

**Exploring the Role of 5-HT in Amphetamine-Induced Depression of Visual Responses in the Superior Colliculus**

**By:**

Timothy Brian Riley

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

The University of Sheffield

Faculty of Science

Department of Psychology

06/12/2018

**Acknowledgements**

I would like to thank my principal supervisor Professor Paul Overton for his (seemingly) unending years of support, wisdom, and especially patience. Apologies that the last of those three was needed in such a quantity, I suspect you didn’t anticipate requiring so much of it when I came to speak you about a PhD in December of 2011. Thanks to Len Hetherington for showing me the ropes and keeping me grounded throughout the PhD. Thanks also for your long chats about academic (and to a greater extent) non-academic exploits. Apologies for any contribution that I may have had to your early retirement. It would be remiss to not also thank Robert Schmidt for stepping in after Len’s retirement. That second supervisor box on all those forms would have looked very empty without you. Please let me win at squash sometime.

Many thanks to too many people to list in Alfred Denny. First, Sam, Paul, and Luke for their friendship and experience. I rarely mentioned this, but it was an excellent experience studying towards a PhD surrounded researchers who set such a high standard, and more importantly, were so generous at the bar. I aim to live up to your examples in only one of those areas. Thanks to Emily for help with the histology, and also to Nat for keeping things running. Thanks to Aneurin, Andy Ham, and Michael Port for technical support in and around the lab. Thanks to Llywelyn for being a great office mate, and to Clare for keeping him employed. Thanks to Myles for support in research, teaching, and allergy prevention, as well as for the odd coffee. Thanks also to Jason for your enthusiasm towards science, and for granting me access to a Man United supporter during these dark times for your club.

I made some friends, but they left the University ahead of me and will never get the chance read this- thanks to Marcel, Colin, Rebecca, Kira, Priya, and Dan- we had some good times. Outside of Sheffield, thanks to John, Danny, and Luke for keeping me distracted and being there to help blow off steam.

Special thanks to my family. Mum and Dad for always instilling the importance of perusing education, and for listless other values and virtues. Thanks to Jon for being my best mate and financial backer, and thanks for Jessica for being so proud. Finally thank you to my beautiful wife Perla, for giving me a reason to stay in Sheffield and for always pushing me and keeping me on track. We did it.

**Exploring the Role of 5-HT in Amphetamine-Induced Depression of Visual Responses in the Superior Colliculus**

**Abstract**

The research presented in the current thesis had two principal motivators. First, to elucidate the mechanism by which d-amphetamine depresses visual responses in the superficial layers of the superior colliculus (SC). Then, to identify a candidate substance that has a similar mechanism of action at the level of the superficial SC to d-amphetamine, but with a safer profile in terms of side effects and abuse potential. d-amphetamine is a current frontline pharmaceutical used in the treatment of ADHD, and when tolerated well, is highly efficacious in improving sustained attention and alleviating the symptoms of ADHD. Despite this efficacy, the use of d-amphetamine in the treatment of ADHD has been criticised due to its abuse potential and aversive side-effects. Thus, there is a need to identify new efficacious interventions for ADHD that lack the abuse potential of psychostimulants. A fruitful strategy when taking this approach is to identify substances that have a common mechanisms of action to psychostimulants in terms of the systems they interact with Data presented in the current thesis show that d-amphetamine induces a depression of the superficial SC response to visual stimuli in vivo, which can be reversed by antagonising 5-HT. The SSRI fluoxetine was identified as a candidate drug with potential to depress SC visual responses with a similar profile to d-amphetamine. It was shown that when administered in the presence of a substance that antagonises autoregulator processes at the level of the dorsal raphe nucleus, fluoxetine depresses aspects of the SC response to visual stimuli in a manner that is comparable to the effects of d-amphetamine. Given the novelty of these results in light of mixed evidence of the efficacy of treating ADHD with SSRIs, it is proposed that the viability of targeting 5-HT transmission in the pharmacotherapy of ADHD should be re-examined.

**Glossary of Terms**

|  |  |
| --- | --- |
| **Term** | **Definition** |
| μg | Microgram |
| μl | Microlitre |
| μm | Micrometre |
| 5-HT | Serotonin |
| ADHD | Attention Deficit Hyperactivity Disorder |
| ADD | Attention Deficit Disorder |
| APA | American Psychiatric Association |
| BG | Basal ganglia |
| BOLD | Blood Oxygenation Level Dependant |
| CMRglu | Cerebral Metabolic Rate for glucose utilisation |
| CN | Caudate Nucleus |
| CNS | Central Nervous System |
| D-amp | d-amphetamine |
| DA | Dopamine |
| DLPFC | Dorsolateral Prefrontal Cortex |
| DMSO | Dimethyl Sulfoxide |
| DRN | Dorsal Raphe Nuclei |
| DSM | Diagnostic and Statistical Manual of Mental Disorders |
| EEG | Electroencephalography |
| ERPs | Event-Related Potentials |
| FLU | Fluoxetine |
| fMRI | Functional Magnetic Resonance Imaging |
| g | Gram |
| Hr | Hour |
| HKD | Hyperkinetic Disorder |
| Hz | Hertz |
| i.v. | Intravenous |
| ICD | International Classification of Mental and Behavioural Disorders |
| kg | Kilogram |
| KHz | Kilohertz |
| LED | light Emitting Diode |
| LFP | Local Field Potentials |
| LGN | Lateral Geniculate Nucleus of the thalamus |
| M | Molar |
| Meter | Metergoline |
| mg | Milligram |
| min | Minutes |
| ml | Millilitre |
| mm | Millimetre |
| MRI | Magnetic resonance imaging |
| MRN | Medial Raphe Nuclei |
| ms | Millisecond |
| MUA | Multi-Unit Activity |
| NA | Noradrenaline |
| nmol | Nanomolar |
| oC | Degrees Celsius |
| PFC | Prefrontal Cortex |
| PSTH | Peri-Stimulus Time Histogram |
| CBF | Cerebral Blood Flow |
| s | Seconds |
| SAL | Saline |
| SAP | Stratum Album Profundum |
| SC | Superior Colliculus |
| SD | Standard Deviation |
| SERT | Serotonin Transporter |
| SGI | Stratum Griseum Intermedial |
| SGP | Stratum Griseum Profundum |
| SGS | Stratum Griseum Superficial |
| SNr | Substantia Nigra Pars Reticularta |
| SO | Stratum Opticum |
| SPECT | Single Photon Emission Computed Tomography |
| SSRI | Selective Serotonin Reuptake Inhibitor |
| SZ | Stratum Zonal |
| TCAs | Tricyclic Antidepressants |
| V | Volts |
| VLPFC | Ventrolateral Prefrontal Cortex |
| VMAT2 | Vesicular Monoamine Transporter |
| WHO | World Health Organisation |

**Thesis Contents**

|  |  |
| --- | --- |
| Title Page | i |
| Acknowledgements | ii |
| Abstract | iv |
| Glossary of Terms | v |
| Thesis Contents | vii |

|  |  |  |
| --- | --- | --- |
| **1** | **Chapter 1 - Introduction** | **1** |
| **1.1** | **Chapter Overview** | **1** |
| **1.2** | **Attention Deficit Hyperactivity Disorder: an Overview** | **1** |
| **1.3** | **A Brief History of ADHD** | **3** |
| **1.4** | **ADHD in Current Diagnostic Criteria** | **5** |
| 1.4.1 | ADHD in the DSM-5 | 5 |
| 1.4.2 | ADHD in the ICD-10 and ICD-11 | 8 |
| **1.5** | **ADHD as a Persistent Lifelong Disorder** | **9** |
| **1.6** | **Interventions for ADHD** | **13** |
| 1.6.1 | Psychostimulants as Frontline Pharmacological Interventions for ADHD | 14 |
| 1.6.2 | Current Alternatives to Psychostimulant Medication for ADHD | 16 |
| **1.7** | **The Neuroscience of ADHD: an Inconclusive search for Pathological Loci** | **18** |
| 1.7.1 | Structural Morphology and Functional Differences in ADHD | 18 |
| **1.8** | **Symptom Based Approach to Drug Development for ADHD: A Focus on Distractibility** | 23 |
| 1.8.1 | Distractibility as a Core Symptom of ADHD | 24 |
| **1.9** | **The Superior Colliculus and its Relevance to Distractibility in ADHD** | **26** |
| 1.9.1 | Structure of the superior colliculus | 26 |
| 1.9.2 | Function and Connectivity of the Superficial SC | 27 |
| 1.9.3 | Evidence of Psychostimulant Action at the Level of the Superior Colliculus | 31 |
| **1.10** | **Summary and Next Steps** | **34** |
| 1.10.1 | Overview of Initial Empirical Chapters | 35 |
| **2** | **Chapter 2 - General Methodology** | **37** |
| **2.1** | **Chapter Summary** | **37** |
| **2.2** | **Construction of Microinjector Coupled Electrodes** | **37** |
| **2.3** | **Surgical procedures** | **38** |
| 2.3.1 | Subjects | 38 |
| 2.3.2 | Femoral Vein Cannulation | 39 |
| 2.3.3 | Craniotomy to Expose the Cortex Overlaying the Superior Colliculus | 40 |
| 2.4 | **Data Preparation** | 41 |
| 2.4.1 | Generic Data Capture Technique | 41 |
| 2.4.2 | Generic Data Preparation and Pre-Processing | 44 |
| **2.5** | **Data Analysis** | **45** |
| 2.5.1 | Local Field Potential | 46 |
| 2.5.2 | Multi-Unit Activity | 47 |
| **2.6** | **Histology** | **49** |
| **3** | **Chapter 3 -Effect of 5-HT Antagonism on Amphetamine-Induced Suppression of Superficial Layer SC Visual Responses** | **52** |
| **3.1** | **Chapter Summary** | **52** |
| **3.2** | **Introduction** | **52** |
| 3.2.1 | SC Hypersensitivity as an Explanation for Increased Distractibility in ADHD | 52 |
| 3.2.2 | Psychostimulant Action at the Level of the SC | 55 |
| 3.2.3 | Aim of the Present Study | 57 |
| **3.3** | **Methods** | **59** |
| 3.2.1 | Subjects | 59 |
| 3.3.2 | Experimental Procedures | 59 |
| 3.3.3 | Data Analysis | 60 |
| **3.4** | **Results** | **65** |
| 3.4.1 | Electrode Placement | 65 |
| 3.4.2 | Effects of Systemically Administered d -amphetamine and Metergoline on Superficial Layer SC LFP Response to Visual Stimuli | 66 |
| 3.4.3 | Effects of Systemically Administered d -amphetamine and Metergoline on Superficial Layer SC MUA Response to Visual Stimuli | 69 |
| 3.4.4 | Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli | 72 |
| **3.5** | **Discussion** | **75** |
| 3.5.1 | Interpretation of Results | 75 |
| 3.5.2 | Implications of Results | 80 |
| 3.5.3 | Future Direction | 81 |
| **4** | **Chapter 4 - Effect of 5-HT Antagonism on Intracranial Amphetamine-Induced Suppression of Collicular Visual Responses** | **82** |
| **4.1** | **Chapter Summary** | **82** |
| **4.2** | **Introduction** | **82** |
| 4.2.1 | Rationale for the Present Study | 82 |
| 4.2.2 | Pharmacological Targets of Psychostimulant Action and Their Relevance to the SC | 83 |
| 4.2.3 | Aim of the Present Study | 88 |
| **4.3** | **Methods** | **89** |
| 4.3.1 | Subjects | 89 |
| 4.3.2 | Experimental Procedures | 89 |
| 4.3.3 | Data Analysis | 91 |
| **4.4** | **Results** | **95** |
| 4.4.1 | Injector Coupled Electrode Placement | 95 |
| 4.4.2 | Effects of Administration of Local d-amphetamine and Systemic Metergoline on Superficial Layer SC LFP Response to Visual Stimul | 96 |
| 4.4.3 | Effects of Administration of Local d-amphetamine and Systemic Metergoline on Superficial Layer SC MUA Response to Visual Stimuli | 97 |
| 4.4.4 | Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli | 101 |
| **4.5** | **Discussion** | **103** |
| 4.5.1 | Summary of Results | 103 |
| 4.5.2 | Interpretation of Results | 104 |
| 4.5.3 | Implications of the Results and Future Direction | 107 |
| **5** | **Chapter 5 - Interim Discussion: Serotonin Transmission as a Potential Target in the Pharmacotherapy of ADHD** | **109** |
| **5.1** | **Chapter Summary** | **109** |
| **5.2** | **Collicular Dysfunction can Explain Distractibility in ADHD** | **109** |
| **5.3** | **Modulation of Oculomotor Activity in the SC** | **111** |
| **5.4** | **Serotonin Based Interventions for ADHD: Disconnect Between Theory and Application** | **115** |
| **5.5** | **Serotonin Based Interventions for ADHD: Autoregulation of Serotonergic Activity** | **120** |
| **5.6** | **Summary and Next Steps** | **123** |
| **6** | **Chapter 6: 5-HT1A auto receptor antagonism enables fluoxetine-induced suppression of superficial layer SC visual responses** | **124** |
| **6.1** | **Chapter Summary** | **124** |
| **6.2** | **Introduction** | **124** |
| 6.2.1 | Rationale for the Present Study | 124 |
| 6.2.2 | Fluoxetine as a Candidate Drug for Research in the Present Study | 126 |
| 6.2.3 | Control of 5-HT1A Mediated Autoregulation in Acute Pharmacological Perpetrations | 127 |
| 6.2.4 | Aim of the Present Study | 129 |
| **6.3** | **Methods** | **130** |
| 6.3.1 | Subjects | 130 |
| 6.3.2 | Antagonism of 5-HT1A Autoreceptors | 130 |
| 6.3.3 | Experimental Procedures | 130 |
| 6.3.4 | Data Analysis | 132 |
| **6.4** | **Results** | **136** |
| 6.4.1 | Electrode Placement | 136 |
| 6.4.2 | Effects of Administration of Systemic NAD-299 and Fluoxetine on Superficial Layer SC LFP Response to Visual Stimuli | 137 |
| 6.4.3 | Effects of Administration of Systemic NAD-299 and Fluoxetine on Superficial Layer SC MUA Response to Visual Stimuli | 141 |
| 6.4.4 | Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli | 146 |
| **6.5** | **Discussion** | **149** |
| 6.5.1 | Summary of Results | 149 |
| 6.5.2 | Interpretation of Results | 150 |
| 6.5.3 | Implications of the Results | 154 |
| **7** | **Chapter 7: Final Discussion and Conclusions** | **156** |
| **7.1** | **Chapter Summary** | **156** |
| **7.2** | **Motivation for Research** | **156** |
| **7.3** | **Principal Findings** | **157** |
| 7.3.1 | Depression of the SC Visual Response Induced by d-amphetamine is Reversed Following 5-HT Antagonism | 158 |
| 7.3.2 | When 5-HT1A Autoreceptors are Antagonised, Fluoxetine acts to Depress the SC Response to Visual Stimuli in a Comparable Manner to d-amphetamine | 159 |
| 7.3.3 | Discrete Phases of the SC MUA Response are Differentially Affected by Both d-amphetamine and Fluoxetine | 160 |
| 7.3.4 | Summary of Findings | 162 |
| **7.4** | **Theoretical Implications** | **163** |
| **7.5** | **Limitations** | **167** |
| 7.5.1 | Limitations Associated with Pharmacology | 167 |
| 7.5.2 | Limitations Associated with Experimental Design | 169 |
| **7.6** | **Future Directions and Remaining Questions** | **170** |
| 7.6.1 | Further Examination of the Effect of Fluoxetine at the Level of the SC | 170 |
| 7.6.2 | Exploration of the Second Phase of the MUA Response | 171 |
| 7.6.3 | Examination of Alternative Neurotransmitter Systems Targeted by d-Amphetamine | 172 |
| **7.7** | **Final Conclusions** | **173** |
|  | **References** | **175** |

Introduction

# **Chapter Overview**

The aim of this chapter is to outline the need to develop new non-addictive pharmacotherapies for Attention Deficit Hyperactivity Disorder, and to discuss strategies to overcome barriers which have prevented progress in this research area. I will first introduce ADHD, and discuss how heterogeneous symptom presentation has acted to confound the search for a pathological basis of this disorder. I will then discuss current pharmacotherapies for ADHD, and argue that focusing on specific core symptoms may be a fruitful strategy for identifying potential new pharmacological interventions. Finally, I will present evidence that the midbrain superior colliculus is uniquely positioned as a locus with relevance to increased distractibility in ADHD, and will outline initial experiments to explore the mechanism of action for psychostimulants at the level of the SC.

# Attention Deficit Hyperactivity Disorder: an Overview

Attention Deficit Hyperactivity Disorder (ADHD) is a common neurodevelopmental disorder that is characterised by the symptomatic triumvirate of inattention, hyperactivity, and impulsivity. The worldwide childhood prevalence rate of ADHD is estimated to be 5.29% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), though in the United States and Europe, the diagnosis rate may be as high as 12% (Biederman, 2005a). These diagnosis rates make ADHD the most commonly diagnosed neurodevelopmental disorder worldwide, and one of the 5 most prevalent mental health disorders across Europe (Wittchen et al., 2011). Though ADHD is associated with core symptoms of inattention, hyperactivity, and impulsivity, presentation of individual symptoms is highly heterogeneous. As such, the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) recognises three distinct sub-types of ADHD based on dominant symptom cluster: predominantly inattentive presentation, predominantly hyperactive/impulsive presentation, and predominantly combined presentation (American Psychiatric Association, 2013). As well as these core symptoms, ADHD is associated with a number of negative financial, social, and academic predicators which persist across the lifespan (Greydanus, Pratt, & Patel, 2007). Whilst previously considered to only have clinical relevance as a childhood disorder (American Psychiatric Association, 2004), clinicians and researchers have long recognised the potential for ADHD to present as a lifelong disorder (Faraone et al., 2000; Murphy & Barkley, 1996). The DSM-5 now includes criteria for adulthood ADHD, recognising that that symptoms persist into adulthood in up to 60% of all ADHD cases (Gentile, Atiq, & Gillig, 2006; Simon, Czobor, Balint, Meszaros, & Bitter, 2009).

In part due to its heterogeneous presentation, the aetiology of ADHD is complex and multifaceted. Research into susceptibility genes for ADHD has produced several candidate genes that contribute to the aetiology, though as with the symptomology, results are inconsistent and heterogeneous (Faraone & Larsson, 2018; Faraone, Perlis, et al., 2005). While no single candidate gene can fully predict ADHD, polymorphisms in several genes make significant contributions to the presentation of ADHD (S. I. Sharp, McQuillin, & Gurling, 2009). Interestingly, the majority of these genes are associated with activity of the monoamine neurotransmitter systems, at either a receptor or transporter level (Gizer, Ficks, & Waldman, 2009). This is reflected in pharmacological interventions for ADHD. Current frontline pharmaceuticals for ADHD consist of the psychostimulant drugs methylphenidate and d-amphetamine, both of which act to increase synaptic availability of monoamine neurotransmitters (Heal, Smith, Kulkarni, & Rowley, 2008; Rothman et al., 2001). When tolerated well, these substances are efficacious in improving sustained attention, and alleviating the symptoms of ADHD (Elia, Ambrosini, & Rapoport, 1999; Konrad, Gunther, Heinzel-Gutenbrunner, & Herpertz-Dahlmann, 2005). Despite their efficacy, the use of psychostimulants has been criticised for their abuse potential and their aversive side-effects, which can lead to discontinuation (Ghuman et al., 2001; Williams, Goodale, Shay-Fiddler, Gloster, & Chang, 2004). Further, in recent years there has been an increasing trend towards stimulant misuse and diversion, particularly amongst adolescents, university students, and children with a familial history of substance use disorders (Colaneri, Keim, & Adesman, 2017; Kollins, 2008). As such, in order to minimise the risk of psychostimulant misuse and discontinuation, a more nuanced approach to the pharmacotherapy of ADHD is required. A potentially fruitful strategy is to identify substances that have common mechanisms of action to psychostimulants, but which lack their addictive qualities. The first step in this process is to identify neural targets of psychostimulants which have relevance to the core symptomology of ADHD (Overton, 2008). To realise this process, we must first consider the symptoms and aetiology of ADHD, and how to approach these from a psychotherapeutic position.

# 1.3 A Brief History of ADHD

Due to the heterogeneous nature of ADHD, no single unifying theory has been able to satisfactorily explain the aetiology of the different symptomatic domains of ADHD (Barkley, 1997; Sagvolden, Johansen, Aase, & Russell, 2005; Willcutt, Doyle, Nigg, Faraone, & Pennington, 2005). As a consequence, there is much discord within the literature as to which research avenues will yield the greatest pharmacotherapeutic benefits. It has thus been proposed that several pathological loci may exist within the ADHD population, and a fruitful approach to developing interventions may be to focus on neural correlates of specific core symptoms (Overton, 2008). While this may be a valid approach, one must first identify a candidate symptom for closer examination. As such, this section will comprise of a brief overview of how ADHD was formalised as a neurodevelopmental disorder, followed by a discussion of current diagnostic criteria for ADHD.

Though ADHD was formalised as a neurodevelopmental disorder in recent decades (DSM III; American Psychiatric Association, 1980), accounts of children presenting with developmentally inappropriate levels of inattention, and impulsivity date back to the late eighteenth century (Crichton, 1798; cited (Lange, Reichl, Lange, Tucha, & Tucha, 2010)). While Crichton’s account provides historical context for ADHD, thus dispelling the myth that ADHD is a creation of recent physicians, many authors agree that the first true clinical description of ADHD was presented by George Still in 1902 (Barkley, 2006; Martinez-Badía & Martinez-Raga, 2015). Research and clinical reports throughout the 1920s to 1940s expanded upon this, and began to link the core triad of ADHD symptoms to a neurological basis. It was noted that brain trauma during childhood could result in symptoms of over-activity, reduced attention span, and impulsivity (D. Ross & Ross, 1976). This was developed through the following decades after observations that inattention and hyperactive symptoms consistent with early brain trauma often presented in the absence of any history of brain damage (Denhoff, Laufer, & Solomons, 1957). The first DSM recognition of ADHD built upon this basis. An increased demand for greater diagnostic specificity resulted in the formalisation of discrete criteria for the attentional and hyperactive symptoms that had previously been associated with early brain trauma. Upon publication of the DSM-II in 1968, a description of hyperactive reactions of childhood was included.

Throughout the 1970’s, there was a movement towards inattention as the defining feature of ADHD following arguments that deficits of attention were the true salient features of the ADHD, as well as being the symptoms that best responded to psychostimulant medication (Douglas, 1972). This paradigm shift was reflected in the DSM-III which redefined hyperactive reaction of childhood as Attention Deficit Disorder (ADD, with or without hyperactivity), emphasising the centrality of attentional symptoms. The DSM-III resembles current diagnostic criteria insomuch as different symptom lists were introduced to reflect the growing understanding of the heterogeneity of the disorder. The DSM-IV expanded upon the DSM-III, again redefining ADD as Attention Deficit Hyperactivity Disorder. The DSM-IV further emphasised the heterogeneity of ADHD with the introduction of subtypes of the disorder. The DSM-IV-TR closely resembles the DSM-V, both of which will be discussed in more depth below.

# 1.4 ADHD in Current Diagnostic Criteria

ADHD is currently described by diagnostic manuals as a disorder of persistent, and developmentally inappropriate patterns of inattention and/or hyperactivity-impulsivity. To be classified as ADHD symptoms must not be context specific (i.e. they must present in at least two discrete environments, such as school and home) and must have a negative impact on social, academic and/or professional attainment. The two most widely used diagnostic manuals for mental health; the DSM-V and ICD-10 largely agree on a core set of symptoms though for ADHD, but differ in the number of symptoms required for full diagnosis.

## 1.4.1 ADHD in the DSM-5

The DSM-5 (American Psychiatric Association, 2013) expanded upon the DSM-IV-TR definition of ADHD to reflect the growing body of research recognising ADHD as a livelong disorder, rather than a purely developmental disorder (e.g. (Asherson, 2005; Barkley, Fischer, Smallish, & Fletcher, 2002; M. D. Weiss & Weiss, 2004). Symptoms of ADHD typically evolve throughout the lifetime; hyperactive symptoms recede in adolescence, while inattentive and impulsive symptoms are retained to varying degrees (Biederman, Mick, & Faraone, 2000; Greydanus et al., 2007). As such, fewer symptoms are required for adults to meet the criteria for a diagnosis of ADHD.

To qualify for a diagnosis of childhood ADHD under the DSM-5 criteria, patients must present with at least six inattentive symptoms and/or 6 hyperactive-impulsive symptoms for a minimum period of six months prior to evaluation. For individuals over the age of 17, a minimum of five inattentive symptoms and/or six hyperactive-impulsive symptoms are required. See table 1 for a full list and classification of symptoms.

Table 1: DSM-5 diagnostic criteria for ADHD (American Psychiatric Association, 2013).   
Symptoms are split into inattentive or hyperactive-impulsive domains.

|  |  |
| --- | --- |
| Inattentive symptoms | Hyperactive-impulsive symptoms |
| 1. Often fails to give close attention to details or makes careless mistakes in schoolwork, at work, or with other activities. 2. Often has trouble holding attention on tasks or play activities. 3. Often does not seem to listen when spoken to directly. 4. Often does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (e.g., loses focus, side-tracked). 5. Often has trouble organizing tasks and activities. 6. Often avoids, dislikes, or is reluctant to do tasks that require mental effort over a long period of time (such as schoolwork or homework). 7. Often loses things necessary for tasks and activities (e.g. school materials, pencils, books, tools, wallets, keys, paperwork, eyeglasses, mobile telephones). 8. Is often easily distracted 9. Is often forgetful in daily activities. | 1. Often fidgets with or taps hands or feet, or squirms in seat. 2. Often leaves seat in situations when remaining seated is expected. 3. Often runs about or climbs in situations where it is not appropriate (adolescents or adults may be limited to feeling restless). 4. Often unable to play or take part in leisure activities quietly. 5. Is often "on the go" acting as if "driven by a motor." 6. Often talks excessively. 7. Often blurts out an answer before a question has been completed. 8. Often has trouble waiting his/her turn. 9. Often interrupts or intrudes on others |

For childhood ADHD, symptoms must present before 12 years of age and must cause impairments in at least two discrete environments, so as to demonstrate a lack of context specificity. Symptoms must be sufficiently debilitating so as to cause clinically significant impairment in social, academic, and/or professional attainment. Additionally, symptoms must not exclusively present while a patient is suffering from schizophrenia or any other psychotic disorder, and must not be more appropriately described by any other mental disorder (including affective disorders, conduct disorders or substance abuse disorders).

Given the array of symptoms and the homogeneous presentation of the disorder, the DSM-V describes three subtypes of ADHD based on dominant categorisation of symptoms.

***Predominantly inattentive:*** At least six inattentive symptoms, but fewer than six hyperactive-impulsive symptoms, for a period of at least 6 months.

***Predominantly hyperactive-impulsive:*** At least six hyperactive-impulsive symptoms, but fewer than six inattentive symptoms, for a period of at least 6 months.

***Predominantly Combined type:***At least six hyperactive-impulsive symptoms and six inattentive symptoms for a period of at least 6 months.

While use of the DSM-5 is widespread within the United States, the International Classification of Mental and Behavioural Disorders 10th revision (ICD-10) is the current diagnostic manual most commonly used by clinicians in countries endorsed by the World Health Organisation (the WHO), including the United Kingdom and Europe.

## 1.4.2 ADHD in the ICD-10 and ICD-11

While the ICD-10 does not include criteria for ADHD, it does include equivalent criteria for hyperkinetic disorder (HKD; (Taylor et al., 2004), consisting of the same symptomatic domains covered in the DSM-5 criteria for ADHD. HKD is defined as “demonstrable abnormality of attention, activity and impulsivity at home, for the age and developmental level of the child (World Health Organization, 1992).” Similar to the DSM-5, impairment must present in two or more contexts, and must present exclusive of other mental disorders. Unlike the DSM, however, a diagnosis of HKD using the ICD-10 requires an age of onset of 6 years or younger, and requires impairment in the symptomatic domains of both attention, and over-activity. As such, a diagnosis of HKD is comparable to a diagnosis of predominantly combined type ADHD using the DSM.

It should be noted that the WHO have recently presented revised diagnostic criteria to be included in the ICD-11. Within these criteria, HKD has been replaced by ADHD, and it has been recognised ADHD may present as the three subtypes used in the DSM-5 criteria. Thus, the ICD-11 will include criteria for the following manifestations of ADHD:

* ADHD-PI: Attention Deficit Hyperactivity Disorder, predominantly inattentive presentation
* ADHD-PHI: Attention Deficit Hyperactivity Disorder, predominantly hyperactive-impulsive presentation
* ADHD-C: Attention Deficit Hyperactivity Disorder, combined presentation

While the ICD-11 will not come into effect until Jan 2022, this revision of diagnostic criteria represents an important paradigm change for the WHO. In previous incarnations of the ICD (ICD-10; (World Health Organization, 1992), the WHO had been reluctant to recognise subtype presentations of ADHD. As such, criteria for HKD excluded individuals who display clinically significant levels of inattention or hyperactivity, but not both. This stricter diagnostic criteria may have contributed to under-diagnosis of ADHD where the ICD is used (Faraone, Sergeant, Gillberg, & Biederman, 2003; Remschmidt, 2005). When children meeting the diagnostic criteria for DSM-IV ADHD were assessed for criteria of ICD-10 HKD, a significant difference in prevalence rates were noted between these criteria (5.0% for DSM-IV; 1.0% for ICD-10; (Döpfner et al., 2008). As such, the introduction of the ICD-11 in 2022 may be associated with increased diagnosis rates of ADHD where the ICD is used as a diagnostic manual. In light of this, there is an increasing need to understand the biological underpinning of ADHD, and to develop a broader spectrum of efficacious pharmacotherapeutic options.

# 1.5 ADHD as a Persistent Lifelong Disorder

It is now accepted that ADHD is not limited to a disorder of childhood, but has a far reaching and persistent impact on the social, academic, and occupational performance of patients (Greydanus et al., 2007). Expression of ADHD symptoms is fluid across the lifespan, with remission of certain childhood symptoms noted during the transition of adulthood (Biederman et al., 2000). Current estimates show an ADHD incidence rate of 5-10% in children and 3-5% in adults worldwide when diagnostic criteria are controlled for (Faraone et al., 2003). As noted above, this number is likely to rise in upcoming years following the additions to diagnostic criteria in DSM-V and upcoming ICD-11. Behavioural expression and comorbidities of ADHD differ based on subtype; patients suffering from inattentional symptoms have lower emotional or behavioural impairment but suffer greater academic impairment when compared with predominantly hyperactive-impulsive patients. Combined type ADHD patients present with higher psychiatric comorbidity and are more likely to develop substance abuse disorders (Wilens, Biederman, Brown, et al., 2002).

ADHD symptoms can be observed across the lifetime, with a change in behavioural expression noted. Preschool children diagnosed with ADHD are described as aggressive, non-compliant, and possessive of poor social skills to the extent that they are frequently rejected by social peer groups (Vierhile, Robb, & Ryan-Krause, 2009). These traits typically persist as the child enters school age, where inappropriate motor activity and poor attentional capabilities also become more apparent (Greydanus et al., 2007; Neef et al., 2005; Woodard, 2006). The middle-school aged ADHD patient exhibits an increase in psychosocial impairment (Barkley, 2006; Trott, 2006) both with respect to familial and peer group relationships. Poor academic attainment is often noted during this period (Cormier, 2008). During the transition to adulthood, a shift in dominant symptom cluster is frequently observed, with an increased prominence of inattentive symptoms and a remission of hyperactive-impulsive symptoms (Biederman et al., 2000; Hurtig et al., 2007). The remission of ADHD symptoms in adulthood will now be discussed in greater details, with focus on inattentive symptoms.

In response to conflicting reports surrounding the persistence of ADHD in later life (Barkley, Fischer, Edelbrock, & Smallish, 1990; G. Weiss, Hechtman, Milroy, & Perlman, 1985), Biederman et al. (2000) explored the age dependent decline of ADHD symptoms. ADHD patients were assessed and symptoms were stratified into hyperactive, impulsive and inattentive clusters. Patients were followed up after 4 years and reassessed for symptom remission as a function of age (see figure 1.1). Remission of symptoms was observed with an average remission for all symptoms of 37%. Significant differences were seen between remission of hyperactive (50%) and impulsive (45%), and inattentive symptoms (20%).

**Figure 1.1:** Remission of ADHD symptoms clusters in patients’ aged 6-19 after a 4 year follow up. Wilens et al., 2002, adapted from Biederman et al., 2000.

In addition to showing higher resistance to remission than hyperactive-impulsive symptoms, inattentive symptoms make up the most frequently presenting symptoms in adult ADHD. Wilens et al. (2009) assessed the prominence of inattentive symptoms in adult ADHD; 93% of patients assessed had clinically relevant levels of inattentive symptoms resulting in diagnosis of either combined type or primarily inattentive ADHD. Similarly high rates of inattention are found in childhood community based samples, with 5.4% of the population presenting with primarily inattentive ADHD, 3.6% with combined type ADHD, and 2.4% with primarily hyperactive-impulsive ADHD (Wolraich, Hannah, Pinnock, Baumgaertel, & Brown, 1996). As well as considering frequency of subtypes, Wilens et al. measured the persistence of individual symptoms of ADHD (see figure 1.2) demonstrating an enhanced presentation rate of inattentive symptoms, particularly “difficultly sustaining attention” and “easily distracted”.

An external file that holds a picture, illustration, etc.
Object name is nihms-236732-f0001.jpg**Figure 1.2:** Frequency of individual ADHD symptoms in adult populations (Wilens et al., 2009)

Other observers have noted the prominence of inattentional symptoms in adolescent and adult ADHD. Similar to Wilens et al., Hurtig et al. (2007) observed a persistence of inattentional symptoms when childhood and adult symptoms of individual patients were compared; these findings are consistent with other authors who have outlined the persistence of inattentional symptoms (Gaub & Carlson, 1997; Smalley et al., 2000). Additionally, (Nahlik, 2004) highlights that the high prevalence of covert inattentional symptoms in adolescent ADHD could act as a diagnostic confound, resulting in both lower referral of ADHD and underestimation of the prevalence of inattentional symptoms. The dominance of inattentive symptoms in later life makes them an ideal target when considering research of a single symptom cluster.

# 1.6 Interventions for ADHD

There is agreement that the symptoms of ADHD are best controlled through pharmacological interventions (Brown et al., 2005). The National Institute for Health and Care Excellence guidelines for ADHD (NICE, 2018) currently recommends pharmacological treatment for adults and children over the age of 5 where symptoms causes significant impairment in at least one domain. Frontline pharmacological interventions for ADHD are comprised of psychostimulant drugs; NICE guidelines recommend methylphenidate as the first line treatment for ADHD, and suggests lisdexamfetamine as an option for patients who are non-responsive to methylphenidate. If lisdexamfetamine is poorly tolerated by the patient, dexamfetamine may instead be offered as a treatment option. While non-pharmacological behavioural interventions have been proposed, evidence strongly suggests that these are significantly less efficacious than treatment with psychostimulants (Brown, Wynne, & Medenis, 1985; R. G. Klein et al., 1997). In a review of the efficacy of interventions for ADHD, A. Miller et al. (1999) concluded that behavioural interventions cause little to no improvement in the core symptoms of ADHD and also appear to cause no improvement in academic attainment. Further, while there may be mild behavioural benefits to combining stimulant medication with behavioural therapies over stimulant medication without behavioural therapies, these benefits do not appear to extend towards meaningful improvement in the presentation of the core symptoms of ADHD. As such, when considering the development of new interventions for ADHD, it is clear that such development should focus on the identification of new efficacious pharmaceutical interventions. Detailed below are the current frontline and alternative therapeutic treatments for ADHD.

## 1.6.1 Psychostimulants as Frontline Pharmacological Interventions for ADHD

Current frontline pharmaceutical interventions for ADHD consist of treatment with stimulant compounds, with specific focus on methylphenidate and amphetamine (Banaschewski, Roessner, Dittmann, Santosh, & Rothenberger, 2004; Wilens, Biederman, & Spencer, 1998). An extensive body of evidence (see Brown et al., 2005 for review) has confirmed the efficacy of psychostimulants in the pharmacotherapy of ADHD. Use of psychostimulant medication in ADHD is associated with significant improvements in the core symptomatic domains of attention, distractibility and impulsivity (Castells et al., 2011; Jadad, Boyle, Cunningham, Kim, & Schachar, 1999). Moderate improvements in social behaviour are also reported, though only mild improvements in academic performance have been observed (Van der Oord, Prins, Oosterlaan, & Emmelkamp, 2008). While psychostimulants are considered to have a safe profile, in some cases, use of may be associated with adverse effects, including sleep disturbances, headaches, fatigue, and motor tics. These side effects are typically mild, and can be controlled for by adjusting dosage or timing of medication administration (Brown et al., 2005). While effective in alleviating the symptoms of ADHD when well tolerated, approximately 10-30% of individuals with ADHD will either not respond to medication, or may otherwise discontinue the course of treatment as a result of aversive side effects (Banaschewski et al., 2004).

Despite the efficacy of psychostimulants, there is still a clear need to develop new pharmaceutical options for ADHD. Although psychostimulant use in ADHD is not associated with increased risk of substance use disorder in later life, and may in fact be associated with reduced risk of substance abuse (Wilens, Faraone, Biederman, & Gunawardene, 2003), prescription of stimulants is associated with diversion and misuse of the substances by other individuals. Compared to subjects taking medication for other disorders, ADHD patients are more likely to report diversion of their medication. 11% of ADHD patients reported having previously sold their stimulants, compared to 0% of a control group comprised of patients prescribed stimulants for other disorders (Wilens, Gignac, Swezey, Monuteaux, & Biederman, 2006). In a 2008 systematic review, Wilens et al., reported that 5% to 9% of school children and 5% to 35% of University students had reported consumption of non-prescribed stimulants. Similarly, 16% to 29% of students who were prescribed stimulant medication had been asked to divert or sell their medication. This phenomenon appears to be persistent. While Wilens et al. remains the most recent review of stimulant use across a range of populations, stimulant misuse amongst school and university students remains prevalent. In recent studies, 6.6% of children ages 10-18, and 10% of University students ages 20-25 reported having misused stimulants prescribed to someone else (Looby, Beyer, & Zimmerman, 2015; Striley, Kelso-Chichetto, & Cottler, 2017).

In summary, psychostimulants are efficacious in treating the core symptoms and social deficits associated with ADHD, whilst having a relatively safe profile. Despite this efficacy, up to 30% of patients will discontinue use of psychostimulants. Further, while use of psychostimulants appears to be protective of later substance use, problems of diversion and misuse of non-prescription stimulants remain prevalent within school and college populations. As such, there is a clear need to develop new pharmacological interventions for ADHD which lack the abuse potential of psychostimulants. For the remainder of the current thesis, d-amphetamine will be the focus of discussion when considering alternatives to psychostimulant medication. While no significant difference have been described in the clinical efficacy between d-amphetamine and methylphenidate in the pharmacotherapy of ADHD (Brown et al., 2005), pharmacological research using anaethetised animal models has revealed that methylphenidate causes significant cortal and sub-cortical EEG desynchronisation, while d-amphetamine does not (Hetherington et al., 2017). As a result, administration of methylphenidate appears to act in a way that alters the anaethetic state of the subject, thus confounding any responses to sensory stimuli. As experiments described in the current thesis will utlisise anaethetised animal models, d-amphetamine is considered to be a more appropriate drug of choice to investigate psychostimulant action.

## 1.6.2 Current Alternatives to Psychostimulant Medication for ADHD

Several substances have been proposed as alternatives to psychostimulants in ADHD, but these either lack the efficacy of psychostimulants, or otherwise possess significant aversive side effects. As such, these substances cannot be considered strong alternative for psychostimulants. Much of the research into alternatives to psychostimulant treatments has focused on atomoxetine and tricyclic antidepressants (TCAs), as detailed below. Where other therapeutics options have been suggested, these are not monotherapeutic options, and instead act to supplement psychostimulant action (e.g. alpha 2 agonists; (Cannon et al., 2009). As the aim of the current thesis is to explore alternatives, rather than supplements, to psychostimulants, these substances will not be discussed.

***Atomoxetine***

The noradrenaline reuptake inhibitor atomoxetine has emerged as a potential drug intervention in ADHD, for patients where psychostimulants are poorly tolerated, or otherwise aversive (Faraone, Biederman, et al., 2005). Atomoxetine is more effective than placebo in treating the core symptoms of ADHD, and is non-inferior to immediate-release psychostimulants. It is, however, significantly less effective than extended-release psychostimulants (Garnock-Jones & Keating, 2009; Kratochvil et al., 2005; Kratochvil, Vaughan, Harrington, & Burke, 2003). While atomoxetine is generally well tolerated, some patients may present with moderate symptoms of nausea, abdominal pain, somnolence and headache. (Garnock-Jones & Keating, 2009; Spencer et al., 1998). Additionally, use of atomoxetine amongst children ages 7-12 years is associated with an increased incidence of suicidal ideation, which has created concerns about use of the substance to treat children within this age range (Wooltorton, 2005). As such, while atomoxetine may have some efficacy when treating the symptoms of ADHD, it cannot be recommended for use in childhood populations, which form a large proportion of ADHD sufferers.

***Tricyclic antidepressants.***

TCAs appear to have limited practical relevance to the pharmacotherapy of ADHD. While TCAs are superior to placebo in clinical trials for ADHD, the improvements in symptom presentations are inferior to those induced by psychostimulants (Pliszka, 2003). TCAs are associated with a range of aversive effects, including dizziness, blurred vision, constipation, and cognitive impairment (Banaschewski et al., 2004). Furthermore, TCA use is associated with a range of cardiovascular risks (Popper, 2000). While the clinical risk associated with TCAs is uncertain (Wilens et al., 1996), TCA use in both children and adults is associated with cardiovascular changes which may be associated with tachycardia (Biederman, Baldessarini, Wright, Knee, & Harmatz, 1989), and increases in systolic and diastolic blood pressure (Kemp et al., 2010). As such, the use of TCAs are not recommended in ADHD.

# 1.7 The Neuroscience of ADHD: an Inconclusive search for Pathological Loci

As shown above, there is a clear need to develop new, non-addictive pharmacotherapies for ADHD. An important step in this process is to understand the pathophysiological changes that underpin ADHD, with a view of identifying potential targets of psychostimulant action. The neurological changes underpinning ADHD are unfortunately, far from well understood (Biederman, 2005b). While recent advances in imaging techniques have allowed for more sophisticated approaches to understanding these changes, results are presently inconclusive (Onnink et al., 2015). As discussed in section 1.4, ADHD is an extensively heterogeneous disorder typified by subtype presentation. As such, it is likely that multiple pathological loci are involved in symptom presentation (Overton 2008), and thus a fruitful strategy may be to identify correlates of specific core symptoms, rather than aiming to identify global neural changes that predict ADHD. The following section will discuss a range of nuclei that have been implicated in ADHD, and will outline the need for a more nuanced approach when considering the pathophysiology and psychopharmacology of ADHD

## 1.7.1 Structural Morphology and Functional Differences in ADHD

The neuroanatomical literature of ADHD reflects the heterogeneous nature of the disorder. A wide array of potential neural correlates have been identified, including the prefrontal cortex, basal ganglia, corpus callosum and cerebellum. As well specific structural differences, a gross 4.0% reduction in intracranial volume for ADHD patients compared to unaffected siblings and matched controls has been described (Durston et al., 2004). Additionally, structural MRI has found ADHD patients to have an average 4.7-8.3% smaller cerebral volume than age and gender matched controls (Castellanos et al., 1996; Mostofsky, Cooper, Kates, Denckla, & Kaufmann, 2002).

***Cortical regions***

Morphological differences in the frontal cortex, a region with intimate links to sustained attention (Biederman, 2005; Wilens, 2009), have been noted in some MRI studies assessing ADHD patients. As with much ADHD neuroscience literature, there is disagreement as to the extent of morphological differences, the location of impairment, as well as the affected cell types. Mostofsky et al. (2002) showed a significant reduction of frontal lobe volume between ADHD patients and matched controls. Lateralisation was found based on cell type; reduction of white matter was specific to the left frontal cortex whereas reduction of grey matter volume was bilateral, though a greater reduction was observed for the right hemisphere. Additional authors have reported that ADHD patients have smaller right anterior frontal volume than matched controls (Castellanos et al., 1996). These reports are contradicted by Castellanos et al. (2002), who did not find evidence of reduced frontal lobe volume in ADHD. Castellanos et al. scanned 152 ADHD patients and 139 matched controls, with an attempted three follow up scans over 10 years (100% of participants scanned once, 40% scanned twice, 13% three times, 7% four times) to allow for the production of predicted longitudinal growth parameters for ADHD and control patients. Castellanos et al. observed a stable 4% reduction in brain volume over time between ADHD patients and non-ADHD control. Significant overall reduction was not found for any single lobe in contrast to previous reports of frontal lobe reduction. Where frontal abnormalities are seen in ADHD, they appear to be limited to the prefrontal cortex (PFC; (Seidman, Valera, & Makris, 2005). Durston et al. (2004) reported up to a 9.1% volumetric decrease of grey matter of the right PFC in ADHD patients and their neurotypical siblings. That this effect is seen in both ADHD patients and unaffected siblings suggests both that there is a strong familial component of ADHD, and that reductions in PFC volume do not wholly contribute to the functional deficits observed in ADHD.

Similar to structural data, functional studies assessing frontal lobe changes in ADHD present an inconsistent picture. In a review of functional data in ADHD, Cubillo, Halari, Smith, Taylor, and Rubia (2012) argue that a consistent pattern of dysfunction in frontal-striatal networks are associated with ADHD. Further, Cubillo et al., presented new data showing that medication naïve adults, who had received a previous childhood diagnosis of ADHD, showed deficits in frontal-striatal networks during a sustained attention task when compared to controls. These data are contradicted by Zang et al. (2007) who found reduced resting state fMRI activity in right inferior frontal cortex of boys with ADHD, but did not find any changes at a striatal level, nor did they find any frontal-striatal dysregulation.

While structural differences in PFC volume between ADHD patients and matched controls appears to be a consistent finding, functional imaging studies investigating this area in ADHD have been less consistent. Rubia et al. (1999) reported reduced ventrolateral PFC (VLPFC) activity during a stop-signal task for ADHD subjects compared to controls. Similarly, Durston et al. (2003) found that while neurotypical participants showed activation of the VLPFC during a Go-NoGo task, ADHD subjects did not activate this area, though comparisons between groups revealed a non-significant difference. In contrast, Bush et al. (1999) showed bilateral VLPFC activation by ADHD participants during a Stroop task, while neurotypical controls did not show bilateral activation. Langleben et al. (2001) demonstrated reduced cerebral blood flow to the DLPFC in ADHD, where greater hypoperfusion was observed in severe ADHD compared to moderate and mild ADHD. As such, dysfunction at the level of the PFC may be symptom specific, with greater symptom presentation associated with greater deactivation of the PFC. This proposition may explain the inconsistent structural and functional findings from investigations into this area.

***Striatum and Basal Ganglia***

A number of observers have noted a reversal of typical caudate nucleus (CN) asymmetry in the brains of ADHD patients. Hynd et al. (1993) postulated that caudate nucleus asymmetry may be predictive of ADHD. MRI results showed that 72.7% of non-ADHD controls featured asymmetry of the CN where the left hemisphere had a great volume than the right. This pattern was reversed in 63.6% of ADHD patients, who showed greater volume of the right CN. Filipek et al. (1997) also found reversed CN asymmetry, with particularly pronounced reduced volume of the head of the left CN. As with the data for frontal morphology in ADHD, data for changes in CN asymmetry are inconsistent. Castellanos et al. (1994) showed slight, but significant, reduction of right CN compared with non-ADHD controls, but no effect on left CN. Further, Castellanos et al. (2002) showed an age dependent effect of CN volume reduction. They demonstrated that while CN volume is initially in reduced children with ADHD compared to neurotypical age matched controls (age 5-15), neurotypical participants also showed a reduction of caudate volume emerging during adolescence, resulting in no significant differences between adult ADHD patients and controls (aged 20+). Thus, while CN morphology may have some contribution to childhood ADHD presentation, it appears to have limited relevance to the adult ADHD phenotype.

In addition to reduced left CN volume, Aylward et al. (1996) also showed significantly reduced left globus pallidus volume when ADHD patients were compared with healthy controls. Taken with the CN data presented above, this suggests that ADHD symptoms may be associated with changes at the level of the striatum, a proposition that has been explored in numerous functional studies. Striatal dysfunction in ADHD appears to be particularly pronounced when performing tasks that assess impulse control. Hypoactivation of the striatum has been reported during inhibition tasks, including the Go-NoGo task, the stop-signal task, and the Stroop task (Emond, Joyal, & Poissant, 2009). Further, ADHD participants and neurotypical controls have been compared in an fMRI paradigm when performing the Monetary Incentive Delay task, which has been shown to reliably increase activity in the ventral striatum of healthy controls. Compared to controls, ADHD patients showed reduced ventral striatum activity, as well as reduced task performance. Ventral striatum activity was negatively associated with parent rated ADHD symptoms, suggesting a potential role for striatal dysfunction in the symptom presentation of ADHD. As noted above, dysfunction in frontal-striatal networks have been implicated in ADHD (Cubillo et al., 2012), presenting a potential pathway whereby altered striatal activity could produce symptoms of impulsivity. A recent diffusion tensor imaging study lends support to this theory, showing that white matter pathways connecting PFC with the striatum may be underdeveloped in ADHD (Silk, Vance, Rinehart, Bradshaw, & Cunnington, 2009). While evidence for altered striatal activity during task performance in ADHD is convincing, data assessing resting state fMRI is currently inconsistent. Two resting state fMRI studies have shown decreased whole brain resting state fMRI activity between ADHD patients and controls, with a negative correlation reported between symptom severity of resting state activity (Sokunbi et al., 2013; Zang et al., 2007). These studies also showed regional changes, where frontal activity, but not striatal activity was, depressed in ADHD. As resting state networks are hypothesised to be involved in the executive control of attention (Lee, Smyser, & Shimony, 2013), a lack of finding of resting state depression at the level of the striatum may suggest that deficits in this region have more relevance to impulsive symptoms than inattentive symptoms. As such, though there appears to be consistent evidence linking the striatum to decreased task performance in ADHD, this may have relevance to some symptoms clusters more than others.

In summary, though several regions of interest have been investigated in ADHD, no single pathological locus has been identified that can reliably predict presentation of all three core symptomatic domains of ADHD. It is likely that the heterogeneous nature of ADHD will make it difficult to identify loci that have relevance to the presentation of each subtype of ADHD. As such, a fruitful strategy may be to focus on neural correlates of specific core symptoms, as discussed below.

# 1.8 Symptom Based Approach to Drug Development for ADHD: A Focus on Distractibility

Despite widespread use in the pharmacotherapy of ADHD, we currently lack a robust understanding of the complete profile of action for psychostimulants (Spencer, Biederman, Wilens, & Faraone, 2002b). This, combined with an incomplete understanding of the pathophysiological changes that underpin ADHD, has acted as a clear obstruction to the development of new non-addictive pharmacotherapies for ADHD. Given the heterogeneity of ADHD, Overton (2008) proposed that multiple pathological loci may lie at the heart of the disorder. Consequently, a more effective approach to the development of new pharmaceutical interventions for ADHD may be to identify neural targets of psychostimulants which have relevance to the symptomology of ADHD. When taking this approach, distractibility emerges as a logical symptom on which to focus. Distractibility is both the single most frequently presenting symptom of ADHD, and the most persistently presenting symptom, as it shows the most resistant to extinction with age and the transition into adulthood (Biederman et al., 2000; Wilens, Biederman, & Spencer, 2002). Even though up to 93% of ADHD patients already display increased levels of distractibility (Wilens et al., 2009), Nahlik (2004) argues that distractibility is likely to be even more prevalent in ADHD cohorts than reported due to the prevalence of covert inattentional symptoms, which may have particular prominence in adolescent ADHD populations.

## 1.8.1 Distractibility as a Core Symptom of ADHD

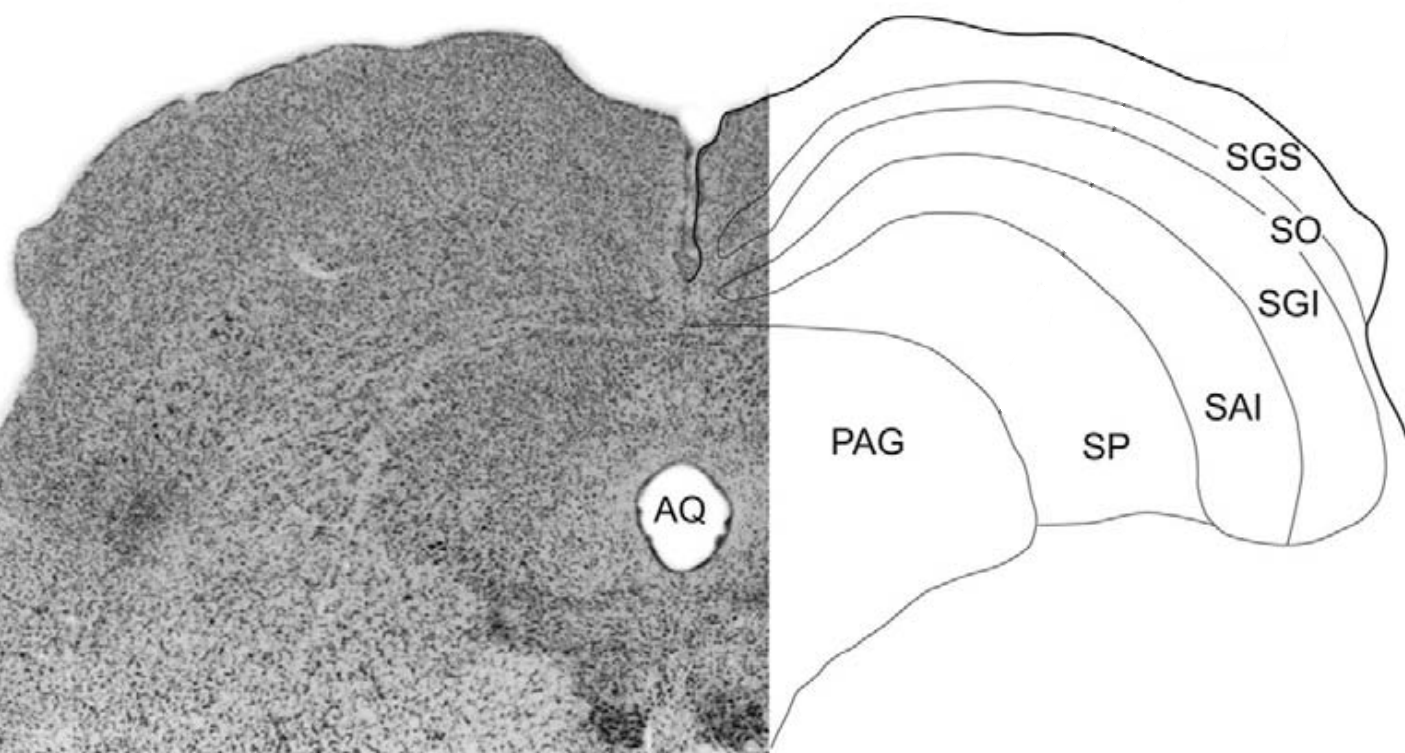
As a core component of the two most common ADHD subtypes, as well as the most commonly presenting single symptom in adult ADHD (Wilens et al., 2009), distractibility is an ideal candidate symptom to investigate when considering potential pathological loci to target for pharmacological intervention. Increased distractibility has long been reported by clinicians as a central deficit frequently presented by individuals with ADHD (Barkley, 2006; Greydanus et al., 2007; Thorley, 1984). Heightened distractibility in ADHD patients has also been observed experimentally, particularly when ADHD patients’ behaviour lies outside their locus of control. Sonuga-Barke, Williams, Hall, and Saxton (1996) observed heightened distractibility in ADHD patients when performing tasks designed to mimic those encountered in typical classrooms. When strict time limits were enforced on tasks, ADHD patients showed shorter response latencies and made more errors than controls. When these time limits were removed, ADHD patients showed reduced distractibility and had response latencies and error rates that were comparable to controls. More recently, Pelletier, Hodgetts, Lafleur, Vincent, and Tremblay (2013) employed an irrelevant sound effect paradigm to assess serial recall performance in adult ADHD patients and controls. They observed impaired recall and increased distractibility in the ADHD group when the recall task was accompanied with unexpected novel sounds. Similar sensitivity to distracting stimuli has been reported in a sub-clinical ADHD population when presented with naturalistic peripheral visual distractors (M. Panagiotidi, P. G. Overton, & T. Stafford, 2017). Behavioural evidence of increased distractibility in ADHD patients is supported by electrophysiological accounts in humans. Gumenyuk, Korzyukov, Alho, Escera, and Naatanen (2004) used a visual discrimination task to compare event-related potentials (ERPs) of children with ADHD to healthy controls following novel auditory distractors. While both groups showed decreased task performance in the presence of distractors, decreased task performance in the ADHD group was more pronounced relative to controls, and was accompanied by increased ERPs evoked by the distractors. Biphasic ERPs were reported for both ADHD and control groups, though ADHD ERPs showed a smaller first phase and larger second phase when compared with controls. These results suggest that distracting stimuli evoke more cortical sensory potentials in ADHD populations that controls. van Mourik, Oosterlaan, Heslenfeld, Konig, and Sergeant (2007) report similar findings, showing that children with ADHD display larger ERPs and increased orienting responses to novel auditory distractors. Furthermore, children with ADHD consistently perform worse than matched controls on psychometric measures of distraction (Seidman, Biederman, Faraone, Weber, & Ouellette, 1997), and adults with ADHD consistently self-report inappropriately high level of behavioural distractibility (De Quiros & Kinsbourne, 2001).

# 1.9 The Superior Colliculus and its Relevance to Distractibility in ADHD

The midbrain superior colliculus (SC), a subcortical sensory structure that plays a central role in the orientation of attention to sensory events, is uniquely placed as a locus of interest when investigating increased distractibility in ADHD. The SC constitutes a critical hub in the orienting network and is strongly involved in the generation and guidance of saccadic eye movements toward biologically salient stimuli, and non-salient distractors (Sparks, 1999; Stein & Meredith, 1993). An emergent body of behavioural and pharmacological evidence suggests that symptoms of distractibility in ADHD may be a result of sensory hyper-responsiveness at the level of the SC, leading to orientation towards stimuli of low biological salience (Overton, 2008). In the following sections the structure and function of the SC will be discussed, followed by evidence linking the SC to distractibility in ADHD.

## 1.9.1 Structure of the Superior Colliculus

The SC is a laminated structure that constitutes a major nucleus of the midbrain of vertebrate animals. The structure and function of the SC are conserved across species, allowing for comparison of data between animal models (May, 2006). A prominent feature of the collicular structure is its laminar arrangement of cellular and fibrous layers. In mammalian species, these layers are subdivided based on function. Layers I-III comprise the superficial SC, consisting of the stratum zonal (SZ, Layer I), the stratum griseum superficial (SGS, layer II) and the stratum opticum (SO, layer III). Layers IV-VII constitute the deep SC, consisting of the stratum griseum intermedial (SGI, layer IV), stratum album intermedial (SAI, layer V) stratum griseum profundum (SGP, layer VI) and stratum album profundum (SAP, layer VII). While the superficial and deep layers of the SC were once thought of as functionally and anatomically distinct, it now understood that intralaminar connections exist between the superficial and deep SC (Hall & Lee, 1993; Saito & Isa, 2005). The focus of the current thesis will be on responsiveness of superficial layers neurons; where function and connectivity of deep SC is discussed, this will be within the context of intralaminar projections from the superficial layers.



**Figure 1.3:** Anatomy of the rat SC. The left side of the image shows a Nissl stained photomicrograph of a section of the SC. The right side of the image shows the divisions within the laminated structure of the SC Adapted from Comoli et al. (2012).

## 1.9.2 Function and Connectivity of the Superficial SC

It has long been known that the colliculus plays a central role in locating novel objects in visual space (Schneider, 1969) and guiding the saccadic eye movements that orient attention towards these objects (Dean, Redgrave, & Westby, 1989; Sparks, 1999; Whittaker & Cummings, 1990). Neurons in the superficial layers of the SC respond almost exclusively to visual stimuli presented in discrete regions of contralateral visual space (Gandhi & Katnani, 2011). The main sources of afferent visual input to the SC arise directly from contralateral retina via the retinotectal pathway, and indirectly from layer V visual cortex neurons via the retino-geniculo-cortical pathway (Boehnke & Munoz, 2008). Retinal and cortical input into the SC frequently synapse onto the same neurons, allowing for the convergence of raw retinal input with information that has already been processed at the level of the cortex and thalamus (Stein & Meredith, 1993). The receptive fields of these superficial layer SC neurons comprise a highly ordered retinotopic map of contra-lateral visual space. This organisation is preserved in the intermediate and deep layers of the SC in register with mapping for other sensory modalities (Chalupa & Rhoades, 1977; Drager & Hubel, 1976), allowing for the intralaminar projections from superficial SC to contribute to the generation of oculomotor responses to salient sensory events (Binns, 1999). Neurons in these deeper layers are believed to play a crucial role in guiding saccadic eye movements to vectors in visual space specified by the SGS (Boehnke & Munoz, 2008). In addition to intralaminar projections, the superficial SC projects to the extrastriate visual cortex, through relays via the lateral geniculate nucleus and pulvinar of the thalamus (see igure 1.4).

Figure 1.4: Connectivity of the orienting network including the retino-geniculo-cortical pathway and the fronto-striatal-tectal (adapted from Boehnke and Munoz, 2008).

LGN

Visual cortex

Parietal cortex

Frontal cortex

SNc

Pulvinar

Anterior thalamus

SCd

SCs

Retina

Striatum

SNr

Results from various experimental paradigms in a range of species imply a role of the SC in distractibility, in both ADHD and neurotypical development. During a guided running task, orienting reflexes to novel distracting stimuli presented in the peripheral visual field were eliminated following bilateral ablation of the SC in rats (Goodale, Foreman, & Milner, 1978). When the visual cortex was instead ablated, no change in orienting reflex was observed. Similar findings have been reported in primates. Macaques showed reduced orientation to irrelevant peripheral stimuli during a visual discrimination task following bilateral SC ablation (Milner, Foreman, & Goodale, 1978). When the frontal eye-fields were ablated instead of the SC, this orienting reflex was maintained, but with reduced duration. Validated rodent models of ADHD are also suggestive of dysfunction of collicular controlled orientation reflexes. The spontaneously hypertensive rat (SHR) displays an impaired ability to reflexively modulate air righting behaviour, which is associated with collicular dysfunction (Dommett & Rostron, 2011)

Thus the function of the SC appears vital for orientation in both rodents and primates. This is perhaps unsurprising given the function of the SC as a novelty detector (Pérez-González, Malmierca, & Covey, 2005), which may help explain findings that ADHD patients are more sensitive to novel distractors than controls (Gumenyuk et al., 2004; van Mourik et al., 2007); see section 8.1 above). In many distraction paradigms, the novelty of a stimulus is a quality associated with the likelihood that a stimulus distracts participants from a given task (Berti, Roeber, & Schroger, 2004; Schroger & Wolff, 1998). As the SC is one of the earliest visual structures recruited to respond to novel stimuli (May, 2006), hypersensitivity of this system may underpin these differences in task performance.

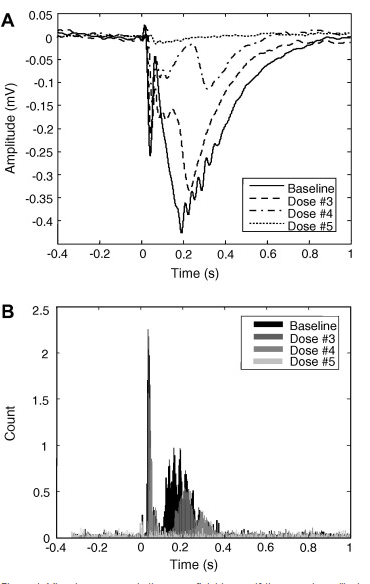
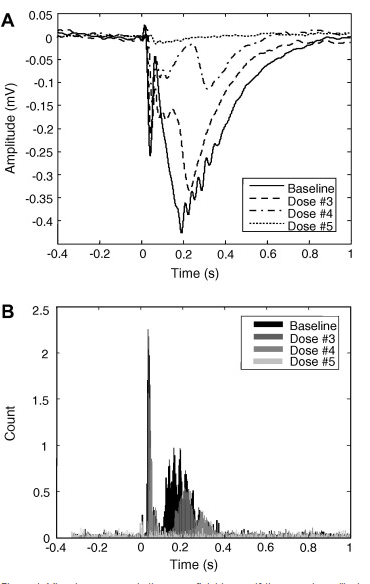
Much evidence for the link between the SC and distractibility stems from collicular involvement in initiating and guiding saccades (Sparks, 1999; Whittaker & Cummings, 1990). Evidence from human and non-human primates show that the SC is an important part of the circuitry involved in a variety of saccadic behaviours, including prosaccades (saccades toward visual targets; (S. Everling, Dorris, Klein, & Munoz, 1999), anti-saccades (saccades away from a visual target; (Dorris, Paré, & Munoz, 1997), and express saccades (rapid stimulus cued saccades; (Stefan Everling, Paré, Dorris, & Munoz, 1998). If altered collicular responsiveness is associated with distractibility in ADHD, we would expect ADHD patients to show impairment on oculomotor tasks that assess these saccadic responses. This position is validated by findings that adults with ADHD have difficulty inhibiting prosaccades to visual distractors during sustained attention tasks (Munoz, Armstrong, Hampton, & Moore, 2003). Similar saccadic impairments are observed when patients with ADHD perform delayed oculomotor response tasks (DOR), which assesses the ability of an individual to manually inhibit reflexive prosaccades following the sudden presentation of distracting visual stimuli (Adams, Roberts, Milich, & Fillmore, 2011). A DOR was also used by Roberts, Fillmore, and Milich (2011) when assessing inhibition of ADHD patients during prosaccadic oculomotor and non-oculomotor tasks, finding that while inhibitory control was impaired during both tasks for ADHD patients relative to a non-ADHD population, greater impairment was found when performing the prosaccadic oculomotor task. Additionally, relative to controls, ADHD patients perform fewer express saccades and have elevated response latencies and error rates when performing anti-saccades (CH Klein, Raschke, & Brandenbusch, 2003).

Orientation deficits in ADHD populations can be normalised through psychostimulant treatment. Folta and Mähler (2010) found that medicated children with ADHD perform similarly to neurotypical children when actively inhibiting prosaccades. However, when compared to unmedicated children with ADHD, neurotypical children performed better on prosaccade inhibition tasks. Congruent findings were reported by Fried et al. (2014) who showed that medicated ADHD-diagnosed adults are better at inhibiting microsaccades towards anticipated visual targets when compared to non-medicated ADHD-diagnosed adults. Further, when performing pro- and anti- saccades tasks, methylphenidate medicated ADHD-diagnosed participants showed improved reaction times and reduced errors, as well as a normalised proportion of express saccades relative to non-medicated controls (CH. Klein, Fischer, Fischer, & Hartnegg, 2002). Given the role of the SC in saccadic behaviour, the evidence of saccadic deficits in ADHD, and reports that psychostimulant medication normalises these deficits, the SC emerges as a potential therapeutic target for psychostimulant action.

## 1.9.3 Evidence of Psychostimulant Action at the Level of the Superior Colliculus

Recall that the mechanism of action of d-amphetamine in ADHD is not completely understood. While a complete pharmacological profile of the effects of d-amphetamine still remains elusive, there is converging evidence that the effects of d-amphetamine are mediated by elevating synaptic levels of the monoamine neurotransmitters dopamine (DA), noradrenaline (NA; (Easton, Steward, Marshall, Fone, & Marsden, 2007) and serotonin (5-HT; (Holmes & Rutledge, 1976; Kuczenski & Segal, 1989). The superficial layers of the SC are extensively innervated by both 5-HT and, to a lesser extent NA (Massey et al., 2013; Wichmann & Starke, 1988), thus the SC expresses the appropriate receptors to allow for action of d‑amphetamine. Several studies have assessed the effect of d-amphetamine administration on the responsiveness of the SC in the anaesthetised rat. Gowan, Coizet, Devonshire, and Overton (2008) recorded visually evoked potentials from the superficial layers of the rat SC following either systemic or intracollicular administrations of d-amphetamine. In the systemic administration condition, extracellular local field potential (LFP) and multi-unit activity (MUA) were recorded to a series of 240 whole field visual flashes following five cumulative systemic doses of d-amphetamine or volumetrically equivalent saline (up to a total dose of 8.0mg/kg). It was revealed that d-amphetamine acts to depress the superficial SC response to visual stimuli in a dose-dependent manner, with the deepest depression observed at the highest dose of d-amphetamine. Similarly when administered locally into the SC, a single bolus injection of d-amphetamine (120nmol) reduced the amplitude and duration of the LFP and MUA response to visual stimuli. As effects of d-amphetamine were revealed when the drug was operating both systemically and locally at the level of the SC, Gowan et al., proposed the SC as a potential locus of d-amphetamine action, where its effects are likely mediated by increasing synaptic availability of monoamine neurotransmitters. Convergent findings have been reported by Clements, Devonshire, Reynolds, and Overton (2014) who showed that systemic administration of d-amphetamine reduces the peak amplitude of the collicular LFP and MUA to whole field light flashes in a validated animal model of ADHD.

**Figure 1.5:** Superficial layer collicular responses to visual stimuli for LFP (A) and MUA (B) following systemic administration of d-amphetamine (adapted from Gowan et al., 2008). Data presented were averaged over responses to 240 whole field visual stimuli. Responses are shown at baseline recording, and following cumulative doses of 2.0mg/kg 4.0mg/kg, 8.0mg/kg of d-amphetamine.



While the data reported above suggests potential d-amphetamine action at the level of the SC, the in vivo methodology utilised means it was only possible to speculate as to the specific neurotransmitter systems involved. Consequently, Dommett, Overton, and Greenfield (2009) used an in vitro preparation to probe the proximal effects of introducing therapeutically appropriate doses of d-amphetamine to the SC. Coronal sections of the SC were submerged in artificial cerebrospinal fluid and evoked potentials of superficial layer SC neurons were recorded in response to direct electrical stimulation of afferent optic fibres at a range of intensities. Responses to optic tract stimulation were recorded when sections were perfused with either d-amphetamine, methylphenidate, or low and high doses of 5-HT. A dichotomous effect was observed, whereby perfusion of d-amphetamine or methylphenidate was found to preferentially inhibit responses to low intensity stimulation, while responses to high intensity stimulation were largely preserved. When psychostimulant perfusion was preceded by the administration of the of the broad spectrum 5-HT antagonist metergoline, no significant effect of either psychostimulant was observed, suggesting that these effects were mediated by 5-HT. This is supported by the results observed when slices were perfused directly with doses of 5-HT. A response similar to the dichotomous effects of the psychostimulants was observed when slices were perfused with low doses of 5-HT, however when slices were perfused with high doses of 5-HT, the collicular response to all stimulation intensities was almost universally depressed.

Dommett et al. thus speculate that d-amphetamine and methylphenidate act to alter the signal-to-noise ratio of visual responses at the level of the SC, suppressing the response to low intensity stimuli whilst preserving the response to high intensity stimuli. As this effect is mimicked following application of 5-HT and blocked by antagonising 5-HT, it is expected that 5-HT acts to mediate this effect. Contextualised to SC hypersensitivity in ADHD, a possible mechanism whereby psychostimulants reduce distractibility could be to increase synaptic levels of 5-HT, thereby mediating the collicular response to visual distractors such that responses to low salience stimuli are depressed, whilst the response to high salience stimuli are preserved, thus reducing the likelihood of foveation towards novel visual distractors. While Dommett et al.’s results are promising, 5-HT mediation of collicular psychostimulant effects have not yet been demonstrated in vivo. As such, a logical extension of this work is to establish if 5-HT antagonism in vivo can reverse the effects of psychostimulants on collicular activity.

# 1.10 Summary and Next Steps

In summation, ADHD is a heterogeneous disorder which is characterised by the triumvirate of attentional, hyperkinetic, and impulsive symptoms. Frontline pharmaceuticals for ADHD consist of psychostimulants which, when tolerated well, are efficacious in improving sustained attention and alleviating the symptoms of ADHD. Despite this efficacy, psychostimulants have a clear abuse potential, and misuse and diversion of these drugs remains a concern, particularly amongst adolescent and university populations. As such, there is a need to identify new, non-addictive pharmacotherapies the ADHD. Two barriers have hampered progress in this area: an incomplete understanding of the neural basis of ADHD, and the lack of a full profile of the mechanism of action of psychostimulants. As such, a more effective strategy in drug development for ADHD may be to understand the neural basis and mechanism by which psychostimulants normalise specific symptoms. When taking this approach, distractibility emerges as a logical symptom on which to focus, as it is the most prevalent and persistent symptom of ADHD. The midbrain SC has been repeatedly linked to distractibility in a range of paradigms in both humans and animal models. It has thus been proposed that the colliculus is hyper-responsive in ADHD, leading to increased foveation towards non-salient stimuli. Convergent pharmacological evidence has revealed that the psychostimulant d-amphetamine acts to depress visually evoked responses in the superficial SC, in a process that is mediated by 5-HT in vivo. However, it is not yet established whether 5-HT mediates the effects of d-amphetamine in living animals. Consequently, a logical extension of extant research is to establish if 5-HT antagonism can reverse the effects of d-amphetamine on collicular activity in vivo.

## 1.10.1 Overview of initial empirical chapters

The following three chapters focus on establishing the effect of 5-HT antagonism on visual responses in the SC following prior administration of d-amphetamine. First, Chapter 2 will outline common methods used throughout this thesis, including surgical procedures, data capture and analysis, and histology. Chapter 3 presents an exploration of potential 5-HT mediation of the effects of d-amphetamine on visual responses in the superficial SC. Data presented in this chapter examines the SC response to whole visual field flashes first following systemic administration of d-amphetamine, and then following subsequent administration of the 5-HT antagonist metergoline. As d-amphetamine was perfused systemically in the Chapter 3, it was then necessary to establish if the effect on the collicular response was a result of operations locally within the SC or whether this was incidental of afferent modulation of the input to the SC. As such, Chapter 4 presents an investigation of the SC response to whole visual field flashes following intra-collicular administration of d-amphetamine, and following subsequent 5-HT antagonism. The findings of Chapters 3 and 4 are discussed in the context of the relevance of 5-HT in the pharmacotherapy of ADHD. This leads into a review of the link between 5-HT ADHD in Chapter 5.

General Methodology

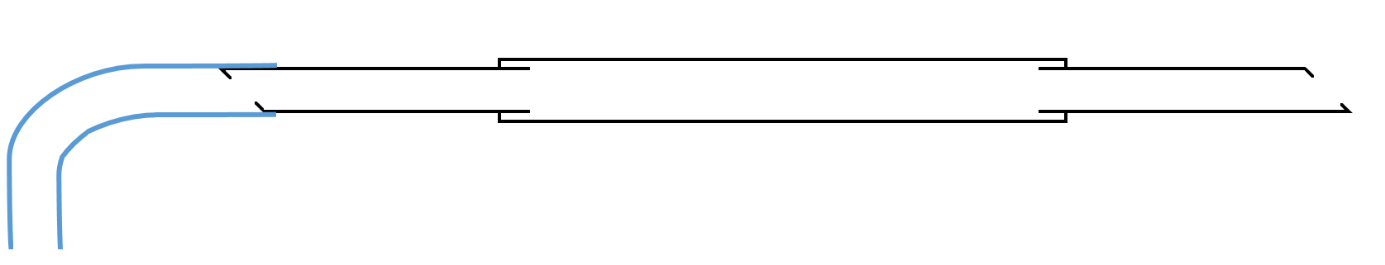
# 2.1 Chapter Summary

This chapter outlines the generic methods that were used within the experimental chapters presented in the current thesis. I will begin by detailing the construction of any non-standard laboratory equipment used. Then, a description will be provided for generic surgical techniques used to prepare the subjects for later drug perfusion, as well as the procedure used to locate the SC in vivo. The data capture process will then be detailed, followed by an overview of generic data preparation techniques. Finally, the histological techniques used to confirm electrode positioning following experimental procedures will be outlined. Variations on drugs used, injection sequencing, and data analysis will be discussed in specific experimental chapters.

# 2.2 Construction of Microinjector Coupled Electrodes

Microinjector coupled electrodes were constructed in house to allow for local injections into the SC, concurrent with electrophysiological recordings. The body of the microinjector was formed of a length of 30 gauge metal capillary tubing. One end of the 30 gauge tubing was soldered to a section of 25 gauge metal capillary, which had been filed to a bevelled tip under microscopic guidance. The opposite end of the 30 gauge tubing was soldered to a section of 25 gauge needle. This needle was attached to approximately 30 cm of 0.40mm internal diameter fine bore polythene tubing. A 2-dimenstional schematic of the microinjector can be seen in figure 2.1. Once constructed, the microinjector was affixed to a Parylene-C-insulated tungsten microelectrode using Araldite adhesive (see figure 2.2). As with previous work (Gowan et al., 2008), the injector and electrode were separated by a lateral distance of 0.2-0.3mm with the electrode positioned marginally ahead of the injector (<0.5mm).

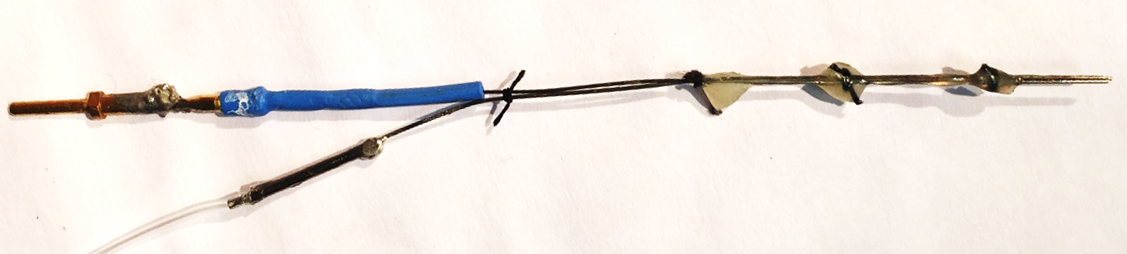
20 gauge needle 20 gauge metal capillary tubing



Polythene tubing 30 gauge metal capillary tubing

Solder points

**Figure 2.1:** A simplified schematic for microinjector construction.



**Figure 2.2:** Microinjector coupled electrode allowing for local injections into the SC concurrent with electrophysiological recordings. Constructed by affixing a single channel Parylene-C-insulated tungsten to the microinjector shown in figure 2.1

# 2.3 Surgical Procedures

## 2.3.1 Subjects

Work in the current thesis represents an exploration of the mechanism of action of current and potential pharmacological interventions for ADHD. While this work is motivated in principle by a need to identify new drug interventions for ADHD, the results of these experiments have relevance beyond of this context. As such, it was appropriate to use a neurotypical mouse model for these investigations, rather than an established murine model of ADHD, such as the New Zealand spontaneously hypertensive rats (SHR, Clements et al., 2014). Future work should aim to replicate the findings detailed in this thesis using the SHR.

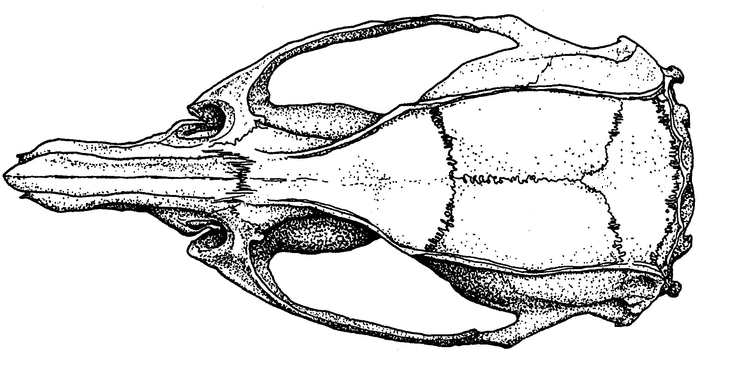
Data were obtained from male Hooded Lister rats. Subjects were housed in groups of 2 to 5, depending on size, in a room that was temperature controlled at 20-22oC on a 12-h light/dark cycle. Data collection for all animals took place during the animals light phase. All procedures conformed to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and the Animals (Scientific Procedures) Act, 1986. Every effort was taken to eliminate unnecessary suffering to the animals, and to reduce the number of subjects used.

## 2.3.2 Femoral Vein Cannulation

Subjects were anaesthetised with a split-dose injection of urethane solution (ethyl carbonate as a 25% aqueous solution; injection into the intraperitoneal cavity) at a dosage of 5.0ml/kg at least one hour prior to surgery. The subject’s temperature was monitored using a rectal thermometer with a coupled homeothermic blanket (Harvard Apparatus Limited) which maintained the subject’s temperature between 36.6 and 37.6°C. Anaesthetic depth was initially assessed by loss of blink reflex following a puff of air to the eye, and then by loss of pedal withdrawal reflex following a hind paw pinch. When both reflexes were eliminated, the subject’s head and upper thigh were shaved for surgical access. Cannulation surgery was then performed in order to affix a 0.40mm internal diameter fine bore polythene catheter (Smith Medical International Ltd.) into the left femoral vein, allowing for later intravenous drug injections. An incision was made high on the thigh of the left hind leg and blunt dissection of tissue was performed to expose the femoral triad. Once exposed, the femoral vein was isolated from the femoral artery and nerve, and clamped with a Moria vessel clamp (Fine Science Tools) in order to minimise blood loss during the introduction of the catheter. Vannas spring scissors (Fine Science Tools) were used to partially bisect the clamped femoral vein. Following partial femoral vein bisection, a catheter filled with heparinised saline (0.1ml heparin sodium in 50.0ml of 0.9% saline) was introduced into the vessel in order to reduce blood clotting prior to drug injection. Correct insertion of the catheter was confirmed by slowly pulling back on the syringe plunger until a small volume of blood was freely expelled into the catheter. The catheter was then secured by twice ligating the catheter within the vein, and then suturing the exposed catheter to the subject’s thigh. Following cannulation, the wound was sutured and secured with cyanoacrylate.

## 2.3.3 Craniotomy to Expose the Cortex Overlaying the Superior Colliculus

Subjects were fixed into a stereotaxic frame (David Kopf Instruments, Tuajanga, CA) with the skull level and secured by ears bars and a bite bar. A midline incision of the scalp was made, and the scalp was reflected to allow for the identification of skull sutures as a reference for craniometry (see figure 2.3). A dental drill was then used to complete a unilateral craniotomy centred on stereotaxic coordinates 6.3mm caudal to bregma, and 2.0mm lateral to midline. This allowed for exposure of the cortex overlaying the right hemisphere SC, contralateral to the eye which would receive later visual stimulation. The subject’s left eye was sutured open and protected with artificial tears (Viscotears liquid gel, Alcon) to ensure light flashes illuminated the whole visual field. The subject was given a 5.0ml split-dose subcutaneous injection of 0.9% saline to avoid dehydration during data collection.



**Bregma**

**Midline**

**Typical right SC craniotomy site**

**Figure 2.3:** Positions of the bregma and midline, skull sutures that were used as guidance for craniotomy. Red circle indicates and the typical craniotomy site for the right SC. Adapted from Paxinos and Watson (1998).

# 2.4 Data Preparation

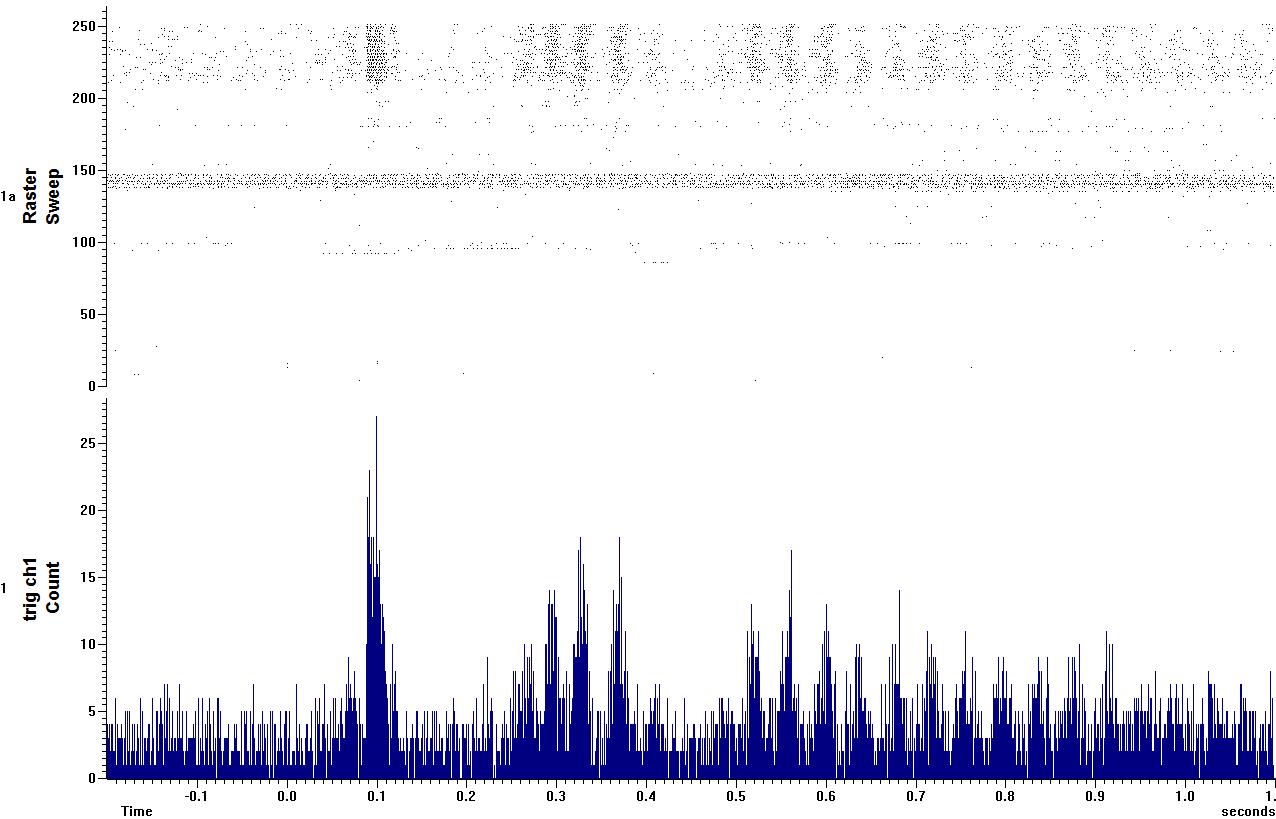
## 2.4.1 Generic Data Capture Technique

A microelectrode, or microinjector coupled electrode, was vertically lowered through the skull until it encountered the cortex overlying the SC (6.3mm caudal, 2.0mm lateral to bregma). Extracellular low frequency (LFP) and high frequency (MUA) signals from the microelectrode were enhanced (gain 1000), band-pass filtered (LFP: 0.1-300 Hz; MUA: 300-10 kHz), digitised at 11 kHz, and recorded to a computer disk via a 1401+ data acquisition system (Cambridge Electronic Design Systems, Cambridge, UK) utilising Spike2 data capture software (Cambridge Electronic Design Systems, Cambridge, UK; version 7.09a). Electrode recordings were monitored via visual feedback from a 20 Hz oscilloscope (Tenma Test Equipment), and auditory feedback from a Grass AM-8 audio monitor (Grass Medical Instruments). An LED was positioned approximately 5.0mm from the left eye to allow for whole visual field light flash stimulation (10ms duration, unfiltered 22 lux white LED, 0.5Hz with 30% jitter). The SC recording electrode was slowly lowered using a micropositioner (Luigs Newman SM-8-V2.0) until strong multi-unit activity was encountered, typical of the superficial layer SC response to visual stimulation. Response was confirmed using a series of 150 light flashes (see figure 2.4 for an example of the stereotypical SC visual response). When a stable response to visual stimuli was established, the subject was left to dark adapt for a minimum of 40min. Light titration was administered subsequent to dark adaptation in order to establish the optimal stimulus luminance for later data collection. This procedure consisted of exposing the subject to sequences of 150 whole visual field flashes, with luminance values ranging from 0.10 to 0.90 lux. The lowest luminance value to produce a detectible multi-unit and local field potential response was selected for the experimental stimulation.

Once the optimum stimulus luminance was established, subjects were exposed to repeated blocks of visual stimuli (150-210 stimuli, 10ms duration, 0.5 Hz with 30% jitter) following the injection of a variety of substances. Sequencing of stimuli and drug injections will be discussed in individual experimental chapters.

**Figure 2.4:** Locating the SC using the stereotypical response to visual stimuli. Part A shows an eight second period of raw high frequency data recorded from the SC. Vertical red lines indicate the timing of visual stimuli. Strong high frequency voltage deflection is observed following presentation of visual stimuli. Part B shows a raster plot and PSTH generated while advancing the electrode from the surface of the cortex towards the SC. Solid vertical red line indicates the timing of the visual stimuli; horizontal dashed red line indicates when the electrode began to encounter the SC. Upon initially encountering the SC, the electrode was slowly advanced until the stereotypical visual response shown in Part A was established. Broad noise encountered from sweep 140 to sweep 150 represents spontaneous activity encountered as the electrode was lowered through the cortex

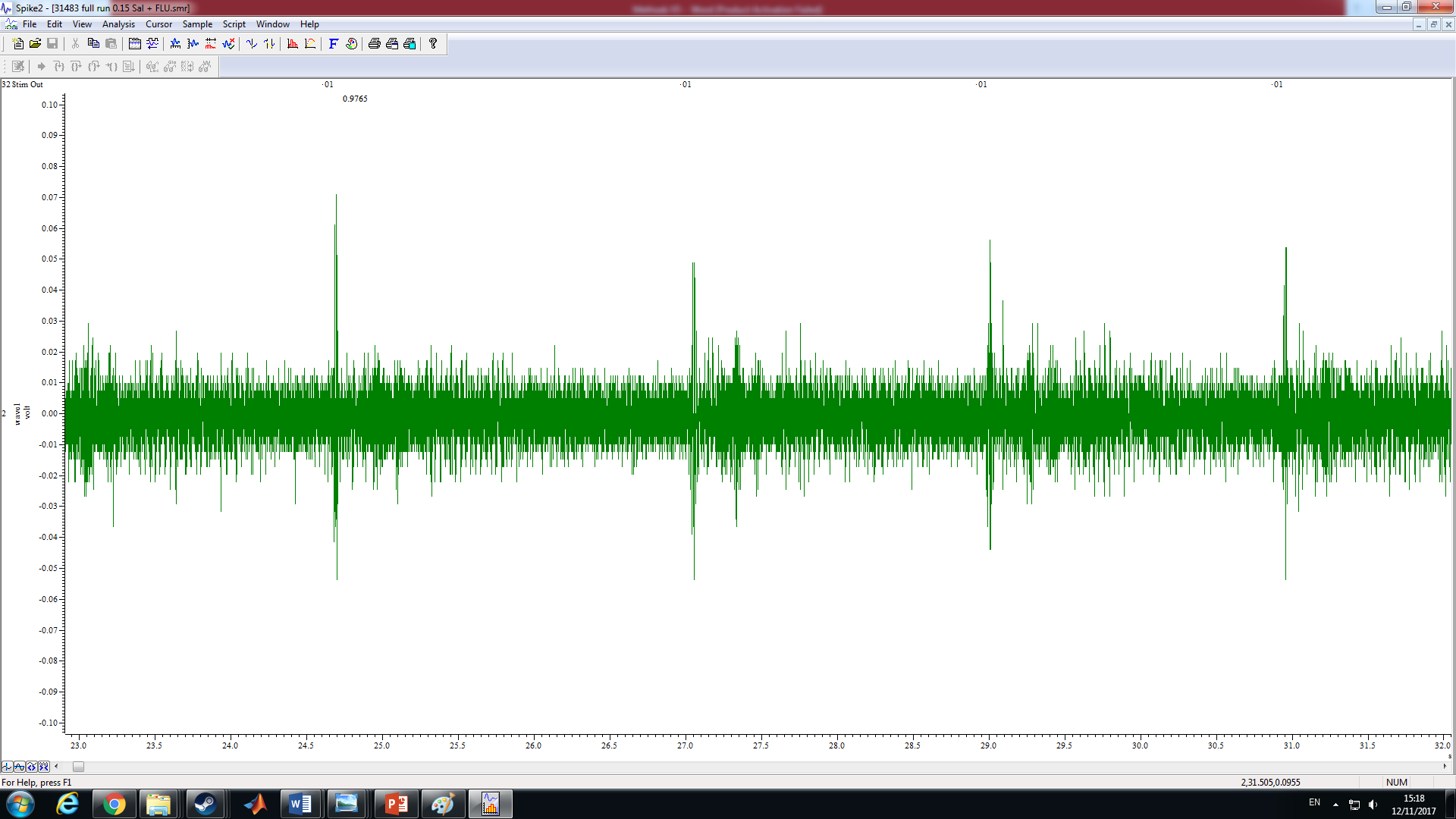
A



Time relative to visual stim (s)

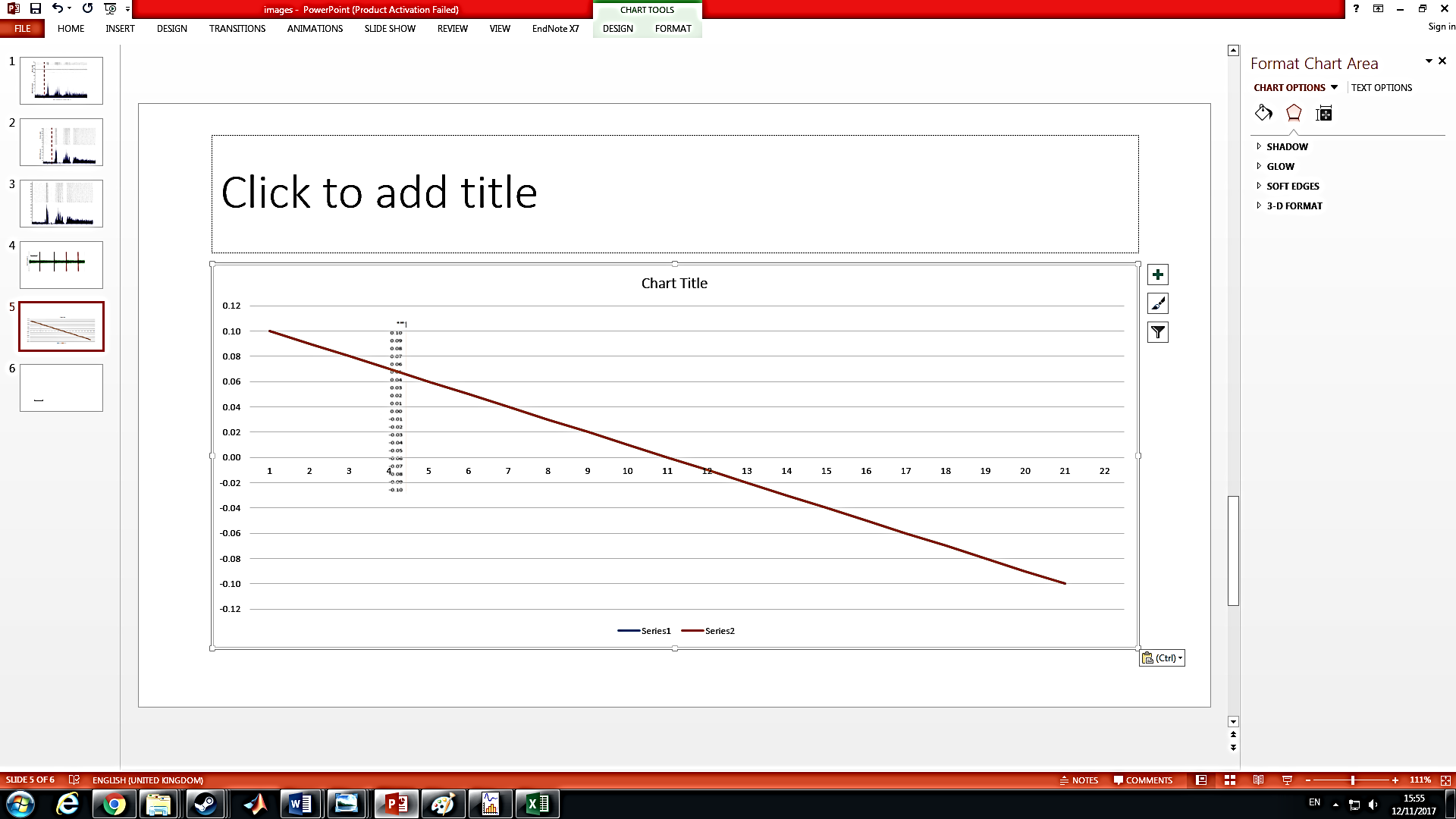
MU PSTH count Raster plot

B



1s

Raw MU response (V)



## 2.4.2 Generic Data Preparation and Pre-Processing

Prior to analysis, data were filtered in Spike 2 to reduce the effect of various sources of noise. Smoothed data for both LFP and MUA were generated by duplicating each channel and applying a median filter with a time constant of 0.1ms for the LFP or 1.0ms for the MUA. Physiologically implausible spikes within the LFP and MUA waveforms were identified by thresholding and marking instances where the gain enhanced voltage exceeded 0.15V. For each of these instances, the marked data points, as well as the preceding and following 1.0ms of waveform, were replaced with time matched data from the appropriate smoothed waveform (see figure 2.5 for an example). This allowed for maintenance of the characteristics of the SC response whilst still eliminating sources of noise; only ~2.0ms of a 2.0s (± 30%) response was altered, rather than removing the whole response and replacing with averaged data. A band-stop filter was then applied to the LFP wave form in order to reduce the effect of 50 Hz noise. Finally, the MUA waveform was de-trended using built in functionality within Spike 2. Data was then converted to a MATLAB file for further analysis



5.0ms

Raw response Smoothed response Filtered response

**Figure 2.5:** Example of filtering of physiologically implausible spiking noise within spike2. The example presented here uses an MUA waveform, though the process is identical for filtering LFP noise. Spiking noise can be clearly seen in the raw waveform. Once detected, the data points comprising the noise, as well as the preceding and following 1.0ms of waveform are overwritten by the smoothed response to generate a waveform primarily consisting of raw data, where only noise is replaced by smoothed data.



# 2.5 Data Analysis

All data were analysed offline using custom written MATLAB scripts (2016; The MathWorks Inc., Natick, MA, USA). For each measure of interest, typically the collicular response to visual stimuli in the presence or absence of a substance of interest, all analyses were performed on averaged data constructed from the response to 150 visual stimuli. Data for onset latency, response duration, and peak amplitude were extracted from both raw MUA and LFP data. Thresholds used were based on previous work (Gowen at al., 2008), and confirmed by visual inspection.

## 2.5.1 Local Field Potential

In order to extract the measures of onset latency and response duration, a response threshold was first constructed. This process comprised of two steps:

1. Calculating the mean and SD of the low frequency voltage trace over the 400ms period prior to stimulus presentation.
2. Constructing a response threshold, defined as any point at which the LFP waveform differed from the pre-stimulus mean by 3SDs.

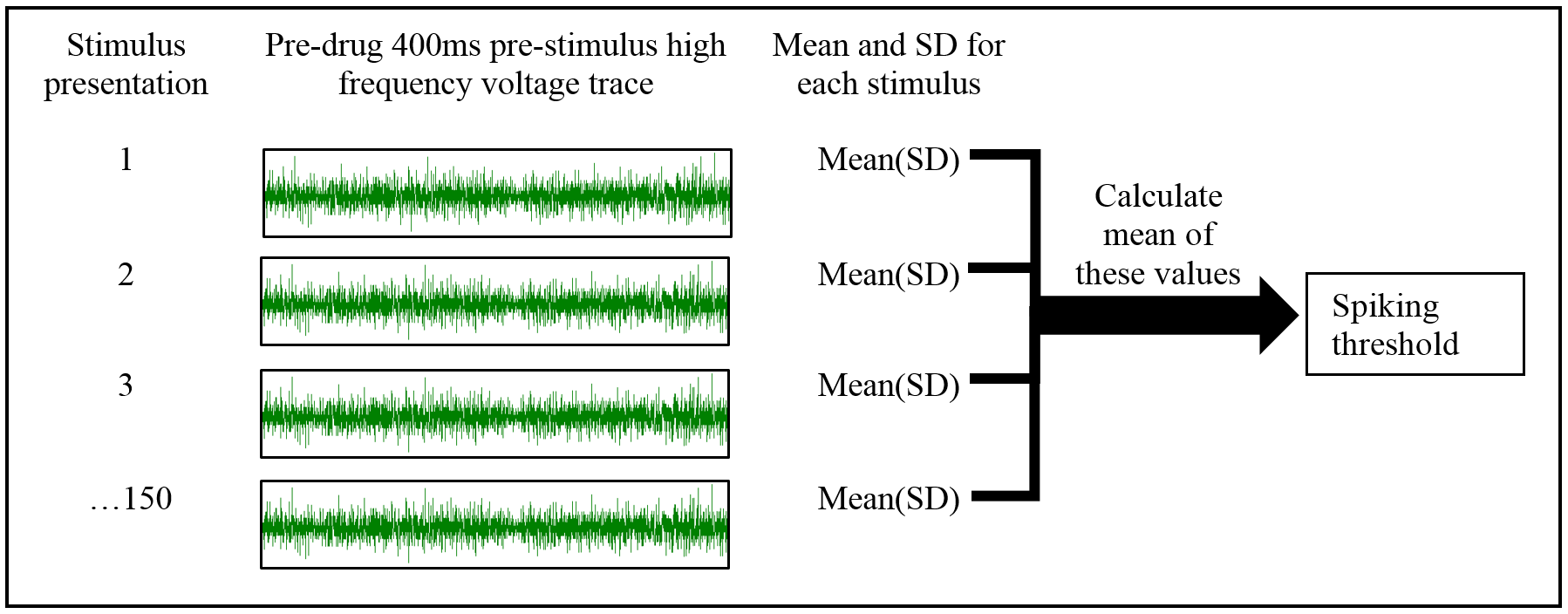
Onset latency was defined as the first time point at which the LFP waveform differed from the pre-stimulus mean by 3SDs. Offset latency was then calculated using a two-step process. First, LFP activity in the post-onset period was divided into epochs of 100ms starting from the first time point immediately post onset. The mean voltage trace was then calculated for each epoch, thus allowing for identification of the 100ms period where the post-onset voltage trace most closely resembled the pre-stimulus mean. Once this 100ms period was identified, onset latency was derived as the first single time point within this period at which the LFP voltage trace was closest to the pre-stimulus mean. Response duration was then derived as the difference between onset and offset latency. See figure 2.6 for depiction of the extraction of onset and duration. Peak-to-peak amplitude was determined as the difference between maximum voltage trace and minimum the voltage trace within the duration of the response.



**Figure 2.6:** Averaged LFP waveform showing extraction of onset latency and duration (x axis = time (s), y axis = voltage (arbitrary units)). The black line indicates mean activity in the 400ms pre-stimulus window, while the red and green lines indicate thresholds 3SD above and below this mean. The red and green circles indicate the time point at which these thresholds are crossed. Onset latency is defined as the first time point that crosses either of these thresholds. The black circle indicates the offset, defined as the post-onset time point that is closest to the pre-stimulus mean.

## 2.5.2 Multi-Unit Activity

Four measures of interest were extracted from the averaged MUA data: baseline activity, onset latency, response duration, and peak amplitude. To derive these measures, it was first necessary to undertake a process to identify and extract spikes. Thresholding was applied to facilitate the extraction of spikes. This was achieved for each individual animal by calculating the mean and SD of the high frequency voltage trace for the 400ms pre-stimulus time period for each of the 150 stimulus presentations in the pre-drug baseline recording. A spiking threshold was then calculated by averaging the mean of the 400ms pre-stimulus voltage trace across all stimulus presentations in the pre-drug baseline recording. Spikes were defined as activity for which the high frequency voltage trace exceeded 2SDs of the spiking threshold (see figure 2.7 for visualisation of this method). This pre-drug spiking threshold was applied across all drug doses so as to avoid any potential confounding effect of drug-induced changes in activity on spike detection.



**Figure 2.7:** Method for calculating the threshold for extraction of spiking activity

Spikes were then extracted from the response to 150 visual stimuli at the time periods defined in each empirical chapter. This data was then used construct a peri-stimulus time histogram for each time point (PSTH; bin width = 10ms, count per bin normalised to number of stimuli), from which values for baseline activity, onset latency, response duration and peak amplitude were extracted. Baseline activity, was derived by calculating the mean bin count in the 400ms pre-stimulus time period. As well acting as a measure of spontaneous neural activity in the SC, baseline activity was also used as a reference point for the construction of a response threshold (defined as any activity that exceeds baseline activity by 5SDs) to facilitate the extraction of onset latency and response duration. Onset latency was thus derived as the first time point subsequent to stimulus presentation where the PSTH bin count exceeded this response threshold. Onset latency was determined as the first time point for which PSTH bin count was equal or less than baseline activity whilst remaining below the response threshold for a period of 400ms. Response duration was then derived as the difference between onset and offset latency. See figure 2.6 for depiction of the extraction of onset and duration. Finally, peak amplitude was calculated as the maximum single bin count within the response duration, normalised to baseline activity.

Figure 2.8: MUA PSTH showing extraction of baseline, onset, and duration (x axis = time (s), y axis = spike count). The green line indicates baseline activity, defined as mean bin count in the 400ms pre-stimulus window. The red line indicates the response threshold, established as 5SDs greater than baseline activity. The first bin to exceed this threshold constitutes the onset. Offset is defined as the first post onset bin which returns to baseline and remains subthreshold for a period of 400ms.



# 2.6 Histology

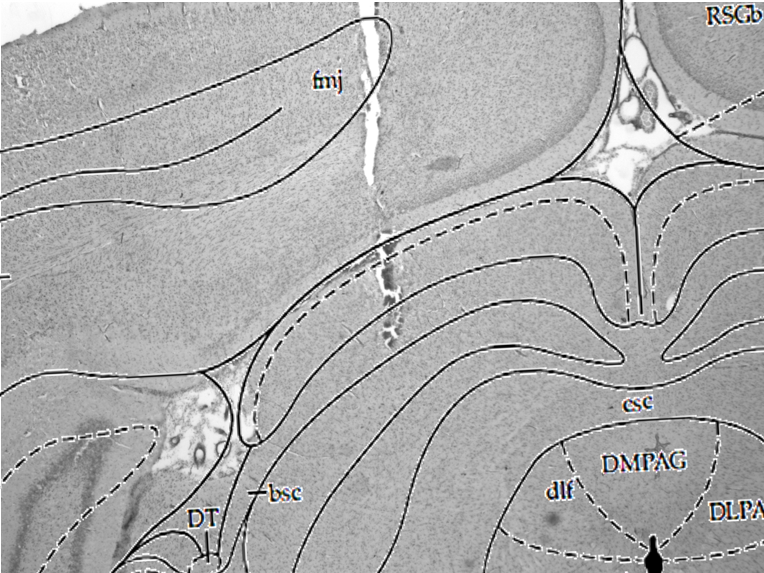
For all experiments, subject’s brains were sectioned and stained with cresyl violet to confirm correct electrode placement. Upon completion of an experiment, brains were immediately removed and placed in fixative solution for a period of at least 72h. Brains were then transferred to a phosphate-sucrose solution (50ml 0.2 M phosphate, 50ml distilled water, 30g sucrose) where they were stored at 3.0-5.0oC until they were ready to be sectioned. Prior to sectioning, brains were submerged in Tissue-Tek optimum cutting temperature compound (Sakura Finetek Europe) and rapidly frozen at -40 oC using a cryostat cooling element (Leica Biosystems (UK) Ltd). Brains were then blocked at skull flat position, removing the cerebellum and brainstem. The blocked brains were mounted within the cryostat and sliced at a thickness of 35μm until the entire SC had been sectioned (using Paxinos and Watson, (1998) as reference). Following sectioning, slices were left to dehydrate overnight. Slices were then loaded into a slice holder and stained with cresyl violet using the following process

1. Slides were cleared by immersing them in xylene for 5min.
2. Slides were rehydrated by immersing slices in decreasing concentrations of ethanol for the following amounts of time
   * 1. 95% ethanol for 15min
     2. 80% ethanol for 2min
     3. 70% ethanol for 2min

**Figure 2.9:** An overview of the process by which sections of the SC were stained with cresyl violet.

1. Slides were immersed in fresh distilled H2O for 2min to rinse off excess ethanol.
2. Slides were stained by immersion in cresyl violet solution (1% in H2O) for 2-10 min, until appropriate staining was achieved.
3. Slides were dehydrated by immersing slices in increasing concentrations of ethanol for the following amounts of time
   * 1. 70% ethanol for 1min
     2. 80% ethanol for 1min
     3. 95% ethanol with 5 drops of glacial acetic acid for 2min
     4. 100% ethanol for 1 min
4. Finally, staining was checked under microscopic guidance. If sections were too darks, steps 4.iii, 4.iv, and 5 were worked through in reverse order until staining level was appropriate.
5. Once appropriate staining was achieved, DPX mounting medium was applied to each slide, and a cover slip was affixed to allow for later light microscopy

Once stained, sections were viewed under light microscopy to identify electrode tracts. Sections were compared to reference drawings of coronal sections of the rat brain from Paxinos and Watson (1998; see figure 2.8). Where electrode tracts could not be clearly defined, the stereotypical superficial SC LFP response to visual stimuli were used as inclusion criteria (Dyer & Annau, 1977).



**1.0mm**

SGS

OP

**Figure 2.8:** An electrode track and recording site from the superficial layers of the SC in a representative animal. Slices were prepared for histological inspection as described above. Image is overlaid with a coronal diagram at stereotaxic coordinates -6.30mm relative to bregma (adapted from (Paxinos & Watson, 1998). Red dotted outline indicates position of the electrode track, which terminated in the opticum.

Effect of 5-HT Antagonism on Amphetamine-Induced Suppression of Superficial Layer SC Visual Responses

# 3.1 Chapter Summary

A key step in developing new interventions for ADHD is elucidating the mechanism of action of current front line pharmaceuticals. The following chapter presents evidence that the SC is a target of frontline ADHD pharmaceuticals, and outlines our recent investigation into a potential mechanism for D-amphetamine at the level of the SC. The results of this study illustrate that the D-amphetamine depresses the SC response to visual stimuli and that this depression is likely mediated, at least in part, by 5-HT.

# 3.2 **Introduction**

## 3.2.1 SC Hypersensitivity as an Explanation for Increased Distractibility in ADHD

Recall from chapter 1 that we currently lack a robust understanding of both the neural pathology of ADHD (Biederman, 2005b; Greydanus et al., 2007) and the therapeutic targets of psychostimulant medication (Spencer et al., 2002b). While several theories attempting to consolidate the pathophysiological changes underpinning ADHD have been advanced (e.g. (Barkley, 1997; Ziegler, Pedersen, Mowinckel, & Biele, 2016), some reviewers point towards the multifactorial aetiology to explain the elusiveness of an accepted unifying theory of ADHD (Himelstein, 2000). Thus, a fruitful strategy may be to instead focus on the neural basis of specific core symptoms of ADHD (Overton 2008). If we are to take this approach, it is logical to focus on inattentive symptoms, specifically distractibility. Inattentive symptoms are the most prevalent symptoms of ADHD presenting in up to 93% of patients, with distractibility being the single most frequently presenting symptom (Wilens et al., 2009). Distractibility shows persistence of presentation as it is the symptom most resistant to extinction with age and the transition into adulthood (Biederman et al., 2000; Wilens et al., 2009). Further, Nahlik (2004) argues that distractibility is likely to be even more prevalent in ADHD cohorts than reported due to the prevalence of covert inattentional symptoms, particularly in adolescent ADHD populations.

In addition to consistent clinical findings (Barkley, 2006; Biederman & Faraone, 2005; Greydanus et al., 2007; Thorley, 1984), experimental accounts of heightened distractibility in ADHD cohorts have also been consistently reported. Children with ADHD commit more errors and are more easily distracted when performing tasks designed to mimic typical classroom activity (Sonuga-Barke et al., 1996). ADHD patients appear to be particularly sensitive to peripherally presented novel stimuli. Adults with a clinical diagnosis of ADHD, and non-ADHD diagnosed adults with high subclinical ADHD-like traits, both show impaired performance on a recall task when presented with peripheral visual or auditory stimuli (M. Panagiotidi et al., 2017). These behavioural accounts are supported by EEG evidence, which shows that children with ADHD display significantly larger event related potentials than neurotypical controls when presented with unexpected distracting sensory stimuli (Gumenyuk et al., 2004; Van Mourik et al., 2007).

The midbrain SC, a subcortical sensory structure that plays a central role in the orientation of attention to biologically salient events, is uniquely placed as a locus of interest when investigating increased distractibility in ADHD. As outlined in chapter 1, behavioural evidence has repeatedly linked distractibility to the SC (Goodale et al., 1978; Milner et al., 1978). In many distraction paradigms, the novelty of a stimulus is a quality associated with the likelihood that a stimulus distracts participants from a given task (Berti et al., 2004; Parmentier, Elsley, Andrés, & Barceló, 2011; Schroger & Wolff, 1998). Given the role of the SC as a novelty detector (Anderson et al., 2012; Pérez-González et al., 2005), the findings of such studies, as well as findings showing that ADHD patients are more sensitive to novel distractors than controls (detailed above, Gumenyuk et al., 2004; Van Mourik et al., 2007), may point towards a potential SC dysfunction in ADHD (Overton 2008). While it is noted above that an accepted global theory of ADHD has remained elusive, a commonality of many these theories is a suspected dysfunction of the basal ganglia (BG; e.g. (Castellanos & Proal, 2012; Castellanos, Sonuga-Barke, Milham, & Tannock, 2006), a group of functionally related subcortical nuclei that play a central role in action selection (Redgrave, Prescott, & Gurney, 1999). This is supported by robust neuroimaging evidence, reviewed by Dickstein, Bannon, Castellanos, and Milham (2006) and Nakao, Radua, Rubia, and Mataix-Cols (2011). As the SC constitutes an important BG input pathway for a variety of sensory modalities (Coizet, Overton, & Redgrave, 2007), altered collicular responsiveness may affect action selection at the level of the BG, potentially explaining the increased distractibility seen in ADHD (Overton, 2008).

Evidence of a link between the SC and distractibility comes from a range of species utilising ablation, behavioural, and pharmacological techniques. (Goodale, Foreman, & Milner, 1977) reported the elimination of an orienting reflex to peripheral novel visual stimuli during a visually guided running task following bilateral collicular ablation in rats. This reduced orientation to novel distractors was not observed when the visual cortex was instead ablated, suggesting a central role for the SC in distraction. Similar findings have been reported in primates (Milner et al., 1978). Stump tailed macaques showed reduced distractibility when performing a visual discrimination task following bilateral SC ablation. Taken together, these results imply a role of the SC in the orientation to novel visual distractors that is preserved between rodents and primates. Much evidence of the link between the SC and distractibility stems from collicular involvement in initiating and guiding saccades (Sparks, 1999; Whittaker & Cummings, 1990). If altered collicular responsiveness is associated with distractibility in ADHD, we would expect ADHD patients to show impairment in oculomotor tasks that assess these saccadic responses. Recall from Chapter 1 that adults with ADHD show deficits in inhibiting saccadic eye movements towards distracting stimuli, particularly for saccades where collicular input guides target selection (Adams et al., 2011; Roberts et al., 2011). These saccadic deficits are normalised in both adult and child ADHD patients treated with psychostimulants, compared to non-medicated ADHD patients (Folta & Mähler, 2010; Fried et al., 2014). Given the role of the SC in saccadic behaviour, the evidence of saccadic deficits in ADHD, and reports that psychostimulant medication normalises these deficits, the SC emerges as a potential therapeutic target for psychostimulant action.

## 3.2.2 Psychostimulant Action at the Level of the SC

In addition to oculomotor evidence linking the SC to psychostimulant action, emergent electrophysiological and psychopharmacological evidence suggests a mechanism of action of d-amphetamine at the level of the colliculus. While a full pharmacological profile of the effects of d-amphetamine remains elusive (Spencer, Biederman, Wilens, & Faraone, 2002a) there is robust evidence that the effects of d-amphetamine are mediated by elevating synaptic levels of the monoamines neurotransmitters DA, NA ((Easton et al., 2007), and 5-HT ((Holmes & Rutledge, 1976; Kuczenski & Segal, 1997). The SC expresses the necessary receptors for d-amphetamine action; evidence suggests that the SC is extensively innervated by both NA and 5-HT (Massey et al., 2013), which preferentially target the superficial visual layers (Wichmann and Starke, 1988). To assess psychostimulant action at the level of the SC, Gowan et al. (2008) recorded visually evoked potentials in the superficial layers of the SC following the injection of systemic or local doses of d-amphetamine, or volumetrically equivalent doses of saline. LFP and MUA were recorded for a series of whole field visual stimuli following five cumulative systemic doses of d-amphetamine (up to a total cumulative dose of 8.0mg/kg). A dose-dependent depression in the amplitude of response was observed for both LFP and MUA following d-amphetamine administration. Similar findings were reported when d-amphetamine was introduced locally into the SC. A single bolus injection of d-amphetamine (120nmol) reduced amplitude and duration for both the LFP and MUA response relative to volumetrically equivalent saline. Similar findings were reported by (Clements et al., 2014) who demonstrated that d-amphetamine reduces peak amplitude components of the collicular LFP and MUA to whole field light flashes in New Zealand Genetically Hypertensive rats, a validated animal model of ADHD.

While Gowan et al., and Clements et al., provide evidence of d-amphetamine action at the level of the SC, they could only speculate as to the neurotransmitter systems involved. Dommett et al. (2009) used an in vitro preparation to probe the proximal effects of introducing therapeutically appropriate doses of d-amphetamine to the SC. Slices were permeated with d-amphetamine, methylphenidate, or 5-HT, and postsynaptic potentials of superficial layer SC neurons were recorded in response to electrical stimulation of afferent optic fibres. A dichotomous effect was observed, whereby both d-amphetamine and methylphenidate were found to preferentially inhibit responses to low intensity optic fibre stimulation, while responses to high intensity stimulation were largely preserved. The effects of d-amphetamine and methylphenidate were mimicked by low doses of 5-HT while high doses of 5-HT almost universally supressed the collicular response to all stimulation intensities. When slices were perfused with psychostimulants subsequent to the prior introduction of the 5-HT antagonist metergoline, no significant effect of psychostimulant action was observed. Dommett et al., thus speculate that d-amphetamine and methylphenidate act to alter the signal-to-noise ratio of visual responses at the level of the SC, suppressing the response to low intensity stimuli whilst preserving the response to high intensity stimuli. As this effect is mimicked following application 5-HT and blocked by antagonising 5-HT, it is expected that 5-HT acts to mediate this effect. Contextualised to SC hypersensitivity in ADHD, a possible mechanism whereby psychostimulants reduce distractibility could be to increase synaptic levels of 5-HT, thereby mediating the collicular response to visual distractors such that responses to low salience stimuli are depressed, whilst the response to high salience stimuli are preserved, thus reducing the likelihood of foveation towards novel visual distractors. While Dommett’s results suggest that 5-HT transmission in the SC may have relevance to the pharmacotherapy of ADHD, this effect has yet to be established in vivo.

## 3.2.3 Aim of the Present Study

The aim of the present study is to explore whether d-amphetamine action at the level of the SC is mediated by 5-HT in vivo. Convergent evidence points towards d-amphetamine reducing the response of superficial layer SC neurons to visual stimuli in vivo (Gowen et al., 2008; Clements et al., 2014), which has shown to be mediated by 5-HT in vitro. It is not yet established whether 5-HT mediates the effects of d-amphetamine in living animals. Consequently, the current study utilises in vivo electrophysiological techniques to record the responses of superficial SC neurons following injections of d-amphetamine and subsequent injections of the 5-HT antagonist metergoline.

# 3.3 Methods

## 3.3.1 Subjects

Data were obtained from 20 male Hooded Lister rats (bred in house; weight 300-550g). Animals were housed together on a 12 hr light/dark cycle with food and water supplied ad libitum. All procedures were performed according to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and the Animals (Scientific Procedures) Act, 1986 (revised 2013). Every effort was made to minimise suffering to subjects and to reduce the number of animals used.

## 3.3.2 Experimental Procedures

Subjects were anaesthetised with urethane (ethyl carbonate as a 25% aqueous solution) and prepared for cannulation and craniotomy surgery as described in chapter 2. Once both surgeries were complete and the cortex overlaying the SC was exposed, an LED was positioned over the contralateral eye to the exposed SC in order to provide whole visual field illumination with which to guide electrode placement. A single channel tungsten parylene C microelectrode was then slowly advanced through the exposed cortex (6.3mm caudal to bregma, and 2.0mm lateral to midline) towards the SC using the multi-unit response to whole-field light flashes as guide (10ms duration, 0.5 Hz with 30% jitter). Once the visually evoked response stereotypical of the superficial layers of the SC was encountered (average placement depth of 3.11mm), the response to a further 150 visual stimuli was then recorded to confirm correct electrode placement. After electrode placement was confirmed, the animal was dark adapted for a minimum of 40min. Following this, optimal stimulus luminance was determined as outlined in chapter 2.

Once an optimal stimulus intensity that reliably evoked a stable LFP and MUA response was established a baseline pre-drug response was recorded to 150 whole visual field light flashes (10ms duration, 0.5 Hz with 30% jitter). This was immediately followed by an intravenous injection of d-amphetamine (0.5 mg/kg in 0.9% saline; experimental group, n =10) or a volumetrically equivalent dose of saline (0.9% saline; control group, n =10). Injections were allowed 2 minutes to take effect, following which the response to a further 150 visual stimuli was acquired. This process was repeated for 5 more injections of d-amphetamine (cumulative dose of 1.0, 2.0, 4.0, 8.0, 16.0 mg/kg. Previous reports have shown that d-amphetamine operates at the level of the SC when administered at the doses used here; Gowan et al., 2008) or volumetrically equivalent doses of saline. Following the full range of d-amphetamine or saline injections, a single bolus injection of 60% dimethyl sulfoxide was delivered (DMSO, 60% in distilled water, 1.0 mg/kg dosage; DMSO was the vehicle used for later injections of metergoline), and the response to a further 150 visual stimuli was recorded. Finally, this process was repeated twice more for two doses of metergoline (both doses 1.0mg/kg in 60% DMSO 40% distilled water, for a total cumulative dose of 2.0mg/kg. Previous reports have demonstrated that, at the doses used in the current study, metergoline acts to block or reverse the effects of systemic d-amphetamine; (Fletcher, 1995; Rech, Borsini, and Samanin, 1984). See figure 3.1 for a simplified view of injection and recording timings. Upon completion of the procedure, animals were immediately euthanised with pentobarbital (1.0ml i.v. injection), following which, brains were immediately removed and placed in fixative for later histological confirmation of electrode placement, as outlined in chapter 2.6.

## 3.3.3 Data Analysis

Data were prepared for analysis as outlined in Chapter 2.4. Data were analysed offline using custom written MATLAB scripts (2016a; The MathWorks Inc., Natick, MA, USA). All analyses detailed below were carried out on averaged data constructed from the response to 150 visual stimuli either at baseline, or following drug/saline perfusion. Data for onset latency, response duration and peak amplitudes were extracted from both raw MUA and LFP data values. For both LFP and MUA there were two comparisons of interest

1. Between doses of d-amphetamine and saline, relative to pre-drug baseline.
2. Between the maximum dose of d-amphetamine or saline, and doses of DMSO and metergoline.

Experimental group

Control group

1. Recording of pre-drug baseline response

2. 0.5 mg/kg d-amphetamine

3. 1.0 mg/kg d-amphetamine

4. 2.0 mg/kg d-amphetamine

5. 4.0 mg/kg d-amphetamine

6. 8.0 mg/kg d-amphetamine

7. 16.0 mg/kg d-amphetamine

Baseline recording (150 stimuli)

2. Saline equivalent to 0.5 mg/kg d-amphetamine

3. Saline equivalent to 1.0 mg/kg d-amphetamine

4. Saline equivalent to 2.0 mg/kg d-amphetamine

5. Saline equivalent to 4.0 mg/kg d-amphetamine

6. Saline equivalent to 8.0 mg/kg d-amphetamine

7. Saline equivalent to 16.0 mg/kg d-amphetamine

Baseline recording (150 stimuli)

8. 1.0 mg/kg 60% DMSO

9. 1.0 mg/kg metergoline

10. 2.0 mg/kg metergoline

Figure 3.1: Sequencing of drug/saline injections throughout the experimental procedure. 2 min after the injection (or immediately for the baseline recording), responses to 150 visual stimuli were recorded (10ms duration, 0.5 Hz with 30% jitter).

N=10

N=10

*Local field potential.* Three measures of interest were extracted from averaged LFP data: onset latency, response duration, and peak-to-peak amplitude. In order to extract these measures, a response threshold was constructed. This process comprised of two steps:

1. Calculating the mean and SD of the low frequency voltage trace over in the 400ms period prior to stimulus presentation.
2. Constructing a response threshold, defined as any point at which the LFP waveform differed from the pre-stimulus mean by 3SDs.

Onset latency was defined as the first time point at which the LFP waveform differed from the pre-stimulus mean by 3SDs. Offset latency was then calculated using a two-step process. First, LFP activity in the post-onset period was divided into epochs of 100ms starting from the first time point immediately post onset. The mean voltage trace was then calculated for each epoch, thus allowing for identification of the 100ms period where the post-onset voltage trace most closely resembled the pre-stimulus mean. Once this 100ms period was identified, onset latency was derived as the single time point within this period at which the LFP voltage trace was closest to the pre-stimulus mean. Response duration was then derived as the difference between onset and offset latency. Peak-to-peak amplitude was then determined as the difference between maximum voltage trace and minimum the voltage trace within the duration of the response.

*Multi-unit activity*. Four measures of interest were extracted from the averaged MUA data: baseline activity, onset latency, response duration, and peak amplitude. To derive these measures, it was first necessary to undertake a process to identify and extract spikes. Thresholding was applied to facilitate the extraction of spikes. This was achieved for each individual animal by calculating the mean and SD of the high frequency voltage trace for the 400ms pre-stimulus time period for each of the 150 stimulus presentations in the pre-drug baseline recording. A spiking threshold was then calculated by averaging the mean of the 400ms pre-stimulus voltage trace across all stimulus presentations in the pre-drug baseline recording. Spikes were defined as activity for which the high frequency voltage trace exceeded 2SDs of the spiking threshold. This pre-drug spiking threshold was applied across all drug doses so as to avoid any potential confounding effect of drug-induced changes in activity on spike detection. Spikes were then extracted from the response to 150 visual stimuli at the time periods shown in figure 3.1. This data was then used construct a peri-stimulus time histogram for each time point (PSTH; bin width = 10ms, count per bin normalised to number of stimuli), from which values for baseline activity, onset latency, response duration and peak amplitude were extracted. Baseline activity, was derived by calculating the mean bin count in the 400ms pre-stimulus time period. As well acting as a measure of spontaneous neural activity in the SC, baseline activity was also used as a reference point for the construction of a response threshold (defined as any activity that exceeds baseline activity by 5SDs) to facilitate the extraction of onset latency and response duration. Onset latency was thus derived as the first time point subsequent to stimulus presentation where the PSTH bin count exceeded this response threshold. Onset latency was determined as the first time point for which PSTH bin count was equal or less than baseline activity whilst remaining below the response threshold for a period of 400ms. Response duration was then derived as the difference between onset and offset latency. Finally, peak amplitude was calculated as the maximum single bin count within the response duration, normalised to baseline activity.

Initial inspection of the data revealed that the superficial layer SC MUA response to visual stimuli is comprised of a multiple phases where the first 100ms of the response constitutes the first phase and all subsequent activity until termination of the response constitutes the second phase. Visual inspection suggested that the amplitude first phase of the MUA response was differently affected both d-amphetamine and metergoline than the second phase. As such, the peak amplitude was calculated for overall MUA response, as well as for phases 1 and 2 of the response.

For statistical purposes, all LFP and MUA data were normalised by dividing post-drug responses by the baseline (pre-drug) responses for each animal. LFP and MUA responses were analysed using mixed analysis of variance (ANOVA) and post-hoc t-tests. Where assumptions of sphericity were violated Greenhouse-Geisser corrections were applied. For all analyses, alpha values of 0.05 were considered significant. For all analyses detailed below, t-tests were used to compare the control injection of DMSO with the doses immediately prior and subsequent. No effect of DMSO was revealed for any measure across all analyses.

# 3.4 Results

## 3.4.1 Electrode Placement

Brains were prepared for histological inspection as described in Chapter 2.6. Inspection of electrode tracks revealed that recording sites were located in the SGS or SO. Due to insult to tissue derived from the histology process, visual inspection could not always reliably distinguish if recording sites were located in SO or SGS. Where this was the case, characteristics of the baseline LFP response were used to aid in distinguishing between the collicular laminar.

For all animals, the baseline superficial SC LFP response to whole visual field stimuli was a complex multi-component phenomena. For the majority of animals where LFP was obtained (15/20; 75.0%) the most salient short-latency LFP component constituted of a short duration negativity (most likely corresponding to components N1-N2 of the superficial SC response to visual stimuli; Dyer and Annau, 1977), followed by a short latency positivity (most likely corresponding to component P2) and then a longer duration, large amplitude negativity (most likely corresponding to components N3 and P3). The initial negative deflection was in some animals (5/20; 25%) preceded by a short duration, low amplitude positivity (P1). Such LFP dynamics are typical of electrode placement in SO. Shallower electrode placement (in SGS) is associated with a reversal of these major field components (Dyer & Annau, 1977; Hirai & Okada, 1995). Thus the majority of animals were considered to have electrode placement in the SO. Where a positive deflection was the most salient short latency LFP aspect (n=5, 25%) electrode placement was considered to be in the SGS.

## 3.4.2 Effects of Systemically Administered d -amphetamine and Metergoline on Superficial Layer SC LFP Response to Visual Stimuli

Intravenous injections of d-amphetamine caused a dose-dependent depression of the peak-to-peak amplitude of the superficial layer SC LFP response to whole visual field flashes (see figure 3.2 for representative animal and figure 3.3 for comparisons between groups for onset, duration, and peak-to-peak amplitude). In contrast, peak amplitude rose over time in the saline condition. Neither DMSO nor metergoline had a significant effect on any aspect of the LFP response.



Amplitude (mV)

Figure 3.2: Effects of d -amphetamine and metergoline on the LFP response to visual stimuli in a representative animal. X axis is time, corresponding to 0.8s of response immediately following stimulus at t0; y axis is voltage deflection in arbitrary units. A significant depressant of amplitude of the LFP is observed following administration of the maximum cumulative dose of d-amphetamine. Though non-significant, administration of metergoline enhanced the previously depressed response towards levels recorded at the pre-drug baseline.

***The effect of d-amphetamine on collicular LFP response to visual stimuli***

A Greenhouse-Geisser (GG) corrected Mixed ANOVA (factors: Dose [N=6], Drug [d-amphetamine or saline]) revealed a significant main effect of Drug on LFP peak-to-peak amplitude (F[1, 16] = 13.06, p = 0.002), but no main effect of Dose (F[2.57, 41.14] = 0.84, p = 0.467), and no Dose\*Drug interaction (F[2.57, 41.14] = 2.23, p = 0.108). Post-hoc independent t-tests revealed significant differences between groups for all doses (0.5mg/kg: t(18) = 3.17, p = 0.005; 1.0mg/kg: t(18) = 2.14, p = 0.046; 2.0mg/kg: t(18) = 2.44, p = 0.025; 4.0mg/kg: t(18) = 2.90, p = 0.009; 8.0mg/kg: t(18) = 3.36, p = 0.005; 16.0mg/kg: t(18) = 4.039, p = 0.001) suggesting that d-amphetamine depresses the superficial SC LFP response to visual stimuli at a range of doses. Further mixed GG-ANOVAs revealed no main effect of Drug or Dose, and no Drug\*Dose interaction for onset (Drug: F(1, 15) = 1.05, p = 0.321; Dose: F(1.15, 14.17) = 0.22, p = 0.950; Dose\*Drug: F(1.15, 14.17) = 1.95, p = 0.181) or duration (Drug: F(1, 16) = 2.06, p = 0.170; Dose: F(1.35, 21.61) = 1.63, p = 0.220; Dose\*Drug: F(1.35, 21.61) = 1.59, p = 0.226).

***The effect of metergoline on collicular LFP responses to visual stimuli***

To assess if antagonising 5-HT attenuated the effect of d-amphetamine on collicular responsiveness, additional t-tests were used to compare onset latency, duration and peak-to-peak amplitude of the maximum dose of d-amphetamine (16.0mg/kg), or saline, with the two 1.0mg/kg doses of metergoline (maximum cumulative dose of 2.0mg/kg). No significant difference was found between the maximum dose of either d-amphetamine or saline and any dose or metergoline. These results suggest that antagonising 5-HT has no effect on d-amphetamine induced depression of the superficial SC LFP response to visual stimuli.

**C1**

**C2**

**A1**

**A2**

**B1**

**B2**

Figure 3.3: The effect of systemic administration of d-amphetamine and metergoline on the superficial layer SC LFP response to visual stimuli: for (A) onset latency, (B) duration, and (C) peak amplitude. Error bars are equal to ±1 SEM. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made initially across rising cumulative doses of d-amphetamine or volumetrically equivalent doses of saline (A1, B1, C1). Comparisons were then made between the maximum dose of d-amphetamine or saline and subsequent doses of metergoline (A2, B2, C2).

## 3.4.3 Effects of Systemically Administered d -amphetamine and Metergoline on Superficial Layer SC MUA Response to Visual Stimuli

Intravenous injections of d-amphetamine caused a dose-dependent depression of the peak amplitude of the superficial layer SC MUA response to whole visual field flashes (see figure 3.4 for representative animal and figure 3.5 for comparisons between groups for onset, duration, and peak-to-peak amplitude). In contrast, peak amplitude rose over time in the saline condition. Administration of metergoline appeared to reverse the depression induced by d-amphetamine, causing the previously depressed amplitude of the response to rise towards levels recorded at the pre-drug baseline. DMSO alone had an effect on any aspect of the MUA response.



**Figure 3.4:** PSTH showing the effects of d -amphetamine on MUA response to visual stimuli of a representative animal. Data presented represent the mean response to 150 visual stimuli presented as whole visual field flashes (stimuli presented at t0). X axis is time; y axis is PSTH count, calculated as described in section 3.3.3.The red vertical line indicates the distinction between the first phase and second phase of the MUA response. A depression of each phase of the MUA response is observed following administration of d-amphetamine. Phase one show a greater absolute depression compared with the second phases. This depression is reversed for both phases following metergoline administration.

***The effect of d-amphetamine on collicular MUA response to visual stimuli***

A Mixed GG-ANOVA (factors: Dose [N=6], Drug [d-amphetamine or saline]) revealed a significant main effect of Drug (F[1, 18] = 21.91, p <0.001) and a significant Dose\*Drug interaction on MUA peak amplitude (F[2.31, 41.62] = 2.87, p = 0.021), but no main effect of Dose (F[2.31, 41.62] = 0.89, p = 0.427). Post-hoc independent t-tests revealed significant differences between groups for all doses (0.5mg/kg: t(18) = 2.15, p = 0.046; 1.0mg/kg: t(18) = 2.68, p = 0.015; 2.0mg/kg: t(18) = 3.27, p = 0.004; 4.0mg/kg: t(18) = 3.38, p = 0.003; 8.0mg/kg: t(18) = 3.88, p < 0.001; 16.0mg/kg: t(18) = 4.91, p < 0.001), suggesting that d-amphetamine depresses the superficial SC MUA response to visual stimuli at a range of doses. Further mixed GG-ANOVAs revealed no main effect of Drug or Dose, and no Drug\*Dose interaction for onset (Drug: F(1, 17) = 0.52, p = 0.477; Dose: F(1.83, 31.10) = 1.16, p = 0.324; Dose\*Drug: F(1.83, 31.10) = 1.74, p = 0.195) or duration (Drug: F(1, 18) = 0.403, p = 0.533; Dose: F(2.32, 41.81) = 1.31, p = 0.265; Dose\*Drug: F(2.32, 41.81) = 1.47, p = 0.241)

***The effect of metergoline on collicular MUA responses to visual stimuli***

To assess if antagonising 5-HT attenuated the effect of d-amphetamine on collicular responsiveness, additional t-tests were used to compare onset latency, duration and peak amplitude of the maximum dose of d-amphetamine (16.0mg/kg), or saline, with the two 1.0mg/kg doses of metergoline (maximum cumulative dose of 2.0mg/kg). A significant difference was revealed for peak amplitude between the maximum dose of d-amphetamine, and the maximum dose of metergoline (t(17) = 2.27, p = 0.036), No difference was revealed between the maximum dose of saline and any dose of metergoline for any measure. These results suggest that the d-amphetamine induced depression of the superficial SC MUA response to visual stimuli can be partially reversed by antagonising 5-HT.

**C1**

**C2**

**A1**

**A2**

**B1**

**B2**

Figure 3.5: The effect of systemic administration of d-amphetamine and metergoline on the superficial layer SC MUA response to visual stimuli: for (A) onset latency, (B) duration, and (C) peak amplitude. Error bars are equal to ±1 SEM. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made initially across rising cumulative doses of d-amphetamine or volumetrically equivalent doses of saline (A1, B1, C1). Comparisons were then made between the maximum dose of d-amphetamine or saline and subsequent doses of metergoline (A2, B2, C2).

## 3.4.4 Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli

Visual inspection revealed a multiphasic profile of the MUA response for 18/20 animals (90%), where the first 100ms of the response post-onset constituted the first phase of the response, and all further activity constituted the second phase of the response (see figure 3.4). Thus further analysis was conducted to extract the peak amplitude of each phase of this response.

Intravenous injections of d-amphetamine caused a dose-dependent depression for both phases of the multi-unit SC response to whole visual field flashes. For phase two, no depression was produced until dose 5 of d-amphetamine (8/.0mg/kg), while phase one of the response was depressed at all doses (see figure 3.6). In contrast, peak amplitude of both phases of the response rose over time in the saline condition. Administration of metergoline reversed the d-amphetamine induced depression of multi-unit SC response for both phases. For phase 1, reversal of the response brought peak amplitude in line with that observed for low doses of d-amphetamine, while for phase 2 peak amplitude was reversed to levels exceeding those recorded at baseline (see figure 3.6).

***The effect of d-amphetamine on discrete phases of the SC MUA response to visual stimuli***

For peak amplitude of phase one of the MUA response, a mixed GG-ANOVA (factors: dose [N=6], Drug [d-amphetamine or saline]) revealed a significant main effect of Drug (F[1,16] = 10.37, p = 0.005), but no main effect of Dose (F[2.37, 37.99] = 0.70, p = 0.524) and no Dose\*Drug interaction (F[2.37, 37.99] = 2.17, p = 0.120). Post-hoc independent t-tests revealed significant differences between groups for doses 2.0 to 16.0mg/kg (2.0mg/kg: t(16) = 2.11, p = 0.050; 4.0mg/kg: t(16) = 2.40, p = 0.028; 8.0mg/kg: t(16) = 2.62, p = 0.018; 16.0mg/kg: t(16) = 3.56, p = 0.002), suggesting that d-amphetamine depresses phase 1 of the superficial SC MUA response to visual stimuli at range of doses.

For peak amplitude of phase one of the MUA response, a mixed GG-ANOVA (factors: dose [N=6], Drug [d-amphetamine or saline]) revealed a significant Dose\*Drug interaction (F[2.07, 28.89] = 5.93, p < 0.001), but no main effect of Dose (F[2.07, 28.89] = 0.69, p = 0.513) or Drug (F[1, 14] = 1.48, p = 0.241). Post-hoc independent t-tests revealed significant differences between groups for doses of 8.0mg/kg (t(15) = 2.27, p = 0.038) and 16.0mg/kg (t(15) = 2.49, p = 0.025), suggesting that d-amphetamine depresses phase 2 of the superficial SC MUA response to visual stimuli only at high doses.

***The effect of metergoline on discrete phases of the SC MUA response to visual stimuli***

To assess if antagonising 5-HT attenuated the effect of d-amphetamine on collicular responsiveness, additional t-tests were used to compare peak amplitude of each phase of the MUA response of the maximum dose of d-amphetamine (16.0mg/kg), or saline, with the two 1.0mg/kg doses of metergoline (maximum cumulative dose of 2.0mg/kg. For both phase 1 and phase 2, a significant difference was revealed between the maximum dose of d-amphetamine condition, and the maximum dose of metergoline (phase 1: t(17) = 2.03, p = 0.032; phase 2: t(15) = 1.84, p = 0.045), suggesting that the d-amphetamine induced depression of the both phases of the superficial SC MUA response to visual stimuli can be reversed, at least partially, by antagonising 5-HT. No significant difference was found between the maximum dose of saline and any dose of metergoline.

**A1**

**A2**

**B1**

**B2**

Figure 3.6: The effect of systemic administration of d-amphetamine and metergoline on the peak amplitude of the superficial layer SC multiphasic MUA response to visual stimuli: for (A) phase 1, and (B) phase 2. Error bars are equal to ±1 SEM. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made initially across rising cumulative doses of d-amphetamine or volumetrically equivalent doses of saline (A1, B1). Comparisons were then made between the maximum dose of d-amphetamine or saline and subsequent doses of metergoline (A2, B2)

# 3.5 Discussion

## 3.5.1 Interpretation of Results

The present study aimed to explore whether d-amphetamine action at the level of the SC is mediated by 5-HT, by following systemic d-amphetamine administration with the administration of the broad spectrum 5-HT antagonist metergoline. The LFP response of superficial layer SC responses to whole field visual stimuli were complex, multicomponent phenomena, consistent with previous reports (e.g. Dyer and Annau, 1977; Gowen et al., 2008; Clements et al., 2014). Based on electrophysiological and histological criteria, electrode placement was considered to be localised to the SGS or SO for the majority of animals. Previous reports have described onset latency of the superficial layer SC LFP response to whole visual filed stimuli as ranging from 28 ms (Dyer and Annau, 1997) to 50-60 ms (Fortin, Itaya, Chemtob, & Molotchnikoff, 1997), which is consistent with our own observations (mean onset latency at baseline for all groups = 51.61 ms).

The major effect of d-amphetamine in the current study was to reduce the amplitude of both the LFP and MUA of visually evoked responses in the superficial layers of the SC. As the major contributing component of the LFP comprises of synchronous synaptic activity (Buzsaki, Anastassiou, & Koch, 2012), while the MUA represents the spiking activity of neurons in an approximate 100μm radius (Buzsaki et al., 2012; Legatt, Arezzo, & Vaughan, 1980), these results suggest that d-amphetamine reduces both the input to (synaptic activity; LFP) and output from (spiking activity; MUA) superficial layer SC neurons. The effects of d-amphetamine on both LFP and MUA amplitude were dose dependent, with the strongest depression recorded at the highest dose (16mg/kg, figures 3.3 and 3.5). When the multi-unit response was split into an initial and later phase (see figure 3.4 for visualisation of phase timings), it was observed for phase 1 that d-amphetamine depressed the amplitude of the response at all doses, while for phase 2 only high doses of d-amphetamine (8.0mg/kg, 16.0mg/kg) depressed the amplitude of the response (figure 3.6). Following the full range of d-amphetamine or saline injections, two doses of metergoline were administered. The major effect of metergoline was to partially reverse the depressant effect of d-amphetamine on the peak amplitude of both phases of the MUA response to whole field visual stimuli. Trends towards reversal were also observed for the LFP subsequent to metergoline administration, but also when DMSO was administered alone, suggesting a potential confound of the vehicle of metergoline on the LFP response. Again, these effects were dose dependent, with the highest reversal observed at 2.0mg/kg of metergoline. For most measures the reversal, while significant, was incomplete. For phase 2 of the MUA, however, the peak amplitude post metergoline administration exceeded the peak amplitude recorded at baseline.

While d-amphetamine produced a depression on the amplitude of visual responsiveness in the colliculus, the true extent of this depression may be masked by the effects of the vehicle. Visually-evoked responses rose over time in the saline condition at equivalent doses to d-amphetamine for both LFP and MUA. While non-significant, trends towards enhancement were observed for onset and duration across doses of saline, though this was not always in contrast to the effect of d-amphetamine. It may therefore be suggested that the depressant effect of d-amphetamine on peak amplitude of the LFP and MUA was produced despite an unknown physiological effect caused by either saline, or time. While this is a somewhat surprising effect, it is not novel. Previous research comparing the effects of visual responsiveness of the SC following administration of either d-amphetamine to saline have similarly reported that an enhancement in the amplitude of visual responsiveness relative to baseline recordings in the control conditions (Gowen et al., 2008). While the current study was naïve to any potential effect of increasing volumes of injectate, Gowen et al., (2008) controlled for this, while still observing an increase in visual responsiveness the saline group, finding that a single injection of saline would cause an increase in the collicular response to visual stimuli over time. In both the present, and Gowen’s et al’s study, urethane anaesthesia was used. While urethane has long been established for use in rodent experimental preparations due to the relatively stable anaesthetic plane produced (Maggi & Meli, 1986), more recent evidence suggests anaesthetic depth when using urethane mimics the full spectrum seen during natural sleep (Clement et al., 2008). Depth of urethane anaesthesia can affect the amplitude of multi-unit (Erchova, Lebedev, & Diamond, 2002) and LFP (Dyer & Rigdon, 1987) responses to sensory stimuli at both a cortical (Devonshire, Grandy, Dommett, & Greenfield, 2010), and subcortical level (Huh & Cho, 2013). As data collection in the present study comprised of a time period of approximately 1.25 hr, it is feasible that a lightening of anaesthetic depth may account for the enhancement observed in the saline group. Thus, while a significant depression of the amplitude of the LFP and MUA were observed following d-amphetamine administration, the true extent of this depression may have been masked by an altering of anaesthetic depth throughout the study. Future work that assesses pharmacological manipulation of sensory responses in the SC should aim to avoid this confound by monitoring physiology that can act as surrogate measures of anaesthetic (e.g. respiratory rate and cortical EEG power, Musizza et al., 2007)

The finding that d-amphetamine depresses visually evoked responses in superficial layers of the SC is consistent with previous reports (Gowen et al., 2008; Dommett et al., 2009; Clements et al., 2014). While Dommett et al., showed that the effects of d-amphetamine can be blocked in vitro by introducing a 5-HT antagonist, the present study is the first to show that d-amphetamine depression of the SC visually evoked response can be reversed by antagonising 5-HT transmission in vivo. Metergoline is a competitive 5-HT antagonist (Cox & Ennis, 1982; Terrón, 1997), that reversibly antagonises the effects of 5-HT at a range of cortical and sub-cortical targets (e.g. (Sastry & Phillis, 1977). While metergoline it is a broad spectrum 5-HT antagonist, its strongest affinity is for 5-HT1, 5-HT2, and 5-HT7 receptors (T. Sharp, Bramwell, Hjorth, & Grahame‐Smith, 1989). As 5-HT1 receptors are the most plentiful serotonergic receptors in the superficial layers of the SC (Shukla, Watakabe, & Yamamori, 2014), it can be suggested that the effects of metergoline to reverse the action of d-amphetamine was likely mediated by 5-HT at the level of the SC. Though metergoline administration significantly reversed the effects of d-amphetamine towards baseline, for most measures this was not a complete reversal. Instead, the amplitude of the response was reversed to levels that resembled low doses of d-amphetamine. Interestingly, for phase 2 of the MUA response d-amphetamine did not begin to have a depressant effect until high doses were administered, yet this was the only measure for which metergoline fully reversed the effects of d-amphetamine. Gowen et al., (2008) postulated that low and high doses may differentially affect NA and 5-HT transmission, with low doses targeting NA and high doses targeting 5-HT. While psychostimulants are believed to target NA at a range of doses (Easton et al., 2007), higher doses are required to target 5-HT transmission (Holmes & Rutledge, 1976; Kuczenski & Segal, 1989). As outlined in the introduction, the superficial layers of the SC are strongly innervated by both NA and 5-HT (Wichmann & Starke, 1988), with previous reports highlighting that NA depresses superficial SC visual responses (Tan, Mooney, & Rhoades, 1999). Taken together with our findings of an incomplete reversal of the effects of d-amphetamine following the introduction of a 5-HT antagonist, it is therefore plausible that in the current study low doses of d-amphetamine targeted NA while higher doses targeted 5-HT. It is also plausible that the effects of d-amphetamine of phase 2 of the MUA responses were wholly mediated by 5-HT.

Though this study is the first to describe differential pharmacological action of d-amphetamine and metergoline on the biphasic SC MUA response, it is not the first to describe the SC response to visual stimuli as biphasic. McPeek and Keller (2002) recorded visual, movement, and visuo-movement neurons in the monkey SC as subjects performed a visual search task. McPeek and Keller observed a subset of visuo-movement neurons that displayed a biphasic response pattern during the search task. This subset, termed visuo-motor burst neurons, had an onset latency of approximately 100ms and was found to modulate target selection. For these neurons phase 1 of the response corresponded to the typical initial SC response to presentation of visual stimuli, while the phase 2 of the response was of more variable strength and discriminated targets from distractors during the visual search task. Thus, phase 2 of the visuo-motor burst neurons is believed to guide saccadic target selection. If phase 2 of the MUA response in our study represents the activity of visuo-motor burst neurons, depression of this aspect of the SC response may serve to reduce foveation towards visual distractors. Thus, within the context of ADHD, one potential mechanism for D-amphetamine may be to enhance sustained attention by reducing the likelihood that visuo-motor burst neurons will fire in response to non-salient stimuli.

## 3.5.2 Implications of Results

There is now converging evidence from pharmacological studies that psychostimulants act to depress visually evoked responses at the level of the superior colliculus (Gowen et al., 2008; Dommett et al., 2009; Clements et al., 2014). Recall from the introduction that robust behavioural evidence links distractibility to the SC, specifically due to the role of the SC in foveation towards distracting stimuli (e.g. Roberts et al., 2011; Fried et al., 2014). If collicular afferent activity is depressed, it follows that diversion of attentional resources towards these distracting stimuli is less likely. The SC constitutes an important input pathway for BG, one of the brain’s major action selection centres (Comoli et al., 2006). The manner by which the BG influences action selection is conceptualised as “bids” for motor expression from any system capable of initiating motor activity (Gurney, Prescott, & Redgrave, 2001; Redgrave et al., 1999). Within this framework enhanced activity is conceived as submitting a stronger “big” to the central action selection system (the BG). It thus follows that the strongest “bid” in the system at a given time is most likely to generate a motor output (for a bid from the SC for example this may be expressed as a saccadic eye movement). By depressing collicular activity a weakened “bid” is submitted for action selection, and thus saccadic eye movements are unlikely to be expressed, with the resultant outcome of reduced distractibility and enhanced sustained attention.

A major finding of the current study is that d-amphetamine action at the level of the SC is, at least partially, mediated by 5-HT. As outlined above visuo-motor burst neurons play a major role in selection of targets for foveating eye movements. As these neurons may be particularly sensitive to manipulation of 5-HT transmission, this could provide a fruitful pathway for development of therapeutic interventions for ADHD. 5-HT transmission has previously been investigated as a potential psychotherapeutic target in ADHD. In an open label preliminary clinical trial assessing the efficacy of treating ADHD with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, (Barrickman, Noyes, Kuperman, Schumacher, & Verda, 1991) demonstrated that 60% of participants showed at least moderate improvement in symptoms. This trial, however, failed to stratify participants by symptom clusters. As the results of the current study suggest that 5-HT transmission at the level of the SC may be a potential pharmacological target for reducing distractibility, it is possible that primarily inattentive ADHD patients may have responded more strongly in this trial than primarily hyperkinetic or combined type. Thus, Chapter 5 will comprise of a review of 5-HT pharmacotherapy in ADHD with a view of identifying a potential candidate drug that may have comparable mechanism of action to d-amphetamine.

## 3.5.3 Future Direction

Before further considering the potential to exploit 5-HT transmission in the pharmacotherapy of ADHD, it is first necessary to confirm the SC as the true locus of action of d-amphetamine and metergoline in the present study. While previous research has confirmed that d-amphetamine depresses visual responsiveness when administered using intra-collicular injections (Gowen et al., 2008), those studies did not attempt to antagonise the effects of 5-HT, and did not consider the biphasic quality of the MUA response. We must first therefore use local intra-collicular injections to confirm that (a) antagonism by metergoline is preserved when d-amphetamine is delivered locally (b) the biphasic properties of the MUA response are preserved when d-amphetamine is administered locally and (c) if the biphasic properties of the MUA response are preserved, then the dichotomous response of each phase to pharmacological manipulation is also preserved.

Effect of 5-HT Antagonism on Intracranial Amphetamine-Induced Suppression of Collicular Visual Responses

# 4.1 Chapter Summary

It was established in the previous chapter that systemic administration of D-amphetamine depresses the superficial layer SC response to visual stimuli. This effect was reversed following the administration of the 5-HT antagonist metergoline. Before we can discuss the potential to further exploit collicular 5-HT transmission in the pharmacotherapy of ADHD we must first confirm the SC as a true target of D-amphetamine and metergoline. The following chapter presents evidence that D-amphetamine operates locally within the SC and that its potential mechanism is mediated, at least in part, by 5-HT.

# 4.2 Introduction

## 4.2.1 Rationale for the Present Study

There is mounting evidence that the SC is well placed as a locus of interest when investigating pharmacological interventions to normalise distractibility in ADHD (e.g. Overton et al., 2008; Clements et al., 2014; see chapter 3.2 for an overview of the literature linking distractibility to the SC). The data presented in the previous chapter adds to this body of evidence by showing that the depressant effect of the D-amphetamine on visual activity in the superficial SC is mediated at least in part by 5-HT in vivo. However, as d-amphetamine and metergoline were perfused systemically in the previous study it was not possible to establish if the effect on the collicular response was a result of these drugs operating locally within the SC or whether it was incidental of afferent modulation of the input to the SC. It has already been substantiated that d-amphetamine operates locally to depress the response to visual stimuli when delivered directly to the superficial layers of the SC (Gowen et al., 2008), but the effect of antagonising 5-HT transmission when d-amphetamine is delivered locally has not been established. As the ultimate aim of this thesis is to explore the potential to exploit collicular 5-HT transmission as a target of therapeutic intervention in ADHD, we must confirm if local operations of d-amphetamine at the level of the SC are modulated by 5-HT in vivo. To do so we will establish the effect of 5-HT antagonism on the collicular response to visual stimuli when D-amphetamine is injected directly into the SC.

## 4.2.2 Pharmacological Targets of Psychostimulant Action and Their Relevance to the SC

To understand why systemic d-amphetamine amine may very well act locally in the colliculus to depress visual activity, we must first discuss the pharmacology and targets of d-amphetamine. It is widely understood that d-amphetamine acts to non-selectively increase synaptic levels of the monoamine neurotransmitters, DA, NA, and 5-HT (Heal, Cheetham, & Smith, 2009; Holmes & Rutledge, 1976; Rothman et al., 2001). Under normal conditions, synaptic monoamine levels are regulated by monoamine reuptake transporters (e.g. the serotonin transporter, SERT), whereby monoamine neurotransmitters are transported back into presynaptic terminals and taken up by local glia. d-amphetamine is a substrate of the monoamine transporters and thus competes with endogenous monoamines for transport into the presynaptic terminal. Once inside the presynaptic terminal, d-amphetamine displays high affinity for the vesicular monoamine transporter (VMAT2; (Erickson, Schafer, Bonner, Eiden, & Weihe, 1996), the complex responsible for transportation of monoamines into vesicles for later exocytosis (Schuldiner, Shirvan, & Linial, 1995). Upon binding with VMAT2 D-amphetamine induces a depletion of synaptic vesicles, increasing intracellular monoamine concentration (Eiden & Weihe, 2011). This change in monoamine concentration reverses the direction of the plasma membrane monoamine transporters, thus promoting retro transportation of monoamines into the synaptic cleft (Robertson, Matthies, & Galli, 2009). While retro transportation is the main mechanism by which d-amphetamine increases extracellular monoamine levels, d-amphetamine also acts a weak inhibitor of monoamine oxidase, the mitochondrial-bound enzyme that catabolises monoamines (H. Miller, Shore, & Clarke, 1980; Robinson, 1985), thus inhibiting the breakdown of monoamines resulting in extended occupation of the synaptic cleft.

Recall from the introduction that the SC the expresses the necessary receptors for d-amphetamine action. The superficial visual layers of the SC are extensively innervated by 5-HT and, to a less extent, NA (Mize & Horner, 1989; Wichmann & Starke, 1988). Notably, dense 5-HT1A and 5-HT1B receptors have been reported on retinotectal axon terminals in the SGS, suggesting that retinal input to the SC is modulated at least in part by 5-HT (May, 2006). The expression of the receptors at the level of the SC, combined with reports that 5-HT antagonism reverses d-amphetamine induced depression of the collicular response to visual stimuli (Dommett et al., 2009, data presented in Chapter 3) presents strong evidence that d-amphetamine targets 5-HT transmission in the SC. While these previous reports are compelling, the effect of antagonising 5-HT when d-amphetamine is operating locally within this SC in vivo has not been established. Thus the results presented in Chapter 3, though unlikely, may have been incidental of afferent modulation of the input to the SC. As outlined in Chapter 1, the SC constitutes a critical hub in the orienting network and plays a key role in the generation of saccadic eye movements to salient stimuli (Sparks, 1999; Stein & Meredith, 1993; Whittaker & Cummings, 1990). Within this network, the main sources of visual input to the SC arise directly from the retinotectal pathway, and indirectly via the retino-geniculo-cortical pathway (Boehnke and Munoz, 2008; see figure 1). Visual input to the SC via the retino-geniculo-cortical pathway may be particularly sensitive to afferent modulation by d-amphetamine as this pathway contains several known targets of psychostimulants (e.g. (Easton et al., 2007; Ferris et al., 2015; Rose et al., 2006); see figure 4.1)

**Figure 4.1:** Connectivity of the orienting network including the retino-geniculo-cortical pathway and the fronto-striatal-tectal (adapted from Boehnke and Munoz, 2008). Extra-collicular areas highlighted in blue are known targets of psychostimulant action

LGN

Visual cortex

Parietal cortex

Frontal cortex

SNc

Pulvinar

Anterior thalamus

SCd

SCs

Retina

Pretectum

Striatum

SNr

Psychostimulants have been shown to target various nuclei along both the retino-geniculo-cortical pathway and the fronto-striatal-tectal pathway the SC, as detailed below (see (Faraone, 2018)8 for review).

***Thalamic nuclei***

Various nuclei of the thalamus, principally the lateral geniculate nucleus (LGN), act as a relays for ascending visual information (Guillery & Sherman, 2002). Evidence from human neuroimaging studies suggests enhanced activity within thalamic nuclei following therapeutically relevant oral doses of D-amphetamine. Increases have been observed in cerebral metabolic rate for glucose utilisation (CMRglu, as measured with PET; (Vollenweider, Maguire, Leenders, Mathys, & Angst, 1998) in the thalamus, and cerebral blood flow (CBF, as measured with SPECT; Devous et al., 2001) in the anterior thalamus. Similar results have been demonstrated in murine models. Easton et al. (2007) showed enhanced BOLD response in the LGN and pretectum of the rat following intraperitoneal injections of d-amphetamine. Additionally, Navarra, Clark, Zitnik, and Waterhouse (2013) recorded responses of LGN neurons to visual stimuli following intraperitoneal injections of the psychostimulant methylphenidate. Methylphenidate acted to enhance both the amplitude and duration of LGN neuron’s response to visual stimuli.

***Basal ganglia***

The reciprocal innervation between the SC and the BG is believed to play a key role in the selection of saccadic targets, and thus, distractibility (Redgrave et al., 1999; Overton, 2008). Rose et al., (2006) employed dynamic susceptibility contrast perfusion MRI to assess haemodynamic changes associated with therapeutically relevant oral doses of d-amphetamine. Following administration of d-amphetamine, significant changes in CBF were observed within striatal structures and across the nigrostriatal circuit. Similar findings have been reported by Schouw et al. (2013). Schouw et al. used arterial spin labelling to observe increased CBF in the striatum following oral ingestion of d-amphetamine. As well as inducing haemodynamic changes within the BG, d-amphetamine has a direct effect on neural excitability. When injected directly into substantia nigra pars reticularta (SNr), d-amphetamine produces a rapid decrease in multi-unit SNr activity (Timmerman & Abercrombie, 1996).

The evidence presented above highlights a major limitation of the data presented in chapter 3. While it was shown that d-amphetamine induced depression of the superficial SC response to visual stimuli can be reversed by antagonising 5-HT in vivo, as these drugs were perfused systemically we cannot be certain of the level at which these drugs operated. As such, it was not possible to identify if the effect of d-amphetamine on the SC response were mediated locally within the SC or if this was incidental of afferent modulation of the input to the SC. The second phase of the collicular MUA response described in chapter 3 may be of particular relevance here. The onset latency of this response is such that it may be especially sensitive to afferent modulation from cortical and BG input (Boehnke & Munoz, 2008; Dorris, Olivier, & Munoz, 2007; Jiang & Stein, 2003). The only other study to consider antagonism of 5-HT following application of d-amphetamine has similar limitations. The effect of d-amphetamine on the retinotectal input to the SC was explored by Dommett et al., (2009), who evoked excitatory postsynaptic potentials in the SC by electrically stimulating afferent optic tract fibres in vitro. While this allowed for delineation of how 5-HT mediates direct retinal input to the SC, modulation of input to the SC via the retino-geniculo-cortical pathway and several other subcortical nuclei was not established. As such, while we can begin to present evidence that d-amphetamine targets 5-HT transmission in the superficial layers of the SC, the evidence for this effect *in vivo* is not yet fully compelling. Thus, the next step is to confirm that intra-collicular administration of d-amphetamine depresses the response of the SC to visual stimuli, and that this depression is reversed when 5-HT transmission is antagonised in vivo.

## 4.2.3 Aim of the Present Study

The aim of the present study is to establish whether the effects of intracollicular administration of d-amphetamine on the visual responses in the superficial SC are mediated by 5-HT in vivo. We have presented evidence showing that d-amphetamine reduces the response of superficial layer SC neurons to visual stimuli in vivo and that this effect appears to be mediated, at least in part, by 5-HT. While this result is consistent with converging evidence, uncertainty remains regarding the level at which d-amphetamine operates to depress the collicular response to visual stimuli. Consequently, the current study utilises in vivo electrophysiological techniques to record the responses of superficial SC neurons following intracollicular injections of d-amphetamine and subsequent systemic injections of the 5-HT antagonist metergoline.

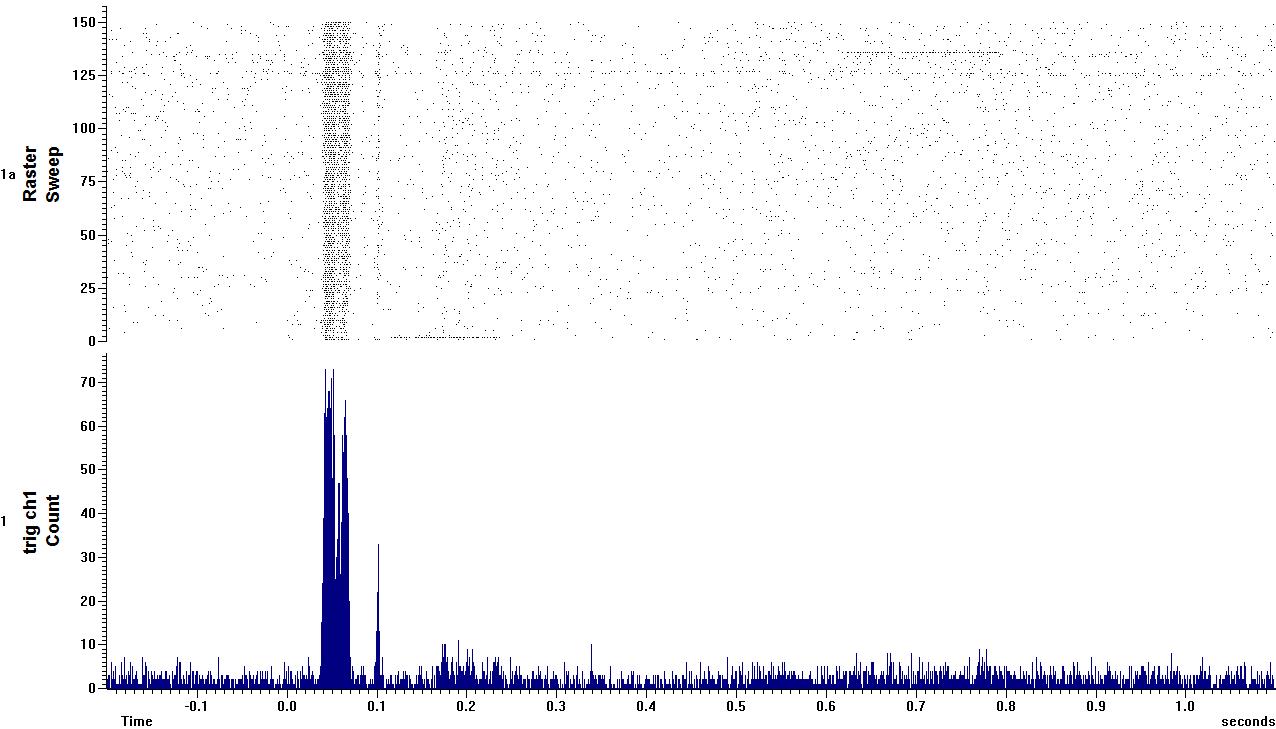
# 4.3 Methods

## 4.3.1 Subjects

Data were obtained from 20 male Hooded Lister rats (bred in house; weight 300-500g). Animals were housed together on a 12 hr light/dark cycle with food and water supplied ad libitum. All procedures were performed according to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and the Animals (Scientific Procedures) Act, 1986 (revised 2013). Every effort was made to minimise suffering to subjects and to reduce the number of animals used.

## 4.3.2 Experimental Procedures

Subjects were anaesthetised with urethane (ethyl carbonate as a 25% aqueous solution) and prepared for cannulation and craniotomy surgery as described in chapter 2. Once both surgeries were complete and the cortex overlaying the SC was exposed, an LED was positioned over the eye contralateral to the exposed SC in order to provide whole field illumination with which to guide electrode placement. A single channel tungsten parylene C microelectrode coupled with a custom built microinjector (see chapter 2.2) was then slowly advanced through the exposed cortex (6.3mm caudal to bregma, and 2.0mm lateral to midline) towards the SC using the multi-unit response to whole-field light flashes as guide (10ms duration, 0.5 Hz with 30% jitter; as described in chapter 3.3.2). Upon encountering the visually evoked response stereotypical of the superficial layers of the SC (average placement depth of 3.06mm) the response to 150 visual stimuli was recorded to confirm response stability (see figure 4.2). Once a stable response to 150 visual stimuli was attained the animal was dark adapted for a minimum of 40min.



**Figure 4.2:** A raster plot and PSTH of the multiunit response to 150 visual stimuli after advancing the electrode towards the SC. Electrode placement is confirmed in vivo by using the stereotypical SC response to visual stimuli.

Following dark adaptation, the optimal stimulus luminance was determined as outlined in chapter 2.4.1. Once an optimal stimulus intensity that reliably evoked a stable LFP and MUA response was established, a baseline pre-drug response was recorded to 150 whole field light flashes (10ms duration, 0.5 Hz with 30% jitter). This was immediately followed by a single intracollicular injection of d-amphetamine (1.0μg in 1.0μl of 0.9% saline, injection rate of 0.5μg/min as controlled by a digital syringe pump; KD Scientific. Doses based on prior work demonstrating that D-amphetamine operates at the level of the SC when administered at the doses used here; Gowan et al., 2008). The local d-amphetamine dose was determined through pilot experiments as the lowest intracollicular dose that reliably depressed the response to visual stimuli. After allowing 2min for the drug to spread from the injection site, the response to 150 visual stimuli was acquired. Following this, a single bolus i.v. injection of metergoline (experimental group, N =10; 2.0mg/kg in 0.3ml of 60% DMSO) or 60% DMSO in distilled water (control group, N = 10; 0.3ml) was administered. After allowing 2 min for the drug to wear on, the response to 150 visual stimuli was acquired. While it may have been preferential to also delivery metergoline via a local route in the present study, it should be noted that the solvent needed for delivery of metergoline, DMSO, has strong potential for disruption when delivered locally. DMSO is known to alter neural excitability (Sawada & Sato, 1975), and when exposed directly to neurons and glia may disrupt their morphology (C. Zhang et al., 2016), and has the potential to induce apoptosis (Hanslick et al., 2009). Thus i.v. injections were used, as established in the previous chapter.

Upon completion of the procedure, animals were immediately euthanised with pentobarbital (1.0ml i.v. injection), following which, brains were immediately removed and placed in fixative for later histological confirmation of electrode placement, as outlined in chapter 2.5.

## 4.3.3 Data Analysis

Data were prepared for analysis as described in chapter 2.4.2. All data were analysed offline using custom written MATLAB scripts (2016a; The MathWorks Inc., Natick, MA, USA). All analyses were carried out on average data constructed from the response to 150 visual stimuli recorded at three time points: (1) pre-drug baseline (2) following local injection of d-amphetamine (3) following systemic infusion of metergoline or DMSO (see figure 4.3).

Experimental group

Control group

1. Recording of pre-drug baseline response

3.a. 2.0mg/kg metergoline i.v.

3.b. 0.3ml DMSO i.v.

**Figure 4.3:** Sequencing of drug injections throughout the experimental procedure. 2 min after each injection (or immediately for the baseline recording), responses to 150 visual stimuli were recorded (10ms duration, 0.5 Hz with 30% jitter).

2. 1.0μg d-amphetamine, intracollicular injection

The main comparisons of interest were between doses of d-amphetamine and metergoline/DMSO (i.e. between time points 2 and 3) and between metergoline and DMSO subsequent to the administration of d-amphetamine. For statistical purposes, all LFP and MUA data used in these comparisons were normalised by dividing post-drug responses by the baseline (pre-drug) responses for each animal. Raw data for pre-drug and d-amphetamine were compared to confirm the expected depression of the SC MUA and LFP response to visual stimuli. Data for onset latency, response duration, and peak amplitude were extracted from both raw MUA and LFP data values for use in these comparisons.

*Local field potential.* Three measures of interest were extracted from averaged LFP data: onset latency, response duration, and peak-to-peak amplitude. In order to extract these measures, a response threshold was constructed. This process comprised of two steps:

1. Calculating the mean and SD of the low frequency voltage trace over in the 400ms period prior to stimulus presentation.
2. Constructing a response threshold, defined as any point at which the LFP waveform differed from the pre-stimulus mean by 3SDs.

Onset latency was defined as the first time point at which the LFP waveform differed from the pre-stimulus mean by 3SDs. Offset latency was then calculated using a two-step process. First, LFP activity in the post-onset period was divided into epochs of 100ms starting from the first time point immediately post onset. The mean voltage trace was then calculated for each epoch, thus allowing for identification of the 100ms period where the post-onset voltage trace most closely resembled the pre-stimulus mean. Once this 100ms period was identified, onset latency was derived as the single time point within this period at which the LFP voltage trace was closest to the pre-stimulus mean. Response duration was then derived as the difference between onset and offset latency. Peak-to-peak amplitude was then determined as the difference between maximum voltage trace and minimum the voltage trace within the duration of the response.

*Multi-unit activity*. Four measures of interest were extracted from the averaged MUA data: baseline activity, onset latency, response duration, and peak amplitude. To derive these measures, it was first necessary to undertake a process to identify and extract spikes. Thresholding was applied to facilitate the extraction of spikes. This was achieved for each individual animal by calculating the mean and SD of the high frequency voltage trace for the 400ms pre-stimulus time period for each of the 150 stimulus presentations in the pre-drug baseline recording. A spiking threshold was then calculated by averaging the mean of the 400ms pre-stimulus voltage trace across all stimulus presentations in the pre-drug baseline recording. Spikes were defined as activity for which the high frequency voltage trace exceeded 2SDs of the spiking threshold. This pre-drug spiking threshold was applied across all drug doses so as to avoid any potential confounding effect of drug-induced changes in activity on spike detection. Spikes were extracted from the response to 150 visual stimuli at the time periods shown in figure 4.3. This data was then used construct a peri-stimulus time histogram for each time point (PSTH; bin width = 10ms, count per bin normalised to number of stimuli), from which values for baseline activity, onset latency, response duration and peak amplitude were extracted. Baseline activity, was derived by calculating the mean bin count in the 400ms pre-stimulus time period. As well acting as a measure of spontaneous neural activity in the SC, baseline activity was also used as a reference point for the construction of a response threshold (defined as any activity that exceeds baseline activity by 5SDs) to facilitate the extraction of onset latency and response duration. Onset latency was thus derived as the first time point subsequent to stimulus presentation where the PSTH bin count exceeded this response threshold. Onset latency was determined as the first time point for which PSTH bin count was equal or less than baseline activity whilst remaining below the response threshold for a period of 400ms. Response duration was then derived as the difference between onset and offset latency. Finally, peak amplitude was calculated as the maximum single bin count within the response duration, normalised to baseline activity.

As established in chapter 3, the superficial layer SC MUA response to visual stimuli comprises of a multi-phasic response, whereby the first 100ms of the response constitutes the first phase and all subsequent activity constitutes the second phase. As such, the peak amplitude of the response was calculated for the MUA response overall, as well as for phases 1 and 2. See figure 4.5 for depiction of MUA PSTH showing the distinction between the phases.

LFP and MUA responses were analysed using t-tests. For all analyses, alpha values of p < 0.05 were considered significant.

# 4.4 Results

## 4.4.1 Injector Coupled Electrode Placement

Brains were prepared for histological inspection as described in Chapter 2.6. Inspection of electrode tracks revealed that recording sites were located in the stratum SGS or SO. Due to insult to tissue derived from the histology process, visual inspection could not always reliably distinguish if recording sites were located in SO or SGS. Where this was the case, characteristics of the baseline LFP response were used to aid in distinguishing between the collicular laminar.

For all animals, the baseline superficial SC LFP response to whole visual field stimuli comprised of a complex multi-component phenomena. For the majority of animals where LFP was obtained (14/20; 70.0%) the most salient short-latency LFP component consisted of a short duration positivity (most likely corresponding to components P1-P3 of the superficial SC response to visual stimuli; Dyer and Annau, 1977), followed by a long duration, large amplitude negativity (most likely corresponding to components N4 and N5). The initial positive deflection was in some animals (6/20; 30%) proceeded by a short duration, low amplitude negativity (N1). Such LFP dynamics are typical of electrode placement in SGS. Deeper electrode placement in SO is associated with a reversal of these major field components (Dyer and Annau, 1977; Hirai and Okada, 1995). Thus the majority of animals were considered to have electrode (and thus injector) placement in the SGS. Where a negative deflection was the most salient short latency LFP aspect (n=6, 25%) electrode and injector placement was considered to be in the SO.

## 4.4.2 Effects of Administration of Local d-amphetamine and Systemic Metergoline on Superficial Layer SC LFP Response to Visual Stimuli

Intracollicular injections of d-amphetamine caused a dose-dependent depression of the peak-to-peak amplitude of the superficial layer SC LFP response to whole visual field flashes (see figure 4.4 for representative animal and figure 4.6 for comparisons between groups for onset, duration, and peak-to-peak amplitude). Both DMSO and metergoline produced a significant enhancement the amplitude of the LFP response, when administered subsequent to d-amphetamine.



AU

Amplitude (mV)

**Figure 4.4:** Effects of d -amphetamine and metergoline on the LFP response to visual stimuli in a representative animal. X axis is time, corresponding to 0.8s of response immediately following stimulus at t0; y axis is voltage deflection in arbitrary units. A significant depressant on amplitude of the LFP is observed following administration of the maximum cumulative dose of d-amphetamine. Following administration of metergoline or DMSO, the previously depresses response is enhances towards levels recorded at the pre-drug baseline.

Paired t-tests revealed that d-amphetamine administration depressed the peak-to-peak amplitude of the LFP response for both the metergoline group (t(8) = 3.46, p = 0.007) and the DMSO control group (t(8) = 4.15, p = .002). d-amphetamine administration had no effect on onset or duration for either group (metergoline group: onset: t(8) = 1.70, p = 0.131; duration: t(8) = 0.35, p = 0.734; DMSO group: onset: (t(9) = 2.05, p = 0.070; duration: (t(9) = 0.42, p = 0.685). Further paired t-tests revealed that administration of either metergoline or DMSO subsequent to d-amphetamine enhanced the peak-to-peak amplitude of the LFP response (metergoline: t(8) = 3.279, p = 0.011; DMSO: t(9) = 5.475 p < 0.001). Neither metergoline nor DMSO had an effect on onset or duration (metergoline group: onset: t(8) = 0.094, p = 0.379; duration: t(8) = 0.58, p = 0.575; DMSO group: onset: t(9) = 1.14, p = 0.282; duration: t(9) = 0.71, p = 0.498). T-tests revealed no difference between onset, duration, or peak-to-peak amplitude for Dose 2 between the metergoline condition and the DMSO condition (Onset: t(17) = 0.54, p = 0.596; Duration: t(17) = 0.83, p = 0.217 peak: t(17) = 0.40, p = 0.791). Thus, in the current study, the main effect of d-amphetamine on the LFP response to visual stimuli was to depress the amplitude responses. This depression was reversed by both metergoline and DMSO.

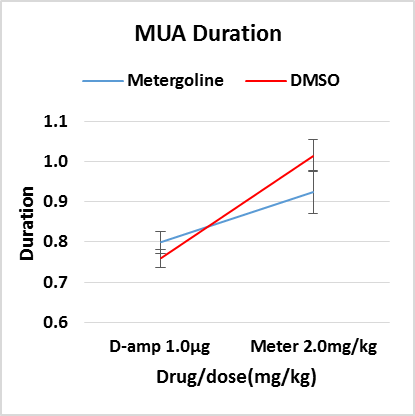
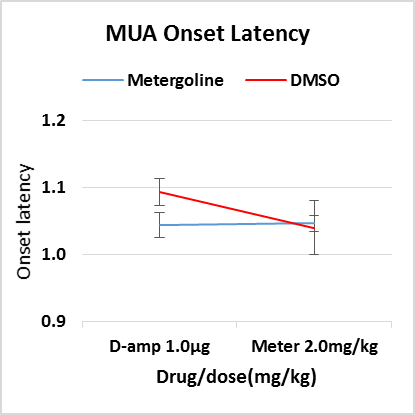
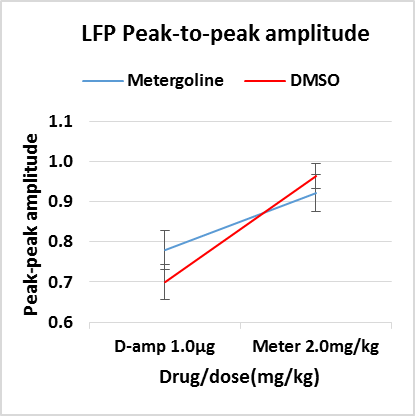
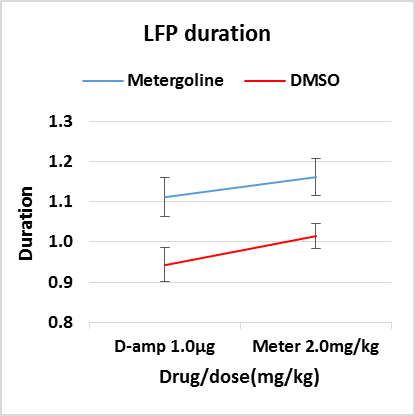
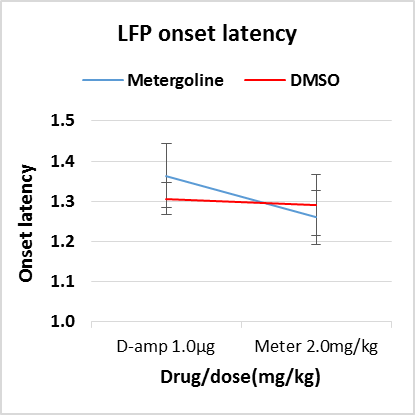
## 4.4.3 Effects of Administration of Local d-amphetamine and Systemic Metergoline on Superficial Layer SC MUA Response to Visual Stimuli

Intracollicular injections of d-amphetamine caused a dose-dependent depression of the peak amplitude and duration of the superficial layer SC MUA response to whole visual field flashes (see figure 4.5 for representative animal and figure 4.6 for comparisons between groups for onset, duration, and peak-to-peak amplitude). Metergoline, but not DMSO alone, produced a significant enhancement the amplitude of the MUA response, when administered subsequent to d-amphetamine.



Figure 4.5: PSTH showing the effects of d -amphetamine and metergoline on the LFP response to visual stimuli in a representative animal. Data presented represent the mean response to 150 visual stimuli presented as a whole visual field flashes (stimuli presented at t0). X axis is time; y axis is PSTH count, calculated as described in section 4.3.3. The red vertical line indicates the distinction between the first phase and second phase of the MUA response. A depression of each phase of the MUA response is observed following administration of D-amphetamine. This depression is reversed for both phases following metergoline administration.

Paired t-tests revealed that d-amphetamine administration depressed the duration and peak amplitude of the MUA response for both the metergoline group (duration: t(8) = 2.87, p = 0.024; peak: t(8) = 2.87, p = 0.021) and the DMSO control group (duration: t(8) = 6.13, p < .001; peak: t(8) = 5.36, p = 0.001). d-amphetamine administration had no effect on onset for either group (metergoline group: t(8) = 0.80, p = 0.447; DMSO group: (t(8) = 1.37, p = 0.207). Further paired t-tests revealed that when administered subsequent to d-amphetamine, metergoline, but not DMSO, enhanced the peak amplitude of the MUA response (metergoline: t(8) = 2.77, p = 0.028; DMSO: t(8) = 2.04 p = 0.076). Neither metergoline nor DMSO had an effect on onset or duration (metergoline group: onset: t(8) = 0.094, p = 0.381; duration: t(8) = 1.43, p = 0.195; DMSO group: onset: t(8) = 0.58, p = 0.581; duration: t(8) = 1.48, p = 0.177). Finally, t-tests revealed a significant difference in peak amplitude for Dose 2 between the metergoline condition and the DMSO condition (t(15) = 2.22, p = 0.042), but no difference between onset, or duration (Onset: t(15) = .06, p = 0.96; Duration: t(15) = 0.47, p = 0.643). Thus, in the current study, the main effect of d-amphetamine on the MUA response to visual stimuli was to depress the amplitude responses. This depression was reversed by metergoline, but not DMSO.



**Figure 4.6:** The effect of intracollicular administration of d-amphetamine, and systemic administration of metergoline or DMSO on the superficial layer SC LFP and MUA response to visual stimuli for: (A) onset latency, (B) duration, (C) peak amplitude. Error bars are equal to ±1 SEM**.** Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle.

B

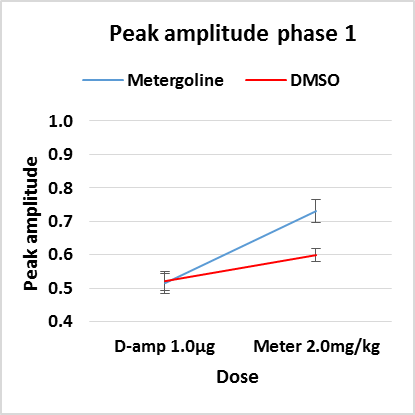
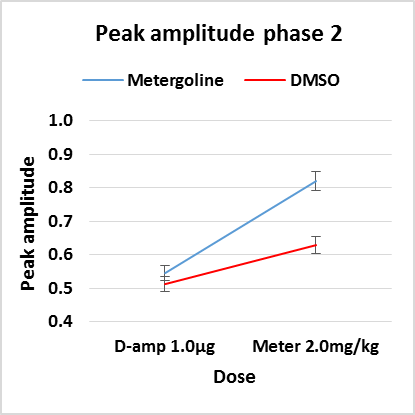
C

A

## 4.4.4 Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli

Visual inspection revealed a multiphasic profile for the MUA response of 16/20 animals (80%), where the first 100ms of the response post-onset constituted the first phase of the response, and all further activity constituted the second phase of the response (as defined in Chapter 3). For both phases of the MUA, intracollicular injections of d-amphetamine depressed the peak amplitude of the response. This depression was reversed by metergoline, but not DMSO, for both phases.

Paired t-tests revealed that d-amphetamine administration depressed the peak amplitude of both phases of the MUA response in both the experimental and control group (metergoline group: phase 1: t(6) = 3.64, p = 0.011; phase 2: t(6) = 3.29, p = .017; DMSO group: phase 1: t(6) = 5.32, p = 0.002; phase 2: t(6) = 6.54, p = 0.001). Further paired t-tests revealed that when administered subsequent to d-amphetamine, metergoline, but not DMSO, enhanced the peak amplitude of both phases of the MUA response (metergoline: phase 1: t(6) = 4.62, p = 0.006; phase 2: t(6) = 4.71, p = 0.005; DMSO: phase 1: t(6) = 2.22, p = 0.068; phase 2: t(6) = 2.33, p = 0.059;). Finally, t-tests revealed a significant difference in peak amplitude for Dose 2 between the metergoline condition and the DMSO condition for phase 2, but not phase 1, of the MUA response (phase 2: t(12) = 2.40, p = 0.035; phase 1: t(12) = 0.85, p = 0.411). Thus, in the current study, the main effect of d-amphetamine on the MUA response to visual stimuli was to depress the amplitude of both phases of the response. The distinct phases of the MUA response were differentially reversed by metergoline, with a strong reversal seen for phase 2, and a moderate reversal seen for phase 1 (see figure 4.7).



**Figure 4.7:** The effect of intracollicular administration of d-amphetamine, and systemic administration of metergoline or DMSO on the superficial layer SC LFP and MUA response to visual stimuli for: (A) Phase 1 peak amplitude, and (B) Phase 2 peak amplitude. Error bars are equal to ±1 SEM**.** Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle.

# 4.5 Discussion

## 4.5.1 Summary of Results

The present study aimed to explore whether d-amphetamine action at the level of the SC is mediated by 5-HT when d-amphetamine is administered locally. The superficial SC LFP responses to whole field visual stimuli were complex, multicomponent phenomena, which is consistent with previous reports (e.g. Dyer and Annau, 1977; Gowen et al., 2008; Clements et al., 2014). Based on electrophysiological criteria, microinjector coupled electrode placement was considered to be localised to the SGS or SO for the majority of animals. Previous reports have described onset latency of the superficial layer SC LFP response to whole visual filed stimuli as ranging from 28 ms (Dyer and Annau, 1997) to 50-60 ms (Fortin et al., 1997), which is consistent with our own observations (mean onset latency at baseline for all groups = 57.01 ms).

Consistent with the effect of systemic injections of d-amphetamine described in Chapter 3, the major effect of intracollicular d-amphetamine in the current study was to reduce the amplitude of the LFP and MUA of visually evoked responses in the superficial layers of the SC. These results suggest that when operating locally, d-amphetamine reduces both the input to (synaptic activity; LFP) and output from (spiking activity; MUA) superficial layer SC neurons. When the multi-unit response was divided into an initial and late phase (phase 1 and phase 2), the peak amplitude of both phases of the response were reduced by d-amphetamine. Following the intracollicular injection of d-amphetamine, a single systemic dose of metergoline (in 60% DMSO) or 60% DMSO was administered. The major effect of metergoline was enhance the amplitude of the overall MUA response and phase two of the multiphasic MUA response, thereby to reversing the depressant effect of d-amphetamine. While significant, this reversal was incomplete for both overall MUA and phase 2 of the MUA response. For phase 1 of the MUA response, the peak amplitude was enhanced following metergoline administration, but this did not significantly differ from the effect of DMSO alone. For the LFP both metergoline and DMSO significantly enhanced the peak-to-peak amplitude of the response, suggesting a potential confounding effect of the vehicle used for metergoline on the LFP.

## 4.5.2 Interpretation of Results

The finding that intracollicular administration of d-amphetamine depresses visually evoked responses in superficial layers of the SC is consistent with previous reports (Gowen et al., 2008; Dommett et al., 2009). Taken together with the results from Chapter 3, we have now shown that d-amphetamine depresses collicular responsiveness to visual stimuli when delivered through systemic and local routes. As such, it is likely that the effects of d-amphetamine on the collicular response are mediated through operations at the level of the SC. Having established this, we can now have more confidence in the possibility that metergoline reverses the effect of d-amphetamine at the level of the colliculus. This is consistent with the findings of Dommett et al., (2009) that the depressant effect of d-amphetamine on the visually evoked SC responses can be blocked by introducing a 5-HT antagonist in vitro. Metergoline is a broad spectrum competitive 5-HT antagonist (Terrón, 1997; Cox and Ennis, 1982; Engel et al., 1983) with strong affinity for 5-HT1, 5-HT2 and 5-HT7 receptors. Of particular relevance for visual processing at the level of the SC is metergoline’s high affinity for 5-HT1A receptors (Peroutka, Newman, & Harris, 1988). Dense distribution of 5-HT1A receptors has been reported in the SO and SGS of the superficial SC (Mooney, Shi, & Rhoades, 1994; Segu, Abdelkefi, Dusticier, & Lanoir, 1986; Shukla et al., 2014). Specifically, in SGS, 5-HT1A receptors are understood to preferentially have a postsynaptic localisation where they are believed to modulate neural excitability (Mooney, Huang, Shi, Bennett-Clarke, & Rhoades, 1996; Rojas & Fiedler, 2016). Antagonism of 5-HT1A receptors is known to block the effects of D-amphetamine; antagonism of 5-HT1A exerts a direct inhibitory effect on amphetamine-induced monoamine release (Ichikawa, Kuroki, Kitchen, & Meltzer, 1995; Kuroki, Ichikawa, Dai, & Meltzer, 1996). Additionally, antagonism of 5-HT1 receptors inhibits self-administration of D-amphetamine (Fletcher & Korth, 1999), and reduces psychostimulant induced locomotion in rats (Borycz, Zapata, Quiroz, Volkow, & Ferré, 2007). Thus, the SC expressed the necessary receptor types for both d-amphetamine and metergoline action.

The proposed mechanisms by which d-amphetamine and metergoline alter visual responsiveness at the level of the SC in both the current study and Chapter 3 are as follows. Recall from the introduction that the therapeutic effects of d-amphetamine are produced by elevating synaptic levels of monoamine neurotransmitters. Given the evidence presented above, it is thus plausible that d-amphetamine acts at the level of the superficial SC to elevate synaptic levels of 5-HT. These elevated levels of 5-HT may then act at postsynaptic retinotectal 5-HT1A receptors to inhibit neural excitability, thus reducing the amplitude of the MUA and LFP response to visual stimuli. By then antagonising 5-HT transmission at 5-HT1A receptors with metergoline, neural excitability may be disinhibited, thus enhancing the response to visual stimuli.

Similar to the data presented in Chapter 3, while metergoline administration significantly reversed the effects of d-amphetamine on the amplitude of visual activity in the SC, this reversal was incomplete. This could potentially be explained by the action of multiple neurotransmitters operating within the SC. The superficial layers of the SC are strongly innervated by NA, though NA receptors are less densely populated than 5-HT receptors (Lindvall & Bjorklund, 1974; Wichmann & Starke, 1988). As amphetamines are largely non-selective in their monoamine targets (Heal, Smith, Gosden, & Nutt, 2013), it is likely that intracollicular administration of d-amphetamine would elevate synaptic levels of NA in addition to 5-HT. Similar to 5-HT, the major effect of NA in the superficial SC is to depress visually evoked responses (Tan et al., 1999). It may therefore be plausible that metergoline reverses any 5-HT mediated depression of the collicular response whilst preserving NA mediated depression. This is contradicted by in vitro evidence showing that 5-HT antagonism with metergoline completely blocks the effect of d-amphetamine at the level of the SC (Dommett et al., 2009). Alternatively then, as metergoline is a competitive antagonist of 5-HT receptors (Terrón, 1997) partial reversal observed in the current study could could be a result of incomplete occupation of 5-HT receptors.

Consistent with the results from Chapter 3, the MUA response in the current study showed a clear biphasic profile consisting of an initial phase (phase 1; the first 100ms post onset) and a late phase (phase 2; any MUA activity with a latency greater than 100ms of onset). In Chapter 3 these phases were differentially affected by d-amphetamine. For phase 1, d-amphetamine induced a depression of the peak amplitude of the visual at all doses administered, with greatest depression seen at the highest doses. For phase 2, only the two highest doses of d-amphetamine administered depressed the amplitude of the response. In the current study d-amphetamine induced a comparable depression for both phases of the MUA response, though only a single dose was administered. The two phases of the MUA response did, however, differ in how they were affected by metergoline. While metergoline induced a strong reversal of the d-amphetamine mediated depression for phase 2, this reversal was much more moderate for phase 1. This is consistent with the data presented in Chapter 3, and suggests that the second phase of the MUA response may be more sensitive to 5-HT manipulation than the first phase. As phase 2 of the MUA may represent the activity of visuo-motor burst neurons (a subset of SC neurons that modulate target selection; McPeek et al., 2002), depression of this aspect of the SC response may serve to reduce foveation towards visual distractors. Within the context of pharmacotherapy of ADHD, one potential mechanism of D-amphetamine may be to enhance sustained attention by reducing the likelihood that visuo-motor burst neurons will fire in response to non-salient stimuli.

## 4.5.3 Implications of the Results and Future Direction

The results of the current study add to converging pharmacological evidence that psychostimulants act to depress visually evoked responses at the level of the SC (Gowen et al., 2008; Dommett et al., 2009; Clements et al., 2014). The SC constitutes a key nucleus in the orienting network (Boehnke and Munoz, 2008) and is heavily implicated in both covert and overt shifts of attention towards salient stimuli (Anderson et al., 2012). The colliculus is also strongly implicated in foveation towards non-salient distracting stimuli (Roberts et al., 2011; Fried et al., 2014), leading to suggestions that enhanced collicular activity may lead to symptoms of distractibility in ADHD (Overton, 2008). Conversely, reduced activity in the SC would be associated with a reduction in distractibility. Thus, in the pharmacotherapy of ADHD, psychostimulants may target the SC to normalise this collicular hypersensitivity and reduce foveation towards distracting stimuli.

The major finding of the current study is that d-amphetamine induced depression of visual activity at the level of the SC is mediated, at least in part, by 5-HT in vivo. As outlined above, the superior colliculus plays a major role in selection of targets for foveating eye movements. As the visual processing in the colliculus appears to be sensitive to manipulation of 5-HT transmission, this may provide a fruitful pathway for identification of potential therapeutic interventions for reducing distractibility in ADHD. 5-HT transmission has previously been investigated as a potential psychotherapeutic target in ADHD. In a preliminary clinical trial assessing the viability of treating ADHD with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, Barrickman et al., (1991) reported that 60% of participants showed at least moderate improvement in symptoms. Reports since this trial, however have been inconclusive, with many prominent authors in the field concluding that 5-HT has little relevance to the pharmacotherapy of ADHD (Kratochvil et al., 2005; Pliszka, 2005). There is therefore a disconnect; if collicular dysfunction underlies distractibility in ADHD, and collicular activity is effectively modulated by 5-HT and normalised by psychostimulants, why are serotonergic drugs not more efficacious in treating ADHD? The next chapter will thus consider the disconnect between pharmacological and biological evidence, which suggests that frontline pharmaceuticals for ADHD target 5-HT transmission, and clinical evidence, which suggests drugs targeting 5-HT may be of limited efficacy in the treatment of ADHD. Following this, a review of 5-HT pharmacotherapy in ADHD will be presented, with a view of identifying a potential candidate drug to reduce distractibility in ADHD.

Interim Discussion: Serotonin Transmission as a Potential Target in the Pharmacotherapy of ADHD

# 5.1 Chapter Summary

The results presented in chapters 3 and 4 add to converging evidence that d-amphetamine acts to depress visual activity at the level of the SC, in a process that is mediated, at least in part, by 5-HT. Given that hypersensitivity of the SC is a candidate explanation for increased distractibility in ADHD, there may be potential to further exploit 5-HT transmission in the pharmacotherapy of ADHD. However, current clinical evidence for the efficacy for treating ADHD by targeting 5-HT transmission is mixed is best. The present chapter will thus consider the disconnect between pharmacological evidence, which suggests that frontline pharmaceuticals for ADHD target 5-HT transmission, and clinical evidence, which suggests drugs targeting 5-HT may be of limited efficacy in the treatment of ADHD.

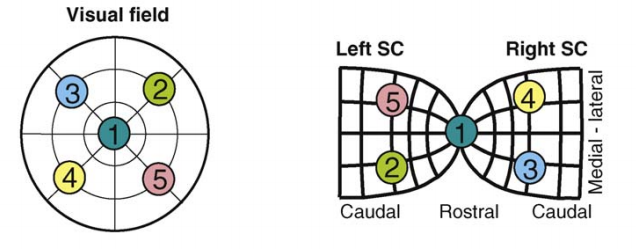
# 5.2 Collicular Dysfunction can Explain Distractibility in ADHD

Recall from Chapter 1 that a fruitful approach to efficacious pharmacotherapy for ADHD may be to understand the pathophysiological changes underpinning specific symptoms (Overton, 2008). Distractibility is an ideal symptom to consider when taking this approach. Increased distractibility is the most frequently presenting symptom of ADHD (Wilens et al., 2009), and is the symptom that shows greatest resistance to extinction during the transition from childhood to adult ADHD (Biederman et al., 2000). By understanding the neural changes that underpin distractibility, we will be well placed to develop more effective pharmacological interventions, and thus improve the quality of life of ADHD patients (Wehmeier, Schacht, & Barkley, 2010).

One system that is intimately linked to distractibility is the SC (see chapter 3.1 for a full account of the evidence linking distractibility in ADHD to the SC). Several oculomotor functions that lie under the control of the SC are impaired in ADHD (Bittencourt et al., 2013; M. Panagiotidi, P. Overton, & T. Stafford, 2017) leading to a reduced ability to supress foveation towards low-salience distracting stimuli (Munoz et al., 2003). Such findings have led to the suggestion that collicular hypersensitivity may explain the increased distractibility observed in ADHD (Overton, 2008). In light of the results presented in chapters 3 and 4, as well as evidence within the extant literature (e.g. (Clements et al., 2014; Dommett et al., 2009), it is apparent that visual processing within the superficial SC is effectively modulated by 5-HT and that d-amphetamine acts to alter this modulation by increasing 5-HT availability. Thus, in the pharmacotherapy of ADHD, d-amphetamine may act to enhance 5-HT mediated modulation of visual activity at the level of the SC, thereby normalising collicular hypersensitivity and reducing symptoms of distractibility. This pharmacological evidence, however, in contradicted by clinical reports that suggest interventions targeting 5-HT are of limited efficacy in the treatment of ADHD (Barrickman et al., 1991; Kratochvil et al., 2005). There is therefore a clear disconnect; if collicular dysfunction underlies distractibility in ADHD, and collicular activity is effectively modulated by 5-HT and normalised by psychostimulants, why are serotonergic drugs not more efficacious in treating ADHD? This question must be addressed before we can consider how to exploit 5-HT transmission to identify effective interventions for ADHD. The following section will consider the role of the SC in foveation towards distracting stimuli, and how this process is potentially moderated by 5-HT in the superficial layers of the SC. After this is established, current evidence assessing the viability of targeting 5-HT transmission will be considered, and an explanation for the mixed efficacy of these drugs will be proposed.

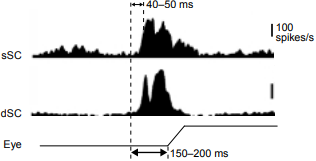
# 5.3 Modulation of Oculomotor Activity in the SC

It has long been known that the colliculus plays a vital role in locating salient objects in visual space (Schneider, 1969) and guiding the saccadic eye movements that orient attention towards these objects (Dean et al., 1989; Sparks, 1999; Whittaker & Cummings, 1990). However, until recently the mechanism by which colliculus biased central action selection systems towards initiating these foveating saccades was obscure (Redgrave et al., 2010). Recall the sources of visual innervation of the superficial SC discussed in Chapter 4.2.2. Neurons in the superficial layers of the SC (stratum zonale, stratum griseum superficiale, and stratum opticum) respond almost exclusively to visual stimuli presented in discrete regions of contralateral visual space (Gandhi & Katnani, 2011). Afferent input to the SGS (the site of electrode placement for the majority of animals in Chapter 4) arise from the contralateral retina and layer V visual cortex neurons (Boehnke & Munoz, 2008). Often, input from retina and visual cortex synapse onto the same neurones in SGS, allowing for the convergence of raw retinal input with information that has already been processed at the level of the cortex and thalamus (B. E. Stein & M. A. Meredith, 1993). The responsiveness of these neurons to visual stimuli is under direct serotonergic modulation; visual responsiveness within the SGS is diminished following intracollicular application of 5-HT (Dommett et al., 2009; Mooney et al., 1996). The receptive fields of these superficial layer SC neurons comprise a highly ordered retinotopic map of contra-lateral visual space (see figure 5.1). This organisation is preserved in the intermediate and deep layers of the SC in register with mapping for other sensory modalities (Chalupa & Rhoades, 1977; Drager & Hubel, 1976), allowing for the generation of oculomotor responses to salient sensory events (Binns, 1999). Neurons in these deeper layers, particularly the SGI, are believed to play a crucial role in guiding saccadic eye movements to specified vectors in visual space (Boehnke & Munoz, 2008).



**Figure 5.1:** Schematic representation of locations in visual space and their expression on the collicular retinotopic map (adapted from Boehnke & Munoz, 2008)

The SGI comprises of both visual and visuomotor neurons, whose firing increases before and during saccades (Marino, Rodgers, Levy, & Munoz, 2008). The major source of visual input to neurons within the SGI arises from intralaminar projections from SGS (Hall & Lee, 1993; Saito & Isa, 2005). Indeed, pre-saccadic activity of SGI neurons is highly synchronous with the activity of the 5-HT modulated visual neurons within the SGS (Isa, 2002; see figure 5.2). The response fields of visuomotor neurons and motor neurons in SGI show strong spatial alignment and organisation into a retinotopically coded visuomotor map (Boehnke & Munoz, 2008). Electrophysiological data from monkeys performing visually guided saccade tasks demonstrates that visuomotor neurons within the SGI specify the location of upcoming saccadic targets within the contralateral visual field (Marino et al., 2008). Activity of these SGI neurons is believed to encode a visuospatial response directly onto tectoreticular neurons and, presumably, tectonigral neurons (May et al., 2009).



**Figure 5.2:** Synchronous activity of superficial and deep SC neurons during saccades to predicted targets. The bottom line indicates the timing of the saccade (Isa, 2002).

While the SGI encodes the vectors in visual space for targets of saccadic eye movements, it is the BG that govern whether a foveating eye movement is initiated. As the principal centre for the shifting of gaze in response to novel sensory events (Dean et al., 1989; Stein & Meredith, 1993), the colliculus possesses an intrinsic predicament of action selection. The SC receives retinotectal input representing the whole of visual space, and as such, the colliculus is regularly presented with multiple visual events, each of which could be a potential target for a saccade. Consequently, there must be a system which assigns priority, or salience, to sensory events when multiple stimuli are presented simultaneously. This system is believed to be the BG. As the BG receives convergent input relaying contextual, motivational, and sensory information from a range of cortical and sub-cortical targets, it is well placed to assess the salience of multiple sensory events and initiate motor programs to best respond to these events (Gurney et al., 2001; Redgrave et al., 1999). The BG and SC form sensorimotor loops, where the SC projects to the input nuclei of the BG via the thalamus, which in turn project back to the SC via the substantia nigra (McHaffie, Stanford, Stein, Coizet, & Redgrave, 2005). It is through this re-entrant looped architecture that the BG is believed to disinhibit tectoreticular neurons within SGI (Ikeda & Hikosaka, 2003), allowing for projection of visuospatial information to premotor circuitry in the reticular formation and brain stem (Rodgers, Munoz, Scott, & Paré, 2006).

Thalamus

Superficial SC

Deep SC

Striatum

Substantia  
Nigra

Visual input

Motor output

**Figure 5.3:** Collicular visuomotor loops through the basal ganglia, adapted from Redgrave et al., 2010.

The manner by which the BG influences action selection is conceptualised as “bids” for motor expression from any system capable of initiating motor activity (Gurney et al., 2001; Redgrave et al., 1999). Within this framework enhanced activity is conceived as submitting a stronger “bid” to the BG. It thus follows that the strongest bid in the system at a given time is most likely to generate a motor output. A bid from the SC may be expressed as a saccadic eye movement, for example. Within the context of ADHD, it is proposed that visual responsiveness in the colliculus is abnormally high (Overton, 2008). At the level of the superficial SC this may be represented by unusually strong responses to visual stimuli that are of low biological salience (i.e. visual distractors). Through intralaminar connections to deeper levels of the SC, this heightened sensory responsiveness may provoke synchronous activity in SGI (Isa, 2002), which in turn projects a bid for motor expression to the BG. If this bid is of sufficient strength, then an eye movement towards this distractor may be initiated. Thus hypersensitivity of the superficial SC has a biologically plausible pathway through which it can increase distractibility. As the response to visual stimuli in the superficial SC is depressed when levels of 5-HT are increased (Dommett et al., 2009; see the results presented in Chapters 3 and 4), there is a clear theoretical pathway through which drugs that enhance 5-HT levels could normalise distractibility in ADHD.

# 5.4 Serotonin Based Interventions for ADHD: Disconnect Between Theory and Application

There is convergent evidence that frontline ADHD pharmaceuticals may reduce distractibility by targeting 5-HT transmission (e.g. Dommett et al., 2009; Chapters 3 and 4 of the current thesis), potentially through the pathway described in the above section. Despite this pharmacological evidence, clinical and behavioural reports assessing the efficacy of 5-HT based medicine in treating the symptoms of ADHD have offered mixed results (Pliszka, 2005). This evidence will be outlined below, and an explanation for disconnect between these reports and the pharmacological and physiological evidence presented above will be considered.

Selective serotonin reuptake inhibitors (SSRIs) are amongst the most widely used interventions for enhancing levels of 5-HT in psychiatric pharmacotherapy (Hiemke & Hartter, 2000; Vaswani, Linda, & Ramesh, 2003). SSRIs are a classification of pharmaceuticals that display very high affinity for 5-HT reuptake sites and low affinity for neurotransmitter sites (Frazer, 1997; Preskorn, 1997). Thus, the therapeutic effects of SSRIs are considered to be mediated by increasing extracellular 5-HT levels by blocking the reuptake of 5-HT into presynaptic terminals (Hyttel, 1994). Of the SSRIs, fluoxetine was the first to become widely available for clinical use (Catterson & Preskorn, 1996; Hiemke & Hartter, 2000), and remains a frontline pharmaceutical for psychiatric disorders, in particular depression, to this day (Selle, Schalkwijk, Vazquez, & Baldessarini, 2014). Consequently, much of the research assessing the viability of targeting 5-HT to normalise clinical symptoms of ADHD populations has utilised fluoxetine (Dalley & Roiser, 2012; Pliszka, 2005), though other SSRIs have also been appraised (e.g. fenfluramine; Donnelly et al., 1989).

Trials assessing the viability of treating ADHD with SSRIs were initially promising. In a six week preliminary open trial, ADHD patients who had been non-responsive to stimulants showed at least a moderate improvements in symptoms when treated with fluoxetine (Barrickman et al., 1991). In other trials, however, SSRIs have shown limited efficacy in treating ADHD. Donnelly et al. (1989) showed that while d-amphetamine produced marked improvements in hyperkinetic and inattentive ADHD symptoms, the SSRI fenfluramine did not significantly alter symptom presentation relative to placebo. Similarly, in patients with comorbid ADHD and major depressive disorder, fluoxetine monotherapy produced a remission of depressive symptoms, but had no significant effect on ADHD symptoms (Findling, 1996). In addition to limited efficacy as a primary treatment for ADHD (Garland, 1998), SSRIs may actually act to exacerbate the symptoms of ADHD. Riddle et al. (1990) found that administering fluoxetine to treat children with comorbid ADHD and depression often aggravated ADHD symptoms in a dose dependant manner. Discontinuing fluoxetine treatment reversed this aggravation. While these trials suggest that SSRIs are of inconsistent therapeutic viability in ADHD (Popper, 1997), it should be noted that each of these studies were comprised of small samples sizes (no trial had more than 20 participants), and that the course of SSRI treatment may have been too short for observable clinical effects (Pittenger & Bloch, 2014). Nevertheless, these early trials have been cited by several major contributors to the field of ADHD psychopharmacology as evidence that the 5-HT system, and drugs that target this system, are of little to no relevance to the pathophysiology of ADHD (Pliszka, 2005; Spencer et al., 2002a). This, combined with the emergence of other viable alternatives to psychostimulant medication, such as the NA reuptake inhibitor atomoxetine (Faraone, Biederman, et al., 2005; Garnock-Jones & Keating, 2009), contributed to a period of reduced interest in researching the viability of 5-HT manipulation in the psychotherapy of ADHD.

In recent years 5-HT transmission in ADHD has been revisited. Much of this renewed interest stems from advances in genetics, which have revealed that ADHD can be reliably predicted by polymorphisms of the SERT (Gizer et al., 2009; McGough et al., 2009). Specifically, ADHD is associated with a significant over expression of the long variant of the promoter region of the SERT gene (the 5HTTLPR; (Faraone, Perlis, et al., 2005; Gizer et al., 2009). The SERT is the carrier protein that is responsible for reuptake of 5-HT into presynaptic terminals, thus terminating 5-HT signalling (Heils et al., 1996). Expression of the long allele of the 5HTTLPR is associated with an approximate two fold higher uptake of 5-HT compared to other genotype expressions (Retz, Retz-Junginger, Supprian, Thome, & Rösler, 2004). As action of the SERT constitutes a fundamental mechanism for the regulation of CNS 5-HT levels, the rapid 5-HT uptake associated with the long variant of 5HTTLPR results in reduced availability of active 5-HT (Lesch et al., 1996). While genetic evidence points towards a dysfunction at the level of the SERT in ADHD, in vivo evidence for altered SERT expression and availability in ADHD patients is currently inconclusive. Early PET investigations assessing binding potentials for ligands with high specificity for SERT showed no difference in SERT availability between ADHD patients and healthy controls (Hesse, Ballaschke, Barthel, & Sabri, 2009; Karlsson et al., 2013). Recently, however, Vanicek et al. (2017) has argued that these studies lacked the required statistical power to detect differences between groups. Vanicek et al. (2017) performed a PET interregional molecular correlational analysis to assess functional connectivity between various regions of interest that are traditionally implicated in the neuropathology of ADHD. Vanicek et al found altered interregional SERT binding between various regions of ADHD patients compared to healthy controls. As SERT expression in vivo is partially regulated by 5-HT release (Benmansour, Owens, Cecchi, Morilak, & Frazer, 2002), these findings point towards altered 5-HT dynamics in ADHD.

There are thus clear contradictions within the literature assessing the contribution of the serotonergic system to ADHD symptoms. Other than a single open- label trial (Barrickman, 1991), clinical reports suggest that monotherapeutic interventions targeting 5-HT in ADHD are either ineffective, or otherwise exacerbate symptoms (Pliszka, 2005; Spencer et al., 2002a). However, genetic and biological evidence point towards dysfunction at the level of the serotonergic system (Gizer et al., 2009). We have argued above that collicular dysfunction underlies distractibility in ADHD and that collicular activity is effectively mediated by 5-HT. Furthermore, we have presented pharmacological evidence showing that psychostimulants may reduce distractibility in ADHD by targeting 5-HT transmission at the level of the SC. In light of this biological and genetic evidence, it is somewhat paradoxical that clinical reports suggest that drugs targeting 5-HT are of limited efficacy when the treating the symptoms of ADHD. One potential explanation for this disconnect is that the contribution of 5-HT to the pathology of ADHD is more complex than an overall increase or decrease in symptom presentation. In a review of serotonergic dysfunction in ADHD, Oades (2007) proposed that while a widespread association of 5-HT dysfunction with a diagnosis of ADHD is unlikely, there may be a differential contribution of 5-HT to the major symptomatic domains of ADHD. Evidence reviewed showed little association between 5-HT and hyperkinetic ADHD symptoms, but did show an association between 5-HT and inattentive symptoms. In particular, Oades proposed that reduced 5-HT availability in ADHD contributes to altered perceptual sensitivity and salience designation, leading to enhanced cognitive impulsivity and distractibility. If 5-HT only has relevance to attentional symptoms, this may explain the mixed results presented in the clinical trials above, where specific symptomatic domains were not controlled for. While this proposition may contextualise why d-amphetamine affects 5-HT transmission in salience designation circuits such as the SC, it does not explain why 5-HT based interventions are not more efficacious in the treatment of ADHD, particularly for inattentive symptoms (Kratochvil et al., 2005).

A more viable explanation for clinical reports suggesting that 5-HT is of limited relevance to the pharmacotherapy of ADHD relates to the regulatory mechanisms that control 5-HT neurotransmission in vivo. It has long been known in depression literature that a minimum of 3-4 weeks of continuous daily SSRI use is required until improvements in clinical symptoms are observed (Briley & Moret, 1993). Similarly, when SSRIs are used in the treatment of obsessive compulsive disorder (OCD), a minimum period 8-12 weeks of continuous daily SSRI use is required until a clinically and statistically significant improvement in symptoms is observed (Issari, Jakubovski, Bartley, Pittenger, & Bloch, 2016; Pittenger & Bloch, 2014). This delay in time to response is believed to result from feedback mechanisms at the level of the raphe nucleus, the major source of ascending 5-HT projections for the CNS (Vertes, 1991; Vertes, Fortin, & Crane, 1999), which act to regulate the rate of 5-HT release in target regions (Briley & Moret, 1993). Consequently, it is only after chronic regular exposure to SSRIs that these regulatory feedback mechanisms are desensitised, allowing for increased levels of synaptic 5-HT and commensurate therapeutic benefits. In the trials assessing SSRI use for ADHD described above, SSRIs were prescribed either for short periods (three weeks for Findling 1996; six weeks for Barrickman et al., 1991), or were otherwise not administered daily (Donnelly et al., 1989; Garland et al., 1998). Given that up to twelve weeks of continuous daily SSRI use is required to desensitise raphe feedback mechanisms and allow for observable clinical improvements in other disorders (e.g. OCD; Issari et al., 2016), it is plausible that participants in the ADHD clinical trials described above were not administered SSRIs for sufficient regularity and length for any potential clinical effect on ADHD symptoms. As these trials represent a basis for which SSRIs are considered to have limited pharmacological relevance to ADHD (e.g. Plizka, 2005), it is possible that by bypassing the mechanisms that regulate 5-HT release, we may observe effects of SSRIs which have clinical relevance to the pharmacotherapy ADHD. The next section will thus consider the principle mechanism that regulates the release of 5-HT, and potential strategies for bypassing this mechanism in order to explore the effects of SSRIs in an in vivo rodent model.

# 5.5 Serotonin Based Interventions for ADHD: Autoregulation of Serotonergic Activity

The delayed onset of the therapeutic benefits of SSRIs observed in both depression and OCD can be explained by regulatory mechanisms of somatodendritic autoreceptors situated in the midbrain raphe nuclei. Serotonergic neurons in the dorsal and medial raphe nuclei (DRN and MRN) form the majority of all 5-HT producing cells within the CNS, with the DRN and MRN accounting for up to 85% and 15% of all serotonergic cell bodies respectively (Hornung, 2003). Neurons in the DRN give rise to ascending projections to a broad range of cortical and subcortical targets, including the cerebral cortex, BG, and limbic system (Cools, Roberts, & Robbins, 2008), and of particular relevance to the present discussion, the SC (Mooney et al., 1996). The firing rate of raphe neurons alters the release of 5-HT in target regions (Maejima, Masseck, Mark, & Herlitze, 2013). Consequently, the mechanisms that regulate that the firing of these DRN neurons thus regulate 5-HT release and availability at afferent targets. The most relevant of these mechanisms to the delayed efficacy SSRIs pharmacotherapy is the down-regulation of DRN firing elicited by somatodendritic 5-HT1A autoreceptors.

Under typical conditions, 5-HT neurones exhibit spontaneous firing at a rate of 1-5 action potentials per second (Aghajanian & Vandermaelen, 1982). When DRN neurons are exposed to 5-HT, either through local dendrodendritic connections or ascending raphe-raphe projections (Adell, Celada, Abellan, & Artigas, 2002), inhibitory post synaptic potentials can be observed, resulting in down-regulation of DRN activity and thus reduced CNS 5-HT release (Morikawa, Manzoni, Crabbe, & Williams, 2000). A major contributor to this down-regulation is negative feedback evoked by 5-HT1A autoreceptors (Fischer, Jocham, & Ullsperger, 2014). 5-HT1A autoreceptors are a class of G protein-coupled receptors which are densely expressed on somatodendritic compartments of raphe nuclei cell bodies (McDevitt & Neumaier, 2011). Upon bindings with 5-HT, these autoreceptors activate G protein-coupled inwardly rectifying potassium channels, thereby increasing permeability to K+ and thus causing membrane hyperpolarisation and a consequent reduction in neural excitability (Penington, Kelly, & Fox, 1993; Stamford, Davidson, McLaughlin, & Hopwood, 2000). It has long been recognised that acute SSRI administration increases extracellular 5-HT availability in the DRN, thereby activating 5-HT1A autoreceptors and reducing the firing rate of neurons mediated by these autoreceptors (Gartside, Umbers, Hajós, & Sharp, 1995). As a result, 5-HT release is inhibited in areas innervated by these DRN neurons (Gardier, Malagie, Trillat, Jacquot, & Artigas, 1996). Consequently, the delayed time to response observed when SSRIs are administered clinically (for e.g. in depression) can be explained by desentisation of 5-HT1A autoreceptors following consistent daily use over a number of weeks (Stahl, 1998). In terms of 5-HT availability at afferent DRN targets, it can be conceptualised that any initial increase in synaptic 5-HT availability caused by SSRI occupying presynaptic 5-HT transporters is counteracted by the down-regulation of DRN firing rate by 5-HT1A autoreceptors. Following desensitisation of these autoreceptors, negative feedback at the level of the raphe is reduced, thereby normalising firing rate and allowing for increased 5-HT availability in afferent target regions. This conceptualisation is supported by evidence that more rapid onset of anti-depressant effects of SSRIs can be obtained when SSRIs are administered concurrently with a 5-HT1A autoreceptor antagonist (Ballesteros & Callado, 2004). Similarly, selective deactivation of 5-HT1A autoreceptors in murine models allows for immediate anti-depressant effect following a single dose of fluoxetine (Bortolozzi et al., 2012). This concept can be expanded to allow for the examination of effects of SSRIs in acute animal preparations. Pre-treatment with 5-HT1A autoreceptors antagonists prior to administration of fluoxetine has allowed for the exploration of a variety of behavioural and pharmacological effects which would have otherwise been obscured by the autoregulation of DRN activity (Cassani, Dorantes-Barron, Novales, Real, & Estrada-Reyes, 2014; Palucha-Poniewiera et al., 2014). Given that we have previously presented pharmacological evidence outlining the potential to exploit collicular 5-HT transmission in the pharmacology of ADHD, we now aim to utilise the process described above to examine the effect of SSRI administration on collicular activity in vivo.

# 5.6 Summary and Next Steps

The present chapter examined the disconnect between pharmacological evidence, which suggests that pharmaceuticals for ADHD target 5-HT transmission, and clinical reports, which suggest that targeting 5-HT transmission is of limited therapeutic relevance to the treatment of ADHD. It was proposed that this disconnect can be explained by inadequate control of autoregulation processes, which paradoxically limit the availability of 5-HT when SSRIs are administered over the irregular periods used in these clinical trials (Findling 1996, Garland et al., 1998). As such, we argue that the participants in these trials were not administered SSRIs for sufficient regularity to fully realise any potential benefits of targeting 5-HT in the pharmacotherapy of ADHD. In light of this, combined with the pharmacological evidence presented in Chapters 3 and 4, there may therefore be an under-researched potential to exploit 5-HT transmission as a potential therapeutic avenue in ADHD. Recent pharmacological research has successfully explored the effects of SSRIs in acute animal preparations by antagonising 5-HT1A autoreceptors prior to the administration of fluoxetine (e.g. Cassani et al., 2014; Palucha-Poniewiera et al., 2014). In the next Chapter, we aim to utilise this technique to examine the effects of fluoxetine administration on collicular responses to visual stimuli. Exploration of this effect will allow us to establish if SSRIs alter 5-HT availability comparably to psychostimulants in systems that are relevant to ADHD symptomology.

5-HT1A Autoreceptor Antagonism Enables Fluoxetine-Induced Suppression of Superficial Layer SC Visual Responses

# 6.1 Chapter Summary

In light of the results presented in Chapters 3 and 4, serotonergic transmission at the level of the SC is an emergent target for exploration in the pharmacotherapy of ADHD. Evidence in the extant literature, however, suggests that targeting 5-HT transmission with SSRIs is of limited therapeutic relevance to the treatment of ADHD. In the previous chapter we reviewed this evidence, and suggested that autoregulation processes mediated by 5-HT1A receptors in the DRN act as to confound these studies by limiting the availability of 5-HT when SSRIs are administered over acute periods. Recent pharmacological research has successfully explored the effects of SSRIs in acute animal preparations by antagonising 5-HT1A autoreceptors prior to the administration of SSRIs. In the following chapter, evidence is presented showing that the SSRI fluoxetine depresses the SC response to visual stimuli only when it is administered subsequent to 5-HT1A antagonism.

# 6.2 **Introduction**

## 6.2.1 Rationale for the Present Study

There is now converging evidence that the SC is well placed as a locus of interest when investigating pharmacological interventions to normalise distractibility in ADHD (e.g. Overton et al., 2008; Clements et al., 2014; see chapter 3.2 for an overview of the literature linking distractibility to the SC). The data presented in Chapters 3 and 4 add to this evidence by showing that the depressant effect of d-amphetamine on visual activity in the superficial SC is quickly and reliably reversed following administration of the 5-HT antagonist metergoline. As such, it is likely that the effects of d-amphetamine on visual responses in the SC are mediated, at least in part, by 5-HT. In Chapter 5, evidence was presented that shows that visual processing within the superficial SC is effectively modulated by 5-HT, and that d-amphetamine acts to alter this modulation by increasing 5-HT availability. Thus, in the pharmacotherapy of ADHD, d-amphetamine may act to enhance 5-HT mediated modulation of visual activity at the level of the SC, thereby normalising collicular hypersensitivity and reducing symptoms of inattention. While it may be inferred from this evidence that serotonergic transmission at the level of the SC could be a potential target for interventions in the pharmacotherapy of ADHD, clinical evidence suggests drugs targeting 5-HT (specifically the SSRI fluoxetine) have little therapeutic relevance to the treatment of ADHD (Pliszka, 2005; Spencer et al., 2002a). In Chapter 5, the disconnect between this pharmacological and clinical evidence was considered. It was proposed that this disconnect can be explained by inadequate control of 5-HT1A mediated autoregulation processes, which paradoxically limit the availability of 5-HT when SSRIs are administered over irregular acute periods (such as in the studies cited which conclude that 5-HT is of limited relevance to the pharmacotherapy of ADHD: e.g. Findling 1996; Garland et al., 1998). Recently, behavioural and pharmacological studies have successfully explored the effect of fluoxetine in acute preparations by first antagonising 5-HT1A autoregulation processes prior to SSRI administration (e.g. Cassani et al., 2014; Palucha-Poniewiera et al., 2014). The current study will extend this approach to examine the effect of acute fluoxetine administration on collicular activity in vivo. Given that the SC is a target of d-amphetamine action, exploration of this effect will allow us to establish if SSRIs alter 5-HT availability in a system with known relevance to the pharmacotherapy of ADHD.

## 6.2.2 Fluoxetine as a Candidate Drug for Research in the Present Study

As highlighted in Chapter 5, much of the literature assessing the viability 5-HT in the pharmacotherapy of ADHD has utilised SSRIs, with a specific focus on fluoxetine. SSRIs are amongst the most widely used interventions for enhancing levels of 5-HT in psychiatric pharmacotherapy (Hiemke & Hartter, 2000; Vaswani et al., 2003). Of this category of drugs, fluoxetine was the first to be made available for clinical use (Wong, Bymaster, & Engleman, 1995), and is still widely prescribed in the treatment of depression, bulimia nervosa, and OCD (Rossi, Barraco, & Donda, 2004; Selle et al., 2014). As a molecule, fluoxetine displays very high affinity for 5-HT reuptake sites and low affinity for neurotransmitter sites (Frazer, 1997; Preskorn, 1997). Thus at therapeutic doses, the effects fluoxetine are mediated principally by increasing synaptic 5-HT availability by the selective blockade of reuptake of 5-HT into presynaptic terminals (Guze & Gitlin, 1994; Hyttel, 1994).

A key motivator for assessing the viability of 5-HT in the pharmacotherapy of ADHD is to identify substances with similar treatment efficacy to psychostimulants, but with a safer profile in terms of side effects and abuse potential (Overton, 2008). In a meta-analysis of the clinical efficacy and viability of fluoxetine, Rossi et al. (2004) concluded that in the treatment of depression, bulimia nervosa, and OCD, fluoxetine has a safe profile of side effects and has efficacy that is comparable to, or greater than, other SSRIs. Of relevance to the pharmacotherapy of ADHD, this safe profile has been observed during efficacious treatment for depression in both children (Emslie et al., 1997) and adolescents (March et al., 2004). Fluoxetine itself is considered to have no addictive qualities, but abrupt interruption of treatment may lead to the emergence of transient withdrawal symptoms, though fewer of these symptoms are observed when compared to other SSRIs (Rosenbaum, Fava, Hoog, Ascroft, & Krebs, 1998). Despite its safe profile, a small minority of patients treated with fluoxetine may present with sexual dysfunction, headache and nausea. Where these symptoms are present, they typically only persist for a period of around two weeks, and are less severe and persistent than for other SSRIs (Addis & Koren, 2000).

In summary, fluoxetine enhances CNS 5-HT levels by selectively blockading the reuptake of 5-HT into presynaptic terminals. Fluoxetine has a safe, non-addictive profile, and has been used to efficaciously treat both adults and children with minimal side effects. For these reasons, fluoxetine is an excellent candidate drug in the present study to consider the potential to exploit 5-HT transmission in the pharmacotherapy of ADHD. While there is limited empirical evidence of fluoxetine directly altering 5-HT levels at the level of the SC, chronic administration of fluoxetine in rats has produced a significant increase in the SERT density in the SC (Hrdina & Vu, 1993). As SERT expression in vivo is partially regulated by 5-HT release (Benmansour et al., 2002), it can be extrapolated that one of the effects of fluoxetine is to increase synaptic levels of 5-HT at the level of the SC.

## 6.2.3 Control of 5-HT1A Mediated Autoregulation in Acute Pharmacological Perpetrations.

In Chapter 5, it was established that acute fluoxetine administration activates negative feedback mediated by 5-HT1A autoreceptors at the level of the DRN, thus reducing the firing rate of neurons mediated by these autoreceptors (Gartside et al., 1995). Consequently, 5-HT release is inhibited in areas innervated by the DRN, including the superficial SC (Gardier et al., 1996). When prescribed chronically (e.g. for depression), daily administration of fluoxetine desensitises these 5-HT1A autoreceptors over a period of several weeks, allowing for improvement in symptom presentation. Following desensitisation of these autoreceptors, negative feedback at the level of the raphe is reduced, thereby normalising firing rate and allowing for increased 5-HT availability in afferent target regions. Thus, to study the effect of fluoxetine in acute animal preparations, it is first necessary to desensitise DRN 5-HT1A autoreceptors.

Desensitisation of DRN 5-HT1A autoreceptors can be achieved through systemic pre-treatment with a 5-HT1A autoreceptor antagonist. Studies utilising this technique have revealed effects of fluoxetine in acute rodent preparations which were otherwise obscured by the down regulation of serotonergic activity at the level of DRN. Microdialysis studies in rats have revealed that fluoxetine alone causes negligible increases in frontal and hippocampal 5-HT levels, but following pre-treatment with WAY 100635 (a potent 5-HT1A autoreceptor antagonist), significant increases in 5-HT levels are observed (Artigas, Romero, de Montigny, & Blier, 1996; Romero, Hervas, & Artigas, 1996; Tordera et al., 2003). Similarly, pre-treatment with either WAY 100635 or NAD-299 (another potent 5-HT1A autoreceptor antagonist), potentiates the effects of fluoxetine on postsynaptic 5-HT activity in frontal and parietal regions, as well as at the caudate and putamen (Tordera et al., 2003). In behavioural studies, pre-treatment with a 5-HT1A autoreceptor antagonist prior to administration of fluoxetine has allowed for the exploration of behavioural effects that were not observed when fluoxetine alone was administered (Cassani et al., 2014; Palucha-Poniewiera et al., 2014). In summation, results from a range of biological, behavioural, and pharmacological studies have revealed the need to antagonise 5-HT1A autoreceptors in order to observe any effects of fluoxetine in acute animal experiments.

## 6.2.4 Aim of the Present Study

The aim of the current study is to utilise in vivo electrophysiological techniques to explore the effect of fluoxetine on the superficial SC response to visual stimuli both in the presence and absence of the 5-HT1A antagonist NAD-299. Convergent evidence shows that the effect of d-amphetamine induced depression of the SC response to visual stimuli is mediated by increasing synaptic availability of 5-HT. In acute in vivo preparations, fluoxetine increases availability of 5-HT at a range of targets innervated by the DRN, but only when administered subsequent to systemic pre-treatment with a 5-HT1A antagonist ([Tordera et al., 2003](#_ENREF_29)). As such, in the current study it is expected that fluoxetine will depress the SC response to visual stimuli, only when 5-HT1A transmission has been previously antagonised by NAD-299.

# 6.3 Methods

## 6.3.1 Subjects

Data were obtained from 20 male Hooded Lister rats (bred in house; weight 300-500g). Animals were housed together on a 12 hr light/dark cycle with food and water supplied ad libitum. All procedures were performed according to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and the Animals (Scientific Procedures) Act, 1986 (revised 2013). Every effort was made to minimise suffering to subjects and to reduce the number of animals used.

## 6.3.2 Antagonism of 5-HT1A Autoreceptors

Antagonism of 5-HT1A receptors was achieved through the administration of NAD-299. NAD-299 was selected as it crosses the blood brain barrier, displays highly selective in vivo antagonism of 5-HT1A autoreceptors at the level of the DRN (Johansson et al., 1997; Martin, Jackson, Wallsten, & Waszczak, 1999), completely reverses the acute inhibitory effects of SSRIs on dorsal raphe firing in rats (Arborelius, Wallsten, Ahlenius, & Svensson, 1999). At doses used in the present study (0.1mg/kg, i.v.) previous PET studies have shown that NAD-299 5-HT1Adisplays occupancy of 70-80% in the DRN (Andrée et al., 2003; Farde, Andree, Ginovart, Halldin, & Thorberg, 2000).

## 6.3.3 Experimental Procedures

Subjects were anaesthetised with urethane (ethyl carbonate as a 25% aqueous solution) and prepared for cannulation and craniotomy surgery as described in Chapter 2.3. Once both surgeries were complete and the cortex overlaying the SC was exposed, an LED was positioned over the eye contralateral to the exposed SC in order to provide whole field illumination by which to guide electrode placement. A single channel tungsten parylene C microelectrode was then slowly advanced through the exposed cortex (6.3mm caudal to bregma, and 2.0mm lateral to midline) towards the SC using the multi-unit response to whole-field light flashes as guide (10ms duration, 0.5 Hz with 30% jitter; as described in chapter 3.3.2). Upon encountering the visual response stereotypical of the superficial layers of the SC (average placement depth of 2.98mm), the response to 150 visual stimuli was recorded to confirm response stability (see chapter 2.4.1 for further detail). Once a stable response to 150 visual stimuli was attained the animal was dark adapted for a minimum of 40min.

Following dark adaptation, the optimal stimulus luminance was determined as outlined in chapter 2.4.1. Once a stimulus intensity that reliably evoked a stable LFP and MUA response was established, a baseline pre-drug response to 150 whole field light flashes was recorded (10ms duration, 0.5 Hz with 30% jitter). This was then followed by a sequence of injections, depending on the experimental condition: either NAD-299 (0.1 mg/kg in 0.9% saline) followed by fluoxetine (cumulative doses 0.5:8.0mg/kg in distilled H2O; experimental condition; N = 7), NAD-299 followed by distilled H2O (NAD-299 control; condition N = 7), or saline followed by fluoxetine (FLU control condition, N = 6). See figure 6.1 for clearer details on the sequencing of drug injections for each group. To control for any unlikely interaction between the vehicles used for both substances, data was collected for final control condition consisting of injections of saline followed by distilled water (saline + H2O condition N = 4). For each dose, 2min were allowed for the drug to circulate, after which the response to 150 visual stimuli was acquired. Upon completion of the procedure, animals were immediately euthanised with pentobarbital (1.0ml i.v. injection), following which, brains were immediately removed and placed in fixative for later histological confirmation of electrode placement, as outlined in Chapter 2.5.

Figure 6.1: Sequencing of drug injections and associated time points for data collection throughout the experimental procedure. Numbers indicate a time at which the response to 150 stimuli were recorded (10ms duration, 0.5 Hz with 30% jitter). Where drugs were infused, 2 min were allowed for the drugs circulate prior to recording of response. Doses at time 2 comprised of 1.0mg/kg of NAD-299 or volumetrically equivalent doses of its vehicle (saline), while doses at times 3:7 comprised of cumulative doses (0.5:8.0mg/kg) of fluoxetine or volumetrically equivalent doses of its vehicle (distilled H2O). Not pictured is the saline+H2O condition, where subjects were administered doses of saline and H2O which were volumetrically equivalent to the relevant NAD-299 and fluoxetine doses. NAD-299 and fluoxetine doses were based on prior research in murine models, which showed that NAD-299 blocks the effects of fluoxetine when these drugs are infused at the doses used in the current study (Eriksson et al., 2012).

3. 0.5 mg/kg fluoxetine

4. 1.0 mg/kg fluoxetine

5. 2.0 mg/kg fluoxetine

6. 4.0 mg/kg fluoxetine

7. 8.0 mg/kg fluoxetine

3. Equivalent H2O dose

4. Equivalent H2O dose

5. Equivalent H2O dose

6. Equivalent H2O dose

7. Equivalent H2O dose

1. Recording of pre-drug baseline response

2. 0.1mg/kg NAD-299

2. Equivalent saline dose

NAD+H2O

NAD+FLU

SAL+FLU

## 6.3.4 Data Analysis

Data were prepared for analysis as described in chapter 2.4.2. All data were analysed offline using custom written MATLAB scripts (2016a; The MathWorks Inc., Natick, MA, USA). All analyses were carried out on average data constructed from the response to 150 visual stimuli recorded at seven time points indicated above (see figure 6.2). For LFP and MUA there were three main comparisons of interest

1. Between post-drug responses in all conditions, relative to pre-drug baseline.
2. Between NAD-299+fluoxetine and NAD-299+H2O.
3. Between NAD-299+fluoxetine and saline+fluoxetine.

Data for onset latency, response duration, and peak amplitude were extracted from both raw MUA and LFP data values. Additional measures of baseline activity and multi-phasic peak amplitude were extracted from the MUA. For statistical purposes, all LFP and MUA data were normalised by dividing post-drug responses by the pre-drug (time 1) responses for each animal.

*Local field potential.* In order to extract the measures of onset latency and response duration, a response threshold was first constructed. This process comprised of two steps:

1. Calculating the mean and SD of the low frequency voltage trace over the 400ms period prior to stimulus presentation.
2. Constructing a response threshold, defined as any point at which the LFP waveform differed from the pre-stimulus mean by 3SD.

Onset latency was defined as the first time point at which the LFP waveform differed from the pre-stimulus mean by 3SD. Offset latency was then calculated using a two-step process. First, LFP activity in the post-onset period was divided into epochs of 100ms starting from the first time point immediately post onset. The mean voltage trace was then calculated for each epoch, thus allowing for identification of the 100ms period where the post-onset voltage trace most closely resembled the pre-stimulus mean. Once this 100ms period was identified, onset latency was derived as the first single time point within this period at which the LFP voltage trace was closest to the pre-stimulus mean. Response duration was then derived as the difference between onset and offset latency. Peak-to-peak amplitude was then determined as the difference between maximum voltage trace and minimum the voltage trace within the duration of the response.

*Multi-unit activity*. Four measures of interest were extracted from the MUA data: baseline activity, onset latency, response duration, and peak amplitude. To derive these measures, it was first necessary to compose a process to identify and extract spikes. Thus, thresholding was applied to facilitate the extraction of spikes. This was achieved for each individual animal by calculating the mean and SD of the high frequency voltage trace for the 400ms pre-stimulus time period for each of the 150 stimulus presentations in the pre-drug baseline recording. A spiking threshold was then calculated by averaging the mean of the 400ms pre-stimulus voltage trace across all stimulus presentations in the pre-drug baseline recording. Spikes were defined as activity for which the high frequency voltage trace exceeded 2 standard deviations of the spiking threshold (see figure 2.6 for visualisation of this method). This pre-drug spiking threshold was applied across all drug doses so as to avoid any potential confounding effect of drug-induced changes in activity on spike detection.

Spikes were extracted from the response to 150 visual stimuli at the time points defined in figure 6.1 above. These data were then used construct a peri-stimulus time histogram for each time point (PSTH; bin width = 10ms, count per bin normalised to the number of stimuli), from which values for baseline activity, onset latency, response duration and peak amplitude were extracted. Baseline activity was derived by calculating the mean bin count in the 400ms pre-stimulus time period. As well acting as a measure of spontaneous neural activity in the SC, baseline activity was also used as a reference point for the construction of a response threshold (defined as any activity that exceeds baseline activity by 5 SD) to facilitate the extraction of onset latency and response duration. Onset latency was thus derived as the first time point subsequent to stimulus presentation where the PSTH bin count exceeded this response threshold. Offset latency was determined as the first time point for which PSTH bin count was equal or less than baseline activity while remaining below the response threshold for a period of 400ms. Response duration was then derived as the difference between onset and offset latency. Finally, peak amplitude was calculated as the maximum single bin count within the response duration, normalised to baseline activity.

As established in Chapters 3 and 4, the superficial layer SC MUA response to visual stimuli is comprised of a multi-phase response, whereby the first 100ms of the response constitutes the first phase and all subsequent activity constitutes the second phase. As such, the peak amplitude of the response was calculated for the MUA response overall, as well as for phases 1 and 2 of the response.

For statistical purposes, all LFP and MUA data were normalised by dividing post-drug responses by the pre-drug (time 1) responses for each animal. LFP and MUA responses were analysed using mixed analysis of variance (ANOVA) and post-hoc t-tests. For statistical purposes, all LFP and MUA data were normalised by dividing post-drug responses by the baseline (pre-drug) responses for each animal. LFP and MUA responses were analysed using mixed analysis of variance (ANOVA) and post-hoc t-tests. Where assumptions of sphericity were violated Greenhouse-Geisser corrections were applied. For all analyses, alpha values of 0.05 were considered significant.

# 6 .4 Results

## 6.4.1 Electrode Placement

Brains were prepared for histological inspection as described in Chapter 2.6. Inspection of electrode tracks revealed that recording sites were located in the stratum SGS or the SO. Due to insult to tissue derived from the histology process, visual inspection could not always reliably distinguish if recording sites were located in SO or SGS. Where this was the case, characteristics of the baseline LFP response were used to aid in distinguishing between the collicular laminar.

For all animals, the baseline superficial SC LFP response to whole visual field stimuli comprised of a complex multi-component phenomena. For the majority of animals (19/20; 95.0%) the most salient short-latency LFP component consisted of a short duration negativity (most likely corresponding to components N1-N2 of the superficial SC response to visual stimuli; Dyer and Annau, 1997), followed by a short latency positivity (most likely corresponding to component P2) and then a longer duration, large amplitude negativity (most likely corresponding to components N3 and P3). The initial negative deflection was in some animals (2/20; 10.0%) preceded by a short duration, low amplitude positivity (P1). Such LFP dynamics are typical of electrode placement in the SO. Shallower electrode placement (in SGS) is associated with a reversal of these major field components (Dyer and Annau, 1977; Hirai and Okada, 1995). Where a positive deflection was the most salient short latency LFP aspect (n = 4, 16.0%), electrode placement was considered to be in the SGS. Thus the majority of animals were considered to have electrode placement in SGS or SO.

## 6.4.2 Effects of Administration of Systemic NAD-299 and Fluoxetine on Superficial Layer SC LFP Response to Visual Stimuli

In the experimental condition, when combined with NAD-299 (0.1 mg/kg), low doses of fluoxetine had little effect on the LFP. However, at its highest dose, NAD-299+fluoxetine (8.0mg/kg, fluoxetine) caused a depression of all major components of the superficial layer SC LFP response to whole visual field flashes relative to both control groups (see figure 6.2 for representative animal, and figure 6.3 for comparisons between groups for onset, duration, and peak-to-peak amplitude). No major effects of dose were observed for the NAD-299 control group, or the FLU control group.



Amplitude (mV)

Figure 6.2: Effect of NAD-299+fluoxetine on the LFP response to visual stimuli in a representative animal. Data presented represent the mean response to 150 visual stimuli presented as a whole visual field flashes (stimuli presented at t0). A depression of the amplitude the LFP is observed following systemic administration of fluoxetine.

Data were analysed initially using two sets of Mixed ANOVAs; one set comparing the experimental group with the NAD-299 control, and one set comparing the experimental group with the FLU control. For all analyses detailed below, comparisons were made across 6 doses, where dose 1 represents 0.1mg/kg NAD-299 in 0.9% saline (experimental group; NAD-299 control) or volumetrically equivalent saline (fluoxetine control), and doses 2:6 represent cumulative doses of 0.5, 1.0, 2.0, 4.0 and 8.0mg/kg of fluoxetine in distilled water (experimental group; fluoxetine control) or volumetrically equivalent doses of distilled water (NAD-299 control). Refer back to figure 6.1 for details.

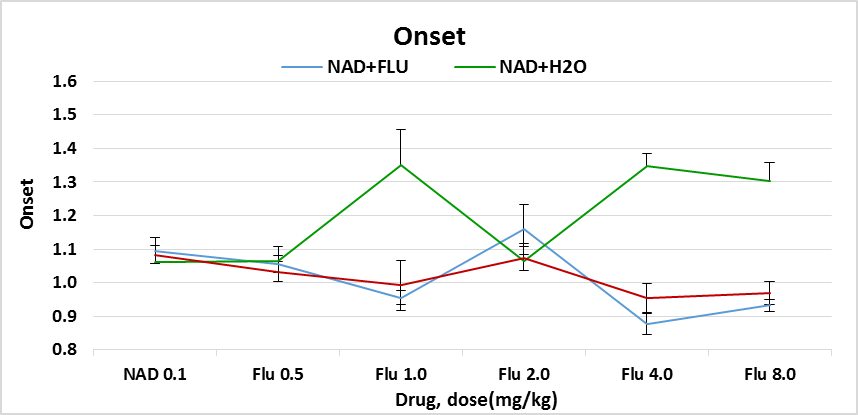
***Effects of NAD-299+Fluoxetine versus NAD-299 + H20***

For peak-to-peak amplitude, Mixed ANOVA (factors: Dose [N=6], Condition [Experimental condition or NAD control]) revealed a significant main effect of Condition (F[1, 8] = 19.25, p = 0.002), but no main effect of Dose (F[5, 40] = 0.79, p = 0.560) and no of Dose\*Condition interaction (F[5, 40] = 1.43, p = 0.23). Post-hoc independent samples t-tests revealed a significant difference between conditions for the maximum fluoxetine dose only (8.0mg/kg; t(9) = 2.28, p = 0.048), suggesting that at high doses, NAD-299+fluoxetine depresses the amplitude of superficial SC LFP responses to visual stimuli. For duration, mixed ANOVA revealed a no main effect of Condition (F[1, 8] = 0.18, p = 0.681), or Dose (F[5, 40] = 1.00, p = 0.429), and no interaction of Condition\*Dose (F[5, 40] =0.05, p = 0.999). No main effect of onset was revealed for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[5, 40] = 0.72, p = 0.617; Condition: F[1, 8] = 3.319, p = 0.106; Dose\*Condition: F[5, 40] = 0.99, p = 0.438).

***Effects of NAD-299+Fluoxetine versus Saline + Fluoxetine***

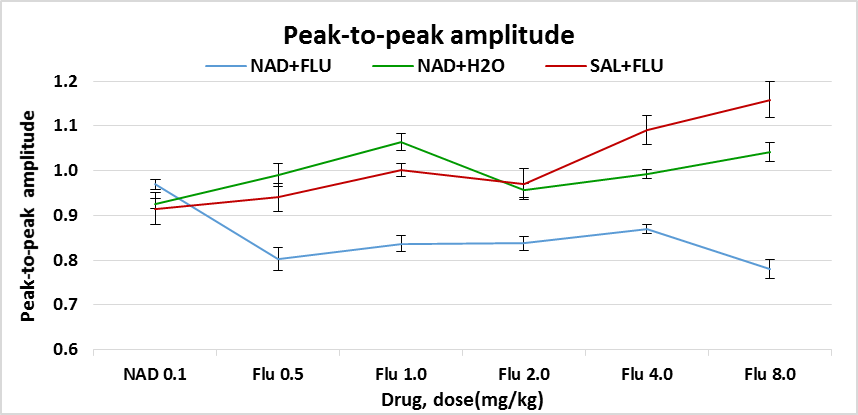
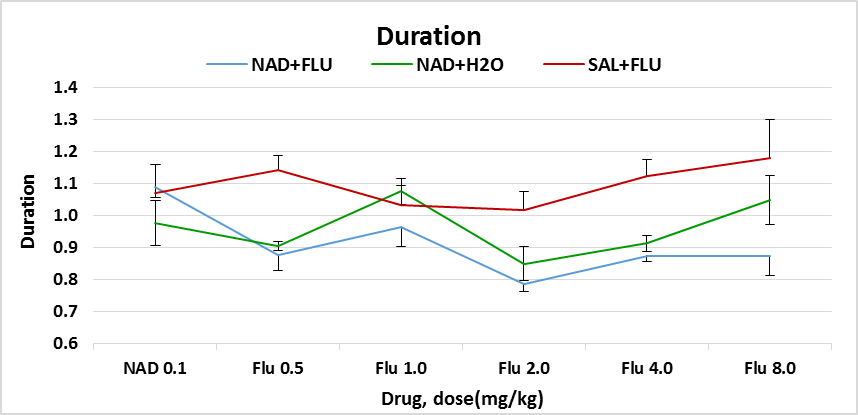
For peak-to-peak amplitude, Mixed ANOVA (factors: Dose [N=6], Condition [Experimental condition or FLU control]) revealed a main effect of Condition (F[1, 9] = 16.44, p = 0.003), but no main effect of Dose (F[5, 45] = 0.31, p = 0.907) and no interaction of Dose\*Condition (F[5, 45] = 1.96, p = 0.103). Post-hoc independent samples t-tests revealed a significant different between conditions for fluoxetine doses of 4.0mg/kg (t(9) = 2.37, p = 0.042) and 8.0mg/kg (t(9) = 3.25, p = 0.010), suggesting that at high doses, fluoxetine depresses the amplitude of superficial SC LFP responses to visual stimuli, but only when administered subsequent to 5-HT1Aautoreceptor antagonism. For onset, no main effect was revealed for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[5, 45] = 1.21, p = 0.324; Condition: F[1, 8] = 1.66, p = 0.233; Dose\*Condition: F[5, 45] = 0.23, p = 0.949). Similarly, for duration, GG-ANOVA revealed no main effect was for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[2.27, 17.806] = 0.37, p = 0.714; Condition: F[1, 8] = 0.14, p = 0.722; Dose\*Condition: F[2.27, 17.806] = 0.88, p = 0.444).

In the saline + H2O control condition, neither substance had any effect on onset, duration, or peak-to-peak amplitude of the LFP response.



**A**

Figure 6.3: The effect of systemic administration of NAD-299+fluoxetine, NAD-299+H2O, and Saline+Fluoxetine on the superficial layer SC LFP response to visual stimuli: for (A) onset latency, (B) duration, (C) peak amplitude. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made across rising cumulative doses of fluoxetine following pre-treatment with either NAD-299 or saline.



**B**

**C**

## 6.4.3 Effects of Administration of Systemic NAD-299 and Fluoxetine on Superficial Layer SC MUA Response to Visual Stimuli

The major effect of NAD-299+Fluoxetine on the MUA was to depress baseline activity at all doses of Fluoxetine relative to NAD-299+H2O and Saline+Fluoxetine. This effect was modest at low to moderate doses (0.5:4.0mg/kg), but considerable at the highest fluoxetine dose (8.0mg/kg). In the experimental condition, no effect of fluoxetine was observed on onset, duration or overall peak amplitude (see figure 6.4 for representative animal, and figure 6.5 for comparisons between groups), though a specific depression of the peak amplitude of the second phase of the response was observed (aspects of the multiphasic response are discussed below in section 6.4.4). Sal+Fluoxetine caused a depression in onset latency across all doses relative to the experimental condition and the NAD-299 control.

As with LFP, all MUA data were analysed initially using two sets of Mixed ANOVAs; one set comparing the experimental group with the NAD-299 control, and one set comparing the experimental group with the FLU control. For all analyses detailed below, comparisons were made across 6 doses, where dose 1 represents 0.1mg/kg NAD-299 in 0.9% saline (experimental group; NAD-299 control) or volumetrically equivalent saline (fluoxetine control), and doses 2:6 represent cumulative doses of 0.5, 1.0, 2.0, 4.0 and 8.0mg/kg of fluoxetine in distilled water (experimental group; fluoxetine control) or volumetrically equivalent doses of distilled water (NAD-299 control).



Figure 6.4: PSTH showing the effects of NAD-299+fluoxetine on the LFP response to visual stimuli in a representative animal. Data presented represent the mean response to 150 visual stimuli presented as a whole visual field flashes (stimuli presented at t0). X axis is time; y axis is PSTH count, calculated as described in section 6.3.4. The red vertical line indicates the distinction between the first phase and second phase of the MUA response. A depression of baseline MUA activity, and peak amplitude of the second phase of the response is observed following systemic administration of fluoxetine. Note that while the first phase of the response appears to be depressed, this is in fact an effect of reduced baseline activity.

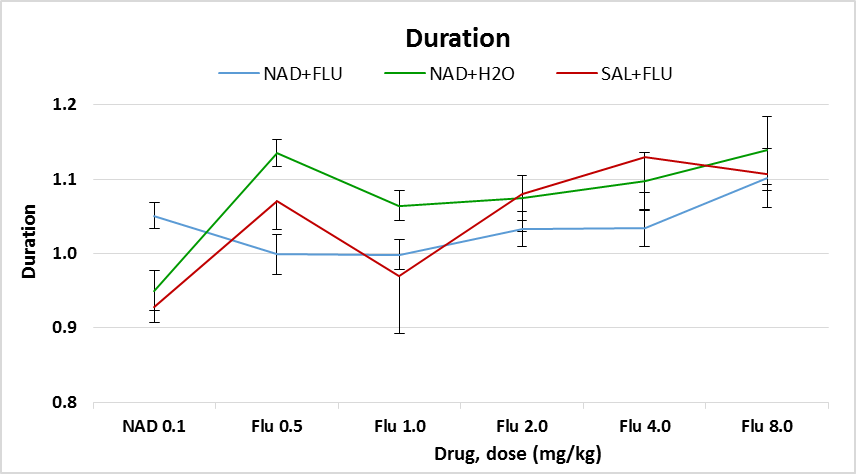
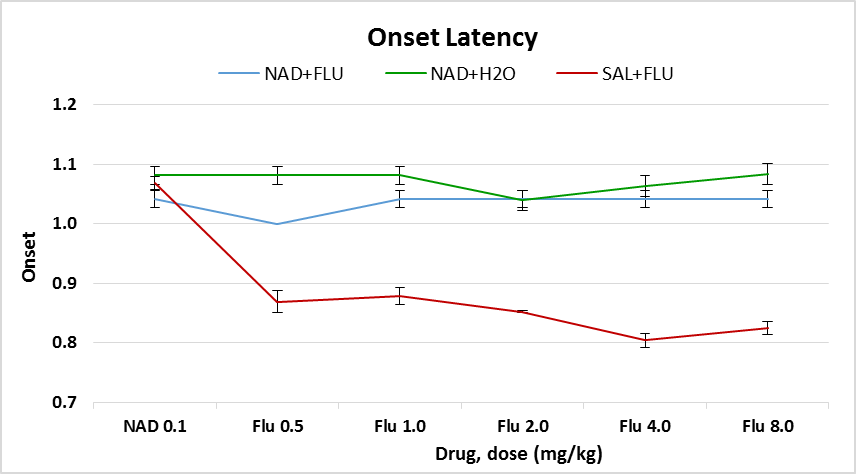
**Effects of NAD-299+Fluoxetine versus NAD-299 + H20**

For baseline activity, Mixed GG-ANOVA (factors: Dose [N=6], Condition [Experimental condition or FLU control]) revealed significant main effects of Dose (F[2.06, 22.66] = 3.57, p = 0.044), Condition (F[1, 11] = 9.32, p = 0.011), but no significant interaction of Dose\*Condition (F[2.06, 22.66] = 2.46, p = 0.107). Post-hoc independent samples t-tests revealed a significant difference between conditions for fluoxetine doses of 1.0mg/kg (t(10) = 2.75, p = 0.019), 4.0mg/kg (t(10) = 3.00, p = 0.012), and 8.0mg/kg (t(10) =3.16, p = 0.009). These data suggest that, at a range doses, NAD-299+fluoxetine depresses spontaneous spiking activity at the level of the SC. For onset, Mixed ANOVA revealed no main effect of Dose or Condition, and no Dose\*Condition interaction (Dose: F[5, 55] = 0.37, p = 0.869; Condition: F[1, 11] = 0.58, p = 0.463; Dose\*Condition: F[5, 55] = 0.67, p = 0.657). Similarly, for duration, mixed ANOVA revealed no main effect of Dose or condition, and no interaction of Condition\*Dose (Dose: F[5, 55] = 1.286, p = 0.283; Condition: F[1, 10] = 0.191, p = 0.67; Dose\*Condition F[5, 55] = 1.22, p = 0.313). Finally, for peak amplitude, GG-ANOVA revealed no main effect for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[1.61, 17.74] = 1.71, p = 0.211; Condition: F[1, 11] = 0.74, p = 0.409; Dose\*Condition: F[1.61, 17.74] = 1.56, p = 0.236).

**Effects of NAD-299+Fluoxetine versus Saline + Fluoxetine**

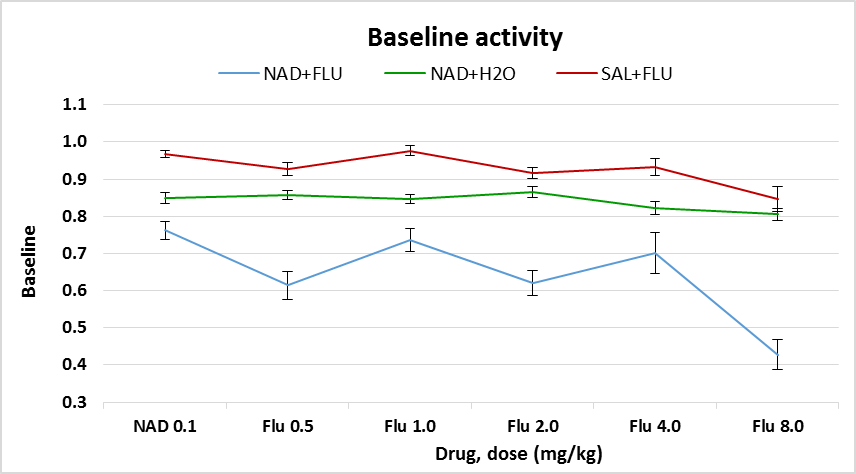
For baseline activity, Mixed ANOVA (factors: Dose [N=6], Condition [Experimental condition or FLU control]) revealed significant main effects of Dose (F[5, 50] = 3.23, p = 0.013), and Condition (F[1, 10] = 8.86, p = 0.014), but a non-significant interaction of Dose\*Condition (F[5, 50] = 0.68, p = 0.638). Post-hoc independent samples t-tests revealed a significant difference between conditions for fluoxetine doses of 1.0mg/kg (t(10) = 3.23, p = 0.009), 4.0mg/kg (t(10) = 2.62, p < .026), and 8.0mg/kg (t(10) = 2.23, p = .045). These data suggest that, at a range doses, fluoxetine depresses spontaneous spiking activity at the level of the SC, but only when administered subsequent to 5-HT1Aautoreceptor antagonism. For onset latency, Mixed GG-ANOVA revealed significant main effects of Dose (F[2.29, 22.90] = 4.89, p = 0.014), Condition (F[1, 10] = 21.90, p < 0.015), and a significant interaction of Dose\*Condition (F[2.29, 22.90] = 4.82, p = 0.001). Post-hoc independent samples t-tests revealed a significant different between conditions for all doses of fluoxetine (0.5mg/kg: t(10) = 2.64, p = 0.025; 1.0mg/kg: t(10) = 2.95, p = 0.015; 2.0mg/kg: t(10) = 4.50, p < 0.001; 4.0mg/kg: t(10) = 4.53, p < 0.001; 8.0mg/kg: t(10) = 4.14, p = 0.002), showing that when administered alone, fluoxetine reduces onset latency of visual MUA in the superficial SC. For duration, mixed GG-ANOVA revealed no main effect of Dose or condition, and no interaction of Condition\*Dose (Dose: F[1.95, 19.57] = 1.13, p = 0.343; Condition: F[1, 10] = 0.76, p = 0.479; Dose\*Condition F[1.95, 19.57] = 1.21, p = 0.322). Similarly, for peak amplitude, GG-ANOVA revealed no main effect was for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[1.71, 17.10] = 1.29, p = 0.295; Condition: F[1, 10] = 0.87, p = 0.375; Dose\*Condition: F[1.71, 17.10] = 1.08, p = 0.352).

In the saline + H2O control condition, neither substance had any effect on onset, duration, or peak amplitude or any aspect of the MUA response.



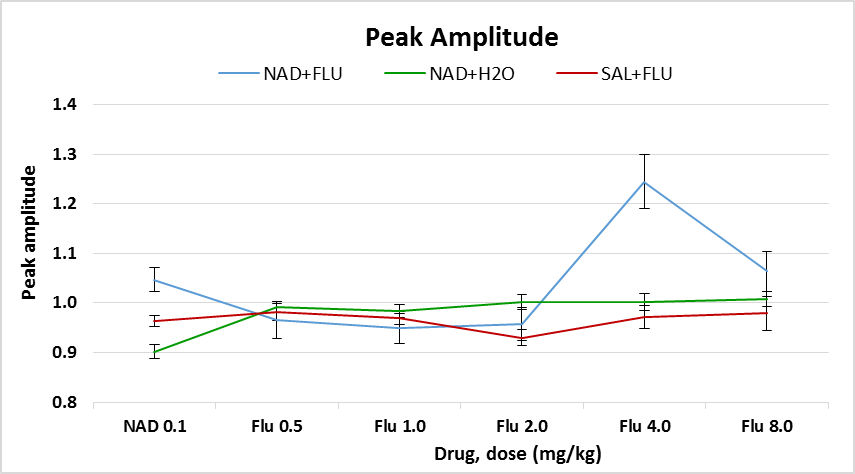
**A**

**B**



**C**

Figure 6.5: The effect of systemic administration of NAD-299+fluoxetine, NAD-299+H2O, and Saline+Fluoxetine on the superficial layer SC MUA response to visual stimuli: for (A) onset latency, (B) duration, (C) baseline activity, (D) peak amplitude. Error bars are equal to ±1 SEM. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made across rising cumulative doses of fluoxetine following pre-treatment with either NAD-299 or saline.



**D**

## 6.4.4 Biphasic Properties of the Superficial Layer SC MUA Response to Visual Stimuli

Visual inspection revealed a multiphasic profile for the MUA response of all animals tested, where the first 100ms of the response post-onset constituted the first phase of the response, and all further activity constituted the second phase of the response (as defined in Chapter 3). In the experimental condition, low doses of NAD-299+fluoxetine had little effect on either phase of the response. At high doses (4.0mg/kg; 8.0mg/kg), NAD-299+fluoxetine depressed the peak amplitude of phase two, but not phase one, of the MUA response. No major effects of dose were observed for the NAD-299 control group, or the FLU control group. Data were analysed as described for MUA above, but with values for peak amplitude extracted from both phases of the response.

**Effects of NAD-299+Fluoxetine versus NAD-299 + H20**

For phase 2 of the MUA response a mixed GG-ANOVA (factors: Dose [N=6], Condition [Experimental condition or FLU control]) revealed significant main effects of Dose (F[1.64, 16.38] = 7.385, p = 0.007), and a significant interaction of Dose\*Condition (F[1.64, 16.38] = 16.40, p < 0.001), but no mean effect of condition (F[1, 10] = 0.124, p = 0.732). Post-hoc independent samples t-tests revealed a significant different between conditions for fluoxetine doses of 4.0mg/kg (t(10) = 3.22, p = 0.009; and 8.0mg/kg (t(10) = 4.81, p < .001), suggesting that at high doses, NAD-299+fluoxetine depresses the amplitude of phase 2 of superficial SC MUA to visual stimuli. For phase 1 of the MUA response a mixed GG-ANOVA revealed no main effect for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[2.19, 24.17] = 0.81, p = 0.465; Condition: F[1, 11] = 2.29, p = 0.158; Dose\*Condition: F[2.19, 24.17] = 1.13, p = 0.346).

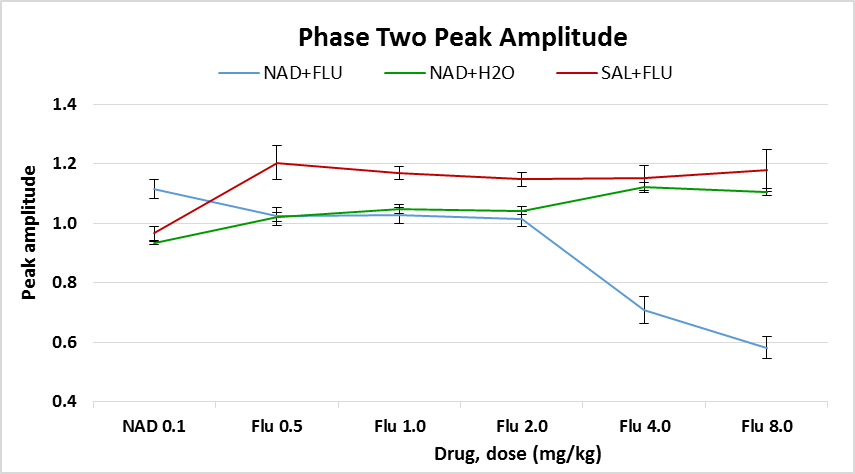
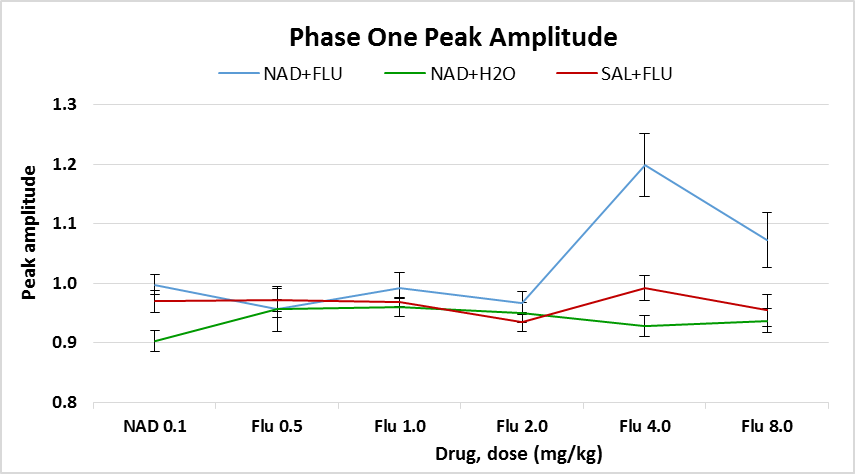
***Effects of NAD-299+Fluoxetine versus Saline + Fluoxetine***

For phase 2 of the MUA response a mixed ANOVA (factors: Dose [N=6], Condition [Experimental condition or FLU control]) revealed significant main effects of Dose (F[5, 50] = 2.76, p = 0.028), Condition (F[1, 11] = 3.73, p = 0.082), and a significant interaction of Dose\*Condition (F[5, 50] = 5.09, p < 0.001). Post-hoc independent samples t-tests revealed a significant different between conditions for fluoxetine dose of 4.0mg/kg (t(10) = 2.54, p = 0.030) and 8.0mg/kg (t(10) = 2.62, p = 0.026), suggesting that at high doses, fluoxetine depresses the amplitude of phase 2 of superficial SC MUA to visual stimuli, but only when administered subsequent to 5-HT1Aautoreceptor antagonism. For phase 1 of the MUA response a mixed GG-ANOVA revealed no main effect was for either Dose, Condition, or a Dose\*Condition interaction (Dose: F[2.21, 22.06]= 0.95, p = 0.411; Condition: F[1, 10] = 1.10, p = 0.318; Dose\*Condition: F[2.21, 22.06] = 0.58, p = 0.583).

Figure 6.6: The effect of systemic administration of NAD-299+fluoxetine, NAD-299+H2O, and Saline+Fluoxetine on the superficial layer SC MUA response to visual stimuli: for (A) phase 1 peak amplitude; (B) phase 2 peak amplitude. Error bars are equal to ±1 SEM. Data for each dose were averaged over a block of 150 visual stimuli, and then normalised to pre-drug activity of 1.0 (represented by bold black line). Thus, deviation from 1.0 represents deviation from pre-drug activity. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Doses represent experimental drug, of volumetrically equivalent doses of vehicle. Comparisons were made across rising cumulative doses of fluoxetine following pre-treatment with either NAD-299 or saline.

**B**

**A**



# 6.5 Discussion

## 6.5.1 Summary of Results

The present study aimed to explore whether fluoxetine depresses visual activity at the level of the SC in the presence and absence of NAD-299. The superficial SC LFP responses to whole field visual stimuli were complex, multicomponent phenomena, which is consistent with previous reports (e.g. Dyer and Annau, 1977; Gowen et al., 2008; Clements et al., 2014). Based on electrophysiological and histological criteria, electrode placement was considered to be localised to the SGS or SO for the majority of animals. Previous reports have described onset latency of the superficial layer SC LFP response to whole visual filed stimuli as ranging from 28 ms (Dyer and Annau, 1997) to 50-60 ms (Fortin et al., 1997), which is consistent with our own observations (mean onset latency at baseline for all groups = 59.12 ms).

The effects of NAD-299+ fluoxetine in the current study differed for the LFP and the MUA. For the LFP, when administered subsequent to NAD-299, the major effect of high doses of fluoxetine (4.0; 8.0mg/kg) was to reduce the amplitude of visual responses in the superficial layers of the SC relative to both NAD-299+H2O and saline+fluoxetine. Neither NAD-299 alone nor fluoxetine alone significantly altered any aspect of the LFP. For the MUA, when administered subsequent to NAD-299, the major effect across all doses of fluoxetine was to reduce spontaneous baseline collicular activity relative to both NAD-299+H2O and Saline+fluoxetine. NAD-299 alone did not significantly alter any aspect of the MUA response, but fluoxetine alone caused a decrease in onset latency at all doses administered.

No effect of NAD-299+fluoxetine was observed for overall peak amplitude, but when the multi-unit response was stratified into an initial and late phase (phase 1 and phase 2), the peak amplitude of phase 2, but not phase 1, was reduced by NAD-299+fluoxetine. Neither NAD-299 alone nor fluoxetine alone significantly altered peak amplitude for either phase of the response. These results suggest that when administered subsequent to 5-HT1A antagonism, fluoxetine reduces the input to (synaptic activity; LFP), and specific aspects of the output from (spiking activity; MUA) superficial layer SC neurons. As no change in peak amplitude or duration were observed when fluoxetine is administered alone, it is suggested that 5-HT1A antagonism is a necessity to observe any effect of fluoxetine on the SC in acute animal psychopharmacology paradigms.

## 6.5.2 Interpretation of Results

There are two major findings in the current study:

1. Pre-treatment with a 5-HT1A antagonist is required for fluoxetine to affect responses in the SC
2. When this pre-treatment is in place, fluoxetine depresses the SC response to visual stimuli.

While finding 1 is a novel observation for the SC, it is consistent with reports in the extant literature that when administered alone in acute preparations, fluoxetine provokes negligible changes in 5-HT mediated transmission in areas innervated by the DRN ([Artigas, Romero, de Montigny, & Blier, 1996](#_ENREF_4); [Romero, Hervas, & Artigas, 1996](#_ENREF_24)). During acute systemic fluoxetine administration, DRN neurons are exposed to increased local availability of 5-HT. This has the effect of down-regulating the spontaneous firing of 5-HT neurons in the DRN, in a process that is largely mediated by 5-HT1A autoreceptors (Fischer et al., 2014; Maejima et al., 2013). As a consequence of this down-regulation 5-HT release at target nuclei is inhibited, resulting in reduction of synaptic 5-HT availibility. Subsequently, while SSRIs may be occupying SERT in target regions of the DRN, the release of 5-HT is itself being inhibited, thus masking any effects of blockading the reuptake of 5-HT. Though we did not directly measure DRN activity in the present study, it can be inferred that administration of the selective 5-HT1A antagonist NAD-299 counteracted the down-regulation of serotonergic activity in the DRN, allowing for the true effects of fluoxetine to be revealed. As such, future explorations of the effect of fluoxetine on collicular activity should ensure be sure to first antagonise 5-HT1A transmission.

Finding 2, that fluoxetine depresses visually evoked responses in superficial layers of the SC is a novel finding, but is consistent with previous reports where 5-HT levels in the SC have been pharmacologically manipulated. (Gowen et al., 2008; Dommett et al., 2009; Clements et al., 2014). At the doses used in the present study, fluoxetine displays very high affinity for SERT and low affinity for NERT (van Harten, 1993; Wong et al., 1995). Though occupancy of fluoxetine at the level of the SC has not been previously established, at both therapeutic and subtherapeutic doses, fluoxetine has a mean occupancy of 76%-85% in other subcortical targets of the DRN, with increased occupancy associated with higher doses (Meyer et al., 2004). As such, the proposed mechanism by which fluoxetine alters visual responsiveness at the level of the SC is though increasing synaptic 5-HT availability by blockading the reuptake of 5-HT into presynaptic terminals. Finding 2 is consistent with the results from Chapters 3 and 4, where d-amphetamine depressed visual responses in the SC in an effect that was shown to be at least partially mediated by 5-HT. In Chapter 4.3 it was proposed that elevated synaptic levels of 5-HT may act at postsynaptic retinotectal 5-HT1A heteroreceptors at the level of the SC to inhibit neural excitability, thus depressing the response to visual stimuli. While the use of a 5-HT1A receptor antagonist in the current study may seem to discount this proposition as a potential explanation for fluoxetine mediated depression of SC activity, it should be noted that NAD-299 alone did not induce changes in any LFP or MUA measure that were statistically different to saline alone. 5-HT1A receptors are expressed either as autoreceptors when located somatodendritically on 5-HT neurons (e.g. in the DRN), or as heteroreceptors when expressed in brainstem and forebrain regions that are the target of 5-HT innervations (e.g. the SC; (Massey et al., 2013). While NAD-299 has been shown to display high affinity for blockading 5-HT1A autoreceptors (S. Ross, B. et al., 2006), its properties with respect to 5-HT1A heteroreceptors are under-researched. It has been demonstrated that other substances that otherwise have very high affinity for DRN 5-HT1A autoreceptors, such as the 5-HT1A agonist 8-OH-DPAT, have no affinity for 5-HT1A when expressed as heterorecptors elsewhere in the brain (Riad, Watkins, Doucet, Hamon, & Descarries, 2001). As such, given that NAD-299 had no effect on the collicular response to visual stimuli in the current study, it is plausible that the antagonistic effects of NAD-299 were specific to 5-HT1A autoreceptors at the level of the DRN. It is thus proposed that the effect of fluoxetine in the current study was to elevate synaptic levels of 5-HT, which then act on postsynaptic retinotectal 5-HT1A heteroreceptors to inhibit neural excitability, thereby depressing the SC response to visual stimuli.

Consistent with the results from Chapters 3 and 4, the MUA response in the current study showed a clear biphasic profile consisting of an initial phase (phase 1; the first 100ms post onset) and a late phase (phase 2; any MUA activity with a latency greater than 100ms of onset). In the current study, when pre-treated with NAD-299, high doses of fluoxetine caused a significant depression in phase 2 of the response, but had no effect on phase 1. In the previous experimental chapters, these phases were differentially affected by d-amphetamine. In Chapter 3, systemic d-amphetamine induced a depression of phase 1 peak amplitude at all doses administered, with greatest depression seen at the highest doses. For phase 2, only the two highest doses of d-amphetamine administered depressed the amplitude of the response. In Chapter 4, systemic d-amphetamine induced a comparable depression for both phases of the response. In both chapters, the two phases of the response differed in how they were affected by the 5-HT antagonist metergoline: antagonism of 5-HT fully reversed the effect of d-amphetamine on phase 2, but only produced a modest reversal for phase 1. It was thus suggested that phase 2 of the MUA response is more sensitive to 5-HT manipulation than the phase 1. This proposition is supported by the results of the current study. The depression of phase 2 MUA peak amplitude observed following administration of high doses of fluoxetine is comparable to the depression observed at high doses of d-amphetamine in chapter 3, which was fully reversed following 5-HT antagonism. At the doses used in this thesis, d-amphetamine enhances the synaptic availability of multiple monoamine neurotransmitters, while fluoxetine enhances only proximal 5-HT availability. As such, it is plausible that phase 1 of the collicular MUA response to visual stimuli is modulated by multiple monoamine systems, while phase 2 is modulated exclusively by 5-HT. It was proposed in Chapter 3 that phase 2 of the MUA may represent the activity of visuo-motor burst neurons, a subset of SC neurons that modulate target selection (McPeek et al., 2002). Depression of this aspect of the SC response may serve to reduce the likelihood that a non-biologically salient target is chosen for a foveating eye movement. Within the context of pharmacotherapy of ADHD, one potential mechanism of fluoxetine may thus be to enhance sustained attention by reducing the likelihood that visuo-motor burst neurons will fire in response to non-salient stimuli.

An additional effect of NAD-299+fluoxetine in the current study was to depress spontaneous multi-unit collicular activity at all doses administered. At low doses, NAD-299+fluoxetine depressed spontaneous collicular activity, whilst retaining the amplitude of visually evoked responses. This finding is comparable to previous in vitro work, where Dommett et al., (2009) demonstrated that low doses of 5-HT differentially affect visually evoked potentials in the superficial SC. Low doses of 5-HT acted to preferentially inhibit responses to low intensity stimulation, while responses to high intensity stimulation were largely preserved. Interestingly, when high doses of 5-HT were introduced to the superficial SC, the collicular response to all stimulation intensities was almost universally depressed. Again, this is comparable to the present study, where high doses of NAD-299+fluoxetine depressed both spontaneous collicular activity, and the amplitude of event related visual responses. While it should be noted that the signals measured in the current study and Dommett et al’s study differ (evoked potentials vs. spontaneous activity), it may be suggested NAD+299 fluoxetine and 5-HT have comparable effects on superficial SC activity. Further examination of this comparison using an in vitro preparation could help illuminate any potential mechanism of fluoxetine at the level of the SC.

## 6.5.3 Implications of the Results

The results of the current study show for the first time that antagonising 5-HT1A autoreceptors allows for fluoxetine to depress responses to visual stimuli in the superficial layers of the SC. This adds to converging pharmacological evidence that 5-HT transmission at the level of the SC may be a relevant target for intervention in the pharmacotherapy of ADHD. The depression of the SC response induced by fluoxetine shows a similar profile to the depression induced by d-amphetamine, a current frontline pharmaceutical for ADHD (see Chapter 3 and Chapter 4). It is proposed that both fluoxetine and d-amphetamine act to elevate synaptic levels of 5-HT, which then act on postsynaptic retinotectal 5-HT1A heteroreceptors to inhibit neural excitability, thereby depressing the SC response to visual stimuli. It has previously been established that 5-HT mediated responses in the superficial layers of the SC can bias action selection via visuomotor loops through the BG (see chapter 5.3). Within the context of ADHD, it is proposed that visual responsiveness in the colliculus is abnormally high (Overton, 2008). As the response to visual stimuli in the superficial SC are depressed when levels of 5-HT are increased (Dommett et al., 2009; see the results presented in Chapters 3 and 4), there is a clear theoretical pathway through which drugs that enhance 5-HT levels could normalise distractibility in ADHD. Given that d-amphetamine may act to enhance sustained attention by normalising 5-HT transmission at the level of the SC, there is thus potential for combined therapy of NAD-299+fluoxetine to emerge as a candidate intervention with relevance to the pharmacotherapy of ADHD.

Final Discussion and Conclusions

# 7.1 Chapter Summary

The previous chapters have described and discussed the research conducted in the present thesis, and have provided an appropriate theoretical and methodological framework to contextualise this research. The current chapter compiles and summarises the results presented in the preceding empirical chapters and discusses the key theoretical implications of the work conducted. Finally remaining questions are considered, and directions for future research are proposed.

# 7.2 Motivation for Research

The research presented in the current thesis had two principal motivators:

1. To elucidate the mechanism by which d-amphetamine depresses visual responses in the superficial layers of the SC.
2. To identify a candidate substance that has a common mechanism of action at the level of the superficial SC as d-amphetamine, but with a safer profile in terms of side effects and abuse potential.

d-amphetamine is a current frontline pharmaceutical used in the treatment of ADHD, and when tolerated well, is highly efficacious in improving sustained attention and alleviating the symptoms of ADHD (Elia et al., 1999; Konrad et al., 2005). Despite this efficacy, the use of d-amphetamine in the treatment of ADHD has been criticised due to its abuse potential and aversive side-effects (Ghuman et al., 2001; Williams et al., 2004). While evidence suggests that clinically relevant levels of stimulant misuse are rare amongst ADHD populations (Kollins, 2007), there is an emergent trend in recent years towards misuse and diversion of prescription d-amphetamine, particularly amongst adolescents, and college students (Colaneri et al., 2017; Weyandt et al., 2013). With recent updates to diagnostic criteria more accurately reflecting the adult ADHD phenotype, this trend is set to continue as prevalence rates of ADHD in adolescent and college populations are expected to rise (Vitola et al., 2017). Thus, it was proposed that in order to minimise the risk of psychostimulant misuse and diversion, there is a need to identify new efficacious interventions for ADHD that lack the abuse potential of psychostimulants. A fruitful strategy when taking this approach is to identify substances that have common mechanisms of action to psychostimulants in terms of the systems they interact with (Overton, 2008).

While a full pharmacological profile of the effects of d-amphetamine remains elusive (Spencer et al., 2002), there is evidence that d-amphetamine acts to depress sensory responsiveness in regions with symptomatic relevance to ADHD. One such locus is the midbrain superior colliculus, where d-amphetamine acts to depress the response to visual stimuli in vivo (Gowan et al., 2008). This depression was shown to be mediated by 5-HT in vitro (Dommett et al., 2009). The research in the current thesis aimed to expand on this work by first identifying the neurotransmitter systems that mediate the effect of d-amphetamine on the SC response in vivo, and then identifying a candidate substance that targets visual responsiveness in the SC via a similar mechanism to d-amphetamine.

# 7.3 Principal Findings

The work presented in the current thesis demonstrates three major findings

1. Depression of the superficial SC visual response induced by d-amphetamine in vivo is reversed following 5-HT antagonism.
2. When 5-HT1A autoreceptors are antagonised, fluoxetine acts to depress the SC response to visual stimuli in a comparable manner to d-amphetamine.
3. Discrete phases of the SC MUA response are differentially affected by both d-amphetamine and NAD-299+fluoxetine.

These results will be considered in more detail below.

## 7.3.1 Depression of the SC Visual Response Induced by d-amphetamine is Reversed Following 5-HT Antagonism

The data presented in Chapters 3 and 4 aimed to examine the effect of 5-HT antagonism following systemic or local administration of d-amphetamine on the superficial SC response to visual stimuli. The major effect of d-amphetamine when administered either systemically or directly into the superficial SC was to reduce the amplitude of both the LFP and MUA of visual responses in the superficial layers of the SC. These results suggest that d-amphetamine reduces both the input to (synaptic activity; LFP) and output from (spiking activity; MUA) superficial layer SC neurons. In chapter 3, the effects of i.v. d-amphetamine on both LFP and MUA amplitude were dose dependant, with the deepest depression recorded at the highest dose of d-amphetamine (16mg/kg). In Chapter 4, only a single bolus injection of d-amphetamine was administered, but the depression induced in the superficial SC visual response was comparable to the highest i.v. dose (see figure 7.1). Following the full range of d-amphetamine injections in both chapters, the broad spectrum 5-HT antagonist metergoline was administered (i.v. administered as two injections in Chapter 3, and a single injection in Chapter 4; equal dose administered in both chapters). The major effect of metergoline in both chapters was to reverse the depressant effect of d-amphetamine on the peak amplitude of the MUA response to whole field visual stimuli to levels approaching those recorded prior to d-amphetamine administration. This reversal was comparable between chapters, regardless of route of administration used for d-amphetamine. While non-significant, trends towards reversal also were observed for the LFP subsequent to metergoline administration.

**Figure 7.1**: The effect systemic (A) and local (B) D-amphetamine administration and subsequent metergoline administration on MUA peak amplitude of the superficial SC response to visual stimuli. All data presented is normalised to a pre-drug baseline response.

**B**

**A**

Thus, data presented in Chapters 3 and 4 show that when 5-HT is antagonised, the effect of d-amphetamine at the level of the superficial SC is partially reversed. Given that a known action of d-amphetamine is to enhance the synaptic availability of monoamine neurotransmitters (Heal et al., 2013), these results suggest that the mechanism by which d-amphetamine depresses visual activity in the SC is at least partially mediated by 5-HT.

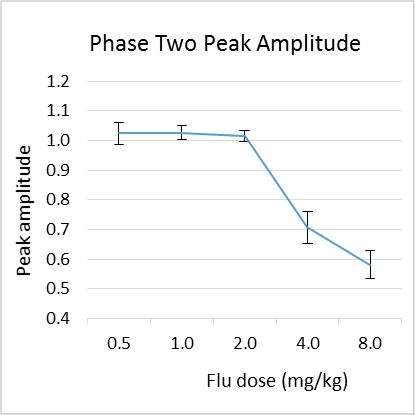
## 7.3.2 When 5-HT1A Autoreceptors are Antagonised, Fluoxetine acts to Depress the SC Response to Visual Stimuli in a Comparable Manner to d-amphetamine.

In light of the results presented in Chapters 3 and 4, it was suggested that there may be greater potential to exploit 5-HT transmission in the pharmacotherapy of ADHD. Following a review of the literature assessing the link between ADHD and 5-HT presented in Chapter 5, it was suggested that fluoxetine is a strong candidate drug for examination when considering the potential to exploit 5-HT transmission in the treatment of ADHD. As such, the data presented in Chapter 6 examine the effects of fluoxetine administration on the response to visual stimuli in the superficial SC. In order to control for 5-HT1A mediated down-regulation of 5-HT release at target areas of the DRN, the effect of fluoxetine on the SC response was assessed with and without systemic pre-treatment with the selective 5-HT1A autoreceptor antagonist NAD-299. The major effect of fluoxetine when administered subsequent to NAD-299 was to reduce the amplitude of the LFP, but not the MUA, of visual responses in the superficial layers of the SC. This depression was dose dependant, with the deepest depression observed at the highest dose of fluoxetine (8.0mg/kg). While overall MUA amplitude was not depressed, a specific depression was observed for phase 2 of the multi-unit response (see section 7.3.3 below). An additional effect of NAD-299+fluoxetine was to suppress spontaneous baseline activity in the SC, which may indicate an altered signal-to-noise ratio.

## 7.3.3 Discrete Phases of the SC MUA Response are Differentially Affected by Both d-amphetamine and Fluoxetine

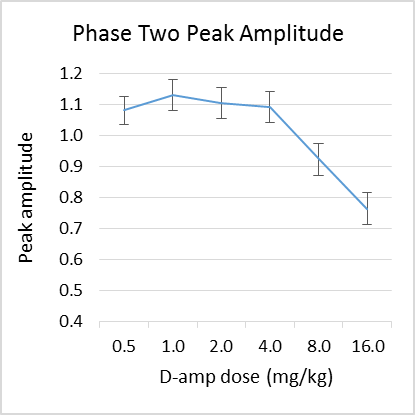
The SC multiunit response to visual stimuli can be divided into an early phase (phase 1), representing the first 100ms of the response, and a late phase (phase 2), representing all other activity until termination of the response. A novel property of d-amphetamine uncovered in the present thesis is its dichotomous effect on phase 1 of the MUA response compared to its effect on phase 2 of the MUA. Though the data presented in the current thesis were not the first to describe the SC response to visual stimuli as biphasic (McPeek & Keller, 2002), these data are the first to describe differential pharmacological action of d-amphetamine on the biphasic SC MUA response. In Chapter 3, it was observed that d-amphetamine depressed the peak amplitude of phase 1 of the MUA at all doses, with the highest depression seen at the highest dose of d-amphetamine. For phase 2, however, low doses of d-amphetamine had little effect on the peak amplitude, but high doses induced a significant depression in peak amplitude. When metergoline was administered, the amplitude of phase 1 of the MUA was partially reversed towards pre-drug levels, while amplitude of phase 2 was reversed to levels above those recorded for the pre-drug baseline. In Chapter 4, when administered locally as a single bolus injection, d-amphetamine induced a comparable depression in both phases of the response, though the reversal induced by metergoline was more significant for phase 2 than for phase 1. As stated above, d-amphetamine is known to enhance the synaptic availability of various monoamine neurotransmitters (Heal et al., 2013), while metergoline selectively antagonises 5-HT transmission (T. Sharp et al., 1989). As such, it was suggested that the second phase of the superficial SC MUA response to visual stimuli is more sensitive to 5-HT manipulation than the phase 1.

Data presented in Chapter 6 shows that, when pre-treated with NAD-299, fluoxetine has no effect on both overall MUA peak amplitude, or phase 1 peak amplitude. However, for phase 2 low doses of NAD-299+fluoxetine had little effect on the peak amplitude response, while high doses induced a significant depression in peak amplitude. This specific depression on phase 2 of the response resembled the depression seen across cumulative doses of d-amphetamine in Chapter 3 (see figure 7.2). At the doses used in this thesis, d-amphetamine enhances the synaptic availability of multiple monoamine neurotransmitters, while fluoxetine enhances proximal 5-HT availability. As such, it is plausible that phase 1 of the collicular MUA response to visual stimuli is modulated by multiple monoamine systems, while phase 2 is modulated almost exclusively by 5-HT.



**Figure 7.2:** The effect systemic d-amphetamine (A) and systemic fluoxetine following NAD-299 pre-treatment (B) on MUA peak amplitude of the superficial SC response to visual stimuli. All data presented is normalised to a pre-drug baseline response

**B**



**A**

# 7.3.4 Summary of Findings

In summation, data presented in the current thesis show for the first time that the depression induced by d-amphetamine on the superficial SC response to visual stimuli can be reversed by antagonising 5-HT in vivo. It was shown that the effect of d-amphetamine on the MUA response is more nuanced than previously reported, with higher doses of d-amphetamine required to depress the peak amplitude of the second phase of the response compared to the first phase. Similarly 5-HT antagonism differentially effects these phases: administration of metergoline reverses the d-amphetamine induced depression amplitude of both phases of responses, though this is a complete reversal for phase 2 and an incomplete reversal for phase 1. Finally a novel effect of fluoxetine on the superficial SC response to visual stimuli has been described. When administered in the presence of the 5-HT1A autoreceptor antagonist NAD-299, Fluoxetine depresses aspects of both the LFP and MUA response, in a manner that is comparable to the effect of d-amphetamine.

# 7.4 Theoretical Implications

The current findings have implications for the viability of targeting 5-HT transmission in the pharmacotherapy of ADHD, and the range of potential treatment strategies available when taking this approach. The data presented in the current thesis add to mounting evidence that d-amphetamine action at the level of the SC is mediated, at least in part, by 5-HT. The findings that systemic and local administration of d-amphetamine depresses visual responses in superficial layers of the SC is consistent with previous reports (Gowen et al., 2008; Dommett et al., 2009; Clements et al., 2014). While it has been previously shown that the depressant effect of d-amphetamine on the visual responses in the superficial SC can be blocked by introducing a 5-HT antagonist in vitro (Dommett et al., 2009), the data presented in Chapters 3 and 4 are the first to show this effect in vivo, when d-amphetamine is administered through both a local and systemic route. Taken together, we can now have more confidence in the possibility that these drugs operate locally within the SC to affect the responsiveness to visual stimuli. It is understood that d-amphetamine acts to increase synaptic levels of the monoamine neurotransmitters, DA, NA, and 5-HT (Heal, Cheetham, Prow, Martin, & Buckett, 1998; Heal et al., 2013; Rothman et al., 2001). As such, previous work has only been able to speculate as to which neurotransmitter systems mediate the effect of d-amphetamine on the SC response in vivo. Having shown than the action of d-amphetamine, when operating both locally and systemically, can be significantly reversed following introduction of a broad spectrum 5-HT antagonist (Terrón, 1997; Cox and Ennis, 1982; Engel et al., 1983), it can be strongly suggested that the depression induced by d-amphetamine on the collicular response is mediated, at least in part, by increased synaptic availability of 5-HT.

Given the relevance of sensory responsiveness in the superficial SC to symptoms of distractibility in ADHD, it has been proposed that a novel path for ADHD drug development may be to identify substances that depress stimulus-related responses in the colliculus in a manner that is comparable to current ADHD pharmaceuticals (Overton 2008). Data presented in the current thesis shows for the first time that, when administered in the presence of NAD-299, the SSRI fluoxetine acts to depress visual responsiveness in the superficial SC in a dose-dependent manner. The depression induced by high doses of NAD-299+fluoxetine for both LFP and phase 2 of the MUA response was comparable to high doses of d-amphetamine. This is particularly notable as high doses of d-amphetamine are required to target 5-HT transmission (Holmes and Rutledge 1976; Kuczenski and Segal 1989). As the effects of fluoxetine at the level of the SC are most likely mediated through increased synaptic 5-HT availability (Meyer et al., 2004), it is proposed that both D-amphetamine and fluoxetine act via a common mechanism to reduce visual sensitivity in the superficial SC. This proposal is legitimised by the dense distribution of postsynaptic 5-HT1A heteroreceptors in the both the opticum and SGS of the superficial SC (Mooney et al., 1994; Segu et al., 1986; Shukla et al., 2014). In the superficial SC, these 5-HT1A heteroreceptors are understood to have a preferential postsynaptic localisation where they are believed to modulate neural excitability (Mooney et al., 1996; Rojas and Fiedler, 2016). As 5-HT1A receptors are the most plentiful serotonergic receptors in the superficial layers of the SC (Shukla et al., 2014) it can be suggested that the effects of d-amphetamine and NAD-299+fluoxetine described in the current thesis are mediated via action at these receptors. It should be noted that while NAD-299 has an antagonist effect at 5-HT1A receptors, when administered alone in Chapter 6, NAD-299 had no effect on any aspect of the SC response to visual stimuli. It is thus plausible that at the doses administered in the current thesis, the effects of NAD-299 were limited to 5-HT1A autoreceptors, and not heteroreceptors ([Riad, Watkins, Doucet, Hamon, & Descarries, 2001](#_ENREF_27)). As such, it is proposed that both d-amphetamine and fluoxetine act at the level of the SC to enhance synaptic availability of the 5-HT, thereby activating postsynaptic retinotectal 5-HT1A heteroreceptors, thus inhibiting neural excitability, and thereby reducing the amplitude of the MUA and LFP response to visual stimuli.

Recall from Chapter 5 the mechanism by which the SC is believed to initiate foveating saccadic eye movements. Visual input to the superficial layers of the SC provokes synchronous activity in SGI (Isa, 2002), which in turn projects a bid for motor expression to the BG. The manner by which the BG influences action selection is conceptualised as “bids” for motor expression from any system capable of initiating motor activity (Gurney et al., 2001; Redgrave et al., 1999), where the strongest bid in the system at a given time is most likely to generate a motor output. A bid from the SC may be expressed as a saccadic eye movement towards a biologically salient stimulus, or a non-salient distracting stimulus. It is proposed that increased distractibility in ADHD is caused by collicular hypersensitivity to incoming sensory stimuli (Overton, 2008). As such, in ADHD, stimuli that lack intrinsic biological salience are more likely to put a strong action selection bid into the BG, and consequently be chosen as a target for a saccade. As the response to visual stimuli in the superficial SC is depressed when levels of 5-HT are increased (Dommett et al., 2009; see the results presented in Chapters 3, 4, and 6), there is a clear pathway through which drugs that enhance collicular 5-HT levels could normalise distractibility in ADHD. Given that d-amphetamine reduces distractibility in ADHD (Elia et al., 1999; Konrad et al., 2005), and that both d-amphetamine and NAD-299+fluoxetine act to modulate 5-HT activity in a system where 5-HT activity has relevance to distractibility, there is therefore strong potential that fluoxetine could act to reduce distractibility in ADHD when administered concurrently with an antagonist of 5-HT1Aautoreceptors. The accelerated onset of anti-depressant onset when SSRIs are combined with 5-HT1A antagonism (Ballesteros & Callado, 2004), suggests this is a potentially fruitful avenue in human pharmacotherapy.

The data presented in the current thesis are the first to describe differential pharmacological action of D-amphetamine and NAD-299+fluoxetine on the different phases of the SC MUA response. While D-amphetamine depressed phase 1 of the MUA response at all doses, high doses of both D-amphetamine and fluoxetine, in the presence of NAD-299, were required to depress phase 2 of the MUA response. Previous reports of the SC response to visual stimuli have described a biphasic profile of the MUA response. McPeek et al., (2002) recorded visual, movement, and visuo-movement neurons in the primate SC as subjects performed a visual search task. It was observed that a subset of visuo-movement neurons, termed visuo-motor burst neurons, displayed a biphasic response pattern during the search task. For these neurons phase 1 of the response corresponded to the typical initial SC response to presentation of visual stimuli. Phase 2 of the response, however, was of variable amplitude and discriminated targets from distractors during the visual search task. When targets were focused on, the amplitude of phase 2 of the MUA response was enhanced, but this response was muted when distractors were focused on. Thus, phase 2 of the SC visuo-motor burst neuron MUA response is believed to guide saccadic target selection by firing emphatically when biologically salient stimuli are presented. It follows that if this aspect of the SC is hypersensitive, then non-salient stimuli would be erroneously chosen as the target of saccades. As such, if phase 2 of the MUA response in the current thesis represents the activity of visuo-motor burst neurons, depression of this aspect of the SC response may serve to reduce foveation towards visual distractors. As this aspect of the SC response appears to be particularly sensitive to 5-HT manipulation, d-amphetamine, and potentially NAD-299+fluoxetine, may act to enhance sustained attention in ADHD by reducing the likelihood that visuo-motor burst neurons will fire in response to non-salient stimuli.

# 7.5 Limitations

Limitations of the work presented in the current thesis can be broadly categorised as limitations associated with the pharmacology, including drug preparation and doses, and limitations associated with the experimental design.

## 7.5.1. Limitations associated with pharmacology

The use of DMSO as vehicle for metergoline in Chapters 3 and 4 may have acted to confound the effects of 5-HT antagonism. While DMSO is the recommended solvent for i.v. administration of metergoline, (Tocris Bioscience, 2019), this solvent is known to alter neural excitability (Sawada & Sato, 1975). As such, despite the results in chapter 3 revealing that a single bolus injection of DMSO did not significantly alter any aspect of the LFP or MUA response, it is possible that the cumulative dose of DMSO used to deliver metergoline may have affected neural excitability in a way that was not possible to deconvolve from the effects of metergoline. Further, when administered locally to neural sites, DMSO may disrupt the morphology of neurons and glia, and has the potential to induce apoptosis (Hansclick et al., 2009; Zhang et al., 2016). As such, it was not feasible to introduce metergoline locally to the SC, and thus it was only possible to speculate as to the level at which metergoline operated to reverse the effects of d-amphetamine in Chapters 3 and 4. While steps were taken to titrate the DMSO dose to the lowest concentration at which metergoline would stay in solution, it is advised that future work should aim to avoid use of this solvent. Metergoline was selected as an appropriate 5-HT antagonist in the current thesis due to its broad antagonism profile, and previous reports of metergoline blocking the effects of d-amphetamine on SC responses *in vitro* (Dommett et al., 2009). While this choice of antagonist was appropriate for the exploratory work outlined in the current thesis, use of an antagonist with more selective affinity for the 5-HT receptors expressed at the level of the SC would have allowed for increased confidence of the level at which 5-HT antagonism acted to reverse the effects of d-amphetamine. Future work using such an antagonist may also be able to avoid the use of DMSO as a solvent, removing this confound and potentially allowing for local administration of the antagonist.

In line with previous work in anaesthetised animal models, the doses of d-amphetamine and fluoxetine used in the current thesis were supra-therapeutic (Gowen et al., 2008; Eriksson et al., 2012). While such doses are often required to induce detectable pharmacological effects in anaesthetised animal preparations (Gowen et al., 2008), it must be conceded that the data presented may not reflect doses that have relevance to human pharmacotherapy. Consequently, it must be established if the effects described in the current thesis are maintained when d-amphetamine and NAD-299+fluoxetine are administered at doses of therapeutic relevance. Such work should first establish doses of d-amphetamine and NAD-299+fluoxetine which produce blood plasma levels in that rat that are comparable to therapeutic doses of these drugs in humans.

## 7.5.2 Limitations associated with experimental design

A persistent limitation across all experiments presented in the current thesis was the use of an anaesthetised animal preparation. Work in the current thesis utilised urethane anaesthesia, which can affect the amplitude of multi-unit (Erchova et al., 2002) and LFP (Dyer & Rigdon, 1987) responses to sensory stimuli at both a cortical and subcortical level (Devonshire et al., 2010; Huh and Cho, 2013). Further, as noted above, supra-physiological drug doses are often necessary to induce detectable drug induced changes in anaesthetised preparations. As such, the data presented in the current thesis may not have accurately represented responses to stimuli or drugs in awake behaving animals. Nevertheless, it should be noted that the use of an anaesthetic preparation allowed for exquisite control and consistency of visual stimuli, and eliminated confounds related to movement or arousal. Thus, while the work described in the current thesis represents important first steps in exploring the viability of targeting 5-HT in the pharmacotherapy of ADHD, such work in the future should aim to use a combination of anaesthetised and awake preparations. Where anaesthetised preparations are used, future work should aim to monitor physiology responses associated with anaesthetic depth (e.g. respiratory rate and cortical EEG power, Musizza et al., 2007), in order to control for any potential confounds of anaesthetic depth on neural responses to sensory events.

An additional limitation of the current thesis was low statistical power as a due to low animal numbers in several groups across experiments, resulting in an increased risk of type II error. Such low numbers are not uncommon in similar work in the field (Gowen et al., 2008; Hetherington et al., 2017), and are in accordance with the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs, 2018) guidelines. Nevertheless, it is crucial to ensure the data presented in the current thesis is replicable. Outlined below are logical extensions of the current work which would both confirm and extend the novel findings presented in this thesis.

# 7.6 Future Directions and Remaining Questions

## 7.6.1 Further Examination of the Effect of Fluoxetine at the Level of the SC

Data presented in the current thesis represent the first time that the effects of fluoxetine on the superficial SC response to visual stimuli have been assessed. In the context of the extant literature, and data presented elsewhere in the current thesis, the results presented in Chapter 6 presents a potentially fruitful new avenue for drug development in ADHD. However, while these results are promising, more rigour must be applied to the examination of this effect before any strong conclusions can be made. A logical first step in this process is to establish whether the effects of NAD-299+fluoxetine on the SC response are preserved when fluoxetine is administered locally into the SC. As described in Chapter 4, the SC is critical hub in of an extensive orienting network, where several afferent and efferent targets express the receptors necessary for monoamine activity (Easton et al., 2007; Schouw et al., 2013). As such, it not possible to be certain at this stage if the effect of fluoxetine on the SC response was mediated locally within the SC, or if this was incidental of afferent modulation of the input to the SC. Additionally, while it is unlikely that neurotransmitter systems other than 5-HT contributed to the depression of the SC response presented in Chapter 6, subsequent antagonism of 5-HT following the introduction of fluoxetine would allow for confirmation of this assumption.

If the depressant effect of NAD-299+fluoxetine on the superficial SC response is maintained when exposed to the above rigour, examination of this effect in awake behaving animals would represent a logical extension of this work. As noted above, examination of the effect of NAD-299+fluoxetine on electrophysiological responses in the superficial SC without the potential confound of anaesthesia would allow for a more valid elucidation of the effect of 5-HT manipulation on the collicular visual response.

A key motivation for the work in the current thesis was to identify a candidate substance that has a common mechanism of action at the level of the superficial SC as d-amphetamine, but with a safer profile in terms of side effects and abuse potential. While NAD-299+fluoxetine has the potential to act as this candidate substance, further examination of its effect on phenomena relevant to ADHD are required before use in humans can be recommended. Recent research has shown that superficial SC LFP and MUAs in the New Zealand Genetically Hypertensive rat, a validated rodent model of ADHD, are elevated compared to control animals (Clements et al., 2014). Administration of d-amphetamine reduces the amplitude of these electrophysiological measures towards levels recorded in control animals. Examination of the effect of NAD-299+fluoxetine on the LFP and MUA of the New Zealand genetically hypertensive rat may allow for further comparison with the effects of d-amphetamine, and may help validate fluoxetine as a candidate drug in the pharmacotherapy of ADHD.

## 7.6.2 Exploration of the Second Phase of the MUA Response

A novel finding presented across all experiments in the current thesis is that discrete phases of the biphasic MUA response in the superficial SC are differentially affected by both d-amphetamine and NAD-299+fluoxetine. Specifically, phase 2 of the biphasic response was shown to be particularly sensitive to 5-HT manipulation when both d-amphetamine and NAD-299+fluoxetine were administered. It was speculated that phase 2 of this biphasic response acts to discriminate targets for saccadic eye movements. Given that an inability to inhibit saccadic eye movements to non-salient stimuli is a common feature of ADHD (e.g. Munoz et al., 2003; Roberts et al., 2011; Fried et al., 2014), this aspect of the SC may have particular relevance to inattentive symptoms in ADHD, and thus warrants further exploration. While there are many potential avenues that such research could take, a logical starting point could be to isolate the second phase of the biphasic response for closer examination, and pharmacological manipulation. Given that the SC acts as a key hub within a wider orienting network, there are a variety of potential afferent and efferent targets that may have relevance to this phase of the MUA response. Such work might best be examined initially in vitro, to allow for greater control and manipulation over the various aspects of input to, and output from, the SC.

## 7.6.3 Examination of Alternative Neurotransmitter Systems Targeted by d-Amphetamine

Work presented in Chapters 3 and 4 of the current thesis shows that D-amphetamine induced depression of the SC response to visual stimuli is mediated, at least in part, by 5-HT. While the superficial SC receives dense serotonergic innervation (Parent, Descarries, & Beaudet, 1981), it also receives dense noradrenergic innervation (Lindvall & Bjorklund, 1974), and light dopaminergic innervation (Campbell, Takada, & Hattori, 1991). When metergoline was administered in Chapters 3 and 4, a reversal of the d-amphetamine induced depression of the superficial SC response was observed, but for most measures (with the exception of phase 2 of the MUA in Chapter 3), this reversal was incomplete. Furthermore, while d-amphetamine induced a depression in phase 1 of the MUA response, this phase was unaffected by fluoxetine. As such, while 5-HT may play a significant role in the modulation of the effects of d-amphetamine at the level of the SC, it is likely that multiple neurotransmitter systems mediate the depression of the SC response observed in Chapters 3 and 4. It has previously been postulated by Gowan et al., (2008) that low and high doses of d-amphetamine differentially affect NA and 5-HT transmission, with low doses targeting NA and high doses targeting 5-HT. Given that NA depresses visual responses in the SC (Y. Zhang, Mooney, & Rhoades, 1999), antagonism of NA transmission following the administration of low doses of d-amphetamine may allow us to elucidate any contribution of NA to the d-amphetamine induced depression of the SC visual response.

# 7.7 Final Conclusions

Due in part to a rising prevalence of psychostimulant misuse and diversion, there is a clear need to identify new efficacious interventions for ADHD, which lack the abuse potential of psychostimulants. An understanding of the mechanism of action of current frontline pharmaceuticals for ADHD is crucial to allow for the identification and development of any new therapeutic interventions. This thesis has presented work which demonstrates that the effect of d-amphetamine on the superficial layer SC response to visual stimuli is reversed following 5-HT antagonism in vivo. It is thus suggested that the mechanism of action of d-amphetamine at the level of the SC is mediated, at least in part, by 5-HT. It was also shown that when 5-HT1A autoreceptors are antagonised, the SSRI fluoxetine induces a dose dependant depression of the superficial SC response to visual stimuli in a manner that is comparable to d-amphetamine. These findings show that there is a currently under-researched potential to exploit 5-HT transmission in ADHD, and that concurrent SSRI administration with 5-HT1A antagonism could be a potential therapeutic avenue in ADHD. Future work must aim to further examine the effect of NAD-299+fluoxetine on visual responsiveness in the superficial SC, with specific focus on phase 2 of the MUA response. Such work could lay the foundation for re-examination of the therapeutic viability of treating ADHD with SSRIs.

# References

Adams, Z. W., Roberts, W. M., Milich, R., & Fillmore, M. T. (2011). Does response variability predict distractibility among adults with attention-deficit/hyperactivity disorder? *Psychological assessment, 23*(2), 427.

Addis, A., & Koren, G. (2000). Safety of fluoxetine during the first trimester of pregnancy: a meta-analytical review of epidemiological studies. *Psychol Med, 30*(1), 89-94.

Adell, A., Celada, P., Abellan, M. T., & Artigas, F. (2002). Origin and functional role of the extracellular serotonin in the midbrain raphe nuclei. *Brain Res Brain Res Rev, 39*(2-3), 154-180.

Aghajanian, G. K., & Vandermaelen, C. P. (1982). Intracellular recordings from serotonergic dorsal raphe neurons: pacemaker potentials and the effect of LSD. *Brain Res, 238*(2), 463-469.

American Psychiatric Association. (2004). *Diagnostic and Statistical Manual of Mental Disorders* (4th text revision ed.).

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.). Washington, DC.

Anderson, S. R., Porrill, J., Pearson, M. J., Pipe, A. G., Prescott, T. J., & Dean, P. (2012). An Internal Model Architecture for Novelty Detection: Implications for Cerebellar and Collicular Roles in Sensory Processing. *PLOS ONE, 7*(9), e44560. doi:10.1371/journal.pone.0044560

Andrée, B., Hedman, A., Thorberg, S.-O., Nilsson, D., Halldin, C., & Farde, L. (2003). Positron emission tomographic analysis of dose-dependent NAD-299 binding to 5-hydroxytryptamine-1A receptors in the human brain. *Psychopharmacology, 167*(1), 37-45. doi:10.1007/s00213-002-1355-0

Arborelius, L., Wallsten, C., Ahlenius, S., & Svensson, T. H. (1999). The 5-HT(1A) receptor antagonist robalzotan completely reverses citalopram-induced inhibition of serotonergic cell firing. *Eur J Pharmacol, 382*(2), 133-138.

Artigas, F., Romero, L., de Montigny, C., & Blier, P. (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends in Neurosciences, 19*(9), 378-383. doi:10.1016/s0166-2236(96)10037-0

Asherson, P. (2005). Clinical assessment and treatment of attention deficit hyperactivity disorder in adults. *Expert Rev Neurother, 5*(4), 525-539. doi:10.1586/14737175.5.4.525

Aylward, E. H., Codori, A. M., Barta, P. E., Pearlson, G. D., Harris, G. J., & Brandt, J. (1996). Basal ganglia volume and proximity to onset in presymptomatic Huntington disease. *Arch Neurol, 53*(12), 1293-1296.

Ballesteros, J., & Callado, L. F. (2004). Effectiveness of pindolol plus serotonin uptake inhibitors in depression: a meta-analysis of early and late outcomes from randomised controlled trials. *J Affect Disord, 79*(1), 137-147. doi:<https://doi.org/10.1016/S0165-0327(02)00404-4>

Banaschewski, T., Roessner, V., Dittmann, R. W., Santosh, P. J., & Rothenberger, A. (2004). Non-stimulant medications in the treatment of ADHD. *Eur Child Adolesc Psychiatry, 13 Suppl 1*, I102-116. doi:10.1007/s00787-004-1010-x

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull, 121*(1), 65-94. doi:10.1037/0033-2909.121.1.65

Barkley, R. A. (2006). *Attention-deficit hyperactivity disorder: A handbook for diagnosis and treatment* (3rd ed.). New York, NY, US: : Guilford Press.

Barkley, R. A., Fischer, M., Edelbrock, C. S., & Smallish, L. (1990). The adolescent outcome of hyperactive children diagnosed by research criteria: I. An 8-year prospective follow-up study. *J Am Acad Child Adolesc Psychiatry, 29*(4), 546-557. doi:10.1097/00004583-199007000-00007

Barkley, R. A., Fischer, M., Smallish, L., & Fletcher, K. (2002). The persistence of attention-deficit/hyperactivity disorder into young adulthood as a function of reporting source and definition of disorder. *J Abnorm Psychol, 111*(2), 279-289.

Barrickman, L., Noyes, R., Kuperman, S., Schumacher, E., & Verda, M. (1991). Treatment of ADHD with fluoxetine: a preliminary trial. *J Am Acad Child Adolesc Psychiatry, 30*(5), 762-767.

Benmansour, S., Owens, W. A., Cecchi, M., Morilak, D. A., & Frazer, A. (2002). Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. *J Neurosci, 22*(15), 6766-6772. doi:20026626

Berti, S., Roeber, U., & Schroger, E. (2004). Bottom-up influences on working memory: behavioral and electrophysiological distraction varies with distractor strength. *Exp Psychol, 51*(4), 249-257. doi:10.1027/1618-3169.51.4.249

Biederman, J. (2005a). Attention-Deficit/Hyperactivity Disorder: A Selective Overview. *Biological Psychiatry, 57*(11), 1215-1220. doi:<https://doi.org/10.1016/j.biopsych.2004.10.020>

Biederman, J. (2005b). Attention-deficit/hyperactivity disorder: a selective overview. *Biol Psychiatry, 57*(11), 1215-1220. doi:10.1016/j.biopsych.2004.10.020

Biederman, J., Baldessarini, R. J., Wright, V., Knee, D., & Harmatz, J. S. (1989). A double-blind placebo controlled study of desipramine in the treatment of ADD: I. Efficacy. *J Am Acad Child Adolesc Psychiatry, 28*(5), 777-784. doi:10.1097/00004583-198909000-00022

Biederman, J., & Faraone, S. V. (2005). Attention-deficit hyperactivity disorder. *The Lancet, 366*(9481), 237-248. doi:10.1016/s0140-6736(05)66915-2

Biederman, J., Mick, E., & Faraone, S. V. (2000). Age-dependent decline of symptoms of attention deficit hyperactivity disorder: impact of remission definition and symptom type. *Am J Psychiatry, 157*(5), 816-818. doi:10.1176/appi.ajp.157.5.816

Binns, K. E. (1999). The synaptic pharmacology underlying sensory processing in the superior colliculus. *Progress in Neurobiology, 59*(2), 129-159. doi:<https://doi.org/10.1016/S0301-0082(98)00099-9>

Bittencourt, J., Velasques, B., Teixeira, S., Basile, L. F., Salles, J. I., Nardi, A. E., . . . Ribeiro, P. (2013). Saccadic eye movement applications for psychiatric disorders. *Neuropsychiatr Dis Treat, 9*, 1393-1409. doi:10.2147/ndt.s45931

Boehnke, S. E., & Munoz, D. P. (2008). On the importance of the transient visual response in the superior colliculus. *Curr Opin Neurobiol, 18*(6), 544-551. doi:10.1016/j.conb.2008.11.004

Bortolozzi, A., Castane, A., Semakova, J., Santana, N., Alvarado, G., Cortes, R., . . . Artigas, F. (2012). Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. *Mol Psychiatry, 17*(6), 612-623. doi:10.1038/mp.2011.92

Borycz, J., Zapata, A., Quiroz, C., Volkow, N. D., & Ferré, S. (2007). 5-HT1B Receptor-Mediated Serotoninergic Modulation of Methylphenidate-Induced Locomotor Activation in Rats. *Neuropsychopharmacology, 33*, 619. doi:10.1038/sj.npp.1301445

Briley, M., & Moret, C. (1993). Neurobiological mechanisms involved in antidepressant therapies. *Clin Neuropharmacol, 16*(5), 387-400.

Brown, R. T., Amler, R. W., Freeman, W. S., Perrin, J. M., Stein, M. T., Feldman, H. M., . . . Wolraich, M. L. (2005). Treatment of attention-deficit/hyperactivity disorder: overview of the evidence. *Pediatrics, 115*(6), e749-757. doi:10.1542/peds.2004-2560

Brown, R. T., Wynne, M. E., & Medenis, R. (1985). Methylphenidate and cognitive therapy: a comparison of treatment approaches with hyperactive boys. *J Abnorm Child Psychol, 13*(1), 69-87.

Bush, G., Frazier, J. A., Rauch, S. L., Seidman, L. J., Whalen, P. J., Jenike, M. A., . . . Biederman, J. (1999). Anterior cingulate cortex dysfunction in attention-deficit/hyperactivity disorder revealed by fMRI and the Counting Stroop. *Biol Psychiatry, 45*(12), 1542-1552.

Buzsaki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. *Nat Rev Neurosci, 13*(6), 407-420. doi:10.1038/nrn3241

Campbell, K. J., Takada, M., & Hattori, T. (1991). Co-localization of tyrosine hydroxylase and glutamate decarboxylase in a subpopulation of single nigrotectal projection neurons. *Brain Res, 558*(2), 239-244.

Cannon, M., Pelham, W. H., Sallee, F. R., Palumbo, D. R., Bukstein, O., & Daviss, W. B. (2009). Effects of clonidine and methylphenidate on family quality of life in attention-deficit/hyperactivity disorder. *Journal of Child and Adolescent Psychopharmacology, 19*(5), 511-517. doi:10.1089/cap.2009.0008

Cassani, J., Dorantes-Barron, A. M., Novales, L. M., Real, G. A., & Estrada-Reyes, R. (2014). Anti-depressant-like effect of kaempferitrin isolated from Justicia spicigera Schltdl (Acanthaceae) in two behavior models in mice: evidence for the involvement of the serotonergic system. *Molecules, 19*(12), 21442-21461. doi:10.3390/molecules191221442

Castellanos, F. X., Giedd, J. N., Eckburg, P., Marsh, W. L., Vaituzis, A. C., Kaysen, D., . . . Rapoport, J. L. (1994). Quantitative morphology of the caudate nucleus in attention deficit hyperactivity disorder. *Am J Psychiatry, 151*(12), 1791-1796. doi:10.1176/ajp.151.12.1791

Castellanos, F. X., Giedd, J. N., Marsh, W. L., Hamburger, S. D., Vaituzis, A. C., Dickstein, D. P., . . . Rapoport, J. L. (1996). Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. *Arch Gen Psychiatry, 53*(7), 607-616.

Castellanos, F. X., Lee, P. P., Sharp, W., Jeffries, N. O., Greenstein, D. K., Clasen, L. S., . . . Rapoport, J. L. (2002). Developmental trajectories of brain volume abnormalities in children and adolescents with attention-deficit/hyperactivity disorder. *Jama, 288*(14), 1740-1748.

Castellanos, F. X., & Proal, E. (2012). Large-scale brain systems in ADHD: beyond the prefrontal-striatal model. *Trends Cogn Sci, 16*(1), 17-26. doi:10.1016/j.tics.2011.11.007

Castellanos, F. X., Sonuga-Barke, E. J., Milham, M. P., & Tannock, R. (2006). Characterizing cognition in ADHD: beyond executive dysfunction. *Trends Cogn Sci, 10*(3), 117-123. doi:10.1016/j.tics.2006.01.011

Castells, X., Ramos-Quiroga, J. A., Rigau, D., Bosch, R., Nogueira, M., Vidal, X., & Casas, M. (2011). Efficacy of methylphenidate for adults with attention-deficit hyperactivity disorder: a meta-regression analysis. *CNS Drugs, 25*(2), 157-169. doi:10.2165/11539440-000000000-00000

Catterson, M. L., & Preskorn, S. H. (1996). Pharmacokinetics of selective serotonin reuptake inhibitors: clinical relevance. *Pharmacol Toxicol, 78*(4), 203-208.

Chalupa, L. M., & Rhoades, R. W. (1977). Responses of visual, somatosensory, and auditory neurones in the golden hamster's superior colliculus. *J Physiol, 270*(3), 595-626.

Clement, E. A., Richard, A., Thwaites, M., Ailon, J., Peters, S., & Dickson, C. T. (2008). Cyclic and Sleep-Like Spontaneous Alternations of Brain State Under Urethane Anaesthesia. *PLOS ONE, 3*(4), e2004. doi:10.1371/journal.pone.0002004

Clements, K. M., Devonshire, I. M., Reynolds, J. N., & Overton, P. G. (2014). Enhanced visual responses in the superior colliculus in an animal model of attention-deficit hyperactivity disorder and their suppression by D-amphetamine. *Neuroscience, 274*, 289-298. doi:10.1016/j.neuroscience.2014.05.054

Coizet, V., Overton, P. G., & Redgrave, P. (2007). Collateralization of the tectonigral projection with other major output pathways of superior colliculus in the rat. *J Comp Neurol, 500*(6), 1034-1049. doi:10.1002/cne.21202

Colaneri, N., Keim, S., & Adesman, A. (2017). Physician practices to prevent ADHD stimulant diversion and misuse. *J Subst Abuse Treat, 74*, 26-34. doi:10.1016/j.jsat.2016.12.003

Comoli, E., Das Neves Favaro, P., Vautrelle, N., Leriche, M., Overton, P., & Redgrave, P. (2012). Segregated Anatomical Input to Sub-Regions of the Rodent Superior Colliculus Associated with Approach and Defense. *Frontiers in Neuroanatomy, 6*(9). doi:10.3389/fnana.2012.00009

Cools, R., Roberts, A. C., & Robbins, T. W. (2008). Serotoninergic regulation of emotional and behavioural control processes. *Trends Cogn Sci, 12*(1), 31-40. doi:10.1016/j.tics.2007.10.011

Cormier, E. (2008). Attention deficit/hyperactivity disorder: a review and update. *J Pediatr Nurs, 23*(5), 345-357. doi:10.1016/j.pedn.2008.01.003

Cox, B., & Ennis, C. (1982). Characterization of 5-hydroxytryptaminergic autoreceptors in the rat hypothalamus. *Journal of Pharmacy and Pharmacology, 34*(7), 438-441. doi:10.1111/j.2042-7158.1982.tb04752.x

Cubillo, A., Halari, R., Smith, A., Taylor, E., & Rubia, K. (2012). A review of fronto-striatal and fronto-cortical brain abnormalities in children and adults with Attention Deficit Hyperactivity Disorder (ADHD) and new evidence for dysfunction in adults with ADHD during motivation and attention. *Cortex, 48*(2), 194-215. doi:10.1016/j.cortex.2011.04.007

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience, 215*, 42-58. doi:<https://doi.org/10.1016/j.neuroscience.2012.03.065>

De Quiros, G. B., & Kinsbourne, M. (2001). Adult ADHD. Analysis of self-ratings on a behavior questionnaire. *Ann N Y Acad Sci, 931*, 140-147.

Dean, P., Redgrave, P., & Westby, G. W. (1989). Event or emergency? Two response systems in the mammalian superior colliculus. *Trends Neurosci, 12*(4), 137-147. doi:10.1016/0166-2236(89)90052-0

Denhoff, E., Laufer, M. W., & Solomons, G. (1957). Hyperkinetic impulse disorder in children's behavior problems. *Psychosom Med, 19*(1), 38-49.

Devonshire, I. M., Grandy, T. H., Dommett, E. J., & Greenfield, S. A. (2010). Effects of urethane anaesthesia on sensory processing in the rat barrel cortex revealed by combined optical imaging and electrophysiology. *Eur J Neurosci, 32*(5), 786-797. doi:10.1111/j.1460-9568.2010.07322.x

Dickstein, S. G., Bannon, K., Castellanos, F. X., & Milham, M. P. (2006). The neural correlates of attention deficit hyperactivity disorder: an ALE meta-analysis. *J Child Psychol Psychiatry, 47*(10), 1051-1062. doi:10.1111/j.1469-7610.2006.01671.x

Dommett, E. J., Overton, P. G., & Greenfield, S. A. (2009). Drug therapies for attentional disorders alter the signal-to-noise ratio in the superior colliculus. *Neuroscience, 164*(3), 1369-1376. doi:10.1016/j.neuroscience.2009.09.007

Dommett, E. J., & Rostron, C. L. (2011). Abnormal air righting behaviour in the spontaneously hypertensive rat model of ADHD. *Exp Brain Res, 215*(1), 45-52. doi:10.1007/s00221-011-2869-7

Donnelly, M., Rapoport, J. L., Potter, W. Z., Oliver, J., Keysor, C. S., & Murphy, D. L. (1989). Fenfluramine and dextroamphetamine treatment of childhood hyperactivity. Clinical and biochemical findings. *Arch Gen Psychiatry, 46*(3), 205-212.

Döpfner, M., Breuer, D., Wille, N., Erhart, M., Ravens-Sieberer, U., & the, B. s. g. (2008). How often do children meet ICD-10/DSM-IV criteria of attention deficit-/hyperactivity disorder and hyperkinetic disorder? Parent-based prevalence rates in a national sample – results of the BELLA study. *Eur Child Adolesc Psychiatry, 17*(1), 59-70. doi:10.1007/s00787-008-1007-y

Dorris, M. C., Olivier, E., & Munoz, D. P. (2007). Competitive Integration of Visual and Preparatory Signals in the Superior Colliculus during Saccadic Programming. *The Journal of Neuroscience, 27*(19), 5053.

Dorris, M. C., Paré, M., & Munoz, D. P. (1997). Neuronal Activity in Monkey Superior Colliculus Related to the Initiation of Saccadic Eye Movements. *The Journal of Neuroscience, 17*(21), 8566.

Douglas, V. (1972). *Stop, look and listen: The problem of sustained attention and impulse control in hyperactive and normal children* (Vol. 4).

Drager, U. C., & Hubel, D. H. (1976). Topography of visual and somatosensory projections to mouse superior colliculus. *J Neurophysiol, 39*(1), 91-101. doi:10.1152/jn.1976.39.1.91

Durston, S., Hulshoff Pol, H. E., Schnack, H. G., Buitelaar, J. K., Steenhuis, M. P., Minderaa, R. B., . . . van Engeland, H. (2004). Magnetic resonance imaging of boys with attention-deficit/hyperactivity disorder and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry, 43*(3), 332-340.

Durston, S., Tottenham, N. T., Thomas, K. M., Davidson, M. C., Eigsti, I. M., Yang, Y., . . . Casey, B. J. (2003). Differential patterns of striatal activation in young children with and without ADHD. *Biol Psychiatry, 53*(10), 871-878.

Dyer, R. S., & Annau, Z. (1977). Flash evoked potentials from rat superior colliculus. *Pharmacol Biochem Behav, 6*(4), 453-459.

Dyer, R. S., & Rigdon, G. C. (1987). Urethane affects the rat visual system at subanesthetic doses. *Physiol Behav, 41*(4), 327-330.

Easton, N., Steward, C., Marshall, F., Fone, K., & Marsden, C. (2007). Effects of amphetamine isomers, methylphenidate and atomoxetine on synaptosomal and synaptic vesicle accumulation and release of dopamine and noradrenaline in vitro in the rat brain. *Neuropharmacology, 52*(2), 405-414. doi:10.1016/j.neuropharm.2006.07.035

Eiden, L. E., & Weihe, E. (2011). VMAT2: a dynamic regulator of brain monoaminergic neuronal function interacting with drugs of abuse. *Ann N Y Acad Sci, 1216*, 86-98. doi:10.1111/j.1749-6632.2010.05906.x

Elia, J., Ambrosini, P. J., & Rapoport, J. L. (1999). Treatment of attention-deficit-hyperactivity disorder. *N Engl J Med, 340*(10), 780-788. doi:10.1056/nejm199903113401007

Emond, V., Joyal, C., & Poissant, H. (2009). [Structural and functional neuroanatomy of attention-deficit hyperactivity disorder (ADHD)]. *Encephale, 35*(2), 107-114. doi:10.1016/j.encep.2008.01.005

Emslie, G. J., Rush, A. J., Weinberg, W. A., Kowatch, R. A., Hughes, C. W., Carmody, T., & Rintelmann, J. (1997). A double-blind, randomized, placebo-controlled trial of fluoxetine in children and adolescents with depression. *Arch Gen Psychiatry, 54*(11), 1031-1037.

Erchova, I. A., Lebedev, M. A., & Diamond, M. E. (2002). Somatosensory cortical neuronal population activity across states of anaesthesia. *European Journal of Neuroscience, 15*(4), 744-752. doi:10.1046/j.0953-816x.2002.01898.x

Erickson, J. D., Schafer, M. K., Bonner, T. I., Eiden, L. E., & Weihe, E. (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci U S A, 93*(10), 5166-5171.

Eriksson, T. M., Holst, S., Stan, T. L., Hager, T., Sjogren, B., Ogren, S. O., . . . Stiedl, O. (2012). 5-HT1A and 5-HT7 receptor crosstalk in the regulation of emotional memory: implications for effects of selective serotonin reuptake inhibitors. *Neuropharmacology, 63*(6), 1150-1160. doi:10.1016/j.neuropharm.2012.06.061

Everling, S., Dorris, M. C., Klein, R. M., & Munoz, D. P. (1999). Role of primate superior colliculus in preparation and execution of anti-saccades and pro-saccades. *J Neurosci, 19*(7), 2740-2754.

Everling, S., Paré, M., Dorris, M. C., & Munoz, D. P. (1998). Comparison of the discharge characteristics of brain stem omnipause neurons and superior colliculus fixation neurons in monkey: implications for control of fixation and saccade behavior. *Journal of Neurophysiology, 79*(2), 511-528.

Faraone, S. V. (2018). The pharmacology of amphetamine and methylphenidate: Relevance to the neurobiology of attention-deficit/hyperactivity disorder and other psychiatric comorbidities. *Neuroscience & Biobehavioral Reviews, 87*, 255-270. doi:<https://doi.org/10.1016/j.neubiorev.2018.02.001>

Faraone, S. V., Biederman, J., Spencer, T., Michelson, D., Adler, L., Reimherr, F., & Glatt, S. J. (2005). Efficacy of atomoxetine in adult attention-Deficit/Hyperactivity Disorder: a drug-placebo response curve analysis. *Behavioral and Brain Functions, 1*(1), 16. doi:10.1186/1744-9081-1-16

Faraone, S. V., Biederman, J., Spencer, T., Wilens, T., Seidman, L. J., Mick, E., & Doyle, A. E. (2000). Attention-deficit/hyperactivity disorder in adults: an overview. *Biol Psychiatry, 48*(1), 9-20.

Faraone, S. V., & Larsson, H. (2018). Genetics of attention deficit hyperactivity disorder. *Mol Psychiatry*. doi:10.1038/s41380-018-0070-0

Faraone, S. V., Perlis, R. H., Doyle, A. E., Smoller, J. W., Goralnick, J. J., Holmgren, M. A., & Sklar, P. (2005). Molecular Genetics of Attention-Deficit/Hyperactivity Disorder. *Biological Psychiatry, 57*(11), 1313-1323. doi:<https://doi.org/10.1016/j.biopsych.2004.11.024>

Faraone, S. V., Sergeant, J., Gillberg, C., & Biederman, J. (2003). The worldwide prevalence of ADHD: is it an American condition? *World psychiatry : official journal of the World Psychiatric Association (WPA), 2*(2), 104-113.

Farde, L., Andree, B., Ginovart, N., Halldin, C., & Thorberg, S. (2000). PET-Determination of robalzotan (NAD-299) induced 5-HT(1A) receptor occupancy in the monkey brain. *Neuropsychopharmacology, 22*(4), 422-429. doi:10.1016/s0893-133x(99)00125-6

Ferris, M. J., Calipari, E. S., Rose, J. H., Siciliano, C. A., Sun, H., Chen, R., & Jones, S. R. (2015). A Single Amphetamine Infusion Reverses Deficits in Dopamine Nerve-Terminal Function Caused by a History of Cocaine Self-Administration. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 40*(8), 1826-1836. doi:10.1038/npp.2015.45

Filipek, P. A., Semrud-Clikeman, M., Steingard, R. J., Renshaw, P. F., Kennedy, D. N., & Biederman, J. (1997). Volumetric MRI analysis comparing subjects having attention-deficit hyperactivity disorder with normal controls. *Neurology, 48*(3), 589-601.

Findling, R. L. (1996). Open-label treatment of comorbid depression and attentional disorders with co-administration of serotonin reuptake inhibitors and psychostimulants in children, adolescents, and adults: a case series. *J Child Adolesc Psychopharmacol, 6*(3), 165-175. doi:10.1089/cap.1996.6.165

Fischer, A. G., Jocham, G., & Ullsperger, M. (2014). Dual serotonergic signals: a key to understanding paradoxical effects? *Trends Cogn Sci*. doi:10.1016/j.tics.2014.11.004

Fletcher, P. J. (1995). Effects ofd-fenfluramine and metergoline on responding for conditioned reward and the response potentiating effect of nucleus accumbensd-amphetamine. *Psychopharmacology, 118*(2), 155-163. doi:10.1007/BF02245834

Fletcher, P. J., & Korth, K. M. (1999). RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT1B receptors. *Behavioural pharmacology, 10*(2), 183-193.

Folta, K., & Mähler, C. (2010). Schnelle Augenbewegungen und visuelle Fixation bei Kindern mit ADHS. *Kindheit und Entwicklung, 20*(1), 21-30. doi:10.1026/0942-5403/a000037

Fortin, S., Itaya, S. K., Chemtob, S., & Molotchnikoff, S. (1997). ON and OFF field potentials in the rat superior colliculus during development. *Vision Research, 37*(22), 3079-3087. doi:<https://doi.org/10.1016/S0042-6989(97)00145-4>

Frazer, A. (1997). Pharmacology of antidepressants. *J Clin Psychopharmacol, 17 Suppl 1*, 2s-18s.

Fried, M., Tsitsiashvili, E., Bonneh, Y. S., Sterkin, A., Wygnanski-Jaffe, T., Epstein, T., & Polat, U. (2014). ADHD subjects fail to suppress eye blinks and microsaccades while anticipating visual stimuli but recover with medication. *Vision Res, 101*, 62-72. doi:10.1016/j.visres.2014.05.004

Gandhi, N. J., & Katnani, H. A. (2011). Motor Functions of the Superior Colliculus. *Annual Review of Neuroscience, 34*, 205-231. doi:10.1146/annurev-neuro-061010-113728

Gardier, A. M., Malagie, I., Trillat, A. C., Jacquot, C., & Artigas, F. (1996). Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic antidepressant drugs: recent findings from in vivo microdialysis studies. *Fundam Clin Pharmacol, 10*(1), 16-27.

Garland, E. J. (1998). Reviews : Pharmacotherapy of adolescent attention deficit hyperactivity disorder: challenges, choices and caveats. *Journal of Psychopharmacology, 12*(4), 385-395. doi:10.1177/026988119801200410

Garnock-Jones, K. P., & Keating, G. M. (2009). Atomoxetine: a review of its use in attention-deficit hyperactivity disorder in children and adolescents. *Paediatr Drugs, 11*(3), 203-226. doi:10.2165/00148581-200911030-00005

Gartside, S. E., Umbers, V., Hajós, M., & Sharp, T. (1995). Interaction between a selective 5-HT1A receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. *Br J Pharmacol, 115*(6), 1064-1070. doi:doi:10.1111/j.1476-5381.1995.tb15919.x

Gaub, M., & Carlson, C. L. (1997). Gender differences in ADHD: a meta-analysis and critical review. *J Am Acad Child Adolesc Psychiatry, 36*(8), 1036-1045. doi:10.1097/00004583-199708000-00011

Gentile, J. P., Atiq, R., & Gillig, P. M. (2006). Adult ADHD: Diagnosis, Differential Diagnosis, and Medication Management. *Psychiatry (Edgmont (Pa. : Township)), 3*(8), 25-30.

Ghuman, J. K., Ginsburg, G. S., Subramaniam, G., Ghuman, H. S., Kau, A. S., & Riddle, M. A. (2001). Psychostimulants in preschool children with attention-deficit/hyperactivity disorder: clinical evidence from a developmental disorders institution. *J Am Acad Child Adolesc Psychiatry, 40*(5), 516-524. doi:10.1097/00004583-200105000-00010

Gizer, I. R., Ficks, C., & Waldman, I. D. (2009). Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet, 126*(1), 51-90. doi:10.1007/s00439-009-0694-x

Goodale, M. A., Foreman, N. P., & Milner, A. D. (1977). The effects of lesions of the superior colliculus on two kinds of visual orientation behaviour in the rat. *Brain Res, 127*(2), 356-357.

Goodale, M. A., Foreman, N. P., & Milner, A. D. (1978). Visual orientation in the rat: A dissociation of deficits following cortical and collicular lesions. *Experimental Brain Research, 31*(3), 445-457. doi:10.1007/BF00237301

Gowan, J. D., Coizet, V., Devonshire, I. M., & Overton, P. G. (2008). D-amphetamine depresses visual responses in the rat superior colliculus: a possible mechanism for amphetamine-induced decreases in distractibility. *J Neural Transm (Vienna), 115*(3), 377-387. doi:10.1007/s00702-007-0858-6

Greydanus, D. E., Pratt, H. D., & Patel, D. R. (2007). Attention deficit hyperactivity disorder across the lifespan: the child, adolescent, and adult. *Dis Mon, 53*(2), 70-131. doi:10.1016/j.disamonth.2007.01.001

Guillery, R. W., & Sherman, S. M. (2002). Thalamic Relay Functions and Their Role in Corticocortical Communication: Generalizations from the Visual System. *Neuron, 33*(2), 163-175. doi:<https://doi.org/10.1016/S0896-6273(01)00582-7>

Gumenyuk, V., Korzyukov, O., Alho, K., Escera, C., & Naatanen, R. (2004). Effects of auditory distraction on electrophysiological brain activity and performance in children aged 8-13 years. *Psychophysiology, 41*(1), 30-36. doi:10.1111/1469-8986.00123

Gurney, K., Prescott, T. J., & Redgrave, P. (2001). A computational model of action selection in the basal ganglia. I. A new functional anatomy. *Biol Cybern, 84*(6), 401-410. doi:10.1007/pl00007984

Guze, B. H., & Gitlin, M. (1994). New antidepressants and the treatment of depression. *J Fam Pract, 38*(1), 49-57.

Hall, W. C., & Lee, P. (1993). Interlaminar connections of the superior colliculus in the tree shrew. I. The superficial gray layer. *J Comp Neurol, 332*(2), 213-223. doi:10.1002/cne.903320206

Hanslick, J. L., Lau, K., Noguchi, K. K., Olney, J. W., Zorumski, C. F., Mennerick, S., & Farber, N. B. (2009). Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system. *Neurobiol Dis, 34*(1), 1-10. doi:10.1016/j.nbd.2008.11.006

Heal, D. J., Cheetham, S. C., Prow, M. R., Martin, K. F., & Buckett, W. R. (1998). A comparison of the effects on central 5-HT function of sibutramine hydrochloride and other weight-modifying agents. *Br J Pharmacol, 125*(2), 301-308. doi:10.1038/sj.bjp.0702067

Heal, D. J., Cheetham, S. C., & Smith, S. L. (2009). The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety. *Neuropharmacology, 57*(7-8), 608-618. doi:10.1016/j.neuropharm.2009.08.020

Heal, D. J., Smith, S. L., Gosden, J., & Nutt, D. J. (2013). Amphetamine, past and present--a pharmacological and clinical perspective. *Journal of psychopharmacology (Oxford, England), 27*(6), 479-496. doi:10.1177/0269881113482532

Heal, D. J., Smith, S. L., Kulkarni, R. S., & Rowley, H. L. (2008). New perspectives from microdialysis studies in freely-moving, spontaneously hypertensive rats on the pharmacology of drugs for the treatment of ADHD. *Pharmacol Biochem Behav, 90*(2), 184-197. doi:10.1016/j.pbb.2008.03.016

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *J Neurochem, 66*(6), 2621-2624.

Hesse, S., Ballaschke, O., Barthel, H., & Sabri, O. (2009). Dopamine transporter imaging in adult patients with attention-deficit/hyperactivity disorder. *Psychiatry Res, 171*(2), 120-128. doi:10.1016/j.pscychresns.2008.01.002

Hetherington, L., Dommett, E. J., Turner, A. C., Riley, T. B., Haensel, J. X., & Overton, P. G. (2017). Effect of methylphenidate on visual responses in the superior colliculus in the anaesthetised rat: Role of cortical activation. *J Psychopharmacol, 31*(10), 1347-1361. doi:10.1177/0269881117730661

Hiemke, C., & Hartter, S. (2000). Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacol Ther, 85*(1), 11-28.

Himelstein, J. (2000). *The neurobiology of attention-deficit hyperactivity disorder* (Vol. 5).

Hirai, H., & Okada, Y. (1995). Adenosine facilitates in vivo neurotransmission in the superior colliculus of the rat. *J Neurophysiol, 74*(3), 950-960. doi:10.1152/jn.1995.74.3.950

Holmes, J. C., & Rutledge, C. O. (1976). Effects of the d- and l-isomers of amphetamine on uptake, release and catabolism of norepinephrine, dopamine and 5-hydroxytryptamine in several regions of rat brain. *Biochem Pharmacol, 25*(4), 447-451.

Hornung, J.-P. (2003). The human raphe nuclei and the serotonergic system. *Journal of Chemical Neuroanatomy, 26*(4), 331-343. doi:<https://doi.org/10.1016/j.jchemneu.2003.10.002>

Hrdina, P. D., & Vu, T. B. (1993). Chronic fluoxetine treatment upregulates 5-HT uptake sites and 5-HT2 receptors in rat brain: an autoradiographic study. *Synapse, 14*(4), 324-331. doi:10.1002/syn.890140410

Huh, Y., & Cho, J. (2013). Urethane anesthesia depresses activities of thalamocortical neurons and alters its response to nociception in terms of dual firing modes. *Frontiers in behavioral neuroscience, 7*, 141-141. doi:10.3389/fnbeh.2013.00141

Hurtig, T., Ebeling, H., Taanila, A., Miettunen, J., Smalley, S. L., McGough, J. J., . . . Moilanen, I. K. (2007). ADHD Symptoms and Subtypes: Relationship Between Childhood and Adolescent Symptoms. *Journal of the American Academy of Child & Adolescent Psychiatry, 46*(12), 1605-1613. doi:<https://doi.org/10.1097/chi.0b013e318157517a>

Hynd, G. W., Hern, K. L., Novey, E. S., Eliopulos, D., Marshall, R., Gonzalez, J. J., & Voeller, K. K. (1993). Attention Deficit- Hyperactivity Disorder and Asymmetry of the Caudate Nucleus. *Journal of Child Neurology, 8*(4), 339-347. doi:10.1177/088307389300800409

Hyttel, J. (1994). PHARMACOLOGICAL CHARACTERIZATION OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIS). *International Clinical Psychopharmacology, 9*, 19-26. doi:10.1097/00004850-199403001-00004

Ichikawa, J., Kuroki, T., Kitchen, M. T., & Meltzer, H. Y. (1995). R(+)-8-OH-DPAT, a 5-HT1A receptor agonist, inhibits amphetamine-induced dopamine release in rat striatum and nucleus accumbens. *European Journal of Pharmacology, 287*(2), 179-184. doi:<https://doi.org/10.1016/0014-2999(95)00624-9>

Ikeda, T., & Hikosaka, O. (2003). Reward-dependent gain and bias of visual responses in primate superior colliculus. *Neuron, 39*(4), 693-700.

Isa, T. (2002). Intrinsic processing in the mammalian superior colliculus. *Curr Opin Neurobiol, 12*(6), 668-677.

Issari, Y., Jakubovski, E., Bartley, C. A., Pittenger, C., & Bloch, M. H. (2016). Early onset of response with selective serotonin reuptake inhibitors in obsessive-compulsive disorder: a meta-analysis. *J Clin Psychiatry, 77*(5), e605-611. doi:10.4088/JCP.14r09758

Jadad, A. R., Boyle, M., Cunningham, C., Kim, M., & Schachar, R. (1999). Treatment of attention-deficit/hyperactivity disorder. *Evid Rep Technol Assess (Summ)*(11), i-viii, 1-341.

Jiang, W., & Stein, B. E. (2003). Cortex Controls Multisensory Depression in Superior Colliculus. *Journal of Neurophysiology, 90*(4), 2123-2135. doi:10.1152/jn.00369.2003

Johansson, L., Sohn, D., Thorberg, S. O., Jackson, D. M., Kelder, D., Larsson, L. G., . . . Westlind-Danielsson, A. (1997). The pharmacological characterization of a novel selective 5-hydroxytryptamine1A receptor antagonist, NAD-299. *J Pharmacol Exp Ther, 283*(1), 216-225.

Karlsson, L., Tuominen, L., Huotarinen, A., Leppamaki, S., Sihvola, E., Helin, S., . . . Karlsson, H. (2013). Serotonin transporter in attention-deficit hyperactivity disorder--preliminary results from a positron emission tomography study. *Psychiatry Res, 212*(2), 164-165. doi:10.1016/j.pscychresns.2013.02.001

Kemp, A. H., Quintana, D. S., Gray, M. A., Felmingham, K. L., Brown, K., & Gatt, J. M. (2010). Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. *Biol Psychiatry, 67*(11), 1067-1074. doi:10.1016/j.biopsych.2009.12.012

Klein, C., Fischer, B., Fischer, B., & Hartnegg, K. (2002). Effects of methylphenidate on saccadic responses in patients with ADHD. *Experimental Brain Research, 145*(1), 121-125. doi:10.1007/s00221-002-1105-x

Klein, C., Raschke, A., & Brandenbusch, A. (2003). Development of pro–and antisaccades in children with attention–deficit hyperactivity disorder (ADHD) and healthy controls. *Psychophysiology, 40*(1), 17-28.

Klein, R. G., Abikoff, H., Klass, E., Ganeles, D., Seese, L. M., & Pollack, S. (1997). Clinical efficacy of methylphenidate in conduct disorder with and without attention deficit hyperactivity disorder. *Arch Gen Psychiatry, 54*(12), 1073-1080.

Kollins, S. H. (2007). Abuse liability of medications used to treat attention-deficit/hyperactivity disorder (ADHD). *Am J Addict, 16 Suppl 1*, 35-42; quiz 43-34. doi:10.1080/10550490601082775

Kollins, S. H. (2008). ADHD, substance use disorders, and psychostimulant treatment: current literature and treatment guidelines. *J Atten Disord, 12*(2), 115-125. doi:10.1177/1087054707311654

Konrad, K., Gunther, T., Heinzel-Gutenbrunner, M., & Herpertz-Dahlmann, B. (2005). Clinical evaluation of subjective and objective changes in motor activity and attention in children with attention-deficit/hyperactivity disorder in a double-blind methylphenidate trial. *J Child Adolesc Psychopharmacol, 15*(2), 180-190. doi:10.1089/cap.2005.15.180

Kratochvil, C. J., Newcorn, J. H., Arnold, L. E., Duesenberg, D., Emslie, G. J., Quintana, H., . . . Biederman, J. (2005). Atomoxetine alone or combined with fluoxetine for treating ADHD with comorbid depressive or anxiety symptoms. *J Am Acad Child Adolesc Psychiatry, 44*(9), 915-924. doi:10.1097/01.chi.0000169012.81536.38

Kratochvil, C. J., Vaughan, B. S., Harrington, M. J., & Burke, W. J. (2003). Atomoxetine: a selective noradrenaline reuptake inhibitor for the treatment of attention-deficit/hyperactivity disorder. *Expert Opin Pharmacother, 4*(7), 1165-1174. doi:10.1517/14656566.4.7.1165

Kuczenski, R., & Segal, D. (1989). Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. *J Neurosci, 9*(6), 2051-2065.

Kuczenski, R., & Segal, D. S. (1997). Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. *J Neurochem, 68*(5), 2032-2037.

Kuroki, T., Ichikawa, J., Dai, J., & Meltzer, H. Y. (1996). R(+)-8-OH-DPAT, a 5-HT1A receptor agonist, inhibits amphetamine-induced serotonin and dopamine release in rat medial prefrontal cortex. *Brain Res, 743*(1), 357-361. doi:<https://doi.org/10.1016/S0006-8993(96)01111-0>

Lange, K. W., Reichl, S., Lange, K. M., Tucha, L., & Tucha, O. (2010). The history of attention deficit hyperactivity disorder. *Attention deficit and hyperactivity disorders, 2*(4), 241-255. doi:10.1007/s12402-010-0045-8

Langleben, D. D., Austin, G., Krikorian, G., Ridlehuber, H. W., Goris, M. L., & Strauss, H. W. (2001). Interhemispheric asymmetry of regional cerebral blood flow in prepubescent boys with attention deficit hyperactivity disorder. *Nucl Med Commun, 22*(12), 1333-1340.

Lee, M. H., Smyser, C. D., & Shimony, J. S. (2013). Resting-state fMRI: a review of methods and clinical applications. *AJNR Am J Neuroradiol, 34*(10), 1866-1872. doi:10.3174/ajnr.A3263

Legatt, A. D., Arezzo, J., & Vaughan, H. G., Jr. (1980). Averaged multiple unit activity as an estimate of phasic changes in local neuronal activity: effects of volume-conducted potentials. *J Neurosci Methods, 2*(2), 203-217.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., . . . Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274*(5292), 1527-1531.

Lindvall, O., & Bjorklund, A. (1974). The glyoxylic acid fluorescence histochemical method: a detailed account of the methodology for the visualization of central catecholamine neurons. *Histochemistry, 39*(2), 97-127.

Looby, A., Beyer, D. L., & Zimmerman, L. (2015). Non-medical prescription stimulant use: Investigating modifiable risk factors. *Addiction Research & Theory, 23*(2), 143-147. doi:10.3109/16066359.2014.946411

Maejima, T., Masseck, O. A., Mark, M. D., & Herlitze, S. (2013). Modulation of firing and synaptic transmission of serotonergic neurons by intrinsic G protein-coupled receptors and ion channels. *Frontiers in Integrative Neuroscience, 7*, 40. doi:10.3389/fnint.2013.00040

Maggi, C. A., & Meli, A. (1986). Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. *Experientia, 42*(2), 109-114.

March, J., Silva, S., Petrycki, S., Curry, J., Wells, K., Fairbank, J., . . . Severe, J. (2004). Fluoxetine, cognitive-behavioral therapy, and their combination for adolescents with depression: Treatment for Adolescents With Depression Study (TADS) randomized controlled trial. *Jama, 292*(7), 807-820. doi:10.1001/jama.292.7.807

Marino, R. A., Rodgers, C. K., Levy, R., & Munoz, D. P. (2008). Spatial Relationships of Visuomotor Transformations in the Superior Colliculus Map. *Journal of Neurophysiology, 100*(5), 2564-2576. doi:10.1152/jn.90688.2008

Martin, L. P., Jackson, D. M., Wallsten, C., & Waszczak, B. L. (1999). Electrophysiological comparison of 5-Hydroxytryptamine1A receptor antagonists on dorsal raphe cell firing. *J Pharmacol Exp Ther, 288*(2), 820-826.

Martinez-Badía, J., & Martinez-Raga, J. (2015). Who says this is a modern disorder? The early history of attention deficit hyperactivity disorder. *World journal of psychiatry, 5*(4), 379-386. doi:10.5498/wjp.v5.i4.379

Massey, C. A., Kim, G., Corcoran, A. E., Haynes, R. L., Paterson, D. S., Cummings, K. J., . . . Commons, K. G. (2013). Development of brainstem 5-HT1A receptor-binding sites in serotonin-deficient mice. *J Neurochem, 126*(6), 749-757. doi:10.1111/jnc.12311

May, P. J. (2006). The mammalian superior colliculus: laminar structure and connections. In J. A. Büttner-Ennever (Ed.), *Prog Brain Res* (Vol. 151, pp. 321-378): Elsevier.

May, P. J., McHaffie, J. G., Stanford, T. R., Jiang, H., Costello, M. G., Coizet, V., . . . Redgrave, P. (2009). Tectonigral Projections in the Primate: A Pathway for Pre-Attentive Sensory Input to Midbrain Dopaminergic Neurons. *The European journal of neuroscience, 29*(3), 575-587. doi:10.1111/j.1460-9568.2008.06596.x

McDevitt, R. A., & Neumaier, J. F. (2011). Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J Chem Neuroanat, 41*(4), 234-246. doi:10.1016/j.jchemneu.2011.05.001

McGough, J. J., McCracken, J. T., Loo, S. K., Manganiello, M., Leung, M. C., Tietjens, J. R., . . . Sugar, C. A. (2009). A candidate gene analysis of methylphenidate response in attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry, 48*(12), 1155-1164. doi:10.1097/CHI.0b013e3181bc72e3

McHaffie, J. G., Stanford, T. R., Stein, B. E., Coizet, V., & Redgrave, P. (2005). Subcortical loops through the basal ganglia. *Trends Neurosci, 28*(8), 401-407. doi:10.1016/j.tins.2005.06.006

McPeek, R. M., & Keller, E. L. (2002). Saccade target selection in the superior colliculus during a visual search task. *J Neurophysiol, 88*(4), 2019-2034. doi:10.1152/jn.2002.88.4.2019

Meyer, J. H., Wilson, A. A., Sagrati, S., Hussey, D., Carella, A., Potter, W. Z., . . . Houle, S. (2004). Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study. *Am J Psychiatry, 161*(5), 826-835. doi:10.1176/appi.ajp.161.5.826

Miller, A., Lee, S., Raina, P., Klassen, A., Zupancic, J., & Olsen, L. (1999). *A review of therapies for attention-deficit/hyperactivity disorder*. Ottawa: Canadian Coordinating Office for Health Technology Assessment (CCOHTA); .

Miller, H., Shore, P., & Clarke, D. (1980). *In vivo monoamine oxidase inhibition by D-amphetamine* (Vol. 29).

Milner, A. D., Foreman, N. P., & Goodale, M. A. (1978). Go-left go-right discrimination performance and distractibility following lesions of prefrontal cortex or superior colliculus in stumptail macaques. *Neuropsychologia, 16*(4), 381-390. doi:<https://doi.org/10.1016/0028-3932(78)90062-3>

Mize, R. R., & Horner, L. H. (1989). Origin, distribution, and morphology of serotonergic afferents to the cat superior colliculus: a light and electron microscope immunocytochemistry study. *Experimental Brain Research, 75*(1), 83-98. doi:10.1007/BF00248533

Mooney, R. D., Huang, X., Shi, M. Y., Bennett-Clarke, C. A., & Rhoades, R. W. (1996). Serotonin modulates retinotectal and corticotectal convergence in the superior colliculus. *Prog Brain Res, 112*, 57-69.

Mooney, R. D., Shi, M. Y., & Rhoades, R. W. (1994). Modulation of retinotectal transmission by presynaptic 5-HT1B receptors in the superior colliculus of the adult hamster. *Journal of Neurophysiology, 72*(1), 3-13. doi:10.1152/jn.1994.72.1.3

Morikawa, H., Manzoni, O. J., Crabbe, J. C., & Williams, J. T. (2000). Regulation of central synaptic transmission by 5-HT(1B) auto- and heteroreceptors. *Mol Pharmacol, 58*(6), 1271-1278.

Mostofsky, S. H., Cooper, K. L., Kates, W. R., Denckla, M. B., & Kaufmann, W. E. (2002). Smaller prefrontal and premotor volumes in boys with attention-deficit/hyperactivity disorder. *Biol Psychiatry, 52*(8), 785-794.

Munoz, D. P., Armstrong, I. T., Hampton, K. A., & Moore, K. D. (2003). Altered control of visual fixation and saccadic eye movements in attention-deficit hyperactivity disorder. *J Neurophysiol, 90*(1), 503-514. doi:10.1152/jn.00192.2003

Murphy, K., & Barkley, R. A. (1996). Attention deficit hyperactivity disorder adults: comorbidities and adaptive impairments. *Compr Psychiatry, 37*(6), 393-401.

Nahlik, J. (2004). Issues in Diagnosis of Attention-Deficit/Hyperactivity Disorder in Adolescents. *Clinical Pediatrics, 43*(1), 1-10. doi:10.1177/000992280404300101

Nakao, T., Radua, J., Rubia, K., & Mataix-Cols, D. (2011). Gray matter volume abnormalities in ADHD: voxel-based meta-analysis exploring the effects of age and stimulant medication. *Am J Psychiatry, 168*(11), 1154-1163. doi:10.1176/appi.ajp.2011.11020281

Navarra, R. L., Clark, B. D., Zitnik, G. A., & Waterhouse, B. D. (2013). Methylphenidate and atomoxetine enhance sensory-evoked neuronal activity in the visual thalamus of male rats. *Exp Clin Psychopharmacol, 21*(5), 363-374. doi:10.1037/a0033563

Neef, N. A., Marckel, J., Ferreri, S. J., Bicard, D. F., Endo, S., Aman, M. G., . . . Armstrong, N. (2005). Behavioral assessment of impulsivity: a comparison of children with and without attention deficit hyperactivity disorder. *Journal of applied behavior analysis, 38*(1), 23-37. doi:10.1901/jaba.2005.146-02

Oades, R. D. (2007). Role of the serotonin system in ADHD: treatment implications. *Expert Rev Neurother, 7*(10), 1357-1374. doi:10.1586/14737175.7.10.1357

Onnink, A. M., Zwiers, M. P., Hoogman, M., Mostert, J. C., Dammers, J., Kan, C. C., . . . Franke, B. (2015). Deviant white matter structure in adults with attention-deficit/hyperactivity disorder points to aberrant myelination and affects neuropsychological performance. *Prog Neuropsychopharmacol Biol Psychiatry, 63*, 14-22. doi:10.1016/j.pnpbp.2015.04.008

Overton, P. G. (2008). Collicular dysfunction in attention deficit hyperactivity disorder. *Med Hypotheses, 70*(6), 1121-1127. doi:10.1016/j.mehy.2007.11.016

Palucha-Poniewiera, A., Branski, P., Wieronska, J. M., Stachowicz, K., Slawinska, A., & Pilc, A. (2014). The antidepressant-like action of mGlu5 receptor antagonist, MTEP, in the tail suspension test in mice is serotonin dependent. *Psychopharmacology (Berl), 231*(1), 97-107. doi:10.1007/s00213-013-3206-6

Panagiotidi, M., Overton, P., & Stafford, T. (2017). Increased microsaccade rate in individuals with ADHD traits. *2017, 10*(1). doi:10.16910/10.1.6

Panagiotidi, M., Overton, P. G., & Stafford, T. (2017). Attention-Deficit Hyperactivity Disorder-Like Traits and Distractibility in the Visual Periphery. *Perception, 46*(6), 665-678. doi:10.1177/0301006616681313

Parent, A., Descarries, L., & Beaudet, A. (1981). Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [3h]5-hydroxytryptamine. *Neuroscience, 6*(2), 115-138. doi:<https://doi.org/10.1016/0306-4522(81)90050-6>

Parmentier, F. B. R., Elsley, J. V., Andrés, P., & Barceló, F. (2011). Why are auditory novels distracting? Contrasting the roles of novelty, violation of expectation and stimulus change. *Cognition, 119*(3), 374-380. doi:<https://doi.org/10.1016/j.cognition.2011.02.001>

Paxinos, G., & Watson, C. (1998). The rat brain atlas in stereotaxic coordinates. *San Diego: Academic*.

Pelletier, M.-F., Hodgetts, H. M., Lafleur, M. F., Vincent, A., & Tremblay, S. (2013). Vulnerability to the Irrelevant Sound Effect in Adult ADHD. *J Atten Disord, 20*(4), 306-316. doi:10.1177/1087054713492563

Penington, N. J., Kelly, J. S., & Fox, A. P. (1993). Whole-cell recordings of inwardly rectifying K+ currents activated by 5-HT1A receptors on dorsal raphe neurones of the adult rat. *J Physiol, 469*, 387-405.

Pérez-González, D., Malmierca, M. S., & Covey, E. (2005). Novelty detector neurons in the mammalian auditory midbrain. *European Journal of Neuroscience, 22*(11), 2879-2885. doi:doi:10.1111/j.1460-9568.2005.04472.x

Peroutka, S. J., Newman, H., & Harris, H. (1988). Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users. *Neuropsychopharmacology, 1*(4), 273-277.

Pittenger, C., & Bloch, M. H. (2014). Pharmacological treatment of obsessive-compulsive disorder. *The Psychiatric clinics of North America, 37*(3), 375-391. doi:10.1016/j.psc.2014.05.006

Pliszka, S. R. (2003). Non-stimulant treatment of attention-deficit/hyperactivity disorder. *CNS Spectr, 8*(4), 253-258.

Pliszka, S. R. (2005). The neuropsychopharmacology of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*(11), 1385-1390. doi:10.1016/j.biopsych.2004.08.026

Polanczyk, G., de Lima, M. S., Horta, B. L., Biederman, J., & Rohde, L. A. (2007). The worldwide prevalence of ADHD: a systematic review and metaregression analysis. *Am J Psychiatry, 164*(6), 942-948. doi:10.1176/ajp.2007.164.6.942

Popper, C. W. (1997). Antidepressants in the treatment of attention-deficit/hyperactivity disorder. *J Clin Psychiatry, 58 Suppl 14*, 14-29; discussion 30-11.

Popper, C. W. (2000). Pharmacologic alternatives to psychostimulants for the treatment of attention-deficit/hyperactivity disorder. *Child Adolesc Psychiatr Clin N Am, 9*(3), 605-646, viii.

Preskorn, S. H. (1997). Clinically relevant pharmacology of selective serotonin reuptake inhibitors. An overview with emphasis on pharmacokinetics and effects on oxidative drug metabolism. *Clin Pharmacokinet, 32 Suppl 1*, 1-21.

Rech, R. H., Borsini, F., & Samanin, R. (1984). Effects of d-amphetamine and d-fenfluramine on performance of rats in a food maze. *Pharmacol Biochem Behav, 20*(4), 489-493.

Redgrave, P., Coizet, V., Comoli, E., McHaffie, J. G., Leriche, M., Vautrelle, N., . . . Overton, P. (2010). Interactions between the Midbrain Superior Colliculus and the Basal Ganglia. *Frontiers in Neuroanatomy, 4*, 132. doi:10.3389/fnana.2010.00132

Redgrave, P., Prescott, T. J., & Gurney, K. (1999). The basal ganglia: a vertebrate solution to the selection problem? *Neuroscience, 89*(4), 1009-1023. doi:<https://doi.org/10.1016/S0306-4522(98)00319-4>

Remschmidt, H. (2005). Global consensus on ADHD/HKD. *Eur Child Adolesc Psychiatry, 14*(3), 127-137. doi:10.1007/s00787-005-0439-x

Retz, W., Retz-Junginger, P., Supprian, T., Thome, J., & Rösler, M. (2004). Association of serotonin transporter promoter gene polymorphism with violence: relation with personality disorders, impulsivity, and childhood ADHD psychopathology. *Behavioral Sciences & the Law, 22*(3), 415-425. doi:10.1002/bsl.589

Riad, M., Watkins, K. C., Doucet, E., Hamon, M., & Descarries, L. (2001). Agonist-induced internalization of serotonin-1a receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). *J Neurosci, 21*(21), 8378-8386.

Riddle, M. A., KING, R. A., HARDIN, M. T., SCAHILL, L., ORT, S. I., CHAPPELL, P., . . . LECKMAN, J. F. (1990). Behavioral Side Effects of Fluoxetine in Children and Adolescents. *Journal of Child and Adolescent Psychopharmacology, 1*(3), 193-198. doi:10.1089/cap.1990.1.193

Roberts, W., Fillmore, M. T., & Milich, R. (2011). Linking impulsivity and inhibitory control using manual and oculomotor response inhibition tasks. *Acta Psychologica, 138*(3), 419-428. doi:<https://doi.org/10.1016/j.actpsy.2011.09.002>

Robertson, S. D., Matthies, H. J., & Galli, A. (2009). A closer look at amphetamine-induced reverse transport and trafficking of the dopamine and norepinephrine transporters. *Mol Neurobiol, 39*(2), 73-80. doi:10.1007/s12035-009-8053-4

Robinson, J. B. (1985). Stereoselectivity and isoenzyme selectivity of monoamine oxidase inhibitors. Enantiomers of amphetamine, N-methylamphetamine and deprenyl. *Biochem Pharmacol, 34*(23), 4105-4108.

Rodgers, C. K., Munoz, D. P., Scott, S. H., & Paré, M. (2006). Discharge Properties of Monkey Tectoreticular Neurons. *Journal of Neurophysiology, 95*(6), 3502-3511. doi:10.1152/jn.00908.2005

Rojas, P. S., & Fiedler, J. L. (2016). What Do We Really Know About 5-HT1A Receptor Signaling in Neuronal Cells? *Frontiers in Cellular Neuroscience, 10*(272). doi:10.3389/fncel.2016.00272

Romero, L., Hervas, I., & Artigas, F. (1996). The 5-HT1A antagonist WAY-100635 selectively potentiates the presynaptic effects of serotonergic antidepressants in rat brain. *Neurosci Lett, 219*(2), 123-126.

Rose, S., Janke, A., Strudwick, M., McMahon, K., B Chalk, J., Snyder, P., & de Zubicaray, G. (2006). *Assessment of dynamic susceptibility contrast cerebral blood flow response to amphetamine challenge: A human pharmacological magnetic resonance imaging study at 1.5 and 4 T* (Vol. 55).

Rosenbaum, J. F., Fava, M., Hoog, S. L., Ascroft, R. C., & Krebs, W. B. (1998). Selective serotonin reuptake inhibitor discontinuation syndrome: a randomized clinical trial. *Biol Psychiatry, 44*(2), 77-87.

Ross, D., & Ross, S. (1976). *Hyperactivity: research, theory and action.* . New York: Wiley.

Ross, S., B., Thorberg, S. O., Jerning, E., Mohell, N., Stenfors, C., Wallsten, C., . . . Öjteg, G. (2006). *Robalzotan (NAD‐299), a Novel Selective 5‐HT 1A Receptor Antagonist* (Vol. 5).

Rossi, A., Barraco, A., & Donda, P. (2004). Fluoxetine: a review on evidence based medicine. *Annals of general hospital psychiatry, 3*(1), 2-2. doi:10.1186/1475-2832-3-2

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V., Rice, K. C., Carroll, F. I., & Partilla, J. S. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse, 39*(1), 32-41. doi:10.1002/1098-2396(20010101)39:1<32::aid-syn5>3.0.co;2-3

Rubia, K., Overmeyer, S., Taylor, E., Brammer, M., Williams, S. C., Simmons, A., & Bullmore, E. T. (1999). Hypofrontality in attention deficit hyperactivity disorder during higher-order motor control: a study with functional MRI. *Am J Psychiatry, 156*(6), 891-896. doi:10.1176/ajp.156.6.891

Sagvolden, T., Johansen, E. B., Aase, H., & Russell, V. A. (2005). A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. *Behav Brain Sci, 28*(3), 397-419; discussion 419-368. doi:10.1017/s0140525x05000075

Saito, Y., & Isa, T. (2005). Organization of Interlaminar Interactions in the Rat Superior Colliculus. *Journal of Neurophysiology, 93*(5), 2898-2907. doi:10.1152/jn.01051.2004

Sastry, B. S., & Phillis, J. W. (1977). Metergoline as a selective 5-hydroxytryptamine antagonist in the cerebral cortex. *Can J Physiol Pharmacol, 55*(1), 130-133.

Sawada, M., & Sato, M. (1975). THE EFFECT OF DIMETHYL SULFOXIDE ON THE NEURONAL EXCITABILITY AND CHOLINERGIC TRANSMISSION IN APLYSIA GANGLION CELLS\*. *Ann N Y Acad Sci, 243*(1), 337-357. doi:10.1111/j.1749-6632.1975.tb25375.x

Schneider, G. E. (1969). Two Visual Systems. *Science, 163*(3870), 895-902.

Schouw, M. L. J., Kaag, A. M., Caan, M. W. A., Heijtel, D. F. R., Majoie, C. B. L. M., Nederveen, A. J., . . . Reneman, L. (2013). Mapping the hemodynamic response in human subjects to a dopaminergic challenge with dextroamphetamine using ASL-based pharmacological MRI. *Neuroimage, 72*, 1-9. doi:<https://doi.org/10.1016/j.neuroimage.2012.12.056>

Schroger, E., & Wolff, C. (1998). Behavioral and electrophysiological effects of task-irrelevant sound change: a new distraction paradigm. *Brain Res Cogn Brain Res, 7*(1), 71-87.

Schuldiner, S., Shirvan, A., & Linial, M. (1995). Vesicular neurotransmitter transporters: from bacteria to humans. *Physiol Rev, 75*(2), 369-392. doi:10.1152/physrev.1995.75.2.369

Segu, L., Abdelkefi, J., Dusticier, G., & Lanoir, J. (1986). High-affinity serotonin binding sites: Autoradiographic evidence for their location on retinal afferents in the rat superior colliculus. *Brain Res, 384*(2), 205-217. doi:<https://doi.org/10.1016/0006-8993(86)91156-X>

Seidman, L. J., Biederman, J., Faraone, S. V., Weber, W., & Ouellette, C. (1997). Toward defining a neuropsychology of attention deficit-hyperactivity disorder: performance of children and adolescents from a large clinically referred sample. *J Consult Clin Psychol, 65*(1), 150-160. doi:10.1037/0022-006x.65.1.150

Seidman, L. J., Valera, E. M., & Makris, N. (2005). Structural brain imaging of attention-deficit/hyperactivity disorder. *Biol Psychiatry, 57*(11), 1263-1272. doi:10.1016/j.biopsych.2004.11.019

Selle, V., Schalkwijk, S., Vazquez, G. H., & Baldessarini, R. J. (2014). Treatments for Acute Bipolar Depression: Meta-analyses of Placebo-controlled, Monotherapy Trials of Anticonvulsants, Lithium and Antipsychotics. *Pharmacopsychiatry, 47*(2), 43-52. doi:10.1055/s-0033-1363258

Sharp, S. I., McQuillin, A., & Gurling, H. M. D. (2009). Genetics of attention-deficit hyperactivity disorder (ADHD). *Neuropharmacology, 57*(7), 590-600. doi:<https://doi.org/10.1016/j.neuropharm.2009.08.011>

Sharp, T., Bramwell, S. R., Hjorth, S., & Grahame‐Smith, D. G. (1989). Pharmacological characterization of 8‐OH‐DPAT‐induced inhibition of rat hippocampal 5‐HT release in vivo as measured by microdialysis. *Br J Pharmacol, 98*(3), 989-997.

Shukla, R., Watakabe, A., & Yamamori, T. (2014). mRNA expression profile of serotonin receptor subtypes and distribution of serotonergic terminations in marmoset brain. *Frontiers in neural circuits, 8*, 52-52. doi:10.3389/fncir.2014.00052

Silk, T. J., Vance, A., Rinehart, N., Bradshaw, J. L., & Cunnington, R. (2009). White-matter abnormalities in attention deficit hyperactivity disorder: a diffusion tensor imaging study. *Hum Brain Mapp, 30*(9), 2757-2765. doi:10.1002/hbm.20703

Simon, V., Czobor, P., Balint, S., Meszaros, A., & Bitter, I. (2009). Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. *Br J Psychiatry, 194*(3), 204-211. doi:10.1192/bjp.bp.107.048827

Smalley, S. L., McGough, J. J., Del'Homme, M., NewDelman, J., Gordon, E., Kim, T., . . . McCracken, J. T. (2000). Familial clustering of symptoms and disruptive behaviors in multiplex families with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry, 39*(9), 1135-1143. doi:10.1097/00004583-200009000-00013

Sokunbi, M. O., Fung, W., Sawlani, V., Choppin, S., Linden, D. E., & Thome, J. (2013). Resting state fMRI entropy probes complexity of brain activity in adults with ADHD. *Psychiatry Res, 214*(3), 341-348. doi:10.1016/j.pscychresns.2013.10.001

Sonuga-Barke, E. J. S., Williams, E., Hall, M., & Saxton, T. (1996). Hyperactivity and Delay Aversion III: The Effect on Cognitive Style of Imposing Delay After Errors. *Journal of Child Psychology and Psychiatry, 37*(2), 189-194. doi:10.1111/j.1469-7610.1996.tb01390.x

Sparks, D. L. (1999). Conceptual issues related to the role of the superior colliculus in the control of gaze. *Current Opinion in Neurobiology, 9*(6), 698-707. doi:<https://doi.org/10.1016/S0959-4388(99)00039-2>

Spencer, T. J., Biederman, J., Wilens, T., Prince, J., Hatch, M., Jones, J., . . . Seidman, L. (1998). Effectiveness and tolerability of tomoxetine in adults with attention deficit hyperactivity disorder. *Am J Psychiatry, 155*(5), 693-695. doi:10.1176/ajp.155.5.693

Spencer, T. J., Biederman, J., Wilens, T. E., & Faraone, S. V. (2002a). Novel treatments for attention-deficit/hyperactivity disorder in children. *J Clin Psychiatry, 63 Suppl 12*, 16-22.

Spencer, T. J., Biederman, J., Wilens, T. E., & Faraone, S. V. (2002b). Overview and neurobiology of attention-deficit/hyperactivity disorder. *J Clin Psychiatry, 63 Suppl 12*, 3-9.

Stahl, S. M. (1998). Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. *J Affect Disord, 51*(3), 215-235.

Stamford, J. A., Davidson, C., McLaughlin, D. P., & Hopwood, S. E. (2000). Control of dorsal raphe 5-HT function by multiple 5-HT(1) autoreceptors: parallel purposes or pointless plurality? *Trends Neurosci, 23*(10), 459-465.

Stein, & Meredith. (1993). *The merging of the senses*. Cambridge, MA, US: The MIT Press.

Stein, B. E., & Meredith, M. A. (1993). *The Merging of the Senses*: MIT Press.

Striley, C. W., Kelso-Chichetto, N. E., & Cottler, L. B. (2017). Nonmedical Prescription Stimulant Use Among Girls 10-18 Years of Age: Associations With Other Risky Behavior. *J Adolesc Health, 60*(3), 328-332. doi:10.1016/j.jadohealth.2016.10.013

Tan, H., Mooney, R. D., & Rhoades, R. W. (1999). Effects of norepinephrine upon superficial layer neurons in the superior colliculus of the hamster: In vitro studies. *Vis Neurosci, 16*(3), 557-570. doi:undefined

Taylor, E., Dopfner, M., Sergeant, J., Asherson, P., Banaschewski, T., Buitelaar, J., . . . Zuddas, A. (2004). European clinical guidelines for hyperkinetic disorder -- first upgrade. *Eur Child Adolesc Psychiatry, 13 Suppl 1*, I7-30. doi:10.1007/s00787-004-1002-x

Terrón, J. A. (1997). Role of 5-ht7 receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat. *Br J Pharmacol, 121*(3), 563-571. doi:10.1038/sj.bjp.0701134

Thorley, G. (1984). PILOT STUDY TO ASSESS BEHAVIOURAL AND COGNITIVE EFFECTS OF ARTIFICIAL FOOD COLOURS IN A GROUP OF RETARDED CHILDREN. *Developmental Medicine & Child Neurology, 26*(1), 56-61. doi:10.1111/j.1469-8749.1984.tb04406.x

Timmerman, W., & Abercrombie, E. D. (1996). Amphetamine-induced release of dendritic dopamine in substantia nigra pars reticulata: D1-mediated behavioral and electrophysiological effects. *Synapse, 23*(4), 280-291. doi:10.1002/(SICI)1098-2396(199608)23:4&lt;280::AID-SYN6&gt;3.0.CO;2-3

Tordera, R., Pei, Q., Newson, M., Gray, K., Sprakes, M., & Sharp, T. (2003). Effect of different 5-HT1A receptor antagonists in combination with paroxetine on expression of the immediate-early gene Arc in rat brain. *Neuropharmacology, 44*(7), 893-902.

Trott, G. E. (2006). Attention-deficit/hyperactivity disorder (ADHD) in the course of life. *Eur Arch Psychiatry Clin Neurosci, 256 Suppl 1*, i21-25. doi:10.1007/s00406-006-1003-5

Van der Oord, S., Prins, P. J., Oosterlaan, J., & Emmelkamp, P. M. (2008). Efficacy of methylphenidate, psychosocial treatments and their combination in school-aged children with ADHD: a meta-analysis. *Clin Psychol Rev, 28*(5), 783-800. doi:10.1016/j.cpr.2007.10.007

van Harten, J. (1993). Clinical pharmacokinetics of selective serotonin reuptake inhibitors. *Clin Pharmacokinet, 24*(3), 203-220. doi:10.2165/00003088-199324030-00003

van Mourik, R., Oosterlaan, J., Heslenfeld, D. J., Konig, C. E., & Sergeant, J. A. (2007). When distraction is not distracting: A behavioral and ERP study on distraction in ADHD. *Clinical Neurophysiology, 118*(8), 1855-1865. doi:10.1016/j.clinph.2007.05.007

Vanicek, T., Kutzelnigg, A., Philippe, C., Sigurdardottir, H. L., James, G. M., Hahn, A., . . . Lanzenberger, R. (2017). Altered interregional molecular associations of the serotonin transporter in attention deficit/hyperactivity disorder assessed with PET. *Human Brain Mapping, 38*(2), 792-802. doi:10.1002/hbm.23418

Vaswani, M., Linda, F. K., & Ramesh, S. (2003). Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 27*(1), 85-102. doi:10.1016/s0278-5846(02)00338-x

Vertes, R. P. (1991). A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *Journal of Comparative Neurology, 313*(4), 643-668. doi:doi:10.1002/cne.903130409

Vertes, R. P., Fortin, W. J., & Crane, A. M. (1999). Projections of the median raphe nucleus in the rat. *Journal of Comparative Neurology, 407*(4), 555-582. doi:doi:10.1002/(SICI)1096-9861(19990517)407:4<555::AID-CNE7>3.0.CO;2-E

Vierhile, A., Robb, A., & Ryan-Krause, P. (2009). Attention-deficit/hyperactivity disorder in children and adolescents: closing diagnostic, communication, and treatment gaps. *J Pediatr Health Care, 23*(1 Suppl), S5-23. doi:10.1016/j.pedhc.2008.10.009

Vitola, E. S., Bau, C. H., Salum, G. A., Horta, B. L., Quevedo, L., Barros, F. C., . . . Grevet, E. H. (2017). Exploring DSM-5 ADHD criteria beyond young adulthood: phenomenology, psychometric properties and prevalence in a large three-decade birth cohort. *Psychol Med, 47*(4), 744-754. doi:10.1017/s0033291716002853

Vollenweider, F. X., Maguire, R. P., Leenders, K. L., Mathys, K., & Angst, J. (1998). Effects of high amphetamine dose on mood and cerebral glucose metabolism in normal volunteers using positron emission tomography (PET). *Psychiatry Research: Neuroimaging, 83*(3), 149-162. doi:<https://doi.org/10.1016/S0925-4927(98)00033-X>

Wehmeier, P. M., Schacht, A., & Barkley, R. A. (2010). Social and Emotional Impairment in Children and Adolescents with ADHD and the Impact on Quality of Life. *Journal of Adolescent Health, 46*(3), 209-217. doi:<https://doi.org/10.1016/j.jadohealth.2009.09.009>

Weiss, G., Hechtman, L., Milroy, T., & Perlman, T. (1985). Psychiatric status of hyperactives as adults: a controlled prospective 15-year follow-up of 63 hyperactive children. *J Am Acad Child Psychiatry, 24*(2), 211-220.

Weiss, M. D., & Weiss, J. R. (2004). A guide to the treatment of adults with ADHD. *J Clin Psychiatry, 65 Suppl 3*, 27-37.

Weyandt, L. L., Marraccini, M. E., Gudmundsdottir, B. G., Zavras, B. M., Turcotte, K. D., Munro, B. A., & Amoroso, A. J. (2013). Misuse of prescription stimulants among college students: a review of the literature and implications for morphological and cognitive effects on brain functioning. *Exp Clin Psychopharmacol, 21*(5), 385-407. doi:10.1037/a0034013

Whittaker, S. G., & Cummings, R. W. (1990). Foveating saccades. *Vision Research, 30*(9), 1363-1366. doi:<https://doi.org/10.1016/0042-6989(90)90009-A>

Wichmann, T., & Starke, K. (1988). Uptake, release, and modulation of release of noradrenaline in rabbit superior colliculus. *Neuroscience, 26*(2), 621-634.

Wilens, T. E., Adler, L. A., Adams, J., Sgambati, S., Rotrosen, J., Sawtelle, R., . . . Fusillo, S. (2008). Misuse and Diversion of Stimulants Prescribed for ADHD: A Systematic Review of the Literature. *Journal of the American Academy of Child & Adolescent Psychiatry, 47*(1), 21-31. doi:<https://doi.org/10.1097/chi.0b013e31815a56f1>

Wilens, T. E., Biederman, J., Baldessarini, R. J., Geller, B., Schleifer, D., Spencer, T. J., . . . Goldblatt, A. (1996). Cardiovascular effects of therapeutic doses of tricyclic antidepressants in children and adolescents. *J Am Acad Child Adolesc Psychiatry, 35*(11), 1491-1501. doi:10.1097/00004583-199611000-00018

Wilens, T. E., Biederman, J., Brown, S., Tanguay, S., Monuteaux, M. C., Blake, C., & Spencer, T. J. (2002). Psychiatric comorbidity and functioning in clinically referred preschool children and school-age youths with ADHD. *J Am Acad Child Adolesc Psychiatry, 41*(3), 262-268. doi:10.1097/00004583-200203000-00005

Wilens, T. E., Biederman, J., Faraone, S. V., Martelon, M., Westerberg, D., & Spencer, T. J. (2009). Presenting ADHD symptoms, subtypes, and comorbid disorders in clinically referred adults with ADHD. *J Clin Psychiatry, 70*(11), 1557-1562. doi:10.4088/JCP.08m04785pur

Wilens, T. E., Biederman, J., & Spencer, T. J. (1998). Pharmacotherapy of Attention Deficit Hyperactivity Disorder in Adults. *CNS Drugs, 9*(5), 347-356. doi:10.2165/00023210-199809050-00002

Wilens, T. E., Biederman, J., & Spencer, T. J. (2002). Attention deficit/hyperactivity disorder across the lifespan. *Annu Rev Med, 53*, 113-131. doi:10.1146/annurev.med.53.082901.103945

Wilens, T. E., Faraone, S. V., Biederman, J., & Gunawardene, S. (2003). Does stimulant therapy of attention-deficit/hyperactivity disorder beget later substance abuse? A meta-analytic review of the literature. *Pediatrics, 111*(1), 179-185.

Wilens, T. E., Gignac, M., Swezey, A., Monuteaux, M. C., & Biederman, J. (2006). Characteristics of Adolescents and Young Adults With ADHD Who Divert or Misuse Their Prescribed Medications. *Journal of the American Academy of Child & Adolescent Psychiatry, 45*(4), 408-414. doi:<https://doi.org/10.1097/01.chi.0000199027.68828.b3>

Willcutt, E. G., Doyle, A. E., Nigg, J. T., Faraone, S. V., & Pennington, B. F. (2005). Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review. *Biol Psychiatry, 57*(11), 1336-1346. doi:10.1016/j.biopsych.2005.02.006

Williams, R. J., Goodale, L. A., Shay-Fiddler, M. A., Gloster, S. P., & Chang, S. Y. (2004). Methylphenidate and dextroamphetamine abuse in substance-abusing adolescents. *Am J Addict, 13*(4), 381-389. doi:10.1080/10550490490483053

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jonsson, B., . . . Steinhausen, H. C. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur Neuropsychopharmacol, 21*(9), 655-679. doi:10.1016/j.euroneuro.2011.07.018

Wolraich, M. L., Hannah, J. N., Pinnock, T. Y., Baumgaertel, A., & Brown, J. (1996). Comparison of diagnostic criteria for attention-deficit hyperactivity disorder in a county-wide sample. *J Am Acad Child Adolesc Psychiatry, 35*(3), 319-324. doi:10.1097/00004583-199603000-00013

Wong, D. T., Bymaster, F. P., & Engleman, E. A. (1995). Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci, 57*(5), 411-441.

Woodard, R. (2006). The diagnosis and medical treatment of ADHD in children and adolescents in primary care: a practical guide. *Pediatr Nurs, 32*(4), 363-370.

Wooltorton, E. (2005). Suicidal ideation among children taking atomoxetine (Strattera). *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 173*(12), 1447-1447. doi:10.1503/cmaj.051474

World Health Organization. (1992). *The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines.* Geneva: World Health Organization.

Zang, Y. F., He, Y., Zhu, C. Z., Cao, Q. J., Sui, M. Q., Liang, M., . . . Wang, Y. F. (2007). Altered baseline brain activity in children with ADHD revealed by resting-state functional MRI. *Brain Dev, 29*(2), 83-91. doi:10.1016/j.braindev.2006.07.002

Zhang, C., Deng, Y., Dai, H., Zhou, W., Tian, J., Bing, G., & Zhao, L. (2016). *Effects of dimethyl sulfoxide on the morphology and viability of primary cultured neurons and astrocytes* (Vol. 128).

Zhang, Y., Mooney, R. D., & Rhoades, R. W. (1999). Effects of norepinephrine on the activity of visual neurons in the superior colliculus of the hamster. *Vis Neurosci, 16*(3), 541-555.

Ziegler, S., Pedersen, M. L., Mowinckel, A. M., & Biele, G. (2016). Modelling ADHD: A review of ADHD theories through their predictions for computational models of decision-making and reinforcement learning. *Neurosci Biobehav Rev, 71*, 633-656. doi:10.1016/j.neubiorev.2016.09.002