion rodent models of schizophrenia (Lyon et al., 2012), further validating this task as a preclinical measure of visual learning and memory. Acute or sub-chronic administration of NMDA receptor antagonists MK-801, PCP, or ketamine induces object recognition impairment in rats and mice (Callahan, Terry, & Tehim, 2014; Damgaard, Plath, Neill, & Hansen, 2011; Grayson et al., 2014; Grayson, Idris, & Neill, 2007; Hashimoto et al., 2008; Horiguchi, Huang, & Meltzer, 2011; Jacklin et al., 2012; Pyndt Jorgensen et al., 2014; White et al., 2014). The object recognition impairment in rodents treated with NMDA receptor antagonists can be seen at long delays, such as 24h, and short delays, such as 1 min (Lyon et al., 2012). In conclusion, the object recognition
Crossmodal object recognition

Crossmodal object recognition is the ability to identify an object in one modality through previous exposure to that object in a different modality. Crossmodal object recognition enables assessment of multisensory integration abilities as they pertain to the representation of real-world objects. Early studies of crossmodal object recognition were performed with monkeys using the Delayed Non-Matching to Sample (DNMS) paradigm (Murray, Malkova, & Goulet, 1998). In this task, monkeys are provided with an object in a sample phase, followed by a choice phase in which they need to discriminate between the sample object and a new object, specifically learning to choose the new object for reward. In the crossmodal version of this task, the sample phase restricts object exploration to the tactile modality and the choice phase provides only visual information; thus, animals must demonstrate tactile-to-visual crossmodal object recognition. It is established that monkeys are capable of performing crossmodal object recognition in the crossmodal DNMS task (Goulet & Murray, 2001; Malkova & Murray, 1996; Murray & Gaffan, 1994; Murray & Mishkin, 1985).

Winters and Reid (2010) developed a variation on the object recognition task to extend crossmodal research to the investigation of crossmodal object recognition (CMOR) in rodents. The rodent CMOR task involves a tactile sample phase and a visual choice phase, which are separated by a variable delay. In the sample phase, rats are limited to exploration of two identical sample objects through touch as they are tested in red light and rats cannot visually discriminate between objects in red light (Jacobs, Fenwick, & Williams, 2001; Winters & Reid, 2010). In the choice phase, rats are limited to exploration of a familiar object from the sample phase and a
novel object using only visual information, as the objects are placed behind transparent Plexiglas barriers. CMOR incorporates the processing of individual modalities (tactile & visual) and exploits a rat’s natural tendency to explore the novel object in order to test object recognition, similar to object recognition (Winters, Saksida, & Bussey, 2008). Rats can successfully perform tactile-to-visual CMOR with a 1h delay (Reid, Jacklin, & Winters, 2012, 2013; Winters & Reid, 2010). In addition, visual-only and tactile-only object recognition tasks are employed to account for any impairments seen in CMOR performance that are not due to multisensory integration (Winters & Reid, 2010).

As noted above, the literature reveals abnormal multisensory integration in schizophrenia patients (Stekelenburg et al., 2013; Stone et al., 2011; Williams et al., 2010), and the CNTRICS initiative has suggested the inclusion of a ‘cross-modal integration’ task in their battery of tests for preclinical models (Butler et al., 2008). In addition, animals sub-chronically treated with NMDA receptor antagonists show severe object recognition impairments at delays as short as 1 min (Lyon et al., 2012). The OR task allows animals to explore all modalities of the objects simultaneously suggesting this impairment could be due to a multisensory deficit. Therefore, Jacklin et al. (2012) investigated the effects of sub-chronic treatment with NMDA receptor antagonists in rats on the CMOR task to assess the possibility of a multisensory deficit. In this study rats were sub-chronically treated with either ketamine for 10-days or MK-801 for 7-days followed by an equal washout period. Rats treated with either NMDA receptor antagonist sub-chronically were impaired on visual-only, tactile-only object recognition and CMOR with a 1h delay (Jacklin et al., 2012). However, when the delay was reduced to an “immediate delay” (approximately 30 sec), to limit mnemonic demand, rats treated with sub-chronic NMDA receptor antagonists were selectively impaired on the CMOR task (Jacklin et al., 2012). Likewise,
the viral mimetic polyinosinic-polycytidylic acid (polyI:C) rat model of schizophrenia displays a selective CMOR deficit at a 1h delay (Ballendine et al., 2014). These findings demonstrate that pharmacological and developmental models of schizophrenia each show a selective CMOR impairment. In particular, the NMDAR antagonist treated animals displayed a severe deficit at a minimal delay, which is suggestive of a perceptual rather than mnemonic deficit, and this will be further investigated in the current thesis. These studies complement the human schizophrenia literature and highlight the validity of using the CMOR task in understanding cognitive dysfunction in animal models of schizophrenia.

**Oddity task**

The object recognition OR and CMOR tasks have allowed for investigation of object memory at short- and long-term memory delays; however, these tasks are not capable of examining object perception in the absence of a memory requirement, even at an “immediate” delay. The oddity task was developed to study object perception in humans and non-human primates (Alvarado, Kazama, Zeamer, & Bachevalier, 2011; Buckley, Booth, Rolls, & Gaffan, 2001; Lee et al., 2006; Overman, Bachevalier, Miller, & Moore, 1996). The oddity task, unlike the object recognition and CMOR tasks, does not have a sample and choice phase. Rather, typically, three objects are presented simultaneously where there are two identical (A) and one odd (B) object. The objective is to determine which object is the odd one (A A B). Research using the perceptual oddity task was extended to rodents to explore perceptual functions. Rats show a natural preference for the odd object in comparison to the identical objects (Bartko, Winters, Cowell, Saksida, & Bussey, 2007a, 2007b; Bartko, Winters, Saksida, & Bussey, 2014). Identification of the odd object is defined by the oddity preference ratio (odd object exploration/total exploration). Thus, if a rat or mouse explores the odd object more than 33% (3
objects) or 20% (5 objects) of the time they demonstrate a preference for the odd object (Bartko et al., 2007a, 2007b). Rats are capable of performing the oddity task with only purely 2D visual stimuli, as well (Forwood, Bartko, Saksida, & Bussey, 2007). Bartko et al. (2007b) used the oddity task to study complex perceptual discrimination in rats using different configurations of object features. In this task objects consisted of two individual features, for example feature A and feature B made up object AB. Five objects were used with two identical objects pairs (AB, AB, CD, CD), and the odd object was a different configuration of the familiar features (AD; see Figure 1). Because the individual features are all represented amongst the different object pairs, the only aspect that distinguishes the odd object is its unique configuration of these features; thus, if a rat displays odd object preference in this task, it is demonstrating an ability to integrate those features and perceptually distinguish between that configuration and all the others.

Figure 1. The oddity task measures object perception in rodents. Five objects are presented simultaneously (two identical pairs and one odd object). From left to right objects: AB, AB, AD, CD, CD. The odd object (AD) is represented by the unique configuration of familiar features. Normal rodents typically prefer to explore the odd object in comparison to the identical objects. Figure duplicated from Bartko et al. 2007b.
The CMOR task is limited to tactile-to-visual recognition and cannot examine other modality combinations, due to the structure of the task. In addition, it can be argued that, at even a minimal delay, the CMOR task still contains a mnemonic component; thus the CMOR task cannot directly examine the perceptual aspect of multisensory integration. Alterations to the oddity task may facilitate examination of multisensory integration at a perceptual level and with multiple modality combinations including and not limited to olfaction. Similar to the task introduced by Bartko et al. (2007b), different configurations of features, but from different modalities, could be employed. Development of multisensory oddity tasks will enable further examination of the severe CMOR impairment found in rats treated with NMDA receptor antagonists (Jacklin et al., 2012), and this is one of the aims of the present thesis.

The Cholinergic System

Acetylcholine (ACh) is the sole neurotransmitter of the cholinergic system. ACh is primarily produced by cholinergic neurons in the basal forebrain and brain stem. These areas project widely – both cortically and subcortically - throughout the brain (Zaborszky, Pang, Somogyi, Nadasdy, & Kallo, 1999). ACh is heavily implicated in cognitive abilities such as attention, memory, and learning (Hasselmo & Sarter, 2011; Sarter, Bruno, & Givens, 2003; Winters, Saksida, & Bussey, 2006).

The cholinergic system consists of two receptor subtypes: the nicotinic and muscarinic acetylcholine receptors. Nicotinic acetylcholine receptors (nAChRs) are ionotropic, linked to ligand-gated ion channels that allow for the movement of Na\(^+\), K\(^+\), and Ca\(^{2+}\) across the cell membrane. These receptors are activated by the endogenous ligand ACh or selectively stimulated by nicotine. nAChRs can be homomeric or heteromeric consisting of five nAChR subunits (\(\alpha_2\)-\(\alpha_{10}\) & \(\beta_2\)-\(\beta_4\)) surrounding a pore (Albuquerque, Pereira, Alkondon, & Rogers, 2009).
The most commonly found nAChR subtypes in the brain are the heteromeric $\alpha_4\beta_2$ and homomeric $\alpha_7$ nAChRs (Albuquerque et al., 2009). These subtypes can be found throughout the brain presynaptically and post-synaptically in areas such as the prefrontal cortex and hippocampus (Albuquerque et al., 2009). Muscarinic acetylcholine receptors (mAChRs) are G-protein coupled receptors that act as either inhibitory or excitatory receptors, depending on subtype and their location in the brain. These receptors are activated by the endogenous ligand ACh or selectively stimulated by muscarine. Five mAChR subtypes exist, consisting of M1-M5.

**Nicotinic acetylcholine receptors and schizophrenia**

Amongst the schizophrenia population there is an extremely high prevalence rate of smoking in comparison to the normal population (de Leon & Diaz, 2005; Leonard et al., 2000). Smoking has been shown to have beneficial effects on cognitive function in schizophrenia patients (Kumari & Postma, 2005; Leonard et al., 2000; Sacco et al., 2005; Smith, Singh, Infante, Khandat, & Kloos, 2002). Furthermore, clinical studies demonstrate that administration of nicotine has pro-cognitive effects in schizophrenia patients (Barr et al., 2008; Smith et al., 2002; Smith et al., 2006). Alterations in specific nAChRs, including the $\alpha_4\beta_2$ and $\alpha_7$ subtypes, have also been shown post-mortem in brains of schizophrenia patients (Breese, Lee, Adams, Sullivan, & Leonard, 1999; Durany, Zo, Boissl, & Paulus, 2000; Esterlis et al., 2014; Freedman, Hall, Adler, & Leonard, 1995; Leonard et al., 2000; Woodruff-Pak & Gould, 2002). However, schizophrenia patients that had previously smoked showed up-regulated expression of nAChRs (Breese et al., 1999; Esterlis et al., 2014).

Clinical trials have been assessing the effects of specific agonists at the $\alpha_4\beta_2$ and $\alpha_7$ nAChR rather than the global effects of nicotine, and the results have been unclear with regards to their pro-cognitive effects (Freedman et al., 2008; Keefe et al., 2015; Lieberman et al., 2013;
Olincy et al., 2006; Radek, Kohlhaas, Rueter, & Mohler, 2010; Umbricht et al., 2014; Velligan et al., 2012; Waldo, Woodward, & Adler, 2010; Zhang et al., 2012). Conversely, in animal models of schizophrenia the benefit of nicotine in terms of cognition is clear (Andreasen, Andersen, Nielsen, Mathiasen, & Mirza, 2006; Jacklin et al., 2012; Rushforth, Steckler, & Shoaib, 2011). In addition, specific agonists of the $\alpha_{4}\beta_{2}$ and $\alpha_{7}$ nAChR subtypes have shown efficacy in their ability to reverse impairments seen in these animal models (Hashimoto et al., 2008; Hauser et al., 2010; Pichat et al., 2007; Radek et al., 2006; Thomsen, Christensen, Hansen, Redrobe, & Mikkelsen, 2009; Timmermann et al., 2009; Wallace et al., 2011; Wildeboer & Stevens, 2008). Development of pharmacological treatments targeting these receptors appears promising for the amelioration of cognitive dysfunction in schizophrenia.

Jacklin et al. (2012) investigated the role of nAChRs in reversing the CMOR impairment seen in rats treated with sub-chronic NMDA receptor antagonists. Pre-sample systemic administration of nicotine (0.05, 0.2 & 0.8 mg/kg) dose-dependently reversed the CMOR deficit (Jacklin et al., 2012). These findings support the growing literature on the involvement of nAChRs in cognitive remediation of schizophrenia symptoms, extending to that of multisensory integration. This thesis will provide further investigation into how these receptors can reverse the severe CMOR impairment in treated animals to understand the underlying cause and potential treatment of this deficit.

**Nicotinic acetylcholine receptor interaction with GABA**

GABA is one neurotransmitter system regulated by nAChR stimulation in areas such as the cortex and hippocampus. Indeed, nAChRs are commonly found presynaptically and somatodendritically on different GABAergic interneurons (Albuquerque et al., 2009). Application of nicotine or ACh in vitro increases GABAergic currents in pyramidal neurons of
rats and mice, and these effects are modulated by both the $\alpha_4\beta_2$ and $\alpha_7$ nAChR subtypes in the cortex and hippocampus; however, the $\alpha_4\beta_2$ nAChR subtype appears to predominately affect GABAergic interneuron activity (Alkondon & Albuquerque, 2001, 2004; Aracri et al., 2010; Banerjee, Alkondon, Pereira, & Albuquerque, 2012; Bloem, Poorthuis, & Mansvelder, 2014; Couey et al., 2007). Contrasting in vitro findings, in vivo knockout of the $\alpha_7$ nAChR gene (CHRNA7) decreases markers of GAD67 and PV in the cortex and hippocampus of mice (Adams et al., 2012; Lin et al., 2014). Furthermore, sub-chronic administration of an $\alpha_4\beta_2$ nAChR agonist, but not a $\alpha_7$ nAChR agonist, increased GAD67 expression in the frontal cortex of mice and this effect was blocked by the non-selective nAChR antagonist mecamylamine (Maloku et al., 2011). A recent study examined the effects of the selective $\alpha_7$ nAChR agonist EVP-6124 on neurotransmitter release in the rat medial prefrontal cortex using in vivo microdialysis, and no significant change in GABA release was found (Huang et al., 2014). Lastly, kainate induced gamma oscillations in vitro, which are regulated by GABAergic activity, are increased by nicotine administration and this is mediated by $\alpha_4\beta_2$ nAChR, and not the $\alpha_7$ nAChR (Zhang et al., 2015). Thus, evidence supports the modulatory effect of nAChR on GABAergic transmission in the cortex of rodents, and the current thesis will investigate the potential role of this interaction in nAChR-mediated reversal of multisensory impairment in ketamine-treated rodents.

**Orbitofrontal Cortex**

The prefrontal cortex (PFC) receives multiple sensory inputs from various cortical regions (Fuster, 1997; Öngür & Price, 2000; Uylings, Groenewegen, & Kolb, 2003), suggesting potential for participation in multisensory integration. Early studies with non-human primates demonstrated that lesions of the PFC impair performance of several crossmodal tasks (Aitken, 1980; Ettlinger & Garcha, 1980; Petrides & Iversen, 1976). Furthermore, ablation of the left, but
not right, PFC disrupts the ability to form visual-auditory associations in monkeys (Gaffan & Harrison, 1991), and ventrolateral PFC lesions impair the ability to form visual-olfactory associations in rats (Whishaw, Tomie, & Kolb, 1992). Electrophysiological studies support these findings, as recordings in the PFC of primates show that there are cells responsible for integrating auditory and visual information (Fuster, Bodner, & Kroger, 2000; Lipton, Alvarez, & Eichenbaum, 1999). Within the orbitofrontal cortex (OFC) of rats, cells were shown to fire prior to presentation of an odour when an animal arrived at a location associated with that odour (Lipton et al., 1999). Thus, previous findings suggest a potentially important role for the PFC in crossmodal cognition.

Reid et al. (2013) investigated the involvement of the PFC, as well as specific PFC regions, in the CMOR task using rats. Large bilateral excitotoxic lesions of the PFC caused a selective impairment in CMOR, sparing tactile and visual object recognition (Reid et al., 2013). Moreover, specific bilateral lesions of the OFC, but not the mPFC, induced a selective CMOR impairment in rats (Reid et al., 2013). These results were suggestive of a central function of the OFC in performance of the CMOR task, similar to other crossmodal tasks (Lipton et al., 1999; Whishaw et al., 1992).

OFC abnormalities in patients with schizophrenia, such as hypofrontality, decreased volume, and connectivity to other regions, are commonly found (Bellani et al., 2010; Eryilmaz et al., 2016; Kanahara et al., 2013; Shenton, Dickey, Frumin, & McCarley, 2001). Furthermore, studies demonstrate specific GABAergic alterations in the OFC of schizophrenia patients, including decreases in GAD67 and PV mRNA expression in layers III and IV (Joshi, Catts, Olaya, & Weickert, 2015; Joshi, Fung, Rothwell, & Weickert, 2012; Thompson, Weickert, Wyatt, & Webster, 2009). Decreases in markers of GAD67 and PV are also found in the OFC of rodent
models of schizophrenia, such as the NMDA receptor hypofunction and neurodevelopmental methylazoxymethanol acetate (MAM) model (Bissonette, Bae, Suresh, Jaffe, & Powell, 2010, 2014; Gastambide et al., 2012; Romon, Mengod, & Adell, 2011; Thomsen, Hansen, & Mikkelsen, 2010). Furthermore, systemic administration of MK-801 or ketamine in rats reduces inhibitory fast-spiking GABAergic interneuron activity in vivo (Homayoun & Moghaddam, 2008; Quirk, Sosulski, Feierstein, Uchida, & Mainen, 2009) and alters gamma oscillatory activity (Wood, Kim, & Moghaddam, 2012) in the OFC. Sufficient evidence therefore supports the assertion that OFC dysfunction could heavily affect cognition in schizophrenia patients and rodent models.

**Current Study**

This thesis expands on the findings of Jacklin et al. (2012) by further investigating the selective multisensory impairment in ketamine-treated rats. The CMOR task was used to further explore the role of specific nAChRs (α7 and α4β2) in remediating the multisensory impairment in ketamine-treated rats. Furthermore, we hypothesized that nAChRs stimulate the GABAergic and/or glutamatergic systems in ketamine-treated rats. Deficiency in these neurotransmitter systems may underlie the multisensory impairment seen in ketamine-treated animals, as they have been linked to dysfunction in schizophrenia (Jentsch & Roth, 1999; Lewis et al., 2012; Lewis et al., 2005; Olney et al., 1999). These hypothesized nicotinic effects were explored in the OFC of ketamine-treated rats.

This thesis also presents a novel task to examine multisensory integration independent of memory demand and which can employ multiple modality combinations, expanding upon the findings with CMOR. This task is a multisensory oddity task (MSO; tactile-visual & olfactory visual). Ketamine-treated rats were examined on these tasks and the remediating role of nAChR
agonism was investigated, as well as the potential interaction between GABAergic transmission and nAChR activation systemically and in the OFC.

Dysfunction in GABA inhibitory transmission has been linked to impaired multisensory integration in mouse models of autism (Gogolla et al., 2014). Rats treated with NMDA receptor antagonists show decreased GABAergic markers, including PV, suggesting that the multisensory impairment in ketamine-treated rats could be caused by dysfunctional GABAergic transmission. It is hypothesized that nAChR activation enhances GABAergic function in ketamine-treated rats reversing their multisensory impairment. Therefore, this thesis also examined baseline GABAergic function within the OFC from ketamine-treated rats using whole-cell electrophysiology and whether ACh application increases GABAergic transmission in a nAChR-dependent manner. Immunohistochemistry was also used to measure GABAergic markers in the prefrontal cortex of ketamine-treated rats. Lastly, DREADDs were used to directly inhibit PV-INs activity in the OFC of mice and investigate their involvement in multisensory integration using the MSO task. The converging evidence provided by these combined techniques should help to clarify the involvement of PV-INs – and their potential interaction with nAChRs - in modulating the multisensory impairment in ketamine-treated rodents. These findings should have significant implications for our understanding of the severe cognitive dysfunction manifesting in schizophrenia and its potential treatment.
Chapter 2

$\alpha_4\beta_2$ Nicotinic Receptor Stimulation of the GABAergic System Within the Orbitofrontal Cortex Ameliorates the Severe Crossmodal Object Recognition Impairment in Ketamine-Treated Rats: Implications for Cognitive Dysfunction in Schizophrenia

Jacob M. Cloke & Boyer D. Winters

Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, ON, Canada N1G 2W1

*Neuropharmacology, 90, 42-52*
Abstract

The neural bases of multisensory integration impairments in schizophrenia are not well understood. Rats treated sub-chronically with NMDA receptor antagonists (e.g., ketamine), which model symptoms of schizophrenia, are severely impaired on a tactile-to-visual crossmodal object recognition (CMOR) task, and this deficit is reversed by systemic nicotine. The current study assessed the receptor specificity of the ameliorative effect of nicotine in the CMOR task, as well as the potential for nAChR interaction with GABA and glutamate, two neurotransmitters with relevance to schizophrenia. Male Long-Evans rats were treated sub-chronically for 10 days with ketamine or saline and then tested on the CMOR task after a 10-day washout. Systemic nicotine given before the sample phase of the CMOR task reversed the ketamine-induced impairment, but this effect was blocked by co-administration of the GABAA receptor antagonist bicuculline at a dosage that itself did not cause impairment. Pre-sample systemic co-administration of the NMDA receptor antagonist MK-801 did not block the remediating effect of nicotine in ketamine-treated rats. The selective α7 nAChR agonist GTS-21 and α4β2 nAChR agonist ABT-418 were tested, with only the latter reversing the ketamine impairment dose-dependently; bicuculline also blocked this effect. Similarly, infusions of nicotine or ABT-418 into the OFC reversed the CMOR impairment in ketamine-treated rats, and systemic bicuculline blocked the effect of intra-OFC ABT-418. These results suggest that nicotine-induced agonism of α4β2 nAChRs within the OFC ameliorates CMOR deficits in ketamine-treated rats via stimulation of the GABAergic system. The findings of this research may have important implications for understanding the nature and potential treatment of cognitive impairment in schizophrenia.
Introduction

Schizophrenia is characterized by severe cognitive dysfunction, which is considered to be a core component of the disorder (Nuechterlein et al., 2004) and is closely linked to functional outcome in patients (Green et al., 2004). Amongst these cognitive deficits, sensory processing and memory impairments are widely acknowledged (Adler, Rose, & Freedman, 1986; Braff, 1989; Ford, Gray, Whitfield, Turken, & Glover, 2004). Multisensory integration represents one facet of this class of symptoms that is receiving increasing research attention (de Gelder et al., 2002; de Gelder et al., 2004; de Jong et al., 2010; de Jong et al., 2009; Pearl et al., 2009; Ross et al., 2007; Stekelenburg et al., 2013; Stone et al., 2011; Williams et al., 2010; Zvyagintsev et al., 2013). Accordingly, the CNTRICS initiative has suggested inclusion of a ‘cross-modal integration’ task in behavioural test batteries assessing cognitive function in pre-clinical models of schizophrenia (Butler et al., 2008). Until recently, the absence of a viable rodent model of multisensory integration has slowed progress in understanding the neural bases of atypical multisensory processing in schizophrenia.

We have recently introduced a paradigm, based on the object recognition task (Ennaceur & Delacour, 1988), which enables assessment of multisensory integration in rodents (Reid et al., 2012, 2013; Winters & Reid, 2010). The crossmodal object recognition (CMOR) task assesses tactile-to-visual crossmodal cognition by asking rats to recognize a visually presented object that they have previously only explored tactiley. Consistent with the common finding that rodent models of schizophrenia are impaired on the standard object recognition task (Grayson et al., 2014; Lyon et al., 2012; Young et al., 2009), we have previously shown that rats treated sub-chronically with NMDA receptor antagonists (MK-801 or ketamine) display object recognition deficits, as well as impairments on tactile- and visual-only unimodal object recognition (Jacklin
et al., 2012). Interestingly, these rats were also severely impaired on the CMOR task, failing to perform successfully even when memory demands were significantly reduced, despite the absence of impairment with the same parameters in the object recognition and unimodal tasks (Jacklin et al., 2012). Hypofunction of the NMDA glutamate receptor has been proposed as an underlying factor in schizophrenia pathology (Jentsch & Roth, 1999; Olney et al., 1999), and administration of NMDA receptor antagonists in humans produces schizophrenia-like symptoms and exacerbates symptoms in schizophrenia patients (Itil et al., 1967; Krystal et al., 1994; Luby, Cohen, Rosenbaum, Gottieb, & Kelley, 1959). Accordingly, sub-chronic administration of these drugs in animals models the disorder, in particular the negative and cognitive symptoms (Gilmour et al., 2012; Neill et al., 2010). Thus, our previous study indicates an acute sensitivity of the CMOR task to cognitive impairment in an established rodent model of schizophrenia.

nAChRs are a clinically relevant target for pharmacological treatment of cognitive dysfunction in schizophrenia. Nicotine and selective nAChR agonists ameliorate cognitive impairment in schizophrenia patients (Freedman et al., 2008; Lieberman et al., 2013; Olincy et al., 2006; Radek et al., 2010; Zhang et al., 2012) and animal models (Hashimoto et al., 2008; Hauser et al., 2010; Jacklin et al., 2012; Pichat et al., 2007; Radek et al., 2006; Rushforth et al., 2011; Thomsen et al., 2009; Timmermann et al., 2009; Wallace et al., 2011; Wildeboer & Stevens, 2008). Consistent with these findings, acute administration of nicotine reversed the severe CMOR impairment in rats treated sub-chronically with NMDA receptor antagonists (Jacklin et al., 2012), further validating this task for pre-clinical modelling of schizophrenia-related cognitive deficits. The current study was concerned with elucidating the underlying mechanisms of this nicotinic facilitation in the CMOR task.
Both the GABAergic and glutamatergic neurotransmitter systems have been implicated in schizophrenia pathology. Specifically, GABAergic PV-INs are thought to be dysfunctional in the disorder (Lewis et al., 2012; Lewis et al., 2005) with a decrease of these interneurons found post-mortem in the brains of schizophrenia patients (Beasley, Zhang, Patten, & Reynolds, 2002a; Beasley & Reynolds, 1997; Zhang & Reynolds, 2002) and animal models (Abdul-Monim et al., 2007; Behrens et al., 2007; Keilhoff, Becker, Greksch, Wolf, & Bernstein, 2004; Lodge, Behrens, & Grace, 2009; Sabbagh et al., 2013). The schizophrenia-like effects of glutamatergic antagonists may be due, in part, to disruption of NMDA receptor signalling on this sub-type of GABAergic interneuron (Carlén et al., 2012; Korotkova et al., 2010). nAChRs are located presynaptically at both GABAergic and glutamatergic synapses (Albuquerque et al., 2009; Radcliffe, Fisher, Gray, & Dani, 1999). Thus, nicotinic stimulation of GABA and/or glutamate release may underlie the remediating effect of nAChR agonists on CMOR task performance by rats treated with NMDA receptor antagonists (Jacklin et al., 2012). This possibility was investigated in the current study; specifically, we predicted that the facilitative effect of nicotine would be prevented by co-administration of the GABA\textsubscript{A} receptor antagonist bicuculline. Furthermore, the subtype of nAChR responsible for the nicotine remediation of CMOR task performance was also assessed. The $\alpha_7$ and $\alpha_4\beta_2$ nAChR subtypes are the two most prevalent nAChRs in the central nervous system (Albuquerque et al., 2009) and have been implicated in the treatment of cognition in schizophrenia (Freedman et al., 2008; Lieberman et al., 2013; Olincy et al., 2006; Radek et al., 2010; Zhang et al., 2012). Selective agonists of these receptor subtypes were therefore used to investigate their potential contribution to the facilitative effects in the CMOR task previously reported following nicotine administration (Jacklin et al., 2012).
One region of the brain that is heavily affected in schizophrenia is the prefrontal cortex (Beasley & Reynolds, 1997; Cochran et al., 2003). Specifically, orbitofrontal cortex (OFC) abnormalities are apparent in schizophrenia patients (Bellani et al., 2010; Kanahara et al., 2013), and acute administration of MK-801 in rats decreases PV mRNA within the OFC (Romon et al., 2011). The OFC has been shown to contribute to the CMOR task, as lesions of this region induce a selective CMOR impairment (Reid et al., 2013). Therefore, the OFC may be a potential target for pharmacological treatment in rats treated with NMDA receptor antagonists. Accordingly, the current study further explored the aforementioned hypotheses within the OFC of treated rats.

**Methods**

**Subjects**

One hundred and forty eight male Long-Evans rats were used as subjects in this study. Rats were housed in pairs, and a reverse light/dark cycle (8 AM lights off; 8 PM lights on) was maintained with testing occurring during the dark cycle. All rats were food restricted throughout the experiment and fed approximately 25g of food each following testing to maintain 85-90% free-feeding weight. Although object recognition tasks do no necessarily rely on food seeking for their performance, it has been consistently found that object exploratory behaviour is more robust when rats are food restricted during testing (Ennaceur, 2010). Water was freely accessible except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and followed the Canadian Council on Animal Care Guidelines.

**Sub-chronic drug administration**

Rats were handled for 1 week prior to treatment and randomly assigned to receive either sub-chronic treatment with ketamine (30 mg/kg; Bioniche, Belleville, ON, Canada) or saline (0.9% NaCl, pH 7.0; Sigma) at a volume of 1 ml/kg of their body weight (Jacklin et al., 2012).
Treatment consisted of twice daily intraperitoneal (i.p.) administration at 8 AM and 8 PM for 10 consecutive days. Following treatment, there was a 10-day washout period during which rats were left undisturbed on free-feed in their homecage. Experimenters were blind to the assigned condition.

Surgery

Following the 10-day treatment period surgery was performed on rats during the 10-day washout period (1 week prior to behavioural testing) for intracranial experiments. Rats were deeply anesthetized with isoflurane (Benson Medical Industries) inhalation anaesthetic prior to and throughout all surgeries. Prior to surgery all animals were administered a systemic subcutaneous injection of the analgesic meloxicam (5 mg/ml; Boehringer Ingelheim). Animals were then positioned in a stereotaxic frame (Kopf Instruments) with the incisor bar set to -3.3 mm. The scalp was cut and retracted to expose the skull. Holes were drilled, and the guide cannulas were implanted bilaterally according to the following coordinates for the orbitofrontal cortex, anteroposterior (AP) +3.4; lateral (L) ± 2.2; dorsoventral (DV) -3.2. The cannulas were secured to the skull using four jeweler screws and dental acrylic. Obturators were cut to extend 1.0 mm beyond the guide cannulas and were kept in the guide cannulas, except during infusions (see below). Following completion of each surgery, the scalp was sutured, and animals recovered on heat pads for 1–2h prior to being returned to their home cages. Rats were allowed to recover for 7–10 days before behavioural testing.

Infusion Procedure

For intracranial experiments, bilateral infusions were performed in a preparation room that was separate from the testing room. Each animal was gently restrained and obturators were removed from the guide cannulas. Infuser cannulas cut to extend 1.0 mm beyond the tips of the
guides were placed in the guide cannulas. Bilateral simultaneous infusions were delivered by two 1µl Hamilton syringes, which were connected to infuser cannulas by propylene tubing. The syringes dispensed 0.5µl of drug by a Harvard Apparatus syringe pump for a total of 1 min. Following this the infuser cannulas were left in the guides for another 90s to allow full diffusion. Infuser cannulas were then removed and replaced by the obturators. Prior to experimentation, all rats underwent two habitation sessions to the infusion procedure; in this case, the infusion procedure was duplicated in every way except that the syringes contained no fluid.

**Histology**

Following behavioural testing, an i.p. injection of 2 ml euthansol was administered to anesthetize the rats. Rats were transcardially perfused with 100 ml of PBS, pH 7.4, followed by 250 ml of 4% neutral buffered formalin, pH 7.4. The brains were then extracted, postfixed in 4% formalin at 4°C for 24 h, and then submerged in 20% sucrose in PBS until they sank. A cryostat was used to cut coronal sections (60 µm) through the OFC, and every fifth section was mounted on a gelatin-coated glass slide and stained with cresyl violet. Slides were examined under a light microscope to confirm cannula placements.

**Object recognition**

**Apparatus**

A Y-shaped apparatus was used to examine object recognition, as described in previous studies (Winters et al., 2004; Winters & Reid, 2010). The Y-shaped apparatus was constructed of Plexiglas with identical white walls measuring 40 cm high; the arms were 27 cm long and 10 cm wide. A start box connected to the two arms, which measured 18 cm from the rear of the arm, included a guillotine door. The start box allowed placement of the rat in the apparatus prior to the
The crossmodal object recognition task (CMOR) measures tactile-to-visual object recognition in rats. In all experiments the CMOR task was tested using a ‘minimal’ retention delay, which was made possible by an alteration of the Y-apparatus. Objects were placed on the feet of opaque inserts during the sample phase, allowing for immediate unveiling of the choice objects. Rats explored two identical objects in the sample phase, which was run in red light; this manipulation restricted object exploration to the tactile features of the objects. Following the sample phase, the inserts were removed, along with the sample objects, and this allowed nearly immediate presentation of the choice objects. In the choice phase, rats explored a novel object and a copy of the sample object. The choice phase was run in white light with Plexiglas barriers placed in front of the objects; this manipulation restricts object exploration to the visual features of the objects. Exploration of the novel object in comparison to the familiar object is typically greater in normal rats, indicating intact object recognition.

start of the sample phase. The apparatus was modified to allow immediate presentation of the choice objects following the sample phase (see Bartko et al., 2007a). Slits were made in the apparatus to allow the placement of object inserts x cm from the ends of the object exploration arms, on which the sample objects were placed (see Figure 2). These inserts measured 40 cm high and 10 cm wide with a platform measuring 9 cm wide and 9 cm long. Sample objects were placed on the inserts, which were merely pulled out of the apparatus at the end of the sample phase, allowing for presentation of the choice objects with a minimal delay between sample and choice phases. Every trial was recorded by a video camera mounted on a tripod above the Y-shaped apparatus. The Y-shaped apparatus was wiped down with a paper towel following each
testing phase, but was not otherwise cleaned. Duplicate ‘junk’ objects were acquired and varied in their tactile and visual features. A reusable adhesive putty was used to fasten all objects to the apparatus in order to prevent them from being knocked over during behavioural testing. Prior to being placed into the apparatus all objects were wiped down with 50% ethanol.

**General procedure**

Animals were habituated to the apparatus following the last day of the 10-day washout period over two consecutive days, which consisted of exploration of the empty Y-apparatus. Animals were transported to the testing room, where they were placed in the start box of the apparatus. The guillotine door was opened, and the animal exited into the arms permitting them to explore the empty apparatus. Each day the animals received two separate habituation sessions in a counterbalanced order, one session in white light with the transparent barriers for 5 min, and one 5-min session in red light with no barriers. Each rat in intracranial experiments also experienced a mock infusion immediately prior to each habituation session.

**Crossmodal object recognition**

Behavioural testing commenced 24h following the second day of habituation. Crossmodal object recognition consisted of two phases: the sample phase and choice phase, which were separated by a ‘minimal’ delay (see Figure 2). Different object pairs were used for each rat and were counterbalanced across all trials and rats. The same object pairs were used across all experiments, but no rat was exposed to the same pair on more than one trial. A computer was used to score and collect all exploratory data.

In the sample phase, two identical objects were placed in each arm of the apparatus on the object inserts. The sample phase was tested in red light with a red-tinted light bulb (40W) mounted to the tripod above the apparatus. This manipulation encourages exclusively tactile
information encoding (Winters & Reid, 2010). Rats were transported to the testing room. They were then placed in the start box of the apparatus, and the guillotine door was opened allowing them to enter the exploration arms. Exploration was defined as when the rat directed its nose at the object at a distance of <2cm and/or touched it with its nose or whiskers. The sample phase was completed when the rat had explored the objects for 25s or when 3 min elapsed, whichever occurred first.

Immediately following the sample phase the object inserts were removed, thereby presenting the choice objects. The red light was turned off and the white light was turned on during this time, as well; this ‘minimal delay’ procedure resulted in an interval of approximately 15s between the sample and choice phases, significantly reducing the mnemonic demands of the task. In the choice phase, a triplicate copy of the sample object and a new object were presented; these objects were placed in the apparatus prior to the start of the sample phase. Location of the objects in the apparatus arms was counterbalanced across all trials and rats. The choice phase was tested in white light with 4 overhead fluorescent lights and clear Plexiglas barriers placed 9 cm from the end of each arm in the apparatus. This manipulation encouraged exclusive use of visual information (Winters & Reid, 2010). Exploration time was recorded for a total time of 2 minutes; however, the first minute of exploration was analyzed, as the highest level of discrimination is seen during this time in normal rats (Dix & Aggleton, 1999). A discrimination ratio was calculated, defined as the difference in time spent exploring the novel versus familiar object divided by the total time spent exploring the objects. Exploration of the novel object in comparison to the familiar object is higher on object recognition tasks in normal rats (Ennaceur & Delacour, 1988; Winters & Reid, 2010).
Experiments

Experiment 1: Nicotinic interaction with the GABAergic system in ketamine-treated rats

Rats (10 saline-treated/10 ketamine-treated) were tested on the CMOR task. Animals were acutely co-administered i.p. nicotine (L-nicotine hydrogen tartrate salt, 0.2 mg/kg; dissolved in 0.9% physiological saline; Sigma) or physiological saline with bicuculline, a GABA<sub>A</sub> receptor antagonist (0.5 mg/kg; dissolved in 50% dimethyl sulfoxide (DMSO); Sigma), or 50% DMSO 20 min prior to the start of each trial. The dose of nicotine was selected based on the dose-response experiment completed in (Jacklin et al., 2012). The dose of bicuculline was selected as a ‘sub-optimal’ dose based on a previous study establishing that this dose does not induce cognitive impairment in rats (Harper, 2000). There were therefore four drug conditions: VEH/VEH, VEH/BIC, NIC/VEH, and NIC/BIC. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.

Experiments 2.1 and 2.2: Nicotinic interaction with the glutamatergic system in ketamine-treated rats

Rats (20 saline-treated/20 ketamine-treated) were tested on the CMOR task. Animals were acutely co-administered i.p. nicotine (0.2 mg/kg) or physiological saline with MK-801, an NMDA receptor antagonist (0.001 or 0.01 mg/kg; dissolved in physiological saline; Sigma), or physiological saline 20 min prior to the start of each trial. Two separate batches of rats were run for the two doses of MK-801. The doses of MK-801 were selected based on a previous study examining the effects of this drug in object recognition (de Lima, Laranja, Bromberg, Roesler, & Schröder, 2005). There were four drug conditions: VEH/VEH, VEH/MK-801, NIC/VEH, and
NIC/MK-801 for each of these experiments. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.

**Experiments 3.1 and 3.2: Involvement of α4β2 and α7 nAChRs in remediation of the CMOR impairment in ketamine-treated rats**

Rats (10 saline-treated/10 ketamine-treated) were tested on the CMOR task. Animals were acutely administered i.p. physiological saline or ABT-418, an α4β2 nAChR agonist (0.06, 0.1, or 0.6 mg/kg, dissolved in physiological saline; Sigma), 20 min prior to the start of each trial. Another batch of rats (10 saline-treated/10 ketamine-treated) was acutely administered i.p. physiological saline or GTS-21, an α7 nAChR agonist (0.01, 0.03, or 0.1 mg/kg, dissolved in physiological saline; Sigma), 20 min prior to the start of each trial. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.

**Experiment 4: α4β2 nAChR interaction with the GABAergic system in ketamine-treated rats**

Rats (10 saline-treated/10 ketamine-treated) were tested on the CMOR task. Animals were acutely co-administered i.p. ABT-418 (0.1 mg/kg) or physiological saline with bicuculline (0.5 mg/kg) or 50% DMSO 20 min prior to the start of each trial. The dose of ABT-418 was selected based on the dose-response in Experiment 3. There were four drug conditions: VEH/VEH, VEH/BIC, ABT/VEH, and ABT/BIC. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.
Experiment 5: Involvement of nAChRs in the OFC in remediation of the CMOR impairment of ketamine-treated rats

Rats (6 saline-treated/6 ketamine-treated) were implanted with bilateral indwelling cannulas in the OFC and tested on the CMOR task. Animals were intracranially infused with physiological saline or nicotine (0.5, 1, or 2 µg), immediately prior to the start of each trial. The doses of nicotine were selected based on a previous study examining the effects of this drug in object recognition (Melichercik, Elliott, Bianchi, Ernst, & Winters, 2012). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.

Experiment 6: Involvement of α4β2 nAChRs in the OFC in remediation of the CMOR impairment of ketamine-treated rats

Rats (8 saline-treated/8 ketamine-treated) were implanted with bilateral indwelling cannulas in the OFC and tested on the CMOR task. Animals were intracranially infused with physiological saline or ABT-418 (0.3, 1, or 3 µg), immediately prior to the start of each trial. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.

Experiment 7: α4β2 nACHR interaction with the GABAergic system within the OFC of ketamine-treated rats

The same cannulated rats (8 saline-treated/8 ketamine-treated) from the previous experiment were tested on the CMOR task. Animals were intracranially infused with physiological saline or ABT-418 (3 µg), immediately prior to the start of each trial, as well as systemically administered i.p. bicuculline (0.5 mg/kg) or 50% DMSO (vehicle) 20 min prior to the start of each trial. The dose of ABT-418 was selected based on the dose-response from
Experiment 6. There were four drug conditions: VEH/VEH, VEH/BIC, ABT/VEH, and ABT/BIC. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.

**Data analysis**

Behaviour was analyzed for ketamine- and saline-treated rats by taking the means of four measures for each drug/dose condition in each experiment. Each phase of behavioural testing was analyzed for two measures: the total object exploration in the sample phase, duration of the sample phase, the total object exploration in the choice phase, and discrimination ratio from the choice phase. As a control measure to confirm there were no general exploratory differences, the total object exploration was assessed in the sample and choice phases. Two-way (Group X Drug or Group X Dose) mixed-factors ANOVAs were used to analyze the means of these four measures, and *post-hoc* independent-samples t-tests were used to assess group differences within each drug/dose condition. A *Bonferroni correction* was applied to account for multiple comparisons. A sample discrimination ratio [(left object-right object/left object + right object)] mean was also calculated and compared to the choice discrimination ratio mean in each condition using paired-samples t-tests. This analysis tests for the presence or absence of novel object preference that is significantly higher than what would be expected by chance, as no object preference is expected in the sample phase (i.e., sample discrimination ratios should be at or around 0). This analysis is more conservative than single-sample t-tests versus zero because the sample discrimination ratio contains variability, thereby decreasing the chance of committing a Type I error (Akkerman, Prickaerts, Steinbusch, & Blokland, 2012). Statistical analyses were conducted with a significance level of $\alpha = 0.05$ using SPSS.
Results

Experiment 1: GABA<sub>A</sub> receptor blockade inhibits nicotinic reversal of the CMOR impairment in ketamine-treated rats

Pre-sample administration of nicotine reversed the CMOR impairment in ketamine-treated rats. However, co-administration of bicuculline, at a dose that otherwise did not cause impairment in saline-treated rats, blocked the effect of nicotine in ketamine-treated rats (Figure 3). A two-way mixed-factors ANOVA revealed a significant interaction between drug and group \( F(3, 54) = 4.286, p < 0.01 \), as well as significant main effects of drug \( F(3, 54) = 9.396, p < 0.01 \) and group \( F(1, 18) = 19.026, p < 0.01 \).

Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated rats in the VEH/VEH and NIC/BIC drug conditions, but not the VEH/BIC or NIC/VEH drug conditions (VEH/VEH: \( t(18) = 3.230, p < 0.01 \); VEH/BIC: \( t(18) = 2.659, p = 0.016 \); NIC/VEH: \( t(18) = 1.402, p = 0.178 \); NIC/BIC: \( t(18) = 2.820, p = 0.011 \)). Furthermore, paired-samples t-tests comparing sample versus choice discrimination ratios also supported this finding, indicating a significant novelty preference in all drug conditions for saline-treated rats (VEH/VEH: \( p < 0.01 \); VEH/BIC: \( p < 0.01 \); NIC/VEH: \( p < 0.05 \); NIC/BIC: \( p < 0.05 \)). Ketamine-treated animals, however, displayed a significant novelty preference only in the NIC/VEH drug condition \( p < 0.01 \). The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects, suggesting no effect of drug condition on general exploratory activity (Tables 1 & 2).
Experiments 2.1 and 2.2: NMDA receptor blockade has no effect on nicotinic reversal of the CMOR impairment in ketamine-treated rats

In Experiment 2.1, pre-sample administration of nicotine reversed the CMOR impairment in ketamine-treated rats, and this effect was not blocked by co-administration of MK-801 (0.001 mg/kg; Figure 4a). A two-way mixed-factors ANOVA revealed a significant interaction between drug and group ($F(3,54) = 20.812, p < 0.01$), as well as significant main effects of drug ($F(3, 54) = 14.641, p < 0.01$) and group ($F(1, 18) = 20.919, p < 0.05$).

Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated rats in the VEH/VEH and VEH/MK-801 drug conditions, but not the other drug conditions (VEH/VEH: $t(18) = 7.223, p < 0.01$; VEH/MK-801: $t(18) = 5.144, p < 0.01$; NIC/VEH: $t(18) = 2.094, p = 0.051$; NIC/MK-801: $t(18) = .853, p = 0.405$). Paired-samples t-
tests comparing sample versus choice discrimination ratios also supported this finding, indicating a significant novelty preference in all drug conditions for saline-treated animals (VEH/VEH: $p < 0.01$; VEH/MK-801: $p < 0.01$; NIC/VEH: $p < 0.01$; NIC/MK-801: $p < 0.05$). Ketamine-treated animals displayed a significant preference only in the NIC/VEH ($p < 0.01$) and NIC/MK-801 ($p < 0.01$) drug conditions.

In Experiment 2.2, pre-sample administration of 0.01 mg/kg MK-801 also failed to reverse the facilitative effect of nicotine; MK-801 at this dose also induced impairment in saline-treated rats (Figure 4b). A two-way mixed-factors ANOVA revealed a significant interaction between drug and group ($F(3,54) = 15.386, p < 0.01$), as well as significant main effects of drug ($F(3, 54) = 33.737, p < 0.01$) and group ($F(1, 18) = 5.406, p < 0.05$).

Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated rats in the VEH/VEH drug condition, but not the other drug conditions.

Figure 4. a) Pre-sample systemic administration of nicotine (0.2 mg/kg) reversed the CMOR impairment in ketamine-treated rats and this effect was not blocked by MK-801 (0.001 mg/kg); b) Similarly, pre-sample systemic administration of nicotine reversed the CMOR impairment in ketamine-treated rats and this effect was not blocked by MK-801 (0.01 mg/kg). However, this dose of MK-801 induced CMOR impairment in control rats. Discrimination ratios displayed for group means (+ SEM). *** $= p < 0.001$, ketamine versus control rats.
(VEH/VEH: \( t(18) = 8.084, p < 0.01; \) VEH/MK-801: \( t(18) = 0.408, p = 0.688; \) NIC/VEH: \( t(18) = 0.156, p = 0.878; \) NIC/MK-801: \( t(18) = 1.339, p = 0.197 \)). Furthermore, Paired-samples t-tests comparing sample versus choice discrimination ratios also supported this finding, indicating a significant novel object preference in saline-treated rats in every drug condition but the VEH/MK-801 condition (VEH/VEH: \( p < 0.01; \) VEH/MK-801: \( p = 0.454; \) NIC/VEH: \( p < 0.01; \) NIC/MK-801: \( p < 0.01 \)). Ketamine-treated rats displayed a significant preference in only the NIC/VEH (\( p < 0.01 \)) and NIC/MK-801 (\( p < 0.01 \)) drug conditions. The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects in either experiment, suggesting no effect of drug condition on general exploratory behaviour (Tables 1 & 2).

**Experiments 3.1 and 3.2: \( \alpha_4\beta_2 \) nAChR, but not \( \alpha_7 \) nAChR, stimulation remediates the CMOR impairment in ketamine-treated rats**

In Experiment 3.1, pre-sample administration of ABT-418 dose-dependently reversed the CMOR impairment in ketamine-treated rats (Figure 5a). A two-way mixed-factors ANOVA revealed a significant interaction between dose and group (\( F(3,54) = 3.680, p < 0.05 \)), as well as significant main effects of dose (\( F(3, 54) = 10.321, p < 0.01 \)) and group (\( F(1, 18) = 11.450, p < 0.01 \)). Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated animals in the VEH condition, but not the other conditions (VEH: \( t(18) = 4.486, p < 0.01; \) 0.06 mg/kg: \( t(18) = 1.903, p = 0.073; \) 0.1 mg/kg: \( t(18) = 0.004, p = 0.997; \) 0.6 mg/kg: \( t(18) = 1.243, p = 0.230 \)). Paired-samples t-tests comparing sample versus choice discrimination ratios corroborated this finding, indicating a significant novel object preference in saline-treated rats in every drug condition (0.06: \( p < 0.05; \) 0.1: \( p < 0.01; \) 0.6: \( p < 0.01 \)), except for the VEH condition (\( p = 0.088 \)). Ketamine-treated rats displayed a significant preference only in
the 0.1 mg/kg ($p < 0.01$) and 0.6 mg/kg ($p < 0.01$) drug conditions. The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects, suggesting no effect of dose condition on general exploration (Tables 1 & 2).

Pre-sample administration of GTS-21 did not alter the CMOR impairment in ketamine-treated rats (Figure 5b). A two-way mixed-factors ANOVA revealed a significant main effect of group ($F(1, 18) = 29.339, p < 0.01$), but no significant interaction effect ($F(3, 54) = 0.518, p = 0.672$) or main effect of dose ($F(3, 54) = 0.413, p = 0.744$). Furthermore, ketamine-treated rats failed to display significant novel object preference in all conditions (all $p > 0.05$). Analysis of total object exploration in the sample and choice phases revealed no significant effects, suggesting no substantial influence of dose condition on general exploratory behaviour (Tables 1 & 2).

Figure 5. a) Pre-sample systemic administration of the $\alpha_4\beta_2$ nAChR agonist ABT-418 (0.06, 0.1 & 0.6 mg/kg) dose-dependently reversed the CMOR impairment in ketamine-treated rats. b) Pre-sample systemic administration of $\alpha_7$ nAChR agonist GTS-21 (0.01, 0.03 & 0.1 mg/kg) had no effect on the CMOR impairment in ketamine-treated rats. Discrimination ratios displayed for group means (+ SEM). *** = $p < 0.001$, ketamine versus control rats.
Experiment 4: GABA\textsubscript{A} receptor blockade prevents \(\alpha_4\beta_2\) nAChR amelioration of the CMOR impairment in ketamine-treated rats

Pre-sample administration of ABT-418 reversed the CMOR impairment in ketamine-treated rats. However, co-administration of bicuculline, at a dose that otherwise did not cause impairment in saline-treated rats, blocked the effect of ABT-418 in ketamine-treated animals (Figure 6). A two-way mixed-factors ANOVA revealed a significant interaction between drug and group \((F(3,54) = 12.955, p < 0.01)\), as well as significant main effects of drug \((F(3, 54) = 10.523, p < 0.01)\) and group \((F(1, 18) = 128.006, p < 0.01)\).

Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated rats in the VEH/VEH, VEH/BIC and ABT/BIC drug conditions, but not the ABT/VEH drug condition \((VEH/VEH: t(18) = 5.433, p < 0.01; VEH/BIC: t(18) = 7.514, p < 0.01; ABT/VEH: t(18) = 0.772, p = 0.450; ABT/BIC: t(18) = 8.497, p < 0.01)\). Moreover, Paired-

![Figure 6](image-url)  

Figure 6. Pre-sample systemic administration of ABT-418 (0.1 mg/kg) reversed the CMOR impairment in ketamine-treated rats; however, bicuculline, at a dose (0.5 mg/kg) that otherwise did not cause impairment in control rats, blocked this effect in ketamine-treated rats. Discrimination ratios displayed for group means (+ SEM). *** = \(p < 0.001\), ketamine versus control rats.
samples t-tests comparing sample versus choice discrimination ratios also supported this finding, indicating a significant preference for the novel object by saline-treated rats in all drug conditions ($p < 0.01$). Ketamine-treated rats, however, displayed a significant novelty preference only in the ABT–VEH drug condition ($p < 0.01$). The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects (Tables 1 & 2).

**Experiment 5: Intra-OFC nAChR stimulation remediates the CMOR impairment in ketamine-treated rats**

Rats included in the analyses for experiments 5-7 had cannulas located bilaterally with injection needle tips terminating in the OFC between 3.72 and 4.20 mm anterior to bregma (Figure 7a). Pre-sample intra-OFC infusion of nicotine reversed the CMOR impairment in ketamine-treated rats (Figure 7b). A two-way mixed-factors ANOVA revealed a significant interaction between dose and group ($F(3, 30) = 11.041, p < 0.01$), as well as significant main effects of dose ($F(3, 30) = 3.711, p < 0.05$) and group ($F(1, 10) = 13.774, p < 0.01$).

*Post-hoc* independent samples t-tests revealed significant differences between ketamine- and saline-treated animals in the VEH and 0.5 µg/µl conditions, but not the other conditions (VEH: $t(10) = 5.956, p < 0.01$; 0.5 µg/µl: $t(10) = 3.189, p < 0.01$; 1 µg/µl: $t(10) = 0.904, p = 0.997$; 2 µg/µl: $t(10) = 1.720, p = 0.116$). Paired-samples t-tests comparing sample versus choice discrimination ratios supported this finding, indicating a significant novel object preference in saline-treated rats in every drug condition ($p < 0.01$), except for the 0.5 µg/ul condition ($p = 0.081$). However, ketamine-treated animals displayed a significant novel object preference only in 1 µg/ul drug condition ($p < 0.01$). The analysis of total object exploration in the sample and choice phases revealed a significant main effect of group ($F(1, 10) = 13.939, p < 0.01$) and dose ($F(3, 30) = 3.139, p < 0.05$), but no significant interaction during the choice phase (Tables 1 & 2).
Figure 7. (a) Cannulation of rat OFC. Schematic representation of the infusion needle tip placements from a typical group of animals (experiment 5; n = 12). These placements are representative of needle tip locations in all animals (closed = saline-treated rats; open = ketamine-treated rats) included in the behavioural analyses of the present study. Cannulas were consistently located between 3.72 to 4.20 mm anterior to bregma. Some needle tips overlap in the figure. (b) Pre-sample intra-OFC administration of nicotine (0.5, 1 & 2 µg/µl) dose-dependently reversed the CMOR impairment in ketamine-treated rats. Discrimination ratios displayed for group means (SEM +). *** = p < 0.001; ** = p < 0.01, ketamine versus control rats.
Experiment 6: Intra-OFC α4β2 nAChR stimulation reverses the CMOR impairment in ketamine-treated rats

Pre-sample intra-OFC infusion of ABT-418 appeared to reverse the CMOR impairment in ketamine-treated rats in a dose-dependent manner (Figure 8a). A two-way mixed-factors ANOVA revealed significant main effects of dose ($F(3, 42) = 4.896, p < 0.01$) and group ($F(1, 14) = 18.364, p < 0.01$), but an interaction that did not quite reach significance ($F(3, 42) = 2.307, p = 0.090$). However, paired-samples t-tests comparing sample to choice discrimination ratios indicated that ketamine-treated rats, while not displaying above chance performance in any other condition, showed significant novel object preference with the 3 μg/μl dose ($p < 0.01$), consistent with the dose-dependent improvement caused by systemic ABT-418. Saline-treated rats displayed significant novelty preference in all conditions (VEH: $p < 0.01$; 0.3 μg/μl: $p < 0.01$; 1 μg/μl: $p < 0.05$; 3 μg/μl: $p < 0.01$). The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects, suggesting no effect of dose condition on general exploration (Tables 1 & 2).

Experiment 7: GABA_A receptor blockade prevents intra-OFC α4β2 nAChR-induced amelioration of the CMOR impairment in ketamine-treated rats

As in Experiment 6, pre-sample intra-OFC infusion of ABT-418 reversed the CMOR impairment in ketamine-treated rats. However, systemic co-administration of bicuculline, at a dose that otherwise did not cause impairment in saline-treated rats, blocked the effect of ABT-418 in ketamine-treated animals (Figure 8b). A two-way mixed-factors ANOVA revealed a significant interaction between drug and group ($F(3, 42) = 3.994, p < 0.05$), as well as significant main effects of drug ($F(3, 42) = 6.147, p < 0.01$) and group ($F(1, 14) = 28.506, p < 0.01$).
Post-hoc independent samples t-tests revealed significant differences between ketamine- and saline-treated rats in the VEH/VEH, VEH/BIC and ABT/BIC drug conditions, but not the ABT/VEH drug condition (VEH/VEH: $t(14) = 4.083, p < 0.01$; VEH/BIC: $t(14) = 5.544, p < 0.01$; ABT/VEH: $t(14) = 0.158, p = 0.877$; ABT/BIC: $t(14) = 3.773, p < 0.01$). Moreover, Paired-samples t-tests comparing sample and choice discrimination ratios corroborated this finding, indicating a significant preference for the novel object by saline-treated rats in all drug conditions (VEH/VEH: $p < 0.05$; VEH/BIC: $p < 0.01$; ABT/VEH: $p < 0.01$; ABT/BIC: $p < 0.01$). Ketamine-treated rats, however, displayed a significant novelty preference only in the ABT–VEH drug condition ($p < 0.01$). The analysis of total object exploration in the sample and choice phases revealed no significant interaction or main effects, suggesting no effect of drug condition on general exploratory behaviour (Tables 1 & 2).

Figure 8. a) Pre-sample intra-OFC administration of ABT-418 (0.3, 1 & 3 µg/µl) dose-dependently reversed the CMOR impairment in ketamine-treated rats. b) Pre-sample intra-OFC administration of ABT-418 (3 µg/µl) reversed the CMOR impairment in ketamine-treated rats; however, systemic administration of bicuculline, at a dose (0.5 mg/kg) that otherwise did not cause impairment in control rats, blocked this effect in ketamine-treated rats. Discrimination ratios displayed for group means (+ SEM). *** = $p < 0.001$; ** = $p < 0.01$, ketamine versus control rats.
Discussion

Abnormal multisensory integration is apparent in schizophrenia (Stekelenburg et al., 2013; Stone et al., 2011; Williams et al., 2010). Likewise, rats treated with sub-chronic NMDA receptor antagonists to model schizophrenia are severely impaired in the CMOR task (Jacklin et al., 2012). The present results replicate and extend these findings, demonstrating that this impairment is seen even when the mnemonic demands of the task are minimized, results suggestive of a deficit in perceptual aspects of crossmodal cognition and multisensory binding. This interpretation is consistent with our previous finding that ketamine-treated rats were equally impaired with relatively long or short retention delays in the CMOR task; interestingly, this contrasts with the apparent delay-dependence of standard and unimodal object recognition task deficits in ketamine-treated rats, a pattern indicative of a specific mnemonic impairment (Jacklin et al., 2012). Acute administration of nicotine reversed the CMOR deficit displayed by ketamine-treated rats, and co-administration of the GABA_A receptor antagonist bicuculline blocked this facilitation at a dose that did not affect CMOR performance in saline-treated rats. Conversely, co-administration of the NMDA receptor antagonist MK-801 did not influence the enhancement produced by nicotine; however, acute MK-801 (0.01 mg/kg) administration impaired CMOR performance in saline-treated rats. Furthermore, the α4β2 nAChR agonist ABT-418, but not the α7 nAChR agonist GTS-21, dose-dependently reversed the impairment in ketamine-treated rats. Co-administration of bicuculline blocked the ameliorative effect of ABT-418 in ketamine-treated rats. In addition, the remediating effect of nicotine and ABT-418 in ketamine-treated rats was replicated through intra-OFC administration of these drugs, and the effect of intra-OFC ABT-418 was blocked by systemic administration of bicuculline. Overall, these findings suggest that
the facilitative effect of nicotine in the CMOR task results from \( \alpha_4\beta_2 \) nAChR mediated stimulation of the GABAergic system within the OFC.

The cholinergic system has been identified as a pharmacological target for the treatment of cognitive dysfunction in schizophrenia (Jones, Byun, & Bubser, 2012). Amongst the candidates, the \( \alpha_4\beta_2 \) and \( \alpha_7 \) nAChR subtypes, which are prevalent throughout the central nervous system, have been implicated. There is debate, however, regarding the effectiveness of specific nAChR agonists in the treatment of cognitive dysfunction in schizophrenia. Studies using such drugs are inconsistent regarding their efficacy in treating the eight cognitive domains (speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, verbal comprehension, and social cognition) outlined by MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) to be impaired in schizophrenia patients (Freedman et al., 2008; Lieberman et al., 2013; Olincy et al., 2006; Radek et al., 2010; Umbricht et al., 2014; Waldo et al., 2010; Zhang et al., 2012). Indeed, a recent study demonstrated that the selective \( \alpha_4\beta_2 \) nAChR agonist AZD3480 failed to improve cognition (speed of processing, attention/vigilance, working memory, verbal learning, reasoning and problem solving) in schizophrenia patients (Velligan et al., 2012). However, there is more consistent evidence from animal models of schizophrenia that acute administration of \( \alpha_4\beta_2 \) and \( \alpha_7 \) nAChR receptor agonists attenuate cognitive impairment (Hashimoto et al., 2008; Hauser et al., 2010; Karamihalev, Prickaerts, & van Goethem, 2014; Pichat et al., 2007; Radek et al., 2006; Thomsen et al., 2009; Timmermann et al., 2009; Wallace et al., 2011; Wildeboer & Stevens, 2008). Our data suggest that nicotine-induced amelioration of the CMOR deficit in ketamine-treated rats results from stimulation of the \( \alpha_4\beta_2 \) nAChR, but not the \( \alpha_7 \) nAChR. Consistent with these findings, post-mortem studies have revealed \( \alpha_4\beta_2 \) nAChRs
to be decreased in the brains of patients with schizophrenia (Durany et al., 2000; Freedman et al., 1995). The inability, however, of an $\alpha_4\beta_2$ nAChR agonist to improve some aspects of cognition in human patients (Velligan et al., 2012) highlights the need for more analysis of nAChR subtype involvement in specific cognitive functions. While this discrepancy may indicate differences between the pathology in schizophrenia and the ketamine rat model, it is also possible that different cognitive abilities respond differentially to specific receptor activation. Clearly, future studies are warranted to assess the generalizability of specific nAChR agonists on different cognitive functions in both animal models and schizophrenia patients.

Evidence shows that nAChRs interact with the GABAergic and glutamatergic systems (Levin, McClernon, & Rezvani, 2006; Timofeeva & Levin, 2011), and alterations of these systems are associated with schizophrenia pathology (Jentsch & Roth, 1999; Lewis et al., 2012; Lewis et al., 2005; Olney et al., 1999). Our results imply that nicotine facilitates CMOR performance in ketamine-treated rats via GABAergic system stimulation that is independent of the glutamatergic system. Although acute administration of MK-801 (0.01 mg/kg) impaired CMOR performance in saline-treated animals, further indicating the sensitivity of the CMOR task to NMDA receptor antagonism, this dose of MK-801 did not prevent the nicotine-induced CMOR task enhancement in ketamine-treated rats. Furthermore, our results suggest that this amelioration is mediated by $\alpha_4\beta_2$ nAChR-induced stimulation of the GABAergic system. Electrophysiological studies support this interpretation, showing that agonism of $\alpha_4\beta_2$ nAChRs on GABAergic interneurons stimulates GABA release (Alkondon & Albuquerque, 2001; Alkondon, Pereira, Eisenberg, & Albuquerque, 2000; Aracri et al., 2010). In addition, sub-chronic administration of the partial $\alpha_4\beta_2$ nAChR agonist varenicline or full agonist A-85380 in mice increased GAD67 expression in the frontal cortex, unlike administration of the $\alpha_7$ agonist
PNU-282987, and this effect was blocked by mecamylamine (Maloku et al., 2011). Varenicline is an approved treatment for smoking cessation and recent work has demonstrated it may have pro-cognitive effects in patients with schizophrenia, as improvements in sensory gating, learning, and memory are found (Hong et al., 2011; Shim et al., 2012; Smith et al., 2009). Given the present results, investigation of the potential efficacy of varenicline, a partial $\alpha_4\beta_2$ nAChR agonist, in schizophrenia-related multisensory deficits may be warranted. Furthermore, the interaction between nAChR subtypes and GABA should be assessed using other tasks, such as spatial memory tasks. It is interesting to note that a recent study reported spatial memory facilitation in MK-801-treated mice administered an $\alpha_7$ nAChR agonist (Karamihalev et al., 2014). Thus, it is possible, as stated above, that different nAChR subtypes mediate improvement on different cognitive tasks, even within very similar animal models; clarification on this point will be required to ensure translational success. Further work is therefore required to evaluate the generalizability of the effects reported here to other animal models and forms of cognition.

Dysfunction of specific GABAergic interneurons appears to be an important aspect of schizophrenia pathology (Lewis et al., 2012; Lewis et al., 2005). Specifically, PV-INs are affected by sub-chronic injections of NMDA receptor antagonists in the prefrontal cortex of animals (Abdul-Monim et al., 2007; Cochran et al., 2003; Pratt et al., 2008), with similar alterations found post-mortem in schizophrenia (Beasley et al., 2002a; Beasley & Reynolds, 1997). These GABAergic interneurons within the prefrontal cortex contribute to cortical gamma oscillatory activity (Sohal et al., 2009), and this activity is strongly linked to cognitive functions, such as multisensory integration, fear conditioning, and spatial integration (Courtin et al., 2014; Olcese et al., 2013; Vaicelinunaite, Erisken, Franzen, Katzner, & Busse, 2013). Previous research implicates the OFC in CMOR task performance (Reid et al., 2013), and the CMOR
deficit apparent in ketamine-treated rats may be associated with GABAergic dysfunction within this region of the prefrontal cortex. The current study supports this hypothesis with $\alpha_4\beta_2$ nAChR-induced stimulation of GABAergic release reversing the impairment in ketamine-treated rats. Furthermore, abnormal oscillatory activity is found within the prefrontal cortex of schizophrenia patients (Uhlhaas & Singer, 2010), and this may disrupt synchronous cortical communication, which is important for the binding of sensory information (Senkowski et al., 2008). Comparably, systemic administration of the NMDA receptor antagonist MK-801 in awake rats reduces inhibitory GABAergic interneuron activity while increasing pyramidal cell activity within the OFC (Homayoun & Moghaddam, 2008), as well as altering gamma oscillatory activity within this region (Wood et al., 2012). The CMOR task relies on contributions from a distributed cortical network that also includes the perirhinal, posterior parietal, and retrosplenial cortices (Hindley, Nelson, Aggleton, & Vann, 2014; Reid et al., 2013; Winters & Reid, 2010). Altered synchronous activity might be expected to disrupt communication within and/or between these brain regions, detrimentally affecting performance on the CMOR task. Functional brain networks appear to be compromised in rats sub-chronically administered the NMDA receptor antagonist PCP (Dawson et al., 2014). Future research is required to clarify the involvement of the OFC, as well as the other regions implicated in CMOR, in the myriad cognitive deficits seen in ketamine-treated rats; such work may help to elucidate the specific mechanism by which $\alpha_4\beta_2$ nAChRs influence the GABAergic system to aid CMOR task performance.

The present study further highlights the pre-clinical value of the CMOR task. An established rodent model of schizophrenia, based on the NMDA receptor hypofunction hypothesis (Jentsch & Roth, 1999; Olney et al., 1999), displays severe impairment in this task, consistent with a growing body of literature demonstrating atypical multisensory integration in
schizophrenia patients (Stekelenburg et al., 2013; Stone et al., 2011; Williams et al., 2010). The fact that this deficit is apparent with even minimal mnemonic demand is also consistent with dysfunction at the stage of relatively early multisensory perceptual binding. This impairment is reversed in ketamine-treated rats by nicotine and ABT-418 (an α4β2 nAChR agonist) systemically and within the OFC, further validating the CMOR task as a potentially valuable addition to behavioural test batteries with relevance to cognitive disruption in schizophrenia. Both nicotinic drug effects were prevented by GABA_A receptor blockade, and this is consistent with the postulated role for GABAergic inhibition in circuit dynamics required for multisensory processing, as well as observed changes to the GABAergic system in the brains of patients with schizophrenia. These findings emphasize the potential value in pursuing nicotinic and/or GABAergic pharmacotherapeutic strategies (Buchanan et al., 2011; Damgaard et al., 2011; Lewis et al., 2008; Menzies et al., 2007) for schizophrenia-related disruption of cognitive abilities requiring coordinated processing across multiple cortical sites.

Acknowledgements

This research was supported by a Natural Sciences and Engineering Research Council (NSERC; 400176) Discovery Grant to BDW and an NSERC Canadian Graduate Scholarship (Master’s) to JMC.
Table 1. Control measures collected from the sample phase for each condition from Experiments 1-7

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Condition</th>
<th>Ketamine Rats</th>
<th>Ketamine Rats</th>
<th>Control Rats</th>
<th>Control Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Object</td>
<td>Total Phase</td>
<td>Total Object</td>
<td>Total Phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exploration</td>
<td>Duration</td>
<td>Exploration</td>
<td>Duration</td>
</tr>
<tr>
<td>1</td>
<td>VEH/VEH</td>
<td>10.71 ± 0.49</td>
<td>180 ± 0</td>
<td>10.02 ± 0.24</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>9.96 ± 0.21</td>
<td>180 ± 0</td>
<td>9.92 ± 0.25</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>10.28 ± 0.26</td>
<td>180 ± 0</td>
<td>9.71 ± 0.34</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC/BIC</td>
<td>13.42 ± 0.17</td>
<td>180 ± 0</td>
<td>11.87 ± 0.37</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>2.1</td>
<td>VEH/VEH</td>
<td>19.9 ± 0.74</td>
<td>180 ± 0</td>
<td>10.44 ± 0.49</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>VEH/MK-801</td>
<td>12.26 ± 0.41</td>
<td>180 ± 0</td>
<td>9.71 ± 0.68</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>10.28 ± 0.36</td>
<td>180 ± 0</td>
<td>12.07 ± 0.20</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC/MK-801</td>
<td>12.28 ± 0.49</td>
<td>180 ± 0</td>
<td>10.24 ± 0.55</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>2.2</td>
<td>VEH/VEH</td>
<td>15.30 ± 0.91</td>
<td>180 ± 0</td>
<td>14.24 ± 0.74</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>VEH/MK-801</td>
<td>14.84 ± 1.24</td>
<td>180 ± 0</td>
<td>16.24 ± 0.80</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>15.88 ± 1.19</td>
<td>180 ± 0</td>
<td>16.06 ± 4.75</td>
<td>177.01 ± 9.27</td>
</tr>
<tr>
<td></td>
<td>NIC/MK-801</td>
<td>15.23 ± 0.86</td>
<td>180 ± 0</td>
<td>14.65 ± 3.73</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>3.1</td>
<td>VEH</td>
<td>9.59 ± 0.56</td>
<td>180 ± 0</td>
<td>9.51 ± 0.56</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT 0.06 mg/kg</td>
<td>9.64 ± 0.31</td>
<td>180 ± 0</td>
<td>9.48 ± 0.48</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT 0.1 mg/kg</td>
<td>9.45 ± 0.68</td>
<td>180 ± 0</td>
<td>8.89 ± 0.73</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT 0.6 mg/kg</td>
<td>9.03 ± 0.33</td>
<td>180 ± 0</td>
<td>9.06 ± 0.39</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>3.2</td>
<td>VEH</td>
<td>13.57 ± 1.87</td>
<td>180 ± 0</td>
<td>12.27 ± 2.04</td>
<td>171.61 ± 17.78</td>
</tr>
<tr>
<td></td>
<td>GTS 0.01 mg/kg</td>
<td>15.63 ± 1.89</td>
<td>179.94 ± 0.59</td>
<td>11.25 ± 1.77</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>GTS 0.03 mg/kg</td>
<td>11.91 ± 1.97</td>
<td>180 ± 0</td>
<td>10.95 ± 1.54</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>GTS 0.1 mg/kg</td>
<td>11.14 ± 0.80</td>
<td>180 ± 0</td>
<td>12.26 ± 1.56</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>4</td>
<td>VEH</td>
<td>15.94 ± 1.08</td>
<td>180 ± 0</td>
<td>14.75 ± 0.97</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>15.43 ± 0.52</td>
<td>180 ± 0</td>
<td>15.45 ± 0.78</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>15.46 ± 0.59</td>
<td>180 ± 0</td>
<td>14.02 ± 0.99</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>15.64 ± 0.38</td>
<td>180 ± 0</td>
<td>15.51 ± 0.59</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>5</td>
<td>VEH</td>
<td>21.53 ± 0.83</td>
<td>172.78 ± 7.36</td>
<td>23.22 ± 1.29</td>
<td>162.59 ± 6.69</td>
</tr>
<tr>
<td></td>
<td>NIC 0.5 µg</td>
<td>23.07 ± 1.50</td>
<td>167.21 ± 7.15</td>
<td>20.22 ± 2.04</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>NIC 1</td>
<td>23.84 ± 0.68</td>
<td>155.64 ± 12.40</td>
<td>19.42 ± 2.63</td>
<td>173.27 ± 6.76</td>
</tr>
<tr>
<td></td>
<td>NIC 2 µg</td>
<td>22.51 ± 0.92</td>
<td>178.61 ± 1.42</td>
<td>19.44 ± 2.00</td>
<td>166.54 ± 13.49</td>
</tr>
<tr>
<td>6</td>
<td>VEH</td>
<td>23.64 ± 0.79</td>
<td>160.47 ± 8.01</td>
<td>19.66 ± 1.53</td>
<td>176.62 ± 3.41</td>
</tr>
<tr>
<td></td>
<td>ABT 0.3 µg</td>
<td>20.98 ± 0.89</td>
<td>178.38 ± 1.65</td>
<td>20.61 ± 1.11</td>
<td>175.78 ± 4.25</td>
</tr>
<tr>
<td></td>
<td>ABT 1 µg</td>
<td>20.97 ± 0.67</td>
<td>175.92 ± 4.12</td>
<td>20.42 ± 1.29</td>
<td>176.19 ± 3.11</td>
</tr>
<tr>
<td></td>
<td>ABT 3 µg</td>
<td>21.39 ± 0.88</td>
<td>178.79 ± 1.24</td>
<td>20.02 ± 0.78</td>
<td>180 ± 0</td>
</tr>
<tr>
<td>7</td>
<td>VEH/VEH</td>
<td>20.02 ± 0.84</td>
<td>175.70 ± 12.26</td>
<td>20.06 ± 1.39</td>
<td>171.91 ± 8.12</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>19.19 ± 1.07</td>
<td>180 ± 0</td>
<td>18.52 ± 0.77</td>
<td>180 ± 0</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>21.56 ± 0.68</td>
<td>178.84 ± 1.19</td>
<td>19.84 ± 1.29</td>
<td>172.84 ± 4.73</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>22.70 ± 1.25</td>
<td>159.39 ± 10.88</td>
<td>20.53 ± 1.59</td>
<td>171.73 ± 7.41</td>
</tr>
</tbody>
</table>

Data are expressed as the mean (± SEM) of the total time in seconds spent exploring the objects and the total duration of the sample phase in seconds.
Table 2. Control measures collected from the choice phase for each condition from Experiments 1-7

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Condition</th>
<th>Ketamine Rats Total Object Exploration</th>
<th>Control Rats Total Object Exploration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VEH/VEH</td>
<td>1.23 ± 0.15</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>1.40 ± 0.10</td>
<td>1.15 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>1.36 ± 0.16</td>
<td>1.46 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>NIC/BIC</td>
<td>1.17 ± 0.14</td>
<td>1.48 ± 0.10</td>
</tr>
<tr>
<td>2.1</td>
<td>VEH/VEH</td>
<td>1.68 ± 0.11</td>
<td>1.71 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>VEH/MK</td>
<td>-</td>
<td>1.51 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>1.71 ± 0.12</td>
<td>1.60 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>NIC/MK-801</td>
<td>1.72 ± 0.22</td>
<td>1.27 ± 0.17</td>
</tr>
<tr>
<td>2.2</td>
<td>VEH/VEH</td>
<td>1.39 ± 0.07</td>
<td>1.54 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>VEH/MK-801</td>
<td>1.49 ± 0.10</td>
<td>1.90 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>NIC/VEH</td>
<td>1.71 ± 0.12</td>
<td>1.72 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>NIC/MK-801</td>
<td>1.68 ± 0.18</td>
<td>1.78 ± 0.18</td>
</tr>
<tr>
<td>3.1</td>
<td>VEH</td>
<td>1.23 ± 0.13</td>
<td>1.18 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>ABT 0.06 mg/kg</td>
<td>1.20 ± 0.12</td>
<td>1.43 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>ABT 0.1 mg/kg</td>
<td>1.30 ± 0.15</td>
<td>1.48 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>ABT 0.6 mg/kg</td>
<td>1.27 ± 0.14</td>
<td>1.23 ± 0.14</td>
</tr>
<tr>
<td>3.2</td>
<td>VEH</td>
<td>2.48 ± 0.34</td>
<td>2.25 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>GTS 0.01 mg/kg</td>
<td>2.30 ± 0.36</td>
<td>2.26 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>GTS 0.03 mg/kg</td>
<td>2.41 ± 0.26</td>
<td>2.54 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>GTS 0.1 mg/kg</td>
<td>2.47 ± 0.29</td>
<td>2.83 ± 0.28</td>
</tr>
<tr>
<td>4</td>
<td>VEH/VEH</td>
<td>1.37 ± 0.18</td>
<td>1.48 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>1.55 ± 0.16</td>
<td>1.23 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>1.64 ± 0.16</td>
<td>1.52 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>1.55 ± 0.16</td>
<td>1.53 ± 0.13</td>
</tr>
<tr>
<td>5</td>
<td>VEH</td>
<td>1.93 ± 0.44</td>
<td>2.92 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>NIC 0.5 µg</td>
<td>1.37 ± 0.18</td>
<td>2.17 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>NIC 1 µg</td>
<td>1.61 ± 0.19</td>
<td>1.92 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>NIC 2 µg</td>
<td>1.31 ± 0.17</td>
<td>1.48 ± 0.13</td>
</tr>
<tr>
<td>6</td>
<td>VEH</td>
<td>2.45 ± 0.22</td>
<td>1.77 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>ABT 0.3 µg</td>
<td>1.67 ± 0.28</td>
<td>1.92 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>ABT 1 µg</td>
<td>2.32 ± 0.29</td>
<td>2.52 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>ABT 3 µg</td>
<td>1.94 ± 0.14</td>
<td>2.19 ± 0.28</td>
</tr>
<tr>
<td>7</td>
<td>VEH/VEH</td>
<td>1.59 ± 0.18</td>
<td>1.85 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>VEH/BIC</td>
<td>1.32 ± 0.17</td>
<td>1.45 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>1.45 ± 0.24</td>
<td>1.54 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>1.76 ± 0.29</td>
<td>1.40 ± 0.24</td>
</tr>
</tbody>
</table>

Data are expressed as the mean (± SEM) of the total time in seconds spent exploring the objects.
Chapter 3

Rat Multisensory Oddity Task Development
Abstract

Multisensory integration is the ability to integrate information from multiple sensory sources into coherent representations, an adaptive form of cognition whose neural bases are poorly understood. There are few viable tasks available to assess multisensory integration in rodents. This chapter details the development of a novel multisensory task called the multisensory oddity (MSO) task, which exploits a rats’ natural tendency to preferentially explore ‘odd’ objects in a group of identical objects. The MSO task enables assessment of multisensory integration independent of memory demand and with multiple modality combinations (tactile-visual & olfactory-visual in the current thesis). In addition, rats are tested on unimodal oddity tasks (tactile-, visual-, & olfactory-only) with the same associative requirements to control for potential effects on basic sensory perception. This chapter demonstrates that rats perform the MSO and unimodal oddity tasks significantly above chance. Performance of the MSO tasks improved across the 5 min of testing. Importantly, rats were only able to identify the visual oddity object features visually and the tactile oddity object features tactiley, but not vice versa. This indicates that rats could only use modality-specific features to differentiate between the oddity objects. This chapter therefore establishes the MSO task as a simple one-trial test of multisensory integration that can be used to assess the neural bases of this complex form of cognition in rats.
Introduction

The brain combines modality-specific information (e.g. visual or tactile) into coherent representations, and this is known as multisensory integration. Research on this cognitive process in humans has primarily been performed using reaction-time tasks, such that a participant responds to visual-only, auditory-only, or visual-auditory stimuli (Collignon et al., 2013; Stone et al., 2011; Williams et al., 2010; Wynn et al., 2014). Participants normally display reduced reaction times to multisensory stimuli (visual-auditory), compared to the unimodal stimuli, suggesting the presentation of a bimodal stimulus facilitates reaction time (Collignon et al., 2013; Stone et al., 2011; Williams et al., 2010; Wynn et al., 2014). These tasks have been effectively used to understand multisensory integration at a basic and applied level in clinical populations, such as schizophrenia and autism (Collignon et al., 2013; Stone et al., 2011; Williams et al., 2010; Wynn et al., 2014).

Due to the nature of human research, there is limited understanding of the underlying neurobiological bases of multisensory integration. The development of multisensory tasks for non-human primates and rodents has facilitated the investigation of the neural underpinnings of this essential cognitive process (Cloke, Jacklin, & Winters, 2015). Winters and Reid (2010) developed the tactile-to-visual CMOR task to study multisensory integration in rodents. In this task, rodents are exposed to two identical objects in a sample phase with red light, limiting exploration to the tactile object features. Following a delay, rodents are exposed to a familiar object from the sample phase and a novel object in a choice phase with white light and Plexiglas barriers placed in front of the object, limiting exploration to the visual object features. The CMOR task exploits a rodent’s natural tendency to explore the novel object, thereby signifying memory for the sample object. Successful performance in this task is presumably based on the
rodents’ ability to map the visual identity of an object in the choice phase onto the previously experienced tactile features of the sample object, which requires the integration of tactile and visual information. The CMOR task has enabled investigation of the neural bases of multisensory integration in rodents using permanent and temporary lesions (Hindley et al., 2014; Jacklin, Cloke, Potvin, Garrett, & Winters, 2016; Reid et al., 2012, 2013; Winters & Reid, 2010), behavioural pharmacology (Jacklin et al., 2015), and rodent models of schizophrenia (Ballendine et al., 2014; Cloke & Winters, 2015; Jacklin et al., 2012).

The CMOR task has proven to be a valuable task to study multisensory integration in rodents; however, certain aspects of the task prevent a comprehensive analysis of this cognitive function. Firstly, this task cannot examine multisensory integration independent of mnemonic demand, because there is always a delay between the sample and choice phases. Modality-specific features are generally experienced simultaneously, rather than independently, enabling our brain to bind this information into a multisensory representation. This is one component of the CMOR task that deviates from the human multisensory tasks described above. Secondly, the structure of the CMOR task limits the modalities that can be examined to tactile and visual, and thus, this task cannot assess other modalities, such as olfactory or auditory. This is particularly important to assess the generalizability of effects across different modality combinations.

This chapter details the development of a novel rodent multisensory task that improves upon the CMOR task by allowing the evaluation of multisensory perception and multiple modality combinations. We have adapted the oddity task that was developed by Bartko et al. (2007b) to investigate the perception of complex objects in rodents. In this task, rodents are presented simultaneously with 5 objects, each consisting of two different features (AB AB AC CD CD); they are allowed to explore these objects in a single session per trial (see Figure 1 in
Chapter 1). In this scenario, rodents preferentially explore the ‘odd’ object, which is the object with the distinct combination of features (AC). We have modified the oddity task to study multisensory integration by using different modality features (tactile, visual, and olfactory), rather than complex object features. This novel multisensory task, called the multisensory oddity (MSO) task, allowed us to investigate tactile-visual and olfactory-visual integration in rats and mice (with minor modifications) in subsequent chapters.

Methods

Subjects

Male Long-Evans rats weighing approximately 350 – 400g at the beginning of experimentation were used as subjects in this study. Rats were housed in pairs, and a reverse light/dark cycle (8 AM lights off; 8 PM lights on) was maintained with testing occurring during the dark cycle. All rats were food restricted throughout the experiment and fed approximately 25g of food each day following testing to maintain 85-90% free-feeding weight. Water was freely accessible except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and followed the Canadian Council on Animal Care Guidelines.

Oddity Tasks

An open field constructed of opaque white plastic (60 cm L X 60 cm W X 50 cm H) was used for all rat oddity tasks. Objects were otherwise indistinct glass jars (10.8 cm tall with 6.35 cm diameter) with distinctive visual, tactile, and/or olfactory features added depending on the specific task (see below). Visual features were stickers with images differing in colour and shape affixed to the front of each jar. Distinct tactile features were provided by sandpapers of different grit sizes (ranging from 60-600) to provide unique textural stimuli; the sandpapers for a given
trial were not dissimilar visually. Recent studies have shown that rats are capable of distinguishing tactually between different sandpaper grits (Ramos, 2014; Wu, Ioffe, Iverson, Boon, & Dyck, 2013). Sandpaper was adhered to the exterior of the glass containers for tactile conditions; for visual-tactile combinations, the visual sticker stimuli were affixed to the sandpaper. Distinct stock odours (e.g., vanilla, apple, lemon) were chosen from Bath and Body Works and diluted to 10% of their initial concentration to reduce their potency. Drops of the odours were placed in a small receptacle that was then placed inside the glass jars. The lids of the glass jars contained holes to allow the rats to sample the olfactory stimuli.

Two types of objects were used: the MSO tasks required multisensory (visual-tactile or visual-olfactory) combinations of features, whereas the control unimodal oddity tasks used objects that combine features from within a single modality (i.e., tactile-, visual-, or olfactory-only). Each unimodal object consisted of two unimodal features (e.g., A and B) to control for the associative nature of the MSO task. So, unimodal tactile objects had combinations of two different sandpaper grits (e.g., AB), unimodal visual objects had two visually distinct stickers, and unimodal olfactory objects contained two different odours. Each multisensory object also consisted of two distinct features, but each feature was from a different modality. So, visual-tactile objects had unique combinations of sandpaper grit and visual sticker, whereas visual-olfactory objects combined stickers and odours (Figure 9a).

Rats were habituated to the apparatus over two consecutive days, which consisted of exploration of the empty open field. Each day the rats received two separate habituation sessions
Figure 9. (a) Examples of objects used in the various oddity tasks. Each object comprises a combination of two different ‘features’, represented here as letters A, B, C, and D. For the unimodal tasks, these features consist of distinct visual, olfactory, or tactile stimuli. So, for example, for the unimodal visual oddity task (middle), object AB is identified by the combination of stickers displaying visual image A and visual image B. An object in the tactile unimodal oddity task (second from right) consists of a jar with two different grits of sandpaper covering its two halves, and an olfactory unimodal object (far right) consists of a jar containing two different odours. For the MSO task, the idea is the same, except that A and B represent features from different sensory modalities; so, for example, an object consists of a visual image affixed to a jar containing a specific odour (second from left) or a specific grit of sandpaper (far left). The different grits used for any given trial are not visually discriminable. (b) The general oddity task procedure. Five objects are presented simultaneously along the back wall of the open field arena. Of these objects, two separate pairs contain identical feature combinations (e.g., AB and CD). The fifth object represents a unique (i.e., ‘odd’) configuration of these features (e.g., AD). The various objects can be presented in any arrangement. Rats are given 5 min to freely explore the objects. Normal rodents preferentially explore the odd object, thereby demonstrating their ability to perceptually discriminate the objects on the basis of their feature combinations. All tasks are identical in their basic associative requirement, but only the MSO task necessitates multisensory integration for its solution.
(5 min for each rat). Behavioural testing commenced 24h following the second day of habituation. The general procedure was identical for the MSO and unimodal oddity tasks. On each trial, five objects were equally spread out along the back wall of the open-field. Two pairs of identical objects (e.g., ABx2 and CDx2) and an ‘odd’ object consisting of a unique combination of features from the other two pairs (e.g., CB) were presented simultaneously (see Figure 9b).

Rats were placed in the open-field and allowed to freely explore the objects. Trials were video recorded and scored using a computer program to tally exploration time at each object. Exploration was scored when the rat directed its nose toward the object at a distance of <2cm and/or touched it with its nose or whiskers. The task was completed when a predetermined amount of time had elapsed (5 min). An oddity preference score was calculated, defined as exploration of the odd object divided by total exploration (Bartko et al., 2007a, 2007b); ‘chance’ exploration is therefore 0.2 for an oddity task with five objects. Normal rats spend a significantly greater proportion of time exploring the single unique object, thereby demonstrating oddity preference based on perceptual binding of the object features (Bartko et al., 2007a, 2007b). Different objects were used for each trial and counterbalanced across all trials and rats.

Object Recognition

Rats were tested in a Y-shaped apparatus for the object recognition experiment (Experiment 3). Object recognition consisted of two phases: the sample phase and choice phase, which were separated by an ‘immediate’ delay (~25s). A discrimination ratio was calculated, defined as the difference in time spent exploring the novel versus familiar object divided by the total time spent exploring the objects For a more detailed description of the object recognition procedure, see Chapter 2 methodology.
Experiments

Experiments 1.1 & 1.2: Rat MSO and unimodal oddity task pilot

Two different sets of rats were tested on either the tactile-visual, tactile-only, and visual-only (n = 3) or olfactory-visual, olfactory-only, and visual-only oddity (n = 10) tasks to examine whether rats can perform these tasks above chance (0.2). Tasks were counterbalanced across all trials. Each rat was run for 3 trials (1 trial per task).

Experiments 2.1 & 2.2: MSO task: Oddity preference analysis by minute

The oddity preference score was assessed for the culmination of each min elapsed (0-1, 0-2, 0-3, 0-4, & 0-5) of the tactile-visual (n = 5) and olfactory-visual (n = 5) MSO task in two different sets of rats. This was completed to examine performance of these tasks across the 5 min of testing. Each rat was run for 1 trial.

Experiment 3: Tactile and visual object control

Rats (n = 8) were tested using object recognition in a Y-shaped apparatus with an immediate delay (~25s). Rats were exposed to the tactile or visual oddity objects in modality specific conditions (tactile- or visual-only). In the tactile-only condition, rats were exposed to the objects in red-light. In the visual-only condition, rats were exposed to objects in white-light with Plexiglas barriers placed in front of the objects. This experiment was performed to clarify that rodents can only use the modality-specific features (i.e. tactile features for tactile objects and visual features for visual objects) to discriminate between the objects used in the oddity experiments. Object type (tactile or visual) and modality condition (tactile- or visual-only) were counterbalanced across all trials. Each rat was run for 4 trials (1 trial per object type/modality condition).
Data Analysis

For Experiments 1 and 2, one-sample t-tests were employed to compare group means versus chance (0.2) to analyze performance of the oddity tasks. For Experiment 3, a mixed ANOVA was performed to examine the interaction and main effects of object type and modality condition. For Experiments 2 and 3 paired-samples t-tests were performed to analyze the differences between group means. Statistical analyses were conducted with a significance level of $\alpha = 0.05$ using SPSS.

Results

Experiments 1.1 & 1.2: Rats can perform the MSO and unimodal oddity tasks

Rats performed the tactile-visual ($t(2) = 23.31, p < 0.01$), tactile-only ($t(2) = 15.20, p < 0.01$), and visual-only ($t(2) = 8.72, p < 0.05$) oddity tasks significantly above chance (0.2; see Figure 10a). Likewise, rats performed the olfactory-visual ($t(9) = 3.89, p < 0.01$), olfactory-only ($t(9) = 2.81, p < 0.05$), and visual-only ($t(9) = 9.36, p < 0.01$) oddity tasks significantly above chance.

![Figure 10](image_url)

Figure 10. Rats were able to perform (a) tactile-visual, (b) olfactory-visual, and unimodal oddity tasks above chance. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM).
chance (see Figure 10b). The analysis of total object exploration revealed no significant differences, suggesting no differences in task exploratory behaviour.

**Experiments 2.1 & 2.2: Performance of the MSO task improves across time**

A repeated measures ANOVA revealed that performance of the tactile-visual ($F(4,16) = 7.19, p < 0.01$) and olfactory-visual ($F(4,16) = 7.43, p < 0.001$) MSO tasks improved across the 5 min of exploration (see Figure 11). *Post-hoc* paired-samples t-test revealed a significant difference between performance at 5 min from 1 min ($t(4) = 3.90, p < 0.05$), 2 min ($t(4) = 3.94, p < 0.05$), and 3 min ($t(4) = 3.56, p < 0.05$) on the tactile-visual MSO task. In addition, *post-hoc* paired-samples t-tests revealed a significant difference between performance at 5 min from 1 min ($t(4) = 3.70, p < 0.05$), 2 min ($t(4) = 4.41, p < 0.05$), and 4 min ($t(4) = 2.99, p < 0.05$) on the olfactory-visual MSO task.

**Experiment 3: Objects used for tactile or visual conditions are not discriminable in other modalities**

Rats were only able to discriminate between tactile objects in red light (tactile-only) and
visual objects in white light with Plexiglas barriers in front of the objects (visual-only)(see Figure 12). A two-way mixed ANOVA revealed a significant interaction ($F(1,7) = 29.60, p < 0.001$) and main effect of object type ($F(1,7) = 24.70, p < 0.01$), but no main effect of modality condition ($F(1,7) = 2.09, p = 0.19$). Post-hoc paired-samples t-tests revealed a significant difference between tactile-only and visual-only conditions within the tactile ($t(7) = 5.64, p < 0.001$) and visual object ($t(7) = 3.73, p < 0.01$) conditions. Furthermore, paired-sample t-tests comparing sample versus choice discrimination ratios also supported this finding, indicating a significant novelty preference in the tactile-only condition with the tactile objects ($t(7) = 5.78, p < 0.001$) and the visual-only condition with the visual objects ($t(7) = 3.32, p < 0.05$), but not the reverse ($p > 0.05$). This finding indicates that rats can only use the distinct tactile properties of the tactile objects and visual properties of the visual objects to distinguish between them in the oddity tasks. Therefore, we can be confident that only visual or tactile modalities are being tested in our oddity tasks with these specific objects.

![Discrimination Ratios](image)

Figure 12. Rats were only able to discriminate between the tactile oddity objects in the tactile-only condition and between the visual oddity objects in the visual-only condition. Dashed line represents chance. Discrimination scores displayed for group means (+SEM). ** = $p < 0.01$, tactile-only versus visual-only.
**Discussion**

This chapter introduces and validates the tactile-visual and olfactory-visual MSO tasks, as well as unimodal oddity tasks, for rats. Performance of the MSO tasks in rats improved across the 5 min of exploration time with the greatest level of performance occurring with 5 min of exploration. This finding suggests that rats require time to explore the objects in order to integrate the tactile, visual, and/or olfactory information to successfully identify the odd object, with above chance performance typically not occurring until 3 min into the exploration session. Importantly, rats appear to discriminate between tactile and visual oddity objects only within their respective modalities, indicating that rats cannot differentiate between ‘tactile’ objects based on visual features or ‘visual’ objects based on tactile features.

This chapter establishes that the MSO task is a robust test of multisensory cognition in rodents. This task allows for multiple modality combinations to be explored, beyond tactile-visual, which is important for establishing the generalizability of findings across different modality combinations. Significantly, the MSO task is more appropriate for assessing multisensory integration in rodents since it does tax memory ability, unlike the CMOR task (Winters & Reid, 2010) and does not require training, unlike other multisensory rodents tasks (Botly & De Rosa, 2007; Siemann et al., 2014). Although the MSO task does not directly mirror the human multisensory reaction time task (Collignon et al., 2013; Stone et al., 2011; Williams et al., 2010; Wynn et al., 2014), it is closer in design to these tasks than the few existing multisensory integration tasks for rodents.

Crucial to this thesis, the MSO task can be used to gain further insight into the nature of multisensory impairment in ketamine-treated rats (Cloke & Winters, 2015; Jacklin et al., 2012). In the following chapter, we assessed whether the immediate delay CMOR impairment in
ketamine-treated rats (Chapter 2) is generalizable to other modality combinations and potentially perceptual, rather than mnemonic, in nature by using the MSO task. This novel multisensory task will contribute greatly to the literature by providing researchers with a simple one-trial task to assess multisensory cognition in rodents.
Chapter 4

Generalized Multisensory Oddity Task Impairment in Ketamine-Treated Rats is Reversed by $\alpha_4\beta_2$ Nicotinic Receptor Stimulation of the GABAergic System Within the Orbitofrontal Cortex

Jacob M. Cloke$^{1,3}$, Robin Nguyen$^4$, David I. Wasserman$^1$, Stephanie De Lisio$^1$, Junchul Kim$^4$, Craig D.C. Bailey$^{2,3}$, & Boyer D. Winters$^{1,3}$

$^1$Department of Psychology, University of Guelph, Guelph, ON, Canada N1G 2W1

$^2$Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1

$^3$Collaborative Neuroscience Program, University of Guelph, Guelph, ON, Canada N1G 2W1

$^4$Department of Psychology, University of Toronto, Toronto, ON, Canada M5S 3GS

Data submitted for publication
Abstract

Atypical multisensory integration is an understudied aspect of the cognitive symptoms in schizophrenia. Appropriate tasks to evaluate multisensory integration in rodent models are lacking. We developed a novel MSO task to assess the mechanistic basis of atypical multisensory integration in ketamine-treated rats, a well-established model of schizophrenia. This study used behavioural pharmacology, whole-cell electrophysiology, and immunohistochemistry to assess the ameliorative effect of nAChR agonists and their interaction with the GABAergic system in ketamine-treated rats performing the MSO task with different combinations of stimulus modalities (visual-tactile and visual-olfactory). Ketamine-treated rats displayed a selective MSO task impairment with both sensory combinations, whereas basic unisensory perception was unaffected. OFC administration of nicotine or ABT-418 (α4β2 nAChR agonist) normalized MSO task performance in ketamine-treated rats; this effect was blocked by GABA_A receptor antagonism. GABAergic currents were also decreased in OFC of ketamine-treated rats and were normalized by activation of α4β2 nAChRs. Furthermore, PV-immunoreactivity was decreased in the OFC of ketamine-treated rats. Together, these findings strongly imply a role for prefrontal GABAergic transmission in the integration of multisensory object features, a cognitive process with relevance to schizophrenia. Accordingly, nAChR agonism, which improves various facets of cognition in schizophrenia, reverses the severe MSO task impairment here and appears to do so via a GABAergic mechanism. Interactions between GABAergic and nAChR receptor systems warrant further investigation for their potential therapeutic value. The novel behavioural paradigm introduced in the current study is acutely sensitive to schizophrenia-relevant cognitive impairment and should prove highly valuable for such research.
Introduction

Cognition is severely affected in people suffering from schizophrenia. This aspect of the disorder is highly connected to functional outcome (Green et al., 2004), highlighting the importance of gaining a better understanding of its behavioural and neural bases. Multisensory integration is a recently acknowledged facet of cognition that is distorted in schizophrenia patients (Stone et al., 2011; Tseng et al., 2015; Williams et al., 2010). Indeed, the CNTRICS initiative recognized the necessity for a ‘cross-modal integration’ task to assess multisensory integration in animal models (Butler et al., 2008).

Our lab developed a tactile-to-visual CMOR task to investigate multisensory integration in rodents (Winters & Reid, 2010), and sub-chronic treatment with the NMDA receptor antagonist ketamine in rats, a well-established model of schizophrenia (Jentsch & Roth, 1999; Olney et al., 1999), selectively impairs performance of the CMOR task with even a minimal retention delay between sample and test phases (Cloke & Winters, 2015; Jacklin et al., 2012). Unfortunately, because of the structure of the task, it is impossible to assess multisensory integration abilities in the absence of at least some minimal mnemonic demand when using CMOR. To further explore the severe multisensory impairment in ketamine-treated rats, we have developed a novel MSO task, which is a variation on the perceptual oddity task previously used to study complex visual perception in rodents (Bartko et al., 2007a, 2007b). The MSO task enables assessment of multiple modality combinations (e.g. olfactory-visual) not possible with the CMOR task, thereby enabling study of the generalizability of multisensory impairment. Moreover, the MSO task can be used to investigate multisensory integration independent of memory demand. The current study used the MSO task to investigate
the neural mechanisms underlying multisensory integration deficits - and their possible remediation - in a rodent model of schizophrenia.

Pharmacological treatments targeting nAChRs have pro-cognitive effects in schizophrenia patients (Barr et al., 2008; Lieberman et al., 2013; Olincy et al., 2006; Smith et al., 2006). Comparably, nicotine and specific nAChR agonists alleviate cognitive deficits in rodent models of schizophrenia (Hashimoto et al., 2008; Pichat et al., 2007; Rushforth et al., 2011; Wildeboer & Stevens, 2008), including the CMOR impairment reported in ketamine-treated rats (Cloke & Winters, 2015; Jacklin et al., 2012). In the current study we evaluated whether the ketamine-induced impairment and its remediation by nAChR agonists extend to the MSO task and generalize beyond visual-tactile modality combinations.

We also wished to delve further into the mechanism underlying these effects. GABAergic markers, including PV and GAD67, are reduced in hippocampus and prefrontal cortex of schizophrenia patients (Lewis et al., 2012; Lewis et al., 2005). Similar decreases in GABAergic markers are found in the brains of rodents sub-chronically treated with NMDA receptor antagonists (Abdul-Monim et al., 2007; Behrens et al., 2007; Cochran et al., 2003; Pratt et al., 2008), suggesting that such antagonism of NMDA receptors disrupts GABAergic function (Gonzalez-Burgos & Lewis, 2012; Lisman et al., 2008). Accordingly, systemic administration of MK-801 or ketamine reduces inhibitory fast-spiking GABAergic interneuron activity in vivo (Homayoun & Moghaddam, 2008), and GABAergic currents are significantly reduced in the mPFC of rodents treated with NMDA receptor antagonists (Jeevakumar & Kroener, 2014; Kjaerby et al., 2014; Zhang et al., 2008). Recently, alterations in GABAergic activity have been linked to impaired multisensory processing (Balz et al., 2016; Gogolla et al., 2014; Olcese et al., 2013). These findings suggest that disrupted GABAergic signalling in ketamine-treated rats
might be linked to impairment in multisensory integration.

We have previously shown that administration of the $\alpha_4\beta_2$ nAChR agonist ABT-418 into the OFC reverses the CMOR impairment in ketamine-treated rats; this effect is blocked by co-administration of the GABA$_A$ antagonist bicuculline (Cloke & Winters, 2015). nAChRs are typically located presynaptically on GABAergic interneurons (Gulledge, Park, Kawaguchi, & Stuart, 2007; Porter et al., 1999) and electrophysiological findings show that activation of nAChRs increases GABAergic currents in the cortex and hippocampus (Alkondon & Albuquerque, 2001; Aracri et al., 2010; Couey et al., 2007). We therefore hypothesize that impairment in multisensory integration is related to disrupted GABAergic interneuron function in the OFC of ketamine-treated rats and that nAChR stimulation restores behaviourally relevant GABAergic function in these animals. The present study provides converging evidence for this hypothesis using behavioural pharmacology, whole-cell electrophysiology, and immunohistochemistry experiments.

**Methods**

**Subjects**

One hundred and sixty one male Long-Evans rats weighing approximately 350 – 400g at the beginning of experimentation were used as subjects in this study. Rats were housed in pairs, and a reverse light/dark cycle (8 AM lights off; 8 PM lights on) was maintained with testing occurring during the dark cycle. All rats were food restricted throughout the experiment and fed approximately 25g of food each following testing to maintain 85-90% free-feeding weight. Water was freely accessible except during testing. All procedures were approved by the Animal Care Committee of the University of Guelph and followed the Canadian Council on Animal Care Guidelines.
Sub-chronic drug administration

Rats were handled for 1 week prior to treatment and randomly assigned to receive either sub-chronic treatment with ketamine (30 mg/kg; Bioniche, Belleville, ON, Canada) or saline (0.9% NaCl, pH 7.0; Sigma) at a volume of 1 ml/kg of their body weight (Jacklin et al., 2012). Treatment consisted of twice daily intraperitoneal (i.p.) administration at 8 AM and 8 PM for 10 consecutive days. Following treatment, there was a 10-day washout period during which rats were left undisturbed on free-feed in their homecage. Experimenters were blind to the assigned condition.

Surgery

Following the 10-day treatment period, surgery was performed on rats during the 10-day washout period (1 week prior to behavioural testing) for intracranial experiments. Rats were anesthetized with isoflurane (Benson Medical Industries) inhalation anaesthetic prior to and throughout all surgeries. Prior to surgery, all rats were administered a systemic subcutaneous injection of the analgesic meloxicam (5 mg/ml; Boehringer Ingelheim). Rats were then positioned in a stereotaxic frame (Kopf Instruments) with the incisor bar set to -3.3 mm. The scalp was cut and retracted to expose the skull. Holes were drilled, and the guide cannulas were implanted bilaterally according to the following coordinates for the OFC: AP +3.4; L ± 2.2; DV -3.2 relative to bregma (Paxinos & Watson, 2005). For the mPFC a bilateral cannula was implanted according to the following coordinates: AP +3.2; L - .75; DV -4.0 relative to bregma. The cannulas were secured to the skull using four jeweler screws and dental acrylic. Following completion of each surgery, the scalp was sutured, and rats recovered on heat pads for 1–2h prior to being returned to their home cages. Behavioural testing began 1 week following surgery.
Infusion Procedure

Each animal was gently restrained and obturators were removed from the guide cannulas. Infuser cannulas cut to extend 1.0 mm beyond the tips of the guides were placed in the guide cannulas. Bilateral simultaneous infusions were delivered by two 1µl Hamilton syringes, which were connected to infuser cannulas by propylene tubing. The syringes dispensed 0.5µl of drug by a Harvard Apparatus syringe pump for a total of 1 min. Following this the infuser cannulas were left in the guides for another 90s to allow full diffusion. Infuser cannulas were then removed and replaced by the obturators. Prior to experimentation, all rats underwent two habitation sessions to the infusion procedure; in this case, the infusion procedure was duplicated in every way except that the syringes contained no fluid.

Histology

Following behavioural testing, an i.p. injection of 2 ml euthansol was administered to anesthetize the rats. Rats were transcardially perfused with 100 ml of PBS, pH 7.4, followed by 250 ml of 4% neutral buffered formalin, pH 7.4. The brains were then extracted, postfixed in 4% formalin at 4°C for 24 h, and then submerged in 20% sucrose in PBS until they sank. A cryostat was used to cut coronal sections (60 µm) through the OFC or mPFC, and every fifth section was mounted on a gelatin-coated glass slide and stained with cresyl violet. Slides were examined under a light microscope to confirm cannula placements.

Immunohistochemistry

For immunohistochemistry analyses, rats were transcardially perfused following the 10-day washout period. Brains were extracted and postfixed in 4% formalin at 4°C for 12h, and then submerged in 30% sucrose in PBS at 4°C until they sank. Brains were sectioned coronally at 40 µm through the prefrontal cortex using a cryostat. Free-floating brain sections were blocked with
16% normal goat serum in 0.1% PBS-Triton for 30 min at room temperature. Sections were then incubated in 1% normal goat serum in PBS containing mouse-anti-rat PV (1:1500; Sigma) for 22h at 4°C. Following this, sections were incubated in 1% normal goat serum in PBS containing goat-anti-mouse Cy2 (1:1200; Abcam) for 75 min at room temperature. Lastly, sections were incubated in DAPI for 5 min at room temperature. Slices according to the placement of OFC cannulas were collected. The sections were mounted and imaged on a confocal laser scanning microscope with a 20× objective. Total PV-immunoreactive (PV-ir) cells were counted in the lateral orbital (LO) and ventral orbital (VO) sub-regions of the OFC.

**Brain Slice Preparation and Electrophysiology**

Coronal slices containing the OFC were prepared from adult rats treated with ketamine or saline following the 10-day washout period. Rats were sacrificed by decapitation under inhalant isoflurane and brains were removed quickly and cooled in 4°C oxygenated sucrose artificial cerebrospinal fluid (ACSF; 254 mM sucrose, 10 mM D-glucose, mM 24 NaHCO$_3$, 2 mM CaCl$_2$, 2 mM MgSO$_4$, 3 mM KCl, and 1.25 mM NaH$_2$PO$_4$, pH 7.4). Brain slices were cut at 400 µM thickness using a Leica VT1200 vibrating microtome (Leica Microsystems, Richmond Hill, ON, Canada) from anterior to posterior and collected from approximately bregma +4.68 to +3.72 mm. Slices were transferred to oxygenated regular ACSF (composition listed above except 128 mM NaCl was substituted for sucrose) maintained at 30°C and allowed to recover for at least 2h. Recordings were made using slices placed within a modified recording chamber (Warner Instruments, Hamden, CT, USA) mounted on the stage of an Axioskop FS2 Microscope (Carl Zeiss Canada, Toronto, ON, Canada). ACSF was bubbled with carbogen (95% oxygen and 5% carbon dioxide) and superfused over the slices at room temperature with a rate of 3-4 ml/min.

Whole-cell recordings were made using borosilicate glass electrodes (2-5 MΩ)
containing 50 mM K-gluconate, 75 mM KCl, 2 mM MgCl₂, 4 mM K₂-ATP, 400 µM Na₂-GTP, 10 mM Na₂-phosphocreatine, 33 µM Alexa Fluor 488 hydrazide (Life Technologies, Burlington, ON, Canada) and 10 mM HEPES buffer, adjusted to pH 7.3 with KOH. The use of this high chloride intracellular solution in neurons held at -75 mV allows for the observation of GABAₐ receptor activation via positive inward currents that result from chloride exiting the open ligand-gated ion channel (Bailey, De Biasi, Fletcher, & Lambe, 2010; Couey et al., 2007). All recordings were performed in layer 2/3 of the OFC, which was visualized by recording immediately dorsal to the apex of layer 1 in the OFC (Van De Werd & Uylings, 2008). Individual pyramidal neurons were visualized using infrared differential interference contrast microscopy and accessed for whole-cell recording. The pyramidal identity was confirmed for each recorded neuron based on its pattern of action potential firing in response to depolarizing current injection and its dendritic morphology, which was visualized using Alexa Fluor 488 hydrazide in the electrode solution and live epifluorescent microscopy. Recordings were made using a Multiclamp 700B amplifier, acquired at 20 kHz and lowpass filtered at 2 kHz using a Digidata 1440A data acquisition system (Molecular Devices, Sunnyvale, California).

Using current clamp mode, changes to membrane potential from rest were first measured in response to positive and negative current steps. This allowed for the determination of neuron basic electrophysiological properties, which were not different between saline- and ketamine-treated rats (see Table 3). For experiments measuring GABAergic function, neurons were held at -75 mV in voltage-clamp mode. Recordings were performed in the continuous presence of 20 µM CNQX to block AMPA and kainate glutamate receptors, 200 nM atropine to block muscarinic receptors, and 10 nM methyllycaconitine (MLA) to block α₇ nAChRs. GABAergic spontaneous postsynaptic currents (sPSCs) were recorded for each neuron during a 30 s baseline.
period and then in the presence of 1 mM acetylcholine (ACh; 15 second application). Under these recording conditions, the observed sPSCs are completely blocked by the addition of the GABA_A receptor competitive antagonist bicuculline at a concentration of 10 µM (Bailey et al., 2010; Couey et al., 2007). Furthermore, ACh with atropine and MLA was applied in the presence of the α4β2 nAChR antagonist dihydro-β-erythroidine hydrobromide (DHβE; 3 µM) as a control. Analysis of GABAergic sPSC frequency and amplitude at baseline and during the application of ACh was performed using Mini Analysis Program (Synaptosoft).

**Oddity Tasks**

Rats were tested in an open-field apparatus for oddity tasks. Each trial for the oddity tasks consisted of a 5 min period of exploration of 5 objects presented simultaneously. An oddity preference score was calculated, defined as exploration of the odd object divided by total exploration (Bartko et al., 2007a, 2007b); ‘chance’ exploration is therefore 0.2 for an oddity task with five objects. For a more detailed description of oddity task procedures, see Chapter 3 methodology.

**Experiments**

**Experiments 1.1. & 1.2: MSO task performance in saline- and ketamine-treated rats**

Two batches of rats were tested. One batch (10 saline-treated/10 ketamine-treated) was tested on the tactile-, visual-only, and tactile-visual oddity tasks. The other batch (9 saline-treated/9 ketamine-treated) was tested on the olfactory-, visual-only, and olfactory-visual oddity tasks. Tasks were counterbalanced across all trials. Each rat was run for 3 trials (1 trial per task).

**Experiments 2.1 & 2.2: Involvement of nAChRs in remediation of the MSO task impairment of ketamine-treated rats**

The same two batches of rats from Experiments 1.1 and 1.2 were tested on the tactile-
visual (10 saline-treated/10 ketamine-treated) or olfactory-visual (9 saline-treated/9 ketamine-treated) MSO task. Rats were acutely administered i.p. physiological saline or nicotine (0.05, 0.2, or 0.8 mg/kg, dissolved in physiological saline; Sigma), 20 min prior to the start of each trial (Jacklin et al., 2012). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.

**Experiments 3.1 & 3.2: Involvement of $\alpha_4\beta_2$ nAChRs in remediation of the MSO task impairment of ketamine-treated rats**

Two batches of rats were tested on the tactile-visual (10 saline-treated/10 ketamine-treated) or olfactory-visual (10 saline-treated/10 ketamine-treated) MSO task. Animals were acutely administered i.p. physiological saline or ABT-418, an $\alpha_4\beta_2$ nAChR agonist (0.06, 0.1, or 0.6 mg/kg, dissolved in physiological saline; Sigma), 20 min prior to the start of each trial (Cloke & Winters, 2015). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.

**Experiments 4.1 & 4.2: Involvement of $\alpha_7$ nAChRs in remediation of the MSO task impairment of ketamine-treated rats**

The same two batches of rats from Experiments 3.1 & 3.2 were tested on the tactile-visual (10 saline-treated/10 ketamine-treated) or olfactory-visual (10 saline-treated/10 ketamine-treated) MSO tasks. Animals were acutely administered i.p. physiological saline or GTS-21, an $\alpha_7$ nAChR agonist (0.3, 1, or 3 mg/kg, dissolved in physiological saline; Abcam), 20 min prior to the start of each trial. We chose to use these higher doses of GTS-21, compared to the doses used in Chapter 2, because a recent study showed these doses of GTS-21 reversed an MK-801-induced impairment in OR (Callahan et al., 2014). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) with a minimum 48h between trials.
Experiments 5.1 & 5.2: Involvement of nAChRs in the OFC in remediation of the MSO task impairment of ketamine-treated rats

Rats (8 saline-treated/9 ketamine-treated) were implanted with bilateral indwelling cannulas in the OFC and tested on both MSO tasks. Animals were intracranially infused with physiological saline or nicotine (0.5, 1, or 2 µg), immediately prior to the start of each trial. The doses of nicotine were selected based on previous studies examining the effects of nicotine on object recognition and CMOR (Cloke & Winters, 2015; Melichercik et al., 2012). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) on each task (tactile-visual and olfactory-visual) with a minimum 48h between trials.

Experiments 6.1 & 6.2: Involvement of nAChRs in the mPFC in remediation of the MSO task impairment of ketamine-treated rats

Rats (8 saline-treated/8 ketamine-treated) were implanted with a bilateral indwelling cannula in the mPFC and tested on both MSO tasks. Animals were intracranially infused with physiological saline or nicotine (0.5, 1, or 2 µg), immediately prior to the start of each trial. This experiment was performed to control for possible spread of the drug to other prefrontal regions. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) on each task (tactile-visual and olfactory-visual) with a minimum 48h between trials.

Experiments 7.1 & 7.2: Involvement of α4β2 nAChRs in the OFC in remediation of the MSO task impairment of ketamine-treated rats

Rats (8 saline-treated/9 ketamine-treated) were implanted with bilateral indwelling cannulas in the OFC and tested on both MSO tasks. Animals were intracranially infused with physiological saline or ABT-418 (0.3, 1, or 3 µg), immediately prior to the start of each trial. The doses of ABT-418 were selected based on our previous study examining the effects of ABT-418
in ketamine-treated rats on the CMOR task (Cloke & Winters, 2015). Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per dose) on each task (tactile-visual and olfactory-visual) with a minimum 48h between trials.

**Experiments 8.1 & 8.2: α_4β_2 nAChR interaction with the GABAergic system within the OFC of ketamine-treated rats**

Two batches of rats were implanted with bilateral indwelling cannulas in the OFC. One batch was examined on the tactile-visual MSO task (8 saline-treated/8 ketamine-treated) and the other was tested on the olfactory-visual MSO task (9 saline-treated/8 ketamine-treated). Rats were intracranially infused with physiological saline or ABT-418 (3 µg), immediately prior to the start of each trial, as well as systemically administered i.p. bicuculline, a GABA_A antagonist (0.5 mg/kg; dissolved in 20% DMSO; Sigma) or 20% DMSO (vehicle) 20 min prior to the start of each trial. The dose of ABT-418 was selected based on the dose-response from Experiments 7.2 & 7.3. The dose of bicuculline was based on our previous study demonstrating that this dose does not affect performance of saline-treated rats (Cloke & Winters, 2015). There were four drug conditions: VEH/VEH, VEH/BIC, ABT/VEH, and ABT/BIC. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.

**Experiment 9: GABAergic currents at baseline and following ACh application in the OFC of ketamine-treated and saline-treated rats**

Whole-cell recordings in 400 µm thick coronal slices containing the OFC were performed in ketamine- (rats: n = 5; neurons: n = 27) and saline-treated (rats: n = 7; neurons: n = 31) rats following the 10-day washout period (Bailey et al., 2010). The frequency and amplitude of GABAergic sPSCs were measured in pyramidal neurons located within layer 2/3 of the OFC at
baseline and following ACh application. All recordings were made in the presence of the $\alpha_7$ nAChR antagonist MLA and muscarinic acetylcholine receptor antagonist atropine, selectively activating $\alpha_4\beta_2$ nAChRs.

To confirm that under these conditions the observed sPSCs were GABAergic, we applied the GABA$_A$ antagonist to examine whether all sPSCs were abolished. In addition, to confirm that under these conditions the observed increase in sPSC frequency following ACh was dependent upon activation of the $\alpha_4\beta_2$ nAChR, we applied ACh with atropine and MLA in the presence of the $\alpha_4\beta_2$ nAChR antagonist DH$eta$E to examine whether the effect of ACh was blocked.

**Experiment 10: PV Expression in the OFC of ketamine- and saline-treated rats**

Fluorescent immunohistochemical staining was performed on the brains of ketamine- and saline-treated rats following the 10-day washout period. These brains were assessed for total PV expression by manual cell counts in the subdivisions of the OFC (LO and VO), which were defined based on the rat brain atlas (Paxinos & Watson, 2005).

**Data Analysis**

Behaviour was analyzed for rats by taking the mean oddity preference score for each task/drug/dose condition in each experiment. As a control measure to confirm there were no general exploratory differences, the total object exploration was also assessed. Two-way mixed-factors or repeated measures ANOVAs were used to analyze the means of these two measures, and *post-hoc* independent- or paired-samples t-tests were used to assess group differences within or between each task/drug/dose condition. A Bonferroni correction was applied to account for multiple comparisons. For electrophysiological experiments, two-way mixed-factor ANOVAs, as well as independent- and paired-samples t-tests were employed. Statistical analyses were conducted with a significance level of $\alpha = 0.05$ using SPSS.
Results

Experiments 1.1. & 1.2: Ketamine-treated rats display a selective MSO task impairment

Ketamine-treated rats were selectively impaired on the tactile-visual (Figure 13a; Group X Task \(F(2,36) = 15.32, p < 0.001\), Group \(F(1,18) = 21.76, p < 0.001\), Task \(F(2, 36) = 12.06, p < 0.001\)) and olfactory-visual (Figure 13b; Group X Task \(F(2,32) = 10.74, p < 0.001\), Group \((F(1,16) = 7.30, p < 0.05), Task (F(2,32) = 7.77, p < 0.01))\) MSO tasks. Post hoc independent-samples t-tests indicated significant differences between ketamine- and saline-treated rats on the tactile-visual \((t(18) = 6.18, p < 0.001)\) and olfactory-visual \((t(16) = 4.98, p < 0.001)\) MSO tasks, but not the unimodal control tasks \((p > .05)\). The analysis of total object exploration for the olfactory-visual MSO experiment revealed a significant main effect of task \((F(2,32) = 8.63, p < 0.01)\) and group \((F(1,16) = 8.80, p < 0.01)\), but no significant interaction. Exploration was significantly lower in the visual-only task \((p < 0.01)\) and higher overall in ketamine-treated rats; however, task performance did not appear affected by these exploration differences (see Table 4).

![Figure 13a](image1.png)  
(a) Tactile-Visual

![Figure 13b](image2.png)  
(b) Olfactory-Visual

Figure 13. Following the 10-day washout period ketamine- and saline-treated rats were tested on the MSO and unimodal oddity tasks. Ketamine treated rats were selectively impaired on the (a) tactile-visual and (b) olfactory-visual MSO tasks, while performance was spared on tactile-, olfactory-, and visual-only oddity task performance. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). *** = \(p < 0.001\), ketamine versus control rats.
Experiments 2.1 & 2.2: Systemic nicotine restores MSO task performance in ketamine-treated rats

Systemic administration of nicotine dose-dependently reversed the tactile-visual (Figure 14a; Group X Dose ($F(3,54) = 3.55, p < 0.05$), Group ($F(1,18) = 32.50, p < 0.001$), Dose ($F(3,54) = 2.26, p = 0.092$)) and olfactory-visual (Figure 14b; Group X Dose ($F(3,48) = 11.58, p < 0.001$), Group ($F(1,16) = 14.84, p < 0.01$), Dose ($F(3,48) = 13.11, p < 0.001$)) MSO task deficits in ketamine-treated rats. Post hoc independent-samples t-tests revealed significant differences between ketamine- and saline-treated rats in VEH condition on tactile-visual ($p < 0.001$) and differences in the VEH ($p < 0.001$) and 0.05 mg/kg nicotine ($p < 0.01$) conditions on the olfactory-visual MSO. The analysis of total object exploration revealed no significant interaction or main effects, suggesting differences in exploratory behaviour (see Table 4).

Figure 14. Following the 10-day washout period ketamine- and saline-treated rats were systemically administered nicotine (0.05, 0.2, & 0.8 mg/kg) 20 min prior to testing. Nicotine dose-dependently restored performance of the (a) tactile-visual and (b) olfactory-visual MSO tasks in ketamine-treated rats. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). ** = $p < 0.01$; *** = $p < 0.001$, ketamine versus control rats.
Experiments 3.1 & 3.2: Systemic ABT-418 reverses the MSO task impairment in ketamine-treated rats

Systemic administration of the selective α4β2 agonist ABT-418 dose-dependently rescued tactile-visual (Figure 15a; Group X Dose (F(3,54) = 4.61, p < 0.01), Group (F(1,18) = 8.47, p < 0.01), Dose (F(3,54) = 3.81, p < 0.05)) and olfactory-visual (Figure 15b; Group X Dose (F(3,54) = 10.25, p < 0.001), Group (F(1,18) = 19.14, p < 0.001), Dose (F(3,54) = 10.34, p < 0.001)) MSO task performance in ketamine-treated rats. Post hoc independent-samples t-tests revealed significant differences between ketamine- and saline-treated rats in VEH condition on the tactile-visual (p < 0.001) and differences in the VEH (p < 0.001) and 0.06 mg/kg ABT-418 (p < 0.001) conditions on the olfactory-visual MSO tasks. The analysis of total object exploration revealed no significant interaction or main effects (see Table 4).

Figure 15. Systemic administration of the α4β2 nAChR agonist ABT-418 20 min prior to testing dose-dependently restored performance of the (a) tactile-visual and (b) olfactory-visual MSO tasks in ketamine-treated rats. Oddity preference scores displayed for group means (+SEM). *** = p < 0.001, ketamine versus control rats.
Experiments 4.1 & 4.2: Systemic GTS-21 does not affect MSO task performance in ketamine-treated rats

Systemic administration of the selective $\alpha_7$ agonist GTS-21 did not restore tactile-visual (Figure 16a; Group X Dose ($F(3,54) = 0.33$, $p = 0.806$), Group ($F(1,18) = 239.64$, $p < 0.001$), Dose ($F(3,54) = 0.50$, $p = 0.684$)) or olfactory-visual (Figure 16b; Group X Dose ($F(3,54) = 0.51$, $p = 0.678$), Group ($F(1,18) = 84.28$, $p < 0.001$), Dose ($F(3,54) = 2.07$, $p = 0.115$)) MSO task performance in ketamine-treated rats. The analysis of total exploration revealed a main effect of group in the tactile-visual MSO experiment ($F(1,18) = 5.53$, $p < 0.05$), such that ketamine-treated rats explored more, but no significant interaction (see Table 4).

**Figure 16.** Systemic administration of the $\alpha_7$ nAChR agonist GTS-21 20 min prior to testing did not affect performance on the (a) tactile-visual or (b) olfactory visual MSO tasks in ketamine-treated rats. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). ** = $p < 0.001$, ketamine versus control rats.

Experiments 5.1 & 5.2: Intra-OFC nicotine dose-dependently reversed the MSO task impairment in ketamine-treated rats

All rats included in the behavioural analyses had guide cannulas located bilaterally with injection needle tips terminating in the OFC (Figures 17a & b). These placements were
consistently located in the OFC (+4.68 mm to +3.72 mm). Intra-OFC nicotine reversed the tactile-visual (Figure 17c; *Group X Dose* (*F*(3,45) = 4.98, *p* < 0.01), *Group* (*F*(1,15) = 45.71, *p* < 0.001), *Dose* (*F*(3,45) = 6.16, *p* < 0.001)) and olfactory-visual (Figure 17d; *Group X Dose* (*F*(3,45) = 28.64, *p* < 0.001), *Group* (*F*(1,15) = 15.71, *p* < 0.001), *Dose* (*F*(3,45) = 5.24, *p* < 0.01)) MSO task impairments in ketamine-treated rats. *Post hoc* independent-samples t-tests revealed significant differences between ketamine- and saline-treated rats in VEH and 0.5 µg nicotine conditions on the tactile-visual (*p* < 0.001) and olfactory-visual (*p* < 0.001) MSO tasks. The analysis of total object exploration revealed a main effect of group in the olfactory-visual MSO experiment (*F*(1,15) = 4.92, *p* < 0.05), but no significant interaction (see Table 4).

**Experiments 6.1 & 6.2: Intra-mPFC nicotine did not affect MSO task performance in ketamine-treated rats**

All rats included in the behavioural analyses had guide cannulas located bilaterally with injection needle tips terminating in the mPFC (Figures 18a & b). These placements were consistently located in the mPFC (+4.20 mm to +3.24 mm). Intra-mPFC nicotine did not affect tactile-visual (Figure 18c; *Group X Dose* (*F*(3,42) = 1.03, *p* = 0.388), *Group* (*F*(1,14) = 109.49, *p* < 0.001), *Dose* (*F*(3,42) = 0.779, *p* = 0.513)) or olfactory-visual (Figure 18d; *Group X Dose* (*F*(3,42) = 1.74, *p* = 0.173), *Group* (*F*(1,14) = 115.50, *p* < 0.001), *Dose* (*F*(3,42) = 0.526, *p* = 0.667)) MSO task performance in ketamine-treated rats. The analysis of total object exploration revealed a main effect of group in the tactile-visual MSO experiment (*F*(1,14) = 17.38, *p* < 0.001), such that ketamine-treated rats explored more, but no significant interaction (see Table 4).
Figure 17. (a) Cannulation of rat OFC. Schematic representation of the infusion needle tip placements from a typical group of animals (intra-OFC nicotine experiment; $n = 17$). These placements are representative of needle tip locations in all animals (closed circles = saline-treated rats; open circles = ketamine-treated rats) included in the behavioural analyses of the present study. Cannulas were consistently located between + 3.72 mm and + 4.68 mm anterior to bregma. Some needle tips overlap in the figure. (b) A representative nissl-stained section of a bilateral OFC cannulation in rat. Following the 10-day washout period ketamine- and saline-treated rats were infused with nicotine (0.5, 1, 2 µg) into the OFC immediately prior to testing. Intra-OFC nicotine dose-dependently restored performance of the (c) tactile-visual and (d) olfactory-visual MSO tasks in ketamine-treated rats. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). ** = $p < 0.01$; *** = $p < 0.001$, ketamine versus control rats.
Figure 18. (a) Cannulation of rat mPFC. Schematic representation of the infusion needle tip placements from all rats ($n = 16$). These placements represent needle tip locations in all rats (closed circles = saline-treated rats; open circles = ketamine-treated rats) included in the behavioural analyses of the present study. Cannulas were consistently located between 3.24 to 4.20 mm anterior to bregma. Some needle tips overlap in the figure. (b) A representative section of a bilateral mPFC cannulation in rat. Following the 10-day washout period ketamine- and saline-treated rats were infused with nicotine (0.5, 1, 2 µg) into the mPFC immediately prior to testing. Intra-mPFC nicotine did not affect performance of the (c) tactile-visual and (d) olfactory-visual MSO tasks in ketamine-treated rats. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM).
Experiments 7.1 & 7.2: Intra-OFC ABT-418 dose-dependently rescued the MSO task impairment in ketamine-treated rats

Replicating our systemic findings, intra-OFC ABT-418 also dose-dependently reversed the tactile-visual (Figure 19a; Group X Dose \(F(3,45) = 6.46, p < 0.001\), Group \(F(1,15) = 22.88, p < 0.001\), Dose \(F(3,45) = 1.83, p = 0.156\)) and olfactory-visual (Figure 19b; Group X Dose \(F(3,45) = 8.62, p < 0.001\), Group \(F(1,15) = 32.26, p < 0.001\), Dose \(F(3,45) = 5.42, p < 0.01\)) MSO task impairments in ketamine-treated rats. Post hoc independent-samples t-tests indicated significant differences between ketamine- and saline-treated rats in VEH and 0.3 µg ABT-418 conditions on the tactile-visual (\(p < 0.001\)) and olfactory-visual (VEH: \(p < 0.001\), 0.3 µg ABT: \(p < .0125\)) MSO tasks, but no significant differences in the other dose conditions (\(p > 0.05\)). The analysis of total object exploration revealed no significant interaction or main effects, suggesting no effect of group or task on general exploratory behaviour (see Table 4).

Experiments 8.1 & 8.2: GABA\(_A\) receptor blockade inhibits the ameliorative effect of intra-OFC ABT-418 on MSO task performance in ketamine-treated rats

Systemic bicuculline blocked the remediating effect of intra-OFC ABT-418 in ketamine-treated rats on tactile-visual (Figure 19c; Group X Drug \(F(3,42) = 16.35, p < 0.001\), Group \(F(1,14) = 121.99, p < 0.001\), Drug \(F(3,42) = 16.68, p < 0.001\)) and olfactory-visual (Figure 19d; Group X Drug \(F(3,45) = 9.63, p < 0.01\), Group \(F(1,15) = 23.91, p < 0.001\), Drug \(F(3,45) = 9.97, p < 0.001\)) MSO task performance. Post hoc independent-samples t-tests indicated that ketamine-treated rats were significantly different in the ABT-BIC condition from saline-treated rats on the tactile-visual (\(t(14) = 7.27, p < 0.001\)) and olfactory-visual (\(t(15) = 4.52, p < 0.001\)) MSO tasks, but not in the ABT-VEH condition (\(p > 0.05\)). The analysis of total object exploration revealed no significant interaction or main effects task (see Table 4).
Figure 19. Following the 10-day washout period ketamine- and saline-treated rats were infused with ABT-418 (0.3, 1, 3 µg) into the OFC immediately prior to testing. Intra-OFC ABT-418 dose-dependently rescued performance of the (a) tactile-visual and (b) olfactory-visual MSO tasks in ketamine-treated rats. Ketamine- and saline-treated rats were infused with ABT-418 (3 µg) into the OFC immediately prior to testing and systemically administered the GABA<sub>A</sub> antagonist bicuculline (0.5 mg/kg) 20 min prior to testing. Administration of bicuculline, at a dose that did not affect saline-treated rat performance, blocked the ameliorative effect of intra-OFC ABT-418 on performance of the (c) tactile-visual and (d) olfactory-visual MSO tasks in ketamine-treated rats. Oddity preference scores displayed for group means (+SEM). * = p < 0.0125; *** = p < 0.001, ketamine versus control rats.
Experiment 9: Decreased GABAergic currents in OFC layer 2/3 of ketamine-treated rats is restored by activation of the α4β2 nAChR

A mixed factor ANOVA for the frequency of GABAergic sPSCs revealed a significant effect of Treatment Group (ketamine or saline; $F(1,56) = 9.18, p < 0.01$) and a significant effect of Drug Application (baseline or ACh; $F(1,56) = 31.52, p < 0.001$), but no significant interaction ($F(1, 56) = 0.518, p = 0.475$). The frequency of GABAergic sPSCs was significantly decreased at baseline in ketamine-treated rats compared with saline-treated rats (Figures 20a & c; $t(56) = 3.90, p < 0.001$). Application of ACh restored the frequency of GABAergic sPSCs in ketamine-treated rats to a level equal to that of saline-treated rats at baseline (Figures 20b & c). The percent change in GABAergic sPSC frequency following ACh application was significantly higher in ketamine-treated rats (Figure 20d; $t(56) = 2.51, p < 0.05$). Furthermore, a mixed factor ANOVA for the amplitude of GABAergic sPSCs revealed a significant Treatment Group ($F(1,56) = 6.89, p < 0.05$) effect, but no significant interaction ($F(1, 56) = 1.09, p = 0.301$) or Drug Application effect ($F(1,56) = 1.95, p = 0.168$)(Figure 20e).

Application of bicuculline completely abolished all measured sPSCs, supporting that specifically GABAergic sPSCs were being assessed. In addition, the ability of ACh to increase GABAergic sPSC frequency in OFC layer 2/3 pyramidal neurons was significantly attenuated by the α4β2 nAChR competitive antagonist DHβE (3 µM) ($t(5) = 3.07, p = 0.028$; data not shown), but did not affect GABAergic sPSC amplitude ($t(5) = 0.17, p = 0.871$; data not shown), suggesting that the remediating effects of ACh are mediated through α4β2 nAChRs located on GABAergic interneuron presynaptic terminals.
Figure 20. Following the 10-day washout period, ketamine- and saline-treated rats were sacrificed for whole-cell electrophysiology. (a) Representative traces from pyramidal neurons located within layer 2/3 of rat OFC showing baseline GABAergic sPSCs in control and ketamine rats. (b) Representative traces from pyramidal neurons located within layer 2/3 of rat OFC showing GABAergic sPSCs at baseline and following 1 mM ACh application (black bar) in control and ketamine rats. (c) The frequency of baseline GABAergic sPSCs was significantly reduced in the OFC of ketamine rats than in control treated rats. Application of 1 mM ACh (in the presence of 200 nM atropine and 10 nM MLA) restored GABAergic sPSC frequency equal to that in control rats at baseline. (d) The percent change in GABAergic sPSC frequency following ACh application was significantly higher in ketamine-treated rats. (e) The amplitude of GABAergic sPSCs was significantly lower overall in ketamine rats than in control rats. Group means (+SEM) are displayed. * = p < 0.05.
Figure 21. Following the 10-day washout period, ketamine- (n = 5) and saline-treated (n = 5) rats were sacrificed to examine PV-immunoreactivity (PV-ir) in the OFC. (a) Representative section stained for PV from a saline-treated rat showing the differentiation of lateral orbital (LO) and ventral orbital (VO) sub-regions of the OFC at 10x magnification. (b) Total PV counts per hemisphere were significantly decreased in ketamine-, but not saline-treated rats. (c) Representative sections from ketamine- and saline-treated rats at 20x magnification demonstrating the difference in PV-ir in LO of the OFC. Group means (+SEM) are displayed. * = p < 0.05.
Experiment 10: Significantly decreased PV expression in the OFC of ketamine-treated rats

PV expression was significantly decreased in LO, but not the VO, subdivision of the OFC of ketamine-treated rats compared to saline-treated rats (see Figure 21). A mixed factor ANOVA revealed a significant interaction \((F(1,16) = 12.13, p < 0.01)\) and main effect of region \((F(1,16) = 99.22, p < 0.001)\), but no main effect of group \((F(1,11) = 1.80, p = 0.199)\). *Post hoc* independent-sample t-test demonstrated a significant difference in PV expression between ketamine- and saline-treated rats in LO \((t(16) = 2.57, p < 0.05)\), but not VO \((t(16) = 0.57, p = 0.575)\).

**Discussion**

The current study, using our newly developed MSO task, demonstrates that ketamine-treated rats, a recognized model of schizophrenia, display a selective multisensory impairment that generalizes across different combinations of sensory modalities; this was in the absence of any obvious unimodal perceptual impairment. Furthermore, systemic and intra-OFC administration of nicotine and the selective \(\alpha_4\beta_2\) nAChR agonist ABT-418 reversed the MSO deficit in treated rats. Co-administration of the GABA\(_A\) antagonist bicuculline blocked the remediating effect of intra-OFC ABT-418 in ketamine-treated rats, suggesting that nAChRs restored MSO task performance by enhancing GABAergic function in the OFC. Using whole-cell electrophysiology, we showed that the frequency of GABAergic currents was significantly decreased in the OFC of ketamine-treated rats in comparison to saline-treated rats; this effect was reversed by activation of \(\alpha_4\beta_2\) nAChRs. Moreover, PV expression was significantly decreased in the OFC of ketamine-treated rats, linking this GABAergic interneuron type to the MSO task deficit reported here. Thus, the present findings provide convergent lines of evidence for a mechanism by which nAChRs ameliorate the MSO task impairment in ketamine-treated rats via stimulation of the GABAergic system in the OFC.
The selective MSO task impairment in ketamine-treated rats supports and extends our previous findings showing that treated rats display a selective tactile-to-visual CMOR deficit (Cloke & Winters, 2015; Jacklin et al., 2012). Importantly, the current study demonstrates that multisensory deficits in ketamine-treated rats are generalizable to modality combinations (e.g. olfactory-visual) beyond tactile-visual and that this impairment is seen with negligible memory demands, suggesting dysfunction at the level of perceptual multisensory binding. Although the MSO task is not identical to the multisensory facilitation task (audio-visual) used in human studies (Stone et al., 2011; Williams et al., 2010), it represents a significant advance over other multisensory tasks for rodents (Botly & De Rosa, 2007; Winters & Reid, 2010) in terms of its relative simplicity and absence of learning and memory components. The multisensory facilitation task for humans, which requires participants to respond to a unimodal (audio- or visual-only) or bimodal target (audio-visual), has been recently used to uncover atypical multisensory integration in schizophrenia (Stekelenburg et al., 2013; Stone et al., 2014; Stone et al., 2011; Williams et al., 2010). These results suggest a basic impairment at the level of multisensory perceptual processing, and this could also explain the deficits we report here in rats.

The nAChR is a clinically relevant target to treat cognitive dysfunction, as administration of nicotine improves cognition in human patients and animal models of schizophrenia (Barr et al., 2008; Hashimoto et al., 2008; Lieberman et al., 2013; Olincy et al., 2006; Pichat et al., 2007; Rushforth et al., 2011; Smith et al., 2006; Wildeboer & Stevens, 2008). Likewise, MSO task performance was rescued by administration of nicotine or an α4β2 nAChR agonist in the OFC of ketamine-treated rats. These findings extend our previous results with the CMOR task (Cloke & Winters, 2015; Jacklin et al., 2012), showing the generalizability of this nicotinic effect to a different multisensory task and multiple sensory modality combinations. Furthermore, the effect
of nAChR agonism was receptor and region specific as the selective α7 nAChR agonist GTS-21 and intra-mPFC nicotine failed to restore MSO task performance in ketamine-treated rats.

A primary goal of the current study was to begin to elucidate the mechanism underlying restoration of MSO task performance in ketamine-treated rats by α4β2 nAChR activation in the OFC. The remediating effect of intra-OFC ABT-418 in ketamine-treated rats was blocked by bicuculline, replicating our previous findings with CMOR (Cloke & Winters, 2015). GABAergic function is severely affected in patients and rodents treated with NMDA receptor antagonists, specifically within the prefrontal cortex (Abdul-Monim et al., 2007; Behrens et al., 2007; Cochran et al., 2003; Lewis et al., 2012; Lewis et al., 2005; Pratt et al., 2008). We hypothesized that decreased GABAergic function in the OFC of ketamine-treated rats was rescued by α4β2 nAChR activation. Our electrophysiological findings demonstrate that the frequency of GABAergic currents are significantly reduced in the OFC of ketamine-treated rats, similar to previous results from the mPFC of rodents treated with NMDA receptor antagonists (Jeevakumar & Kroener, 2014; Kjaerby et al., 2014; Zhang et al., 2008). Activation of α4β2 nAChRs in the OFC restored GABAergic function equal to that of saline-treated rats at baseline, suggesting that α4β2 nAChRs rescue GABAergic function. In support of this, previous studies show that α4β2 nAChRs predominantly enhance GABAergic currents in the cortex and hippocampus (Alkondon & Albuquerque, 2001; Aracri et al., 2010; Couey et al., 2007), and these receptors are localized presynaptically on GABAergic interneurons (Gulledge et al., 2007; Porter et al., 1999). These findings reinforce our behavioural findings, suggesting that an optimal level of GABAergic function in the OFC is required for MSO task performance and that α4β2 nAChR activation restores this essential inhibitory transmission in ketamine-treated rats.
A vital finding in the schizophrenia literature is a decrease in PV found post-mortem in the prefrontal cortex (Lewis et al., 2012; Lewis et al., 2005). In the current study, we found a significant decrease in PV expression in the OFC of ketamine-treated rats, replicating previous findings (Abdul-Monim et al., 2007; Cochran et al., 2003; Pratt et al., 2008), and further establishing the validity of studying this model. PV-IN dysfunction has been linked to impaired multisensory integration (Gogolla et al., 2014; Olcese et al., 2013). Thus, impairment in ketamine-treated rats may be linked to deficient PV-IN activity since decreased GABAergic currents and PV are found in the OFC of our treated rats. However, the techniques used in the current experiments did not allow for direct manipulation of specific interneurons populations. Therefore, the next chapter aimed to elucidate the direct involvement of GABAergic PV-INs in MSO performance.
Table 3. Basic electrophysiological properties for neurons recorded from ketamine- and saline-treated animals. Data are expressed as the mean (± SEM).

<table>
<thead>
<tr>
<th></th>
<th>Saline-Treated Rats</th>
<th>Ketamine-Treated Rats</th>
<th>Independent-Samples T-Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Rats</td>
<td>7</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Number of Neurons</td>
<td>31</td>
<td>27</td>
<td>-</td>
</tr>
<tr>
<td>Input Resistance (MΩ)</td>
<td>697.75 ± 30.6</td>
<td>733.58 ± 31.42</td>
<td>( t(56) = .813, p = 0.419 )</td>
</tr>
<tr>
<td>Resting Membrane Potential (mV)</td>
<td>-77.66 ± 0.37</td>
<td>-77.30 ± 0.61</td>
<td>( t(56) = .512, p = 0.610 )</td>
</tr>
<tr>
<td>Spike Amplitude (mV)</td>
<td>104.42 ± 1.05</td>
<td>104.51 ± 0.84</td>
<td>( t(56) = .064, p = 0.949 )</td>
</tr>
</tbody>
</table>
Table 4. Control measures collected from rat oddity experiments for each condition.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Condition</th>
<th>Control Rats</th>
<th>Ketamine Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC-VIS MSO + Controls</td>
<td>TAC-VIS</td>
<td>14.03 ± 1.33</td>
<td>14.64 ± 1.32</td>
</tr>
<tr>
<td>TAC-VIS only</td>
<td>TAC-VIS</td>
<td>13.59 ± 1.79</td>
<td>12.61 ± 1.42</td>
</tr>
<tr>
<td></td>
<td>VIS</td>
<td>12.11 ± 2.63</td>
<td>12.51 ± 1.40</td>
</tr>
<tr>
<td>OLF-VIS MSO + Controls</td>
<td>OLF-VIS</td>
<td>14.31 ± 1.48</td>
<td>19.74 ± 1.69</td>
</tr>
<tr>
<td>OLF-VIS only</td>
<td>OLF-VIS</td>
<td>14.87 ± 1.48</td>
<td>17.33 ± 1.58</td>
</tr>
<tr>
<td></td>
<td>VIS</td>
<td>10.36 ± 0.92</td>
<td>14.09 ± 0.74</td>
</tr>
<tr>
<td>TAC-VIS MSO: Systemic Nicotine</td>
<td>VEH</td>
<td>10.67 ± 0.56</td>
<td>11.31 ± 1.00</td>
</tr>
<tr>
<td>0.05 mg/kg</td>
<td>10.10 ± 0.79</td>
<td>11.65 ± 1.02</td>
<td></td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>11.71 ± 0.77</td>
<td>12.39 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>0.8 mg/kg</td>
<td>12.27 ± 1.07</td>
<td>12.05 ± 0.93</td>
<td></td>
</tr>
<tr>
<td>OLF-VIS MSO: Systemic Nicotine</td>
<td>VEH</td>
<td>10.37 ± 1.10</td>
<td>12.46 ± 1.83</td>
</tr>
<tr>
<td>0.05 mg/kg</td>
<td>12.38 ± 0.96</td>
<td>11.75 ± 1.29</td>
<td></td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>11.91 ± 0.98</td>
<td>11.19 ± 1.01</td>
<td></td>
</tr>
<tr>
<td>0.8 mg/kg</td>
<td>11.73 ± 0.66</td>
<td>12.68 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>TAC-VIS MSO: Systemic ABT-418</td>
<td>VEH</td>
<td>8.92 ± 0.43</td>
<td>8.51 ± 0.92</td>
</tr>
<tr>
<td>0.06 mg/kg</td>
<td>7.05 ± 0.60</td>
<td>8.53 ± 1.07</td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>8.30 ± 0.93</td>
<td>7.99 ± 0.67</td>
<td></td>
</tr>
<tr>
<td>0.6 mg/kg</td>
<td>7.88 ± 0.43</td>
<td>8.44 ± 0.94</td>
<td></td>
</tr>
<tr>
<td>OLF-VIS MSO: Systemic ABT-418</td>
<td>VEH</td>
<td>16.33 ± 1.63</td>
<td>21.06 ± 1.89</td>
</tr>
<tr>
<td>0.06 mg/kg</td>
<td>17.60 ± 1.56</td>
<td>22.10 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>0.1 mg/kg</td>
<td>17.31 ± 2.00</td>
<td>19.66 ± 2.29</td>
<td></td>
</tr>
<tr>
<td>0.6 mg/kg</td>
<td>19.30 ± 2.07</td>
<td>19.79 ± 2.57</td>
<td></td>
</tr>
<tr>
<td>TAC-VIS MSO: Systemic GTS-21</td>
<td>VEH</td>
<td>9.37 ± 0.63</td>
<td>11.62 ± 1.12</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>10.10 ± 0.91</td>
<td>11.20 ± 1.44</td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>11.99 ± 1.29</td>
<td>12.25 ± 1.09</td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>11.45 ± 0.87</td>
<td>11.59 ± 0.84</td>
<td></td>
</tr>
<tr>
<td>OLF-VIS MSO: Systemic GTS-21</td>
<td>VEH</td>
<td>19.22 ± 2.79</td>
<td>17.42 ± 2.56</td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>16.22 ± 1.67</td>
<td>17.50 ± 1.65</td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>18.05 ± 1.23</td>
<td>15.97 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>19.00 ± 2.18</td>
<td>20.37 ± 2.39</td>
<td></td>
</tr>
<tr>
<td>TAC-VIS MSO: Intra-OFC Nicotine</td>
<td>VEH</td>
<td>13.69 ± 1.07</td>
<td>12.67 ± 0.70</td>
</tr>
<tr>
<td>0.05 µg</td>
<td>12.82 ± 2.00</td>
<td>13.69 ± 1.96</td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>14.07 ± 0.99</td>
<td>12.92 ± 1.10</td>
<td></td>
</tr>
<tr>
<td>2 µg</td>
<td>13.14 ± 0.99</td>
<td>11.95 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>OLF-VIS MSO: Intra-OFC Nicotine</td>
<td>VEH</td>
<td>33.44 ± 2.90</td>
<td>26.22 ± 2.87</td>
</tr>
<tr>
<td>0.05 µg</td>
<td>33.72 ± 2.98</td>
<td>25.51 ± 1.90</td>
<td></td>
</tr>
<tr>
<td>1 µg</td>
<td>26.28 ± 0.64</td>
<td>24.93 ± 2.32</td>
<td></td>
</tr>
<tr>
<td>2 µg</td>
<td>32.38 ± 3.50</td>
<td>27.79 ± 2.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td><strong>TAC-VIS MSO:</strong></td>
<td>VEH</td>
<td>10.26 ± 1.54</td>
<td>15.13 ± 1.73</td>
</tr>
<tr>
<td>Intra-mPFC</td>
<td>0.05 µg</td>
<td>11.02 ± 1.13</td>
<td>15.00 ± 1.11</td>
</tr>
<tr>
<td>Nicotine</td>
<td>1 µg</td>
<td>9.10 ± 1.25</td>
<td>13.10 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>2 µg</td>
<td>11.61 ± 0.93</td>
<td>12.43 ± 1.40</td>
</tr>
<tr>
<td><strong>OLF-VIS MSO:</strong></td>
<td>VEH</td>
<td>17.60 ± 1.84</td>
<td>20.64 ± 3.00</td>
</tr>
<tr>
<td>Intra-mPFC</td>
<td>0.05 µg</td>
<td>16.49 ± 1.45</td>
<td>19.13 ± 1.79</td>
</tr>
<tr>
<td>Nicotine</td>
<td>1 µg</td>
<td>18.03 ± 2.60</td>
<td>20.38 ± 2.07</td>
</tr>
<tr>
<td></td>
<td>2 µg</td>
<td>16.87 ± 2.54</td>
<td>18.32 ± 1.84</td>
</tr>
<tr>
<td><strong>TAC-VIS MSO:</strong></td>
<td>VEH</td>
<td>18.07 ± 1.93</td>
<td>14.57 ± 1.63</td>
</tr>
<tr>
<td>Intra-OFC ABT-418</td>
<td>0.3 µg</td>
<td>14.21 ± 0.86</td>
<td>15.63 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>1 µg</td>
<td>13.12 ± 1.94</td>
<td>13.03 ± 1.70</td>
</tr>
<tr>
<td></td>
<td>3 µg</td>
<td>14.44 ± 3.37</td>
<td>12.07 ± 1.49</td>
</tr>
<tr>
<td><strong>OLF-VIS MSO:</strong></td>
<td>VEH</td>
<td>23.33 ± 2.15</td>
<td>26.18 ± 2.26</td>
</tr>
<tr>
<td>Intra-OFC ABT-418</td>
<td>0.3 µg</td>
<td>27.29 ± 1.48</td>
<td>23.44 ± 2.13</td>
</tr>
<tr>
<td></td>
<td>1 µg</td>
<td>27.43 ± 1.83</td>
<td>22.42 ± 3.08</td>
</tr>
<tr>
<td></td>
<td>3 µg</td>
<td>26.83 ± 3.00</td>
<td>25.06 ± 2.17</td>
</tr>
<tr>
<td><strong>TAC-VIS MSO:</strong></td>
<td>VEH/VEH</td>
<td>8.54 ± 0.95</td>
<td>11.82 ± 1.20</td>
</tr>
<tr>
<td>Intra-OFC ABT-418 + Bic</td>
<td>VEH/BIC</td>
<td>8.51 ± 0.76</td>
<td>11.68 ± 1.28</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>8.67 ± 0.72</td>
<td>8.12 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>8.40 ± 0.88</td>
<td>10.01 ± 1.43</td>
</tr>
<tr>
<td><strong>OFC-VIS MSO:</strong></td>
<td>VEH/VEH</td>
<td>22.69 ± 1.67</td>
<td>21.49 ± 2.19</td>
</tr>
<tr>
<td>Intra-OFC ABT-418 + Bic</td>
<td>VEH/BIC</td>
<td>21.94 ± 1.59</td>
<td>21.17 ± 1.95</td>
</tr>
<tr>
<td></td>
<td>ABT/VEH</td>
<td>21.52 ± 2.75</td>
<td>18.95 ± 1.71</td>
</tr>
<tr>
<td></td>
<td>ABT/BIC</td>
<td>20.18 ± 1.79</td>
<td>18.61 ± 2.26</td>
</tr>
</tbody>
</table>

Data are expressed as the mean (± SEM) of the total time in seconds spent exploring the objects.
Chapter 5

Pharmacogenetic Inhibition of Orbitofrontal GABAergic Parvalbumin-Interneurons Impairs Multisensory Oddity Task Performance in Mice: Remediation by $\alpha_4\beta_2$ Nicotinic Receptor Activation

Jacob M. Cloke$^{1,3}$, Robin Nguyen$^4$, David I. Wasserman$^1$, Stephanie De Lisio$^1$, Junchul Kim$^4$, Craig D.C. Bailey$^{2,3}$, & Boyer D. Winters$^{1,3}$

$^1$Department of Psychology, University of Guelph, Guelph, ON, Canada N1G 2W1
$^2$Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1
$^3$Collabirative Neuroscience Program, University of Guelph, Guelph, ON, Canada N1G 2W1
$^4$Department of Psychology, University of Toronto, Toronto, ON, Canada M5S 3GS

Data submitted for publication
Abstract

Dysfunction of the GABAergic system is heavily linked to cognitive impairment in schizophrenia. In particular, one specific type of GABAergic interneuron, PV-INs, is affected in the prefrontal cortex of patients. These effects are mimicked in rodents sub-chronically treated with ketamine, suggesting a crucial link between NMDA receptor hypofunction and PV-IN dysfunction in the disorder. PV-INs have recently been shown to contribute to multisensory integration, and therefore, this chapter explored the direct involvement of PV-INs in multisensory integration using the MSO task and DREADDs. Control mice were able to perform the tactile-visual MSO task and unimodal oddity tasks above chance. Systemic administration of ketamine selectively impaired tactile-visual MSO performance in mice, similar to rats. Systemic administration of DREADD receptor specific drug CNO in wild type mice did not affect tactile-visual MSO task performance, indicating that the drug is inert without a DREADD receptor available. Silencing of OFC PV-INs in PV-Cre mice expressing the inhibitory DREADD receptor hM4D selectively impaired tactile-visual MSO performance, sparing unimodal oddity task performance. Administration of the selective $\alpha_4\beta_2$ nAChR agonist ABT-418 reversed the MSO task impairment induced by OFC PV-IN inhibition in PV-Cre mice. These findings provide novel evidence for the role of prefrontal PV-INs in multisensory integration and further support the remediating role of nAChRs on cognitive impairment induced by GABAergic dysfunction.


**Introduction**

The aetiology of schizophrenia is not well understood; however, the literature recognizes that alterations in the GABAergic system are apparent in patients with schizophrenia (Lewis et al., 2012; Lewis et al., 2005). In particular, the main GABAergic interneuron marker PV and GABA synthesizing enzyme GAD67 are commonly decreased in the PFC and hippocampus of schizophrenia patients (Lewis et al., 2012; Lewis et al., 2005). Dysfunction in GABAergic function may be linked to decreased cognitive function in the disorder (Gonzalez-Burgos et al., 2015). In support of this, inhibition of GABA\textsubscript{A} receptors in the mPFC of rats causes similar behavioural and cognitive deficits, and changes in dopaminergic tone to that of schizophrenia patients (Auger & Floresco, 2014; Enomoto et al., 2011; Piantadosi & Floresco, 2014). Further research is required to elucidate how GABAergic dysfunction contributes to cognition in schizophrenia.

Regulation of GABAergic PV-IN activity in schizophrenia may be important for the components of the disorder, as knockout or silencing of PV-INs in mice induces a schizophrenia-like phenotype (Brown et al., 2015; Fujihara et al., 2015; Lazarus et al., 2015; Nguyen et al., 2014). Our results demonstrate that GABAergic function and PV is decreased in the OFC of ketamine-treated rats (Chapter 4), suggesting that dysfunction of GABAergic PV-INs impairs multisensory performance. Indeed, a link between PV-INs and multisensory integration has previous been shown (Gogolla et al., 2014; Olcese et al., 2013); however, the effect of direct PV-IN silencing on multisensory integration has not been tested. Therefore, the first objective of the current study was to use the inhibitory DREADD receptor hM4D to silence PV-INs in the OFC of PV-Cre mice to study their involvement in MSO task performance.

We have shown that GABA regulates nAChR-induced remediation of the selective
CMOR (Chapter 2) and MSO (Chapter 4) task impairments in ketamine-treated rats. Similarly, using whole-cell electrophysiology we demonstrated that activation of α₄β₂ nAChRs restored GABAergic function in ketamine-treated rats (Chapter 4). Together, these results imply that nAChRs rescue the multisensory impairment in ketamine-treated rats through modulation of GABAergic activity and this notion is consistent with the localization of nAChRs presynaptically on GABAergic interneurons (Gulledge et al., 2007; Porter et al., 1999). Thus the second objective of the current study was to examine whether activation of α₄β₂ nAChRs reverses the multisensory impairment induced by PV-IN inhibition in PV-Cre mice. This chapter therefore explored the direct involvement of PV-INs in MSO performance.

**Methods**

**Subjects**

C57BL/6 mice, as well as PV-Cre (B6;129P2-Pvalbtm1(cre)Arbr/J; JAX#008069) mice, obtained from The Jackson Laboratory and bred as homozygotes, approximately 2- to 3-months of age were used as subjects in this study. Food and water was freely accessible except during testing. All procedures adhered to the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committees at the University of Guelph and University of Toronto.

**AAV Vector Construction**

The recombinant AAV-hSyn-FLEX-hM4D-mCherry plasmid (Krashes et al., 2011) was obtained from Dr. Bryan Roth at the University of North Carolina at Chapel Hill and packaged in serotype 2/8 by the University of Pennsylvania Vector Core service; titers were 1 × 10¹² particles/ml.
Surgery

Mice were anesthetized with isoflurane and mounted onto a stereotaxic frame. The AAV vector containing doubly floxed hM4D-mCherry (AAV2/8-hsyn-FLEX-hM4D-mCherry) was infused into the OFC (AP: 2.50 mm, DV: -2.60, ML: ±1.40 mm). A volume of 0.3 µL was infused via an internal cannula connected by Tygon tubing to a 10 µL Hamilton needle syringe by pressure ejection at a rate of 0.1 µL/min. Following infusion, the internal cannula was left in place for 10 minutes to prevent solution backflow. After surgery, mice were single-housed.

Drugs

Ketamine was administered at 20 mg/kg systemically in mice 30 min prior to testing. Clozapine-N-oxide (CNO; dissolved in 20 % DMSO and saline; obtained from the NIH as part of the Rapid Access to Investigate Drug Program funded by the National Institute of Neurological disorders and Stroke (NINDS)) was administered at 3 mg/kg systemically 15 min prior to testing. ABT-418 was administered systemically at 0.2 mg/kg in mice 15 min prior to testing. Mice were administered each drug at a volume of 10 ml/kg. All systemic drugs were administered ip.

Immunohistochemistry

Mice were transcardially perfused with PBS, pH 7.4, followed by 4% paraformaldehyde. Brains were extracted and post-fixed overnight in 4% paraformaldehyde at 4°C and then cryoprotected with PBS containing 30% sucrose. Brains were sectioned coronally at 40μm thickness using a cryostat (CM 1520; Leica). For PV immunostaining, free-floating brain sections were blocked with 5% normal donkey serum in PBS-T (0.1%) for 1 h. Sections were then incubated with PBS-T containing rabbit polyclonal anti-PV antibody (1:1000; Abcam) for 48 h at 4°C, followed by Alexa Fluor 488–conjugated donkey anti-rabbit secondary antibody
(1:1000 in PBS-T; Invitrogen) for 2 h at room temperature. For cell-counting experiments, every third section between bregma 2.50 mm to 2.00 mm was collected and immunostained for PV. The sections were mounted and imaged on a confocal laser scanning microscope with a 20× objective. mCherry-positive and PV-ir cells were counted in a 500 × 500 µm area. The percentage transduction selectivity [(total number of double labelled cells/total number of mCherry-positive cells) × 100] and the percentage transduction efficacy [(total number of double labelled cells/total number of PV-ir cells) × 100] were calculated.

**Oddity Tasks**

A trapezoid-shaped open field constructed of opaque white plastic (front wall 39 cm, side wall 14 cm, angled side wall 10 cm, back wall 28 cm) was used for mouse oddity task experiments. Each trial for the oddity tasks consisted of a 10 min exploration period of 5 objects presented simultaneously. An oddity preference score was calculated, defined as exploration of the odd object divided by total exploration (Bartko et al., 2007a, 2007b); ‘chance’ exploration is therefore 0.2 for an oddity task with five objects. For a more detailed description of the oddity task procedures, see Chapter 3 methodology.

**Experiments**

**Experiment 1: Mouse tactile-visual MSO and unimodal oddity task pilot**

C57BL/6 mice (n = 4) were tested on the tactile-visual, tactile-only, and visual-only oddity tasks to examine whether mice can perform these tasks above chance (0.2). Tasks were counterbalanced across all trials. Each mouse was run for 3 trials (1 trial per task).

**Experiment 2: Effect of acute ketamine administration in wild type mice on tactile-visual MSO task performance**

C57BL/6 mice (n = 6) were tested on the tactile-visual, visual-, and tactile-only oddity
tasks. Mice were systemically administered either ketamine (20 mg/kg) or saline 30 min prior to testing. This experiment was performed to confirm that ketamine administration in mice selectively impairs MSO task performance, sparing visual- and tactile-only performance, similar to sub-chronic ketamine administration in rats. Drug administration and tasks were counterbalanced across all trials. Each mouse was run for 6 trials (1 trial per task/drug) with a minimum 48h between trials.

**Experiment 3: Effect of CNO administration in wild type mice on tactile-visual MSO task performance**

C57BL/6 mice (n = 7) were tested on the tactile-visual MSO task. Mice were acutely administered CNO (3 mg/kg) or vehicle 15 min prior to testing. This experiment was performed to confirm that CNO alone does not affect MSO task performance in wild type mice. Drug administration counterbalanced across all trials. Each mouse was run for 2 trials (1 trial per drug).

**Experiment 4: Effect of silencing PV-INs in OFC of PV-CRE on MSO task performance**

PV-Cre mice (n = 11) were infused with AAV-hSyn-FLEX-hM4D-mCherry in the OFC. This virally expresses the inhibitory DREADD receptor hM4D in PV-INs in the OFC of mice. Mice were left undisturbed for 3 weeks to enable maximum virus expression. PV-Cre mice were tested on tactile-visual MSO, visual-, and tactile-only oddity tasks. Mice were acutely administered CNO (3 mg/kg) or vehicle 15 min prior to testing. Drug administration and tasks were counterbalanced across all trials. Each mouse was run for 6 trials (1 trial per task/drug).

**Experiment 5: Effect of systemic ABT-418 administration on tactile-visual MSO task impairment induced by OFC PV-IN silencing**

The same PV-Cre mice from Experiment 3 were tested on the tactile-visual MSO task to
examine whether activation of the α4β2 nAChR would reverse the CNO-induced impairment. Mice were administered CNO (3 mg/kg) or vehicle in combination with the selective α4β2 nAChR agonist ABT-418 (0.2 mg/kg) or vehicle 15 min prior to testing. There were four drug conditions: VEH/VEH, VEH/CNO, ABT/VEH, and ABT/CNO. Drug administration was counterbalanced across all trials. Each rat was run for 4 trials (1 trial per drug condition) with a minimum 48h between trials.

Data Analysis

Behaviour was analyzed by taking the mean oddity preference score for each task/drug/dose condition in each experiment. As a control measure to confirm there were no general exploratory differences, the total object exploration was also assessed. Repeated measures ANOVAs were used to analyze the means of these two measures, and post-hoc paired-samples t-tests were used to assess significant differences within each task/drug condition. A Bonferroni correction was applied to account for multiple comparisons. Statistical analyses were conducted with a significance level of α = 0.05 using SPSS.

Results

Experiment 1: Mice can perform the MSO and unimodal oddity tasks

C57BL/6 mice performed the tactile-visual (t(3) = 5.27, p < 0.05), tactile-only (t(3) = 9.30, p < 0.01), and visual-only (t(2) = 5.46, p < .05) oddity tasks significantly above chance (0.2; see Figure 22a). The analysis of total object exploration revealed a significant main effect of task (F(2,6) = 12.95, p < 0.01), such that mice explored the objects greater in the tactile-only task than the tactile-visual (p < 0.05) and visual-only (p < 0.05) oddity tasks; however, task performance was not affected by these exploratory differences (see Table 5).
Experiment 2: Acute ketamine administration in wild type mice selectively impairs tactile-visual MSO performance

Acute ketamine (20 mg/kg) selectively impaired tactile-visual MSO task performance in C57BL/6 mice, sparing tactile- and visual-only performance (see Figure 22b). A repeated measures ANOVA revealed a significant interaction effect ($F(2,10) = 19.29, p < 0.001$) and main effect of Task ($F(2,10) = 11.30, p < 0.01$), but no significant main effect of Drug ($F(1,5) = 0.78$, $p = 0.418$). Post-hocs paired-samples t-tests revealed a significant difference between saline and ketamine administration on the tactile-visual oddity task ($t(5) = 5.96, p < 0.001$), but not the unimodal tasks ($p > 0.05$). The analysis of total object exploration revealed a main effect of Drug ($F(1,5) = 24.26, p < 0.01$) and Task ($F(2,10) = 18.45, p < 0.001$). Administration of ketamine significantly increased exploration in mice and this is consistent with the literature demonstrating acute ketamine increases locomotor activity (Chan et al., 2008; Hou et al., 2013). Also, mice explored significantly more in the tactile-visual oddity task than the tactile- ($t(11) = 3.50, p < 0.01$) and visual-only tasks ($t(11) = 3.98 , p < 0.01$), as well mice explore significantly more in the tactile-only than the visual only task ($t(11) = 5.90, p < 0.001$)(see Table 5).

Experiment 3: CNO administration in wild type mice does not affect tactile-visual MSO performance

Systemic administration of CNO in C57BL/6 mice did not affect tactile-visual MSO task performance (see Figure 22c). A paired-samples t-test revealed no significant difference between vehicle and CNO ($t(6) = 0.03, p = 0.98$). The analysis of total object exploration revealed no significant interaction or main effects, suggesting no effect of group or task on general exploratory behaviour (see Table 5).
Figure 22. (a) C57BL/6 mice (n = 4) can perform the tactile-visual MSO task, as well as unimodal tasks, above chance. (b) Systemic administration of ketamine (20 mg/kg) in C57BL/6 mice (n = 6) selectively impaired tactile-visual MSO task performance, sparing tactile- and visual-only unimodal oddity task performance. (c) CNO (3 mg/kg) administration in C57BL/6 mice (n = 7) did not affect tactile-visual MSO task performance. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). *** = p < 0.01, ketamine versus saline.
Figure 23. (a) Representative image of hM4D-mCherry expression in the orbitofrontal cortex (OFC) of a PV-Cre mouse overlaid on a section from the Paxinos and Watson (2001) mouse brain atlas (Bregma +2.6mm). Dashed lines indicate the boundaries of the OFC. 0.3 µl of the AAV was infused into the OFC bilaterally approximately two weeks before behavioural testing. hM4D-mCherry expression was observed consistently within the ventral and lateral OFC with minimal spread to adjacent areas. (b) High magnification (20x) confocal image of boxed area in (a). (c) Representative confocal images (40x) of mCherry expression (left), parvalbumin (PV) immunoreactivity (ir)(middle) and merged images (right) from OFC of a PV-Cre mouse administered the hM4D AAV. (d) Percentage of cells (mean ±SEM) double-labelled for hM4D-mCherry and PV-ir in PV-Cre mice infused with hM4D AAV (n=6). Red bar indicates that approximately 72% of hM4D-mCherry expressing cells were also immunopositive for PV. Green bar shows that approximately 73% of PV-immunopositive cells also expressed hM4D-mCherry.
Experiment 4: Silencing PV-INS in OFC of PV-Cre mice selectively impairs tactile-visual MSO performance

PV-Cre mice were infused with the cre-dependent AAV targeting PV neurons with the pharmacogenetic activity silencer hM4D in the OFC (see Figures 23a & b). Targeting of hM4D in the OFC was selective to PV since 73% of hM4D expressing neurons were positive for PV-ir. Furthermore, 73% of the total PV-ir neuron population in the OFC expressed hM4D, demonstrating high efficacy in PV neuron transduction (Figures 23c & d).

CNO (3 mg/kg) administration in PV-Cre mice selectively impaired tactile-visual MSO task performance in comparison to vehicle administration, sparing tactile- and visual-only performance (Figure 24a; Drug X Task \(F(2,20) = 27.24, p < 0.001\), Drug \(F(1,10) = 5.26, p < 0.05\), Task \(F(2,20) = 4.13, p < 0.05\)). Post-hoc paired-samples t-tests demonstrated significant differences between vehicle and CNO infused mice in tactile-visual MSO \(t(10) = 6.06, p < 0.001\), but not visual- or tactile-only oddity performance \((p > 0.05)\). The analysis of total object exploration revealed a significant main effect of task \(F(2,20) = 8.69, p < 0.01\), but no significant interaction or main effect of drug. Exploration was significantly lower in the visual-only task \((p < 0.01)\); however, task performance was not affected by lower exploration (see Table 5).

Experiment 5: Administration of ABT-418 reverses the effect of silencing PV-INS in the OFC of PV-Cre mice

ABT-418 (0.2 mg/kg) was administered in PV-Cre mice and reversed the MSO impairment induced by CNO (Figure 24b; \(F(3,30) = 14.04, p < 0.001\)). Post-hoc paired-samples t-tests demonstrated a significant difference between CNO-VEH and VEH-VEH \((t(10) = 6.91, p < 0.001)\), as well as CNO-VEH and ABT-CNO \((t(10) = 10.46, p < 0.001)\). The analysis
of total object exploration revealed no significant main effect of drug, suggesting no effect on general exploratory behaviour (see Table 5).

**Discussion**

The current study demonstrated that mice could perform the tactile-visual, tactile-only, and visual-only oddity tasks above chance, similar to rats. Acute administration of the NMDA receptor antagonist ketamine in wild type mice selectively impaired MSO, sparing unimodal performance. Furthermore, CNO-induced inhibition of PV-INs in the OFC of PV-Cre mice using the inhibitory DREADD receptor hM4D selectively impaired MSO performance, paralleling our results with acute ketamine in wild type mice. Administration of CNO did not affect performance of wild type mice, establishing that this drug is inert without the DREADD receptor virally expressed in the brain of transgenic mice. The CNO-induced MSO impairment in PV-Cre

Figure 24. (a) PV-INs were pharmacogenetically silenced in the OFC of PV-Cre mice (n = 11). Both experiments were within-subjects. Administration of CNO (3 mg/kg) 15 min prior to testing selectively impaired tactile-visual MSO task performance, sparing tactile- and visual-only unimodal oddity performance in PV-Cre mice. (b) Systemic co-administration of ABT-418 (0.2 mg/kg) with CNO rescued tactile-visual MSO performance in PV-Cre mice. Dashed line represents chance oddity preference score. Oddity preference scores displayed for group means (+SEM). *** = p < 0.001, CNO versus vehicle.
mice was reversed by systemic administration of the α4β2 nAChR agonist ABT-418. These findings suggest that prefrontal cortex PV-INs are critical for MSO performance and that functioning of these interneurons may be restored by activation of the α4β2 nAChR.

Acute administration of ketamine in wild type mice selectively impaired MSO performance, sparing unimodal oddity performance, replicating our findings with sub-chronic treatment of ketamine in rats. This finding further establishes the selective multisensory deficit induced by NMDA receptor antagonism that is generalizable to mice. In the previous chapter, it was established that GABAergic inhibitory transmission and PV markers were decreased in the OFC of ketamine-treated rats. Therefore, it was hypothesized that PV-IN dysfunction was responsible for the MSO task impairment in ketamine-treated rats. This is supported by Gogolla et al. (2014), who measured overlapping auditory and somatosensory response fields in the insular cortex of mice using in vivo imaging and showed that decreased markers of PV were associated with impaired multisensory integration; this deficit was reversed by pharmacologically restoring PV levels, suggesting that these interneurons are crucial for multisensory integration. To test this hypothesis we inhibited PV-INs using PV-Cre mice virally expressing inhibitory DREADD receptor hM4D in the OFC. Inhibition of PV-INs in the OFC selectively impaired MSO task performance, mirroring our findings with ketamine-treated rats and mice. This is the first evidence to our knowledge that direct inhibition of PV-INs induces impairment in multisensory integration, supporting the importance of these interneurons in this cognitive function.

Surprisingly, systemic administration of ABT-418 in mice restored the deficit induced by PV-IN inhibition. The α4 nicotinic subunit has been shown to be co-localized on PV-INs in the cortex of mice (Aracri et al., 2010), humans (Krenz et al., 2001), and rats (Dehkordi et al., 2007;
Liu, Mohila, Gong, Govindarajan, & Onn, 2005), and cholinergic projections from the basal forebrain innervate PV-INs in the rat cortex (Henny & Jones, 2008). Thus, it is likely, given our other results, that ABT-418 stimulates GABA release from PV-INs to restore MSO task performance. However, nAChRs are also found on other GABAergic interneurons (Gulledge et al., 2007; Porter et al., 1999), and we cannot conclude from the current data which specific GABAergic interneuron nAChRs stimulate in the OFC. Future studies should aim to specifically target nAChRs on PV-INs.

These findings may have important implications for atypical multisensory integration in schizophrenia patients. PV is commonly reduced in the PFC of schizophrenia patients (Lewis et al., 2012; Lewis et al., 2005), and our findings indicate a direct connection between PV-IN dysfunction and abnormal multisensory integration. This may also be associated with abnormal gamma oscillatory activity in the disorder (Cho et al., 2006; McNally et al., 2013; Uhlhaas & Singer, 2010), which is regulated by PV-IN activity (Cardin et al., 2009; Sohal et al., 2009). Activity in the gamma range appears important for cognition (Senkowski & Gallinat, 2015), as well as multisensory integration in schizophrenia (Stone et al., 2014). The current study establishes the importance of PV-IN activity in multisensory processes and suggests potential targets for pharmacotherapies treating cognition in schizophrenia, which include the GABAergic and cholinergic systems.
Table 5. Control measures collected from mouse oddity experiments for each condition.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition</th>
<th>Total Object Exploration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC-VIS MSO + Controls Pilot</td>
<td>TAC-VIS</td>
<td>13.01 ± 1.72</td>
</tr>
<tr>
<td></td>
<td>TAC</td>
<td>19.16 ± 2.58</td>
</tr>
<tr>
<td></td>
<td>VIS</td>
<td>8.89 ± 0.36</td>
</tr>
<tr>
<td>TAC-VIS MSO + Controls</td>
<td>VEH (TAC-VIS)</td>
<td>14.35 ± 1.29</td>
</tr>
<tr>
<td>(TAC- &amp; VIS-only): Systemic Ketamine in Type Mice</td>
<td>VEH (TAC)</td>
<td>23.02 ± 3.77</td>
</tr>
<tr>
<td></td>
<td>VEH (VIS)</td>
<td>11.57 ± 1.28</td>
</tr>
<tr>
<td></td>
<td>KET (TAC-VIS)</td>
<td>20.70 ± 1.32</td>
</tr>
<tr>
<td></td>
<td>KET (TAC)</td>
<td>26.80 ± 3.17</td>
</tr>
<tr>
<td></td>
<td>KET (VIS)</td>
<td>15.31 ± 1.52</td>
</tr>
<tr>
<td>TAC-VIS MSO:</td>
<td>VEH</td>
<td>9.45 ± 1.80</td>
</tr>
<tr>
<td>CNO in Wild Type Mice</td>
<td>CNO</td>
<td>6.96 ± 1.36</td>
</tr>
<tr>
<td>TAC-VIS MSO + Controls</td>
<td>VEH (TAC-VIS)</td>
<td>24.15 ± 1.92</td>
</tr>
<tr>
<td>(TAC- &amp; VIS-only): Systemic CNO in PV-Cre Mice</td>
<td>VEH (TAC)</td>
<td>24.18 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>VEH (VIS)</td>
<td>19.14 ± 2.14</td>
</tr>
<tr>
<td></td>
<td>CNO (TAC-VIS)</td>
<td>27.37 ± 2.65</td>
</tr>
<tr>
<td></td>
<td>CNO (TAC)</td>
<td>23.88 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>CNO (VIS)</td>
<td>17.17 ± 1.33</td>
</tr>
<tr>
<td>TAC-VIS MSO:</td>
<td>VEH/VEH</td>
<td>16.46 ± 1.63</td>
</tr>
<tr>
<td>Systemic CNO + Systemic ABT-418 in PV-Cre Mice</td>
<td>VEH/ABT</td>
<td>15.49 ± 2.12</td>
</tr>
<tr>
<td></td>
<td>CNO/VEH</td>
<td>19.31 ± 1.70</td>
</tr>
<tr>
<td></td>
<td>CNO/ABT</td>
<td>17.34 ± 2.23</td>
</tr>
</tbody>
</table>

Data are expressed as the mean (± SEM) of the total time in seconds spent exploring the objects.
Chapter 6

General Discussion
This thesis advances the understanding of deficient multisensory integration in a rodent model of schizophrenia (Jacklin et al., 2012) and details the mechanism through which prefrontal nAChRs restore multisensory cognition by normalizing GABAergic function in ketamine-treated rats. This mechanism was first suggested in chapter 2, in which co-administration of the GABA\textsubscript{A} antagonist bicuculline blocked the remediating effect of nicotine on the CMOR impairment in ketamine-treated rats; however, the NMDA receptor antagonist MK-801 did not. The effect of nicotine appeared to be dependent on the $\alpha_4\beta_2$ nAChR, but not the $\alpha_7$ nAChR, and the facilitative effect of $\alpha_4\beta_2$ nAChR agonism in ketamine-treated rats was also blocked by bicuculline. Furthermore, intra-OFC administration of nicotine and ABT-418 reversed the CMOR impairment in ketamine-treated rats, and this was also blocked by bicuculline. Using behavioural pharmacology, chapter 2 established a link between prefrontal nAChRs and GABAergic function in regulating the selective multisensory impairment in ketamine-treated rats.

One goal of this thesis was to further specify the nature of the selective multisensory impairment in ketamine-treated rats at a behavioural level. Thus, chapter 3 documented the development of the MSO task to study multisensory integration independent of mnemonic demand and using multiple modality combinations, such as tactile-visual and olfactory-visual configurations, in rodents. It was demonstrated that rats were able to perform both MSO tasks and unimodal control oddity tasks above chance. Chapter 4 used the MSO task to reveal that ketamine-treated rats display a selective tactile-visual and olfactory-visual MSO task impairment, with spared unimodal performance. These results suggest that the selective multisensory impairment initially revealed by the CMOR task in ketamine-treated rats is likely perceptual in nature, generalizable to multiple modality combinations, and extends to multisensory tasks beyond CMOR.
Chapter 4 further explored the interaction between nAChRs and GABA in ketamine-treated rats using the MSO task. Replicating the CMOR effects, nicotine and the selective $\alpha_4\beta_2$ nAChR agonist ABT-418, but not the selective $\alpha_7$ nAChR agonist GTS-21, rescued the tactile-visual and olfactory-visual MSO task impairments in ketamine-treated rats. Moreover, intra-OFC, but not intra-mPFC, nicotine reversed the MSO task impairments in ketamine-treated rats. These results demonstrate the receptor and regional specificity of the remediating effect of nAChR activation in ketamine-treated rats. Intra-OFC ABT-418 also rescued the MSO task impairment in ketamine-treated rats. This was blocked by administration of the GABA$_A$ antagonist bicuculline, which mirrors the CMOR results from chapter 2 and further suggests that altered GABAergic transmission plays a crucial role in the multisensory impairment in ketamine-treated rats.

It was hypothesized that decreased GABAergic function in the OFC of ketamine-treated rats was linked to the MSO impairment and that $\alpha_4\beta_2$ nAChRs could restore this essential inhibitory transmission. To test this hypothesis, whole-cell electrophysiology was used to record GABAergic currents in the OFC of ketamine- and saline-treated rats. The frequency and amplitude of GABAergic currents onto postsynaptic pyramidal cells in the OFC of ketamine-treated rats were significantly decreased compared to saline-treated rats. Application of ACh, in the presence of the muscarinic antagonist atropine and selective $\alpha_7$ nAChR antagonist MLA, restored the frequency of GABAergic currents without affecting amplitude in ketamine-treated rats to the same level of saline-treated rats at baseline. This hypothesis was further supported by decreased PV expression, a marker of a specific subpopulation of GABAergic interneurons, in the OFC of ketamine-treated rats. Chapter 4 extended findings from chapter 2 by demonstrating that GABAergic function, potentially mediated by PV-INs, is severely affected in the OFC of
ketamine-treated rats and $\alpha_4\beta_2$ nAChR activation appears to reverse the MSO impairment by restoring inhibitory transmission.

Chapter 5 explored the direct involvement of prefrontal PV-INs in multisensory integration by expressing inhibitory DREADD receptor hM4D in the OFC of PV-Cre mice. We first demonstrated that wild type mice were able to perform the tactile-visual MSO and unimodal oddity tasks above chance and that acute ketamine administration selectively impaired MSO performance, replicating our results with rats. Silencing of OFC PV-INs in PV-Cre mice selectively impaired tactile-visual MSO, sparing unimodal oddity task performance. The MSO impairment induced by PV-IN inhibition was reversed by systemic administration of the selective $\alpha_4\beta_2$ nAChR agonist ABT-418. The results from chapter 5 strongly suggest that prefrontal PV-INs are necessary for multisensory integration and that dysfunction of these interneurons can be compensated for by $\alpha_4\beta_2$ nAChR activation.

This thesis proposes that GABAergic PV-IN dysfunction in the OFC of ketamine-treated rats underlies their selective multisensory impairment and that presynaptic $\alpha_4\beta_2$ nAChR activation reverses this behavioural impairment by enhancing PV-IN inhibitory transmission. This thesis details the severe multisensory impairment in a rodent model of schizophrenia at a behavioural and cellular level. These findings may have important implications for understanding the neural bases of multisensory cognition in schizophrenia.

Viable Tasks to Assess Multisensory Cognition in Rodents

There are few viable behavioural tasks available to assess multisensory cognition in rodents. The multisensory tasks available for rodents include: the CMOR task (Winters & Reid, 2010), the crossmodal associative forced digging task (Botly & De Rosa, 2007), and the operant reaction time multisensory task (Siemann et al., 2014). These rodent tasks differ in their modality
combinations and design. Firstly, the CMOR task evaluates *tactile-visual* processing, the crossmodal associative forced digging task evaluates *tactile-olfactory* processing, and the operant multisensory task evaluates *auditory-visual* processing. Secondly, the crossmodal associative forced digging task and operant multisensory task present rodents with both modalities simultaneously (tactile-olfactory or auditory-visual) and thus, rodents can form an association between stimulus features. Conversely, the CMOR task does not enable this process to occur, since tactile and visual information is presented independently, rather than simultaneously, and rodents must infer the visual representation of objects from the previously experienced tactile representation. One important similarity across all these rodent multisensory tasks is the inclusion of unimodal control tasks (tactile-only, olfactory-only, visual-only, or auditory-only) to account for unimodal impairment, rather than a selective deficit in multisensory integration. All these tasks equally evaluate different forms of multisensory integration in rodents, but require further refinement.

The current thesis aimed to design a novel multisensory task for rodents that compliments and improves upon the CMOR task. The design of the MSO task was based on the perceptual oddity task used to assess complex object perception in rats (Bartko et al., 2007b). The oddity task exploits a rodent’s natural tendency to show preferred exploration for the ‘odd’ object and requires no prior training or reinforcement to perform. The MSO task uses objects composed of different modality feature combinations, such as olfactory-visual and tactile-visual configurations, to assess multisensory integration in rodents. We have also created unimodal (tactile-only, visual-only, and olfactory-only) tasks that control for unimodal impairments and the associative nature of the MSO task. This novel multisensory task removes the non-associative and mnemonic components of the CMOR task, which is more in line with human
tests of multisensory integration (Jacklin et al., 2012; Stone et al., 2014; Stone et al., 2011; Williams et al., 2010), and enables inference about the nature of direct multisensory perceptual binding processes. A significant aspect of the MSO task is the ability to assess multiple modality combinations (tactile-visual & olfactory-visual), which improves upon the other available multisensory rodent tasks that are limited to one specific modality combination. This is particularly important because it enables researchers to probe the multisensory functions of specific neural mechanisms and their generalizability across multiple modality combinations. Although it was not conducted in the current thesis, it is also worth noting that the MSO task could be adapted in the future to assess additional modality combinations (e.g., olfactory-tactile).

This thesis demonstrates that both rats and mice robustly perform the MSO and unimodal control oddity tasks above chance. Thus, the MSO task appears to be a simple one-trial test of multisensory cognition in rodents that can be used to evaluate this cognitive ability at a basic and applied level. Future studies should aim to develop a tactile-olfactory version of the MSO task to enable assessment of a third modality combination. Also, the neural bases of MSO task performance in rodents requires examination, in particular the potential involvement of OFC and perirhinal cortex, which are both known to contribute to CMOR performance (Bartko et al., 2007b; Jacklin et al., 2016; Reid et al., 2013; Winters & Reid, 2010).

**Generalized Multisensory Impairment in a Rodent Model of Schizophrenia**

An acknowledged rodent model of schizophrenia is the NMDA receptor hypofunction model, which can be induced by treatment with non-competitive NMDA receptor antagonists (MK-801, PCP, or ketamine). This model produces negative and cognitive symptoms, as well as cellular changes, that are highly similar to those reported for schizophrenia (Gilmour et al., 2012; Neill et al., 2010). Rodents treated with these NMDA receptor antagonists display severe object
recognition deficits (Lyon et al., 2012), a recognized test of the visual learning and memory domain in preclinical schizophrenia models (Young et al., 2009). During object recognition, rodents are able to explore objects in multiple modalities simultaneously, such as visual and tactile, to form a multisensory representation of these objects. Therefore, a multisensory integration impairment in rodents treated with NMDA receptor antagonists may account for this object recognition deficit. This possibility is supported by atypical multisensory integration being documented in schizophrenia patients (Stone et al., 2014; Stone et al., 2011; Tseng et al., 2015; Williams et al., 2010). Jacklin et al. (2012) explored this hypothesis and showed that rats subchronically treated with ketamine or MK-801 exhibited a selective CMOR impairment, sparing visual-only and tactile-only object recognition performance, with a minimal delay between sample and choice. Likewise, the neurodevelopmental polyI:C rat model of schizophrenia also displays a selective CMOR impairment (Ballendine et al., 2014). These findings show that two models of schizophrenia have impaired multisensory integration that is not influenced by a unimodal deficit. The selective multisensory impairment in rats treated with NMDA receptor antagonists supports using this model to study the neural basis of multisensory cognition in schizophrenia.

Due to the design of the CMOR task, aspects of the selective multisensory impairment in ketamine-treated rats remained unclear and required further exploration. This thesis used the newly developed MSO task to expand upon the CMOR findings. Ketamine-treated rats displayed a selective tactile-visual and olfactory-visual MSO impairment, sparing unimodal oddity performance. As well, acute administration of ketamine in mice selectively impaired tactile-visual MSO performance, demonstrating the generalizability of this effect to other rodents. These results have three important implications for understanding the multisensory impairment in
ketamine-treated rodents. Firstly, the selective MSO impairment suggests that the multisensory deficit in ketamine-treated rats is independent of memory and may be more perceptual in nature. This is an important finding as it parallels the human schizophrenia literature that uses perceptual multisensory tests (Stekelenburg et al., 2013; Stone et al., 2014; Stone et al., 2011; Williams et al., 2010). In particular, patients show selective deficits in different tests of multisensory integration, similar to our findings. Although the MSO task does not mirror human multisensory tests, it establishes a similar impairment in ketamine-treated rats to human schizophrenia patients. The discrepancy in the MSO impairment is whether it is due to perceptual or attention deficits, as attention impairments are apparent in ketamine-treated rats. Likewise, attention deficits are apparent in schizophrenia patients (Laurent et al., 1999; Luck & Gold, 2008), and this may be linked to atypical multisensory integration (de Jong et al., 2010; Zvyagintsev et al., 2013). However, schizophrenia patients lack a N100 component (Stekelenburg et al., 2013) and show a decreased P50 component (Magnée et al., 2009; Roa Romero et al., 2016), which are correlated with multisensory integration, and these findings support impairment at a perceptual level. Therefore, this question requires additional exploration to determine whether the impairment is due to attention or perception, but it is clear that this impairment is relatively selective to multisensory integration. Secondly, a deficit in tactile-visual and olfactory-visual integration suggests that the multisensory impairment is generalizable to other modality combinations, rather than limited to tactile-visual integration. Schizophrenia patients display atypical integration with multiple modality combinations, which includes auditory-visual, tactile-visual, and olfactory-visual (de Gelder et al., 2002; Ferri et al., 2013; Pearl et al., 2009; Peled et al., 2003; Peled et al., 2000; Seubert et al., 2010; Stone et al., 2014; Stone et al., 2011; White et al., 2014; Williams et al., 2010). Lastly, the MSO impairment in ketamine-treated rats demonstrates the generalizability
of this deficit to other multisensory tasks beyond CMOR. This is in line with human literature showing that schizophrenia patients display abnormal multisensory integration with different tests, such as the multisensory reaction-time task, rubber hand illusion, and McGurk effect (de Gelder et al., 2002; Ferri et al., 2013; Pearl et al., 2009; Peled et al., 2003; Peled et al., 2000; Stone et al., 2014; Stone et al., 2011; White et al., 2014; Williams et al., 2010).

The multisensory impairments in the NMDA receptor hypofunction rat model closely mirror those found in the human schizophrenia literature. Evidence strongly supports using the MSO task as a measure of multisensory cognition in preclinical models of schizophrenia. Future studies need to explore the generalizability of the MSO impairment in other rodent models of schizophrenia, such as genetic and neurodevelopmental models, which encapsulate different aspects of the disorder. Similar multisensory deficits, or differences, in other preclinical models will help to elucidate the underlying neurobiology of atypical multisensory integration in schizophrenia. These findings may have implications for other aspects of the disorder, including hallucinations and social cognition, which have been previously linked to abnormal multisensory integration (Green et al., 2005; Jardri et al., 2009). Early atypical multisensory integration may even be a potential predictor of onset of the disorder (Gamma et al., 2014) and thus, future research should focus on this aspect of the disorder.

**Prefrontal α_4β_2 nAChRs Restore Multisensory Cognition in Ketamine-Treated Rats**

The cholinergic system has been a key pharmacological target for treating cognitive impairment in schizophrenia due to the established role of ACh in regulating attention, learning, and memory (Hasselmo & Sarter, 2011; Sarter et al., 2003; Winters et al., 2006). In particular, this system has been a focus because schizophrenia patients commonly exhibit drug use, including cigarettes (de Leon & Diaz, 2005; Leonard et al., 2000), and patients who smoke
display reduced cognitive impairment (Kumari & Postma, 2005; Leonard et al., 2000; Sacco et al., 2005; Smith et al., 2002). These findings have led researchers to investigate the efficacy of nicotine in reducing specific cognitive symptoms of the disorder. Accordingly, clinical results have clearly demonstrated the pro-cognitive effects of nicotine in patients (Barr et al., 2008; Smith et al., 2002; Smith et al., 2006). Administration of nicotine also restores aspects of cognition (attention, memory, multisensory integration etc.) in preclinical rodent models of schizophrenia (Andreasen et al., 2006; Jacklin et al., 2012; Rushforth et al., 2011). Thus, researchers have targeted the nAChR, in particular specific receptor subtypes, as a potential pharmacotherapy for cognitive dysfunction in schizophrenia. Clinical trials examining selective nAChR compounds acting on the \( \alpha_4\beta_2 \) or \( \alpha_7 \) nAChR have had less clear results with unreliable effects on cognition in patients (Freedman et al., 2008; Keefe et al., 2015; Lieberman et al., 2013; Olincy et al., 2006; Radek et al., 2010; Umbricht et al., 2014; Velligan et al., 2012; Waldo et al., 2010; Zhang et al., 2012). \( \alpha_7 \) nAChR agonists encenicline, DMXB-A, and tropisetron have been shown to improve aspects of cognition in schizophrenia, including memory, auditory gating, and attention (Keefe et al., 2015; Olincy et al., 2006; Zhang et al., 2012). Other studies using \( \alpha_7 \) nAChR agonists DMXB-A and RG3487 suggested no beneficial cognitive effects in the disorder (Freedman et al., 2008; Umbricht et al., 2014). The \( \alpha_4\beta_2 \) agonist ADZ3480 failed to improve cognition in schizophrenia patients (Velligan et al., 2012). However, the partial \( \alpha_4\beta_2 \) agonist varenicline, which is currently used for smoking cessation, has shown pro-cognitive effects in schizophrenia in domains such as verbal learning and memory, sensory gating, executive function, and visuospatial working memory (Hong et al., 2011; Shim et al., 2012; Smith et al., 2009; Wing, Wass, Bacher, Rabin, & George, 2013). At the same time, other studies have shown no beneficial effects of varenicline on cognition (Waldo et al., 2010). Our findings support the
efficacy of nAChRs alleviating multisensory impairments; however, the effect of nAChR agonists on atypical multisensory integration in human patients has yet to be tested. Further work is necessary to clarify the role of nAChRs in treating cognitive impairment in schizophrenia. Ongoing clinical work is developing more selective nAChR agonists, partial agonists, and allosteric modulators to examine whether these drugs show promise in reducing cognitive symptoms in the disorder.

In the CMOR task, systemic administration of nicotine reverses the multisensory impairment in rodents treated sub-chronically with NMDA receptor antagonists (Jacklin et al., 2012), and this finding was replicated in chapter 2 of this thesis with ketamine-treated rats. This thesis extended this finding by demonstrating that systemic administration of the selective $\alpha_4\beta_2$ nAChR agonist ABT-418, but not the selective $\alpha_7$ nAChR agonist GTS-21, ameliorates the CMOR impairment and MSO task impairment (tactile-visual and olfactory-visual) in ketamine-treated rats. A recent study demonstrated that administration of a selective $\alpha_4\beta_2$ nAChR or $\alpha_7$ nAChR agonist reversed an object recognition deficit in PCP-treated rats with a 1 min delay between the sample and choice phases (Miyauchi, Neugebauer, Oyamada, & Meltzer, 2016), and GTS-21 has been shown to ameliorate an object recognition impairment induced by acute MK-801 with a 3h delay (Callahan et al., 2014). Our results, along with previous results, suggest that $\alpha_4\beta_2$ nAChRs may have a specific role in restoring multisensory integration, unlike $\alpha_7$ nAChRs. To further support the selective effect of $\alpha_4\beta_2$ nAChRs, future studies should administer selective $\alpha_4\beta_2$ and $\alpha_7$ nAChRs antagonists in conjunction with nicotine or selective nAChRs agonists to examine whether the remediating effect on CMOR and MSO in ketamine-treated rats is blocked.

The ameliorative ability of nAChRs on CMOR and MSO performance in ketamine-treated rats was further explored in the brain to understand the brain region involved in this
multisensory impairment. The PFC, in particular the OFC region, was targeted in our studies because the OFC is affected in schizophrenia (Bellani et al., 2010; Eryilmaz et al., 2016; Kanahara et al., 2013; Shenton et al., 2001) and this region of the brain has previously been shown to contribute to multisensory processes (Aitken, 1980; Ettlinger & Garcha, 1980; Fuster et al., 2000; Gaffan & Harrison, 1991; Lipton et al., 1999; Petrides & Iversen, 1976; Whishaw et al., 1992), including the CMOR task (Reid et al., 2013). Infusion of nicotine or ABT-418 into the OFC reversed the CMOR and MSO task impairments in ketamine-treated rats. Conversely, infusion of nicotine into the mPFC did not remediate the MSO task impairment in ketamine treated rats, suggesting that the effect of nAChRs is regionally specific within the PFC. This finding is consistent with previous work by Reid et al. (2013), which demonstrated that selective OFC, but not selective mPFC lesions, selectively impaired CMOR, sparing unimodal performance in rats. These findings suggest that the OFC dysfunction in ketamine-treated rats may be connected to CMOR/MSO impairments and this requires further exploration to understand the role of the OFC in ketamine-treated rats. Although similar lesions studies have yet to be performed with the MSO tasks used here, the current result strongly indicate a role for the OFC in MSO task performance.

$\alpha_4\beta_2$ nAChRs appear to play a predominate role in restoring multisensory cognition in ketamine-treated rats. This is an interesting finding as most studies have documented that activation of the $\alpha_7$ nAChR restores different forms of cognition in preclinical models of schizophrenia, with little research exploring the involvement of $\alpha_4\beta_2$ nAChRs (Hashimoto et al., 2008; Hauser et al., 2010; Pichat et al., 2007; Radek et al., 2006; Thomsen et al., 2009; Timmermann et al., 2009; Wallace et al., 2011; Wildeboer & Stevens, 2008). In support of the potential role of $\alpha_4\beta_2$ nAChRs, it has been shown that the $\beta_2$ nAChR subunit is upregulated in the
cortex and hippocampus of schizophrenia patients with a history of smoking, compared to controls (Breese et al., 1999; Esterlis et al., 2014). Upregulation of the β2 nAChR subunit has been linked to lower negative symptoms and increased executive function in schizophrenia patients, suggesting that the remediating effect of nicotine may be due to the α4β2 nAChR (Esterlis et al., 2014). Furthermore, one study showed that there was greater radioligand binding for the α4β2 nAChR than the α7 nAChR in the rat OFC (Mendez, Damborsky, Winzer-Serhan, Bizon, & Setlow, 2013). This was also seen with radioligand binding in human schizophrenia patients (Marutle et al., 2001), suggesting that increased expression of the α4β2 nAChR in the OFC may contribute to their role in remediating this form of cognition. Future studies need to examine the generalizability of these nAChR effects in other forms of cognition that are affected in both schizophrenia patients and preclinical rodent models. Furthermore, while nAChR activation reversed these multisensory deficits in ketamine-treated rats, neither nicotine nor ABT-418 enhanced CMOR or MSO performance in saline-treated rats, suggesting that nAChR activity differentially regulates cognition and behaviour in these treated rats. This may be due, in part, to the fact that chronic ketamine administration in rodents upregulates nAChR expression in the cortex (Chatterjee et al., 2012), potentially explaining the reason nAChRs have greater efficacy improving cognition in ketamine-treated rats versus saline-treated rats. Further examination of the effects of ketamine on nAChR receptor function and expression in the prefrontal cortex with our treatment regimen is required to understand the tremendous efficacy of nicotine to reverse cognitive impairment in ketamine-treated rats.
A GABAergic-nAChR Mechanism Restores Multisensory Cognition in Ketamine-Treated Rats

GABAergic inhibitory transmission is compromised in schizophrenia, and this has been linked to aspects of the symptomology (Lewis et al., 2012; Lewis et al., 2010; Lewis et al., 2005). Post-mortem examination of the brains of schizophrenia patients shows decreased expression of GAD67, GAT1, VGAT, and GABA receptors in the prefrontal cortex and hippocampus (Akbarian et al., 1995; Beneyto et al., 2011; Duncan et al., 2010; Hashimoto et al., 2003; Hoftman et al., 2015; Lewis et al., 2010; Volk et al., 2001; Volk et al., 2000; Volk et al., 2002). Recent in vivo studies using radioligands in schizophrenia patients have demonstrated that cortical GABA is decreased (Frankle et al., 2015; Thakkar et al., 2016), supporting post-mortem findings. Reduced prefrontal GABA may have specific implications for cognitive dysfunction in schizophrenia as antagonism of the GABA$_A$ receptor in the mPFC of rats produces similar cognitive deficits (Auger & Floresco, 2014; Enomoto et al., 2011; Piantadosi & Floresco, 2014). Furthermore, it has been hypothesized that NMDA receptor antagonism or dysfunction is associated with or causes the decrease in GABA function found in schizophrenia. Recent genetic evidence shows that both glutamatergic and GABAergic signalling is disrupted in the disorder (Pocklington et al., 2015). Moreover, chronic treatment with NMDA receptor antagonists ketamine or PCP in rodents replicates decreases in GABAergic function and markers in the prefrontal cortex and hippocampus (Abdul-Monim et al., 2007; Behrens et al., 2007; Cochran et al., 2003; Jeevakumar & Kroener, 2014; Kjaerby et al., 2014; Pratt et al., 2008; Zhang et al., 2008), and these rodents display severe cognitive impairment (Gilmour et al., 2012; Neill et al., 2010). Several researchers suggest that reduced GABAergic function induced by NMDA receptor antagonism may be central to the cognitive impairment in schizophrenia (Gonzalez-
Burgos & Lewis, 2012; Lisman et al., 2008). Thus, we examined the involvement of decreased GABAergic transmission in ketamine-treated rats on multisensory cognition and the potential for nAChRs to restore performance by facilitating GABAergic function.

The remediating effect of nicotine and ABT-418 on CMOR performance in ketamine-treated rats was blocked by the GABA\textsubscript{A} receptor antagonist bicuculline. Similarly, GABA\textsubscript{A} receptor antagonism blocked the ameliorative effect of intra-OFC ABT-418 on CMOR and MSO performance in ketamine-treated rats. These results suggested that nAChRs restore multisensory cognition in the prefrontal cortex of ketamine-treated rats by enhancing GABA release, which may be decreased in this model. It has previously been shown that GABAergic currents are decreased in the mPFC of rodents treated with NMDA receptor antagonists (Jeevakumar & Kroener, 2014; Kjaerby et al., 2014; Zhang et al., 2008) and acute administration of MK-801 disrupts OFC GABAergic interneuron activity \textit{in vivo} (Homayoun & Moghaddam, 2008; Quirk et al., 2009); however, it has yet to be examined whether GABAergic release is decreased in the OFC of ketamine-treated rats. This may be particularly important for multisensory integration, as a recent paper demonstrated that GABAergic activity is correlated with audio-visual multisensory integration in humans (Balz et al., 2016). Therefore, we explored the hypothesis that decreased OFC GABAergic function in ketamine-treated rats was linked to the MSO impairment. We tested this hypothesis by sacrificing ketamine- and saline-treated rats following the washout period and performing whole-cell electrophysiology in brain slices. The frequency of GABAergic sPSCs (reflecting presynaptic GABAergic release) onto postsynaptic pyramidal neurons in OFC layer 2/3 of ketamine-treated rats was significantly decreased compared to saline-treated rats. This result parallels previous findings demonstrating that GABAergic sPSCs are decreased in layer 2/3 of the mPFC, not but layer 5 of ketamine-treated rodents (Jeevakumar
& Kroener, 2014; Kjaerby et al., 2014), although GABAergic function in layer 5 of the OFC was not examined in these experiments. Furthermore, we found that the amplitude of GABAergic sPSCs was also decreased compared to saline-treated rats, suggesting that postsynaptic GABA receptor function may also be affected in this rodent schizophrenia model.

Application of ACh, in the presence of the muscarinic antagonist atropine and selective \( \alpha_7 \) nAChR antagonist MLA, restored GABAergic sPSC frequency in ketamine-treated rats to the same level of saline-treated rats without drug application. Application of the selective \( \alpha_4\beta_2 \) nAChR antagonist DH\( \beta \)E in combination with ACh blocked this effect. Activation of the \( \alpha_4\beta_2 \) nAChR and blockade by DH\( \beta \)E did not alter GABAergic sPSC amplitude demonstrating that the observed effects are likely due to presynaptic, rather than postsynaptic, nAChR activation. This is consistent with \( \alpha_4\beta_2 \) nAChRs being located presynaptically on GABAergic interneurons and enhancing GABA release in the cortex and hippocampus (Alkondon & Albuquerque, 2001, 2004; Aracri et al., 2010; Banerjee et al., 2012; Bloem et al., 2014; Couey et al., 2007; Porter et al., 1999). This is a novel finding showing that \( \alpha_4\beta_2 \) nAChR activation restores OFC GABAergic function in ketamine-treated rats. These results with whole-cell recordings support our behavioural pharmacology findings that demonstrate that multisensory cognition is restored in ketamine-treated rats by enhancing GABAergic release through activation of the presynaptic \( \alpha_4\beta_2 \) nAChRs.

Sub-chronic administration of ketamine in rats decreased expression of PV in the OFC, replicating previous findings (Abdul-Monim et al., 2007; Cochran et al., 2003; Pratt et al., 2008; Romon et al., 2011; Thomsen et al., 2009). Reduced expression of PV and GAD67 post-mortem in the PFC of schizophrenia patients is commonly found (Beasley & Reynolds, 1997; Beasley et al., 2002b; Hashimoto et al., 2003; Hoftman et al., 2015; Joshi et al., 2015; Reynolds et al., 2002;
Volk et al., 2012; Zhang & Reynolds, 2002) and has been linked to cognitive impairment in the disorder (Lewis et al., 2012; Lewis et al., 2005). Inhibition or knockout of PV-INs in different areas of the brain has been shown to induce a schizophrenia-like phenotype, supporting this hypothesis (Brown et al., 2015; Fujihara et al., 2015; Lazarus et al., 2015; Nguyen et al., 2014). Furthermore, knockout of the NMDA receptor on PV-INs induces cognitive impairment, establishing a link between NMDA receptors and PV-INs in mediating cognition (Carlén et al., 2012; Korotkova et al., 2010). Our results demonstrate that GABAergic transmission and PV is decreased in the OFC, suggesting that dysfunction of the PV GABAergic interneuron subtype may be connected to the MSO impairment. To test this hypothesis, we used PV-Cre mice and virally expressed inhibitory DREADD receptor hM4D in the OFC. This provided us with a method of directly inhibiting OFC PV-INs in vivo to test their involvement in MSO performance. Silencing of OFC PV-INs impaired performance of the tactile-visual MSO task, sparing tactile-only and visual-only performance. This finding provides direct evidence for the involvement of PFC PV-INs in a novel form of cognition, multisensory integration. A previous study supports our findings showing that decreased PV expression in mouse models of autism was associated with multisensory impairment (Gogolla et al., 2014). Conversely, Olcese et al. (2013) demonstrated that optogenetic activation of PV-INs impairs multisensory enhancement in adjacent pyramidal cells. These findings suggest that bidirectional regulation of PV-IN activity may be crucial for multisensory integration. Our study provides novel support by directly inhibiting PV-INs using DREADDs. Silencing of PV-INs mirrors the results seen in ketamine-treated rats. These findings suggest that reduced activity of OFC PV-INs may lead to the selective MSO impairment in ketamine-treated rats, although further support is required to directly link PV-INs to this deficit.
An important implication of the involvement of PV-INs in multisensory integration is the generalizability across other rodent model of schizophrenia. The neurodevelopmental poly I:C rat model displays a selective CMOR impairment (Ballendine et al., 2014), and alterations in GAD67 and PV expression in the PFC and hippocampus are apparent in this rodent model (Cassella et al., 2016; Meyer, Nyffeler, Yee, Knuesel, & Feldon, 2008; Nyffeler, Meyer, Yee, Feldon, & Knuesel, 2006; Piontkewitz et al., 2012), similar to the our ketamine model. A recent study showed that GAD67, but not PV, expression was reduced within PV-INs in the mPFC of the offspring of Poly I:C treated pregnant mice (Canetta et al., 2016). As well, GABAergic transmission was reduced in the mPFC of these mice, specific to PV-INs, and not calretinin-expressing interneurons (Canetta et al., 2016). These results replicate our findings with a different rodent model and propose that PV dysfunction may be a common mechanism underlying the multisensory impairment in schizophrenia. Future studies need to investigate a common mechanism amongst multiple schizophrenia models to gain a better understanding of atypical multisensory integration in schizophrenia.

Because we had demonstrated that activation of the α4β2 nAChR restored GABAergic function in ketamine-treated rats, we examined whether ABT-418 would reverse the impairment induced by PV-IN silencing in PV-Cre mice. Systemic administration of the selective α4β2 nAChR agonist in PV-Cre mice ameliorated the MSO deficit induced by PV-IN silencing. These results suggest that α4β2 nAChRs, potentially on PV-INs, restore MSO performance by facilitating GABAergic function. The α4 nicotinic subunit has been shown to be co-localized on PV-INs in the cortex of mice in the terminal region (Aracri et al., 2010), humans (Krenz et al., 2001), and rats (Dehkordi et al., 2007; Liu et al., 2005), and cholinergic projections from the basal forebrain innervate the terminal region of PV-INs in the rat cortex (Aracri et al., 2010;
Henny & Jones, 2008), supporting this hypothesis. However, nAChRs are also located presynaptically on various types of GABAergic interneurons, including somatostatin- (SOM) or vasoactive intestinal peptide (VIP)- expressing GABAergic interneurons (Porter et al., 1999). It is possible that presynaptic nAChRs on SOM-interneurons (SOM-INs) restored the behavioural deficit, as this interneuron acts directly on pyramidal neurons in the cortex (Gentet et al., 2012; Lovett-Barron et al., 2012; Pfeffer, Xue, He, Huang, & Scanziani, 2013), like PV-INs, and activation of SOM-INs may compensate for the lack of inhibition provided by PV-IN activity. It is unlikely that VIP-interneurons (VIP-INs) are involved in this effect since they mainly inhibit other interneurons subtypes and would be less likely to enhance GABAergic release onto pyramidal neurons (Pfeffer et al., 2013; Pronneke et al., 2015). Moreover, PV expression has been shown primarily to be affected in the brains of schizophrenia patients, independent of SOM; however, a recent study showed that PV and SOM mRNA expression was decreased in the OFC of patients (Joshi et al., 2015), suggesting that changes to both these interneuron subtypes could contribute to the disorder. PV-INs and SOM-INs differentially modulate pyramidal input-output activity, with PV-INs inhibiting the soma and SOM-INs inhibiting the distal dendrites (Chiu et al., 2013; Lovett-Barron et al., 2012). Therefore, it would be of interest to examine whether inhibition of SOM-INs would induce a similar MSO deficit to see if specific types of inhibitory transmission onto pyramidal neuron inhibition (somatic or dendritic) are important for multisensory cognition. In the current study, due to systemic administration of ABT-418 these results lack regional specificity, as well as interneuron specificity, and we cannot draw firm conclusions regarding the localization of nAChRs on the targeted interneurons. However, it is clear that activation of the α4β2 nAChR reverses the behavioural deficit induced by PV-IN silencing, and this must be followed up with subsequent experiments.
Future Directions

This thesis introduces a putative mechanism by which PFC nAChRs restore MSO performance in ketamine-treated rats. In particular, we propose that decreased PV-IN function in the OFC of ketamine-treated rats, linked to the MSO impairment, is restored by nAChR activation. However, due to the nature of the techniques used in these studies it cannot be determined which GABAergic interneuron subtype nAChRs are located or acting on. Future studies should perform all experiments using PV-Cre mice to enable direct manipulation or imaging of PV-expressing cells. In these experiments, we could sub-chronically treat mice with ketamine or saline, similar to rats, and these transgenic mice would allow us to target PV-INs.

The first step for future experiments is to examine the expression of the $\alpha_4$, $\beta_2$, and $\alpha_7$ nAChR subunits in PV-INs of the OFC to clarify localization of these nAChRs on PV-INs. Secondly, using PV-Cre mice we can express a fluorescent protein to allow imaging and direct recording of PV-INs in slice using whole-cell electrophysiology. We can measure PV-IN GABAergic sPSCs in ketamine- versus saline-treated mice, and we can measure the activity of PV-INs in the presence of $\alpha_4\beta_2$ activation. These studies would provide us with more direct evidence for the involvement $\alpha_4\beta_2$ on PV-INs in regulating activity in the NMDA receptor hypofunction schizophrenia model.

Importantly, we can manipulate the activity of PFC PV-INs using DREADDs in PV-Cre mice. Behaviourally, it would be important to replicate these findings showing that silencing of PV-INs in the OFC, which selectively impairs tactile-visual MSO performance, also affects the olfactory-visual MSO task. Then we can examine whether ABT-418 reverses this impairment, importantly infusing the drug directly in the OFC and co-administering DHβE to see if this is blocked. These experiments would further strengthen our findings, but still lacks specificity of
the drug acting on PV-INs. To examine the involvement of $\alpha_4\beta_2$ nAChR on PV-INs, we could infuse a Cre-dependent virus in the OFC of PV-Cre mice that knocks down expression of the $\beta_2$ subunit (Mineur et al., 2011). This would enable us to see whether the effect of an $\alpha_4\beta_2$ nAChR agonist on MSO performance would be eliminated. However, these mice would have to be characterized to examine whether there are any cognitive or behavioural impairments produced by knockout of this receptor subunit.

Lastly, we hypothesize that decreased PFC PV-IN activity in ketamine-treated rodents is connected to the selective multisensory impairment. We could test this by expressing excitatory DREADD receptor hM3D in the OFC following ketamine treatment in PV-Cre mice, enabling us to activate PV-INs in vivo. I hypothesize that activation of OFC PV-INs in ketamine-treated mice would restore MSO performance, strongly supporting the idea that PV-INs in ketamine-treated rodents are critical for multisensory integration. Recent studies have shown that stimulation of PV-INs restores attention and cognitive inflexibility impairments that are induced by PV-IN dysfunction (Cho et al., 2015; Kim, Ahrlund-Richter, Wang, Deisseroth, & Carlen, 2016).

**Conclusions**

The current study reveals that ketamine-treated rats display a generalized multisensory integration impairment, the nature of which appears to be independent of memory function and basic perceptual processing. Schizophrenia patients appear to display multisensory integration deficits at the level of perceptual binding (Stone et al., 2011; Tseng et al., 2015; Williams et al., 2010), and the novel MSO task provides a simple and robust method for assessing similar functions in rodents, providing a potentially valuable pre-clinical tool for cognitive assessment in animal models (Butler et al., 2008). Accordingly, we used the MSO task here to assess the mechanistic basis of nAChR-induced remediation of multisensory deficits in a rodent model of
schizophrenia. Our results strongly suggest that nAChRs – specifically α4β2 nAChRs – can restore impaired GABAergic transmission within the OFC of ketamine-treated rats to facilitate multisensory integration. We also identify the PV-IN class of interneurons as a likely target of ketamine-mediated disruption, consistent with findings from animal models and schizophrenia patients (Abdul-Monim et al., 2007; Behrens et al., 2007; Cochran et al., 2003; Homayoun & Moghaddam, 2008; Jeevakumar & Kroener, 2014; Kjaerby et al., 2014; Lewis et al., 2012; Lewis

Figure 25. (a) GABAergic PV-INs inhibit the soma of pyramidal neurons (PYR) in the cortex. Other subtypes of GABAergic interneurons (IN) also inhibit PYR in the cortex through different forms of inhibition. (b) This thesis proposes that activation of presynaptic α4β2 nAChRs on PV-INs in the OFC enhances presynaptic release of GABA. GABA binds to GABA_A receptors on the postsynaptic PYR to provide inhibition.
et al., 2005; Pratt et al., 2008; Zhang et al., 2008). In particular, we postulate that treatment of ketamine leads to decreased presynaptic GABA release from PV-INs onto the postsynaptic pyramidal neuron and activation of presynaptic $\alpha_4\beta_2$ nAChRs facilitates GABA release, binding to postsynaptic GABA$_A$ receptors on pyramidal neurons in the prefrontal cortex (Figure 25). Future pharmacological therapies targeting the GABAergic system and/or GABAergic-nicotinic interactions may show promise in treating specific aspects of cognition in schizophrenia (Damgaard et al., 2011; Lewis et al., 2008; Menzies et al., 2007). The current findings may have future implications for understanding the neurobiology of atypical multisensory integration in schizophrenia, an understudied aspect of cognition in the disorder.
References


Bailey, CD, De Biasi, M, Fletcher, PJ, & Lambe, EK. (2010). The nicotinic acetylcholine receptor α5 subunit plays a key role in attention circuitry and accuracy. *Journal of Neuroscience, 30*(27), 9241-9252.


Beasley, CL, Zhang, ZJ, Patten, I, & Reynolds, GP. (2002b). Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biological Psychiatry, 52*, 708-715.


Cloke, JM, & Winters, BD. (2015). α4β2 Nicotinic receptor stimulation of the GABAergic system within the orbitofrontal cortex ameliorates the severe crossmodal object recognition impairment in ketamine-treated rats: implications for cognitive dysfunction in schizophrenia. *Neuropsychopharmacology, 90*, 42-52.


Dawson, N, Xiao, X, McDonald, M, Higham, DJ, Morris, BJ, & Pratt, JA. (2014). Sustained NMDA Receptor Hypofunction Induces Compromised Neural Systems Integration and
d Esterlis, I, Ranganathan, M, Bois, F, Pittman, B, Picciotto, MR, Shearer, L, . . . D'Souza, DC. (2014). In vivo evidence for beta2 nicotinic acetylcholine receptor subunit upregulation
in smokers as compared with nonsmokers with schizophrenia. Biological Psychiatry, 76(6), 495-502.


Jentsch, JD, & Roth, RH. (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology, 20*, 201-225.


Joshi, D, Catts, VS, Olaya, JC, & Weickert, CS. (2015). Relationship between somatostatin and death receptor expression in the orbital frontal cortex in schizophrenia: a postmortem brain mRNA study. *npj Schizophrenia, 1*. doi: 10.1038/npjschz.2014.4


Kinney, JW, Davis, CN, Tabarean, I, Conti, B, Bartfai, T, & Behrens, MM. (2006). A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and


Lieberman, JA, Dunbar, G, Segreti, AC, Girgis, RR, Seoane, F, Beaver, JS, ... Hosford, DA. (2013). A randomized exploratory trial of an α-7 nicotinic receptor agonist (TC-5619) for cognitive enhancement in schizophrenia. *Neuropsychopharmacology, 38*(6), 968-975.


Reid, JM, Jacklin, DL, & Winters, BD. (2013). Delineating prefrontal cortex region contributions to crossmodal object recognition in rats. Cerebral Cortex, 24(8), 2108-2119.


Ross, LA, Saint-Amour, D, Leavitt, VM, Molholm, S, Javitt, DC, & Foxe, JJ. (2007). Impaired multisensory processing in schizophrenia: deficits in the visual enhancement of speech


Senkowski, D, & Gallinat, J. (2015). Dysfunctional prefrontal gamma-band oscillations reflect working memory and other cognitive deficits in schizophrenia. *Biological Psychiatry, 77*(12), 1010-1019.


Thompson, M, Weickert, CS, Wyatt, E, & Webster, MJ. (2009). Decreased glutamic acid decarboxylase(67) mRNA expression in multiple brain areas of patients with schizophrenia and mood disorders. *Journal of Psychiatric Research, 43*(11), 970-977.
Thomsen, MS, Christensen, DZ, Hansen, H H, Redrobe, JP, & Mikkelsen, JD. (2009). alpha(7) Nicotinic acetylcholine receptor activation prevents behavioral and molecular changes induced by repeated phencyclidine treatment. *Neuropharmacology, 56*, 1001-1009.


Volk, DW, Austin, MC, Pierr, JN, Sampson, AR, & Lewis, DA. (2000). Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. *Archives of General Psychiatry, 57*, 237-245.

Volk, DW, Pierri, JN, Fritschy, JM, Auh, S, Sampson, AR, & Lewis, DA. (2002). Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cell inputs to pyramidal neurons in schizophrenia. *Cerebral Cortex, 12*, 1063-1070.


Zhang, ZJ, & Reynolds, GP. (2002). A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophrenia Research, 55*, 1-10.