The Efficacy of Specific Activation of D1-class Dopamine Receptors to Enhance Motor Recovery in Mice Following Cortical Photothrombotic Stroke

Annette Gower

This thesis is submitted to the Faculty of Graduate and Postdoctoral Studies as a partial fulfillment of the M.Sc. program in Neuroscience.

Department of Cellular and Molecular Medicine

Faculty of Medicine

University of Ottawa

February 26, 2018

Abstract

Stroke is a widespread condition, which often leaves survivors with lasting deficits in motor function, however, physical rehabilitation is the only treatment available after the acute period. A large body of preclinical literature suggests dopamine-augmenting drugs, could enhance motor recovery following stroke. Unfortunately, mixed clinical results have prevented the implementation of such treatments, possibly due to the wide variety of G protein-coupled receptors these drugs can activate. Using a mouse photothrombosis stroke model and a battery of motor and sensorimotor behavioural tests, the current study aims to demonstrate proof of principle for the use of D1-class dopamine receptor agonists to enhance poststroke motor recovery and to evaluate the role of aerobic exercise rehabilitation in an asynchronous study design. The effect of light-dark cycle on behavioural outcome (horizontal ladder test, adhesive removal test, cylinder test) and histological outcome (infarct size) in photothrombotic stroke was evaluated in order to optimize the stroke model, but no there was no evidence of differences between strokes occurring during the light or dark period of a mouse's circadian rhythm. A bioactive, suboptimal dose of D1-agonist dihydrexidine, was determined by evaluating its effect on locomotor activity and its ability to increase expression of immediate early gene c-fos. Using the determined dose, studies evaluating the efficacy of 7-days and 2-days of dihydrexidine administration on poststroke motor recovery, were performed, indicating efficacy of a 7-days, but not of a 2-days, course of treatment. The 7-days dihydrexidine treatment resulted in accelerated recovery as compared to a control group receiving saline. This work demonstrates, for the first time, proof of principle for the use of specific activation of D1-class dopamine receptors to enhance motor recovery following stroke.

Table of Contents

Abstract ii
List of Tables vii
List of Figures viii
List of Abbreviations x
Acknowledgements xiii
Introduction 1
1. Characteristics and Incidence of Stroke 2
2. The Dopamine System 3
3. Effect of Stroke on Functioning of the Dopaminergic System 4
3.1 Stroke Triggers Early, Massive Dopamine Release into the Striatum 4
3.2 Evidence Indicates a Decrease in Dopamine Receptors Following Stroke 7
3.3 Striatal Ischemia Leads to Development of Secondary Exofocal Degeneration in the Substantia Nigra 8
3.4 Stroke can impact the response of the DA system to DA modulating drugs 9
4. Animal Studies on the Effect of Dopamine-boosting Drugs in Post-Stroke Motor Recovery 11
4.1 Recovery Paradigms Showing Rapid Improvement of Motor Skills 11
4.1.1 Beam Walk Test 11
4.1.2 Tactile Placing in Cats 12
4.2 Paradigms Demonstrating Recovery after a Delay 13
4.2.1 Tactile Placing in Response to Vibrissae or Forelimb Stimulation in Rats 13
4.2.2 The Foot Fault Test and The Horizontal Ladder 14
4.2.3 Skilled Reaching Tests 14
4.2.4 Other Tests 15
4.2.5 DA-boosting Pharmacotherapy and Forced Use of the Impaired Limb 16
5. Potential Mechanisms of Action 16
5.1 Reversal of Catecholamine Diaschisis 16
5.2 Enhanced Synaptic Plasticity, Axonal Sprouting and Cortical Map Reorganization. 17
5.3 Growth Factors 20
5.4 Modulation of Immune and Inflammatory Responses 22

5.5 Vascular Recovery 23
6. Clinical Evidence for the Use of DA-Augmenting Drugs in Motor Recovery from Stroke 25
6.1 Amphetamine Studies 25
6.2 L-Dopa Studies 26
6.3 Methylphenidate Studies 28
6.4 Direct Dopamine Agonists 29
7. Rationale and Statement of the Problems 29
7.1 Why have clinical results been so mixed? 29
7.2 Rehabilitation in Animal Models of Pharmacotherapy for Post-Stroke Motor Recovery 33
7.3 D1-class Agonists and Antagonists 36
7.4 The Effect of Light-Dark Cycle on Functional Outcome in Rodent Stroke Models .. 38
7.5 Objectives and Hypotheses 39
Methods 41
1. General Procedures 42
1.1 Animal Housing and Behavioural Protocols 42
1.2 Photothrombosis Stroke Induction 43
1.3 Magnetic Resonance Imaging (MRI) Procedure 46
1.4 Adhesive Removal Test 46
1.5 Horizontal Ladder Test 48
1.6 Cylinder Test 49
1.7 Drug Preparation and Administration 50
1.8 Running Wheels 51
1.9 Perfusion and Tissue Processing 51
1.10 Cresyl Violet Staining and Quantification of Infarct Size 52
1.11 MRI Quantification of Infarct Size 53
1.12 Locomotor Activity Test 54
1.13 Immunohistochemistry 54
1.14 Statistical Analysis and Randomization 56
2. Outlines of Specific Experiments 57
2.1 Light-Dark Stroke 57
2.2 Acute DHX Dosage 60

2.3 Chronic DHX Dosage 60
2.4 Acute Dosage Determination for SCH23390 60
2.5 Co-administration of SCH23390 and DHX 61
2.6 Seven-Days DHX Stroke Recovery 61
2.7 Two-Days DHX Stroke Recovery 62
Results 67
1. Light-Dark Stroke Study 68
1.1 Sensorimotor Behaviour Following Stroke 68
1.1.1 Cylinder Test 68
1.1.2 Horizontal Ladder Test 68
1.1.3 Adhesive Removal Test 69
1.2 Infarct Size as Measured from Cresyl Violet Stained Slices 74
1.3 Amount of c-fos Positive Cells 74
2.Dosage Determination for DHX and SCH23390 In Reverse Light-Dark Housed Mice 77
2.1 Twenty-Four Hour Locomotor Activity Over Seven Days of DHX Administration. 77
2.2 Analysis of Short-Term Locomotor Response Following Chronic DHX Injection ... 78
2.3 Acute Dosage Testing for SCH23390 81
2.4 Blocking the Locomotor Response Induced by 4 mg/kg DHX with SCH23390 81
2.5 c-fos Expression Levels in the Striatum after Acute DHX Injection 82
3. The Seven-Day DHX Stroke Recovery Study 86
3.1 Motor and Sensorimotor Recovery from Stroke 86
3.1.1 Cylinder Test 86
3.1.2 Horizontal Ladder Test 86
3.1.3 Adhesive Removal Test 86
3.2 Wheel Usage 93
3.3 Infarct Sizes 93
4. Two-Days DHX Stroke Recovery Study 96
4.1 Motor Recovery from Stroke 96
4.1.1 Cylinder Test 96
4.1.2 The Horizontal Ladder Test 96
4.1.3 Adhesive Removal Test 99
4.2 Wheel Usage 99

4.3 Infarct Sizes 99
Discussion 104
1. Light-Dark Stroke 105
2. DHX and SCH23390 Dosage 107
3. DHX Stroke Recovery Studies 113
References 125
Appendices 145
Appendix 1: Results of the Left Paws during the Seven-Days and Two-Days DHX Studies 146
Appendix 2: Additional Dosage Experiments 151
Appendix 3: Injection Histories of Mice That Underwent Multiple Drug Dosage Experiments. 156
Appendix 4: Summary Tables of the Literature of Animal and Clinical Studies Employing Dopamine-Augmenting Drugs in Motor Recovery from Stroke 160

List of Tables

Table 1: Injection History of Mice Used in the Acute DHX with Short Acclimation Experiment and In the Acute DHX and Perfusion Experiment……...158

Table 2: Injection History of Mice in the Chronic DHX experiment and the Blocking DHX with SCH23390 Experiment……159

Table 3: Injection History of Mice Used in The Acute SCH23390 and SKF81297 Dosage Experiment and the Blocking SKF81297 with SCH23390 Experiment……..160

Table 4: Summary of Studies Using AMPH or L-dopa in Animal Stroke Models……161

Table 5: Summary of Clinical Trials Using Dopamine-Receptor Stimulating Drugs to Improve Motor Recovery from Stroke……...168

List of Figures

Figure 1: Concept Map of the Most Likely Mechanisms of Action for DA Boosting Pharmacotherapies in Stroke……..24

Figure 2: Outline of the Light-Dark Stroke Study……59

Figure 3: Mouse Outcomes during the Light-Dark Stroke Study…….59

Figure 4: Timeline of the DHX 7-days Stroke Recovery Experiment…….63

Figure 5: Mouse Outcomes and Complications from the 7-Days DHX Study……64

Figure 6: Timeline of the DHX 2-days Stroke Recovery Experiment…….65

Figure 7: Two-Days DHX Study Mouse Outcomes and Complications……..66

Figure 8: Light-Dark Stroke Study Percent Time Spent with the Right Forepaw on the Cylinder Wall during Rears……..70

Figure 9: Light-Dark Stroke Study Change in Percent Missteps on the Horizontal Ladder……71

Figure 10: Light-Dark Stroke Study Adhesive Removal Test Training……...72

Figure 11: Light-Dark Stroke Study Adhesive Removal Test Results…….73

Figure 12: Light-Dark Stroke Study Infarct Sizing Results…….75

Figure 13: Levels of c-fos+ Striatal Cells in Light and Dark group animals……...76

Figure 14: Locomotor Activity throughout 7 Days of DHX Administration……...79

Figure 15: Total Distance Traveled in One Hour After Injection of DHX During A Seven Day Injection Regimen……..80

Figure 16: Locomotor Activity Following a Single Injection of SCH23390……...83

Figure 17: SCH23390 Blocked the Locomotor Activity Enhancing Effect of 4 mg/kg DHX….84

Figure 18: Amount of c-fos+ Cells in the Striatum Following Injection of 4 mg/kg DHX**…….**85

Figure 19: Use of the Right Forepaw during the Cylinder Test during the 7-Days DHX Study.88

Figure 20: Right Paw Performance on the Horizontal Ladder, 7-Days DHX Study……...89

Figure 21: Training on the Adhesive Removal Test 7-Days DHX Study……90

Figure 22: Seven-Days DHX Study Right Paw Adhesive Removal Test..……..91

Figure 23: Results of the Adhesive Removal Test in Groups Stratified by Poststroke Deficit for the 7-Days DHX Study…….92

Figure 24: Wheel Usage between Day 2 and 8 of Wheel Access, 7-Days DHX Study……...94

F**igure 25:** Infarct Sizes, 7-Days DHX Study……...95

Figure 26: Time Spent Using the Right Paw during the Cylinder Test, 2-Days DHX Study…..97

Figure 27: Results of the Horizontal Ladder Task, 2-Days DHX Study……..98

Figure 28: Adhesive Removal Training for the 2-Days DHX Treatment Cohorts…….100

Figure 29: Adhesive Removal Test for the 2-Days DHX Study……101

Figure 30: Wheel Usage during the 2-Days DHX Study……102

Figure 31: Cresyl Violet Infarct Sizes for the 2-Days DHX Study……103

Figure 32: Mice with No Deficit on One or More Outcome Measure……...118

Figure 33: Results of the Horizontal Ladder Test for the Left Fore- and Hind-paws during the Seven-Days DHX Study.....147

Figure 34: Results of the Adhesive Removal Test for the Left Paw throughout the Seven-Days DHX Study…….....148

Figure 35: Results of the Left Paws on the Horizontal Ladder Test throughout the Two-Days DHX Study……..149

Figure 36: Results of the Left Paw on the Adhesive Removal Test during the Two-Days DHX Study……150

Figure 37: Locomotor Activity after a Single DHX Injection with Short Acclimation…….152

Figure 38: Acute Dosage Determination for SKF81297……153

Figure 39: Both 0.1 mg/kg and 0.5 mg/kg SCH23390 Block the Locomotor Stimulating Effect of SKF81297……154

Figure 40: Chronic SCH23390 Administration followed by an SKF81297 Challenge…….155

List of Abbreviations

ADL: Activities of daily living

AI: Activity Index

AMPH: Amphetamine

ANOVA: Analysis of variation

BDNF: Brain derived neurotrophic factor

BI: Barthel index

cAMP: Cyclic AMP

CIMT: Constraint-induced movement therapy

CMSA: Chedoke-McMaster Stroke Assessment

CNS: Central nervous system

CNS: Canadian Neurological Scale

COMT: Catechol-O-methyltransferase

CREB: cAMP response element binding protein

D1R: D1 dopamine receptor

D2R: D2 dopamine receptor

D3R: D3 dopamine receptor

D4R: D4 dopamine receptor

D5R: D5 dopamine receptor

DA: Dopamine

DAB: 3,3'-diaminobenzidine

DARPP-32: Dopamine and cAMP responsive phosphoprotein of molecular weight 32 kDa

DAT: Dopamine transporter

DHX: Dihydrexidine

DOPAC: Dihydrophenylacetic acid

EE: Environmental enrichment

ET-1: Endothelin-1

EtOH: Ethanol

FGF-2: Fibroblast growth factor 2

FIM: Functional independence measure

FM: Fugl-Meyer

GABA: Gamma Aminobutyric acid

GAP-43: Growth associated protein 43

GDNF: Glial derived neurotrophic factor

GPCR: G protein-coupled receptor

HVA: Homovanilic acid

ICH: Intracerebral hemorrhage

IP: Intraperitoneal injection

K^+: Potassium

L-dopa: Levodopa

LMAC: Lindmark motor assessment chart

LTD: Long term depression

LTP: Long term potentiation

MAO-A: Monoamine oxidase A

MAO-B: Monoamine oxidase B

MCA: Middle cerebral artery

MCAO: Middle cerebral artery occlusion

MHCII: Major histocompatibility class II

MPH: Methylphenidate

MRI: Magnetic resonance imaging

NAc: Nucleus accumbens

NDS: Normal donkey serum

NE: Norepinephrine

NIHSS: National Institute of Health Stroke Scale

NMDA: N-methyl-D-asparate

OFC: Orbital frontal cortex

PBS: Phosphate buffered saline

PFA: Paraformaldehyde

PFC: Prefrontal cortex

PKA: Protein kinase A

RMA: Rivermead motor assessment

SE: Standard error of the mean

SN: Substantia nigra

SSRI: Selective serotonin reuptake inhibitor

SSS68: Scandinavian Stroke Scale 68

TAAR1: Trace amine-associated receptor 1

TBS: Tris buffered saline

TEMPA : Test Evaluant les Membres superieurs des Persones Agees

TH: Tyrosine hydroxylase

TMS: Transcranial magnetic stimulation

TrkB: Tropomyosin receptor kinase B

VTA: Ventral tegmental area

α1AR: Alpha-1 adrenergic receptor

α2AR: Alpha-2 adrenergic receptor

β1AR: Beta-1 adrenergic receptor

β2AR: Beta-2 adrenergic receptor

Acknowledgements

I would firstly like to express my deep appreciation and gratitude for my thesis supervisor Dr. Mario Tiberi. The door to Dr. Tiberi's office was always open whether I had a trivial question or a tricky one, and I truly value his collaborative, discussion-based approach to problem-solving. His guidance and scientific knowledge were invaluable to the completion of this project. Dr. Tiberi has been a fantastic mentor to me on both a scientific and professional level, and I feel truly lucky to have worked with him.

I would also like to thank my advisory committee. Dr. Diane Lagace, who was not only a member of my Thesis Advisory Committee, but also a close collaborator on this project, contributed hugely to helping get this project off the ground and provided endless wisdom throughout the process. Dr. Jing Wang, the other half of my advisory committee, provided helpful discussion and critical assessment, making this project better. I am thankful to have had input from such excellent scientists.

I want to express my gratitude to those who taught me skills and techniques. My thanks to Anthony Carter, for help with the photothrombosis strokes, Mirella Barclay and Christine Luckhart, for help with animal behavioural testing, Keren Leviel Kumar and Dr. Maheen Cezair for help with tissue processing and Marc Vani for help with immunohistochemistry. Additionally, I am thankful for the tireless efforts of the University of Ottawa Vivarium staff. I would also like to thank past and current members of the Tiberi lab, Awatif Albaker, Boyang Zhang, Xiaodi Yang, Bradley Mischuk and Bassam Albrady. I am especially thankful for the help provided by Tiberi lab members Aida Said, Binhui Liang, Olivia Dupius and Oana Mirel, for their help with surgeries, behavioural testing, immunohistochemistry and/or behavioural and

infarct data analysis. I am also grateful for the financial support provided by the Canadian Partnership for Stroke Recovery Trainee Scholarship, the Ontario Graduate Scholarship and the University of Ottawa Entrance and Excellence Scholarships.

Lastly, I would like to thank my personal support system, my parents and my boyfriend, without their support and encouragement this accomplishment would not have been possible.

Introduction

1. Characteristics and Incidence of Stroke

Stroke refers to an interruption of blood supply to some regions of the brain. This can be either ischemic (about 85% of strokes) or hemorrhagic (about 15% of strokes) (Auriat and Colbourne, 2008). Stroke is a major cause of disability in adults. In 2008 stroke was the cause of 5.7 million deaths and 46.6 disability-adjusted life years worldwide (Oczkowski, 2013). Stroke survivors are left with damage to their brain resulting in deficits and dysfunction in various domains of function. Among the most common of these is motor deficits, affecting up to 80% of patients (Langhorne et al., 2009). Currently available interventions for stroke include clot-busting approaches such as thrombolytic drugs or angioplasty. These interventions are only available in select cases and only in the hyperacute stage of stroke (Oczkowski, 2013). In 2009 3.4-5.2% of patients in the United States received tissue plasminogen activator (Adeoye et al., 2011). Once the patient has stabilized, a combination of spontaneous recovery and rehabilitation allow them to regain some function. While rehabilitation methods such as physical therapy have been shown to be more useful than no rehabilitation at all, no one physiotherapy treatment has been shown to be more efficacious than the others (Oczkowski, 2013). Additionally, physical rehabilitation is only available to individuals who are well enough to complete it. Unfortunately, with currently available treatments, 40-60% of stroke patients present with motor deficit in the chronic stage, after recovery has plateaued (Acler and Manganotti, 2013). There is a need for more strategies to enhance stroke recovery. One approach, pharmacotherapy, uses drugs and physical rehabilitation to boost recovery. Several classes of drugs have been investigated for this purpose, including selective serotonin reuptake inhibitors (SSRIs), cholinergic agents and dopamine-augmenting drugs (Rösser and Flöel, 2008; Viale et al., 2018). However, none of these therapies have been approved for clinical use in stroke recovery.

2. The Dopamine System

Dopamine (DA) is released from cells originating in the substantia nigra and the ventral tegmental area. Once released DA interacts with six G protein-coupled receptors (GPCRs) separated classically into the D1-class (D1R and D5R) and the D2-class (short and long isoform of D2R, D3R and D4R) subtypes, activating and inhibiting adenylyl cyclase, respectively (Beaulieu and Gainetdinov, 2011). Aside from their canonical Gs/olf and Gi/o-linked primary signaling pathways, studies have suggested a number of alternate G protein-dependent and independent signaling routes and downstream effects for these receptors (Beaulieu and Gainetdinov, 2011). There are many drugs which can modulate the activation of these receptors in a nonspecific and class-specific fashion, but none exist that can bind to only one subtype. Studies in both animal and human stroke have mostly been done using indirect dopamine agonists, mainly amphetamine (AMPH), methylphenidate (MPH) and levodopa (L-dopa). Amphetamine works by increasing the release, and preventing the reuptake of DA, norepinephrine (NE) and to a lesser extent serotonin (Barbay and Nudo, 2009). Methylphenidate works by blocking the reuptake and thus increasing extrasynaptic dopamine and norepinephrine levels, although not as dramatically as AMPH (Kuczenski and Segal, 1997; Challman and Lipsky, 2000). L-dopa is the dopamine precursor. Unlike DA, L-dopa can cross the blood brain barrier. It can be given systemically and, once it enters the brain it is converted to DA (Barbeau et al., 1972). The dopamine molecule is in the synthesis pathway for NE, so L-dopa can increase NE levels, however it has been reported that only 5% of L-dopa ends up as norepinephrine (Nutt and Fellman, 1984), although this finding remains to be further clarified.

3. Effect of Stroke on Functioning of the Dopaminergic System

Stroke impacts the dopamine system in a variety of ways, the implications of which are still somewhat unclear. For obvious reasons this is a difficult topic to study in humans, especially in the acute phase. In animal studies, differences in species and model can shift the timeline and magnitude of results, and this can make it difficult to translate these findings to humans. Add to this the importance of considering not just levels of DA and DA metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA)), but also levels of enzymes regulating DA synthesis (tyrosine hydroxylase or TH, and DOPA decarboxylase) and degradation (monoamine oxidases MAO-A and MAO-B, and catechol-O-methyltransferase (COMT)), DA reuptake (dopamine transporter or DAT) and receptor proteins. Further complicating matters is that in the wake of stroke, any of these factors could be up or downregulated as part of the damage or as part of intrinsic attempts at recovery, making it difficult to determine how best to respond to these natural changes. Nonetheless, the background of the DA system remains an important consideration for those looking to modulate it to promote motor recovery from stroke.

3.1 Stroke Triggers Early, Massive Dopamine Release into the Striatum

A massive release of DA into the ipsilateral striatum almost immediately following the onset of ischemic stroke in the middle cerebral artery (MCA) territory has been repeatedly observed in many experimental models (Kogure et al., 1975; Ahagon et al., 1980; Brannan et al., 1987; Globus et al., 1988; Kawano et al., 1988; Slivka et al., 1988; Akiyama et al., 1991; Delbarre et al., 1992; Richards et al., 1993; Hashimoto et al., 1994; Toner and Stamford, 1996). Although this finding has been remarkably consistent, dissenting evidence does exist (Cvejic et al., 1980).

The return to DA baseline levels is likely dependent upon the model used and the time that the ischemic period lasts, seeming to range from 30 minutes after reperfusion in bilateral middle cerebral artery occlusion (MCAO) models (Akiyama et al., 1991), to several hours after microsphere injection (Kogure et al., 1975), and to take longer in permanent ligation or occlusion methods than in transient methods. All the studies discussed here used stroke models which significantly impact the striatum, so it remains unclear if this is a general reaction to ischemia or only to ischemia affecting the striatum. Additionally, evidence suggests that the magnitude of the DA efflux correlates with the severity of the ischemia (Richards et al., 1993).

Previous studies have shown that this DA release is not caused by action potentials (Akiyama et al., 1991). It cannot be attenuated with antagonists for D1R, D2R or both (Hashimoto et al., 1994). Prior lesioning of the substantia nigra (SN) on the side of the stroke prevents this massive DA release (Globus et al., 1988; Hashimoto et al., 1994). There is evidence which links the dopamine response to ischemia to local release of nitric oxide (Weinberger, 2002). Levels of DA metabolites DOPAC and HVA decrease during ischemia but rise after, likely indicating efforts to return to baseline DA levels (Globus et al., 1988; Kawano et al., 1988; Slivka et al., 1988). DAT is overwhelmed during this release and drugs targeting it are unable to modulate the level of DA efflux, however it does seem to be active in the return to baseline (Akiyama et al., 1991; Toner and Stamford, 1996).

Interestingly, there appears to be differences in the degree of this response between animals of different ages, with aged animals having a more dramatic DA release after infarct and a more severe outcome from stroke (Delbarre et al., 1992). It has been suggested that the massive DA release contributes to neurodegeneration over the course of ischemia, as prior lesions to the SN were observed to be neuroprotective in the striatum in several studies (Globus et al., 1987;

Globus et al., 1988; Buisson et al., 1992; Wirkner et al., 2004). Additionally, use of D2-class antagonists, though not D1-class antagonists, have been reported to have a protective effect on neurons in the striatum, though this (Hashimoto et al., 1994; Okada et al., 2005; Yulug et al., 2006a; Yulug et al., 2006b).

The massive DA release is followed by a period of reduced DA levels in the hemisphere ipsilateral to the stroke, appearing as early as 2 hours after stroke, or as late as 24 hours (Zervas et al., 1974; Harrison et al., 1979; Ahagon et al., 1980). Some studies have suggested a loss or inefficiency in DA nerve terminals, with animals with more severe strokes having 30%, and moderately affected animals having 66%, of the number of DA nerve terminals of the contralateral hemisphere (Weinberger et al., 1983). In a rat photothrombosis model employing less intensive but longer duration irradiation to simulate a penumbra zone, levels of MAO-B were increased 2.75 fold in the penumbra as compared to contralateral tissue at 4 hours post stroke and 2.01 fold at 24 hours post stroke (Uzdensky et al., 2017). DOPA decarboxylase and TH were down regulated. Additionally, levels of DAT were lower than the contralateral side (Uzdensky et al., 2017). These regulatory changes in the penumbra suggest lower levels of DA are available after the immediate stroke period. An attenuated DA release response to high K^+ stimulation, which was at about 40% of the response of non-ischemic controls and was not modulated by nomifensine (a DAT inhibitor) treatment, has also been observed. This attenuation lasted at least 48 hours after stroke, but was corrected by 98 hours post stroke (Akiyama et al., 1991). It is clear that the massive DA release during the period of ischemia leaves the DA system altered, which must be taken into account when considering the use of dopamine-augmenting therapies following stroke. In the contralateral hemisphere the concentration of DA, but not DA

metabolites, has been found to be higher than sham operated animals at one-week post-stroke, but not at two weeks post-stroke in a mouse photothrombosis model (Obi et al., 2018).

There is no data in humans regarding DA levels immediately following stroke, however two studies have used non-human primates. One study injected a shower of microemboli followed by one large embolus into the middle cerebral artery of baboons. Animals were sacrificed 1 hour after injection. DA was found to be increased in the cortical grey matter, significantly so in the frontal and occipital regions, which would be largely spared in this model. DA was found to be decreased in the caudate nucleus (Ishihara et al., 1979). Additionally, in squirrel monkeys subjected to MCA ligation and sacrificed after 3 hours, there was a decrease in hemispheric DA on the side ipsilateral to the stroke as compared to the contralateral side (Zervas et al., 1974). It is possible that these decreases came after an initial increase in DA much sooner after stroke, like what is seen in rodent models. In the absence of earlier data, it is difficult to draw a conclusion as to whether primate stroke follows the same pattern as rodents, though it does seem certain that the DA system is modulated in the wake of stroke.

3.2 Evidence Indicates a Decrease in Dopamine Receptors Following Stroke

Less information is available regarding the effect of stroke on levels of dopamine receptors. Several studies suggest reduced D2R expression in the hemisphere ipsilateral to the stroke around 2-14 days after stroke (Dawson et al., 1994; Martin et al., 2013; Sieber et al., 2014). Less clear are the levels of D1 receptor expression. In two studies employing MCAO, which leads to large infarcts, D1R expression decreased in the ipsilateral hemisphere, with evidence indicating this was due to down regulation and not simply loss of D1R expressing cells (Abe et al., 2004; Sieber et al., 2014). Meanwhile, a study using a rat photothrombotic model found a decrease in D1R levels in the infarct core but not in the penumbra (Rogozinska and Skangiel-

Kramska, 2010). One study which looked at degree of expression for many genes following stroke, including genes related to DA function, saw a trend of younger animals showing a greater down- (or up-) regulatory gene response to stroke, when a difference in the response of different age animals was present, leading the authors to speculate that the younger mice may be more severely impacted early in stroke, but ultimately recover better (Sieber et al., 2014).

3.3 Striatal Ischemia Leads to Development of Secondary Exofocal Degeneration in the Substantia Nigra

Another well documented phenomenon is the delayed degeneration of the ipsilateral SN following striatal stroke. The literature suggests a progression of events from the time of stroke to the delayed degeneration. It seems that this exofocal degeneration is triggered by the loss of GABAergic inputs to the substantia nigra (Yamada et al., 1996; Zhao et al., 2001). The loss of inhibitory inputs resulted in "burn out" of the cells in the SN leading to neuronal swelling and the start of apoptosis processes, which are visible in some cells as early as 24 hours post stroke (Zhao et al., 2002; Rodriguez-Grande et al., 2013). This development leads to the gathering of microglia and astrocytes in the region (Rodriguez-Grande et al., 2013; Prinz et al., 2015). The cellular edema, due to the increase in inflammatory immune processes and cellular swelling is visible on T2 weighted magnetic resonance images (MRI) as early as 4 days post stroke in animal models (Zhao et al., 2001; 2002; Kronenberg et al., 2012; Prinz et al., 2015). Sometime after the developing degeneration is visible using MRI, a decrease in TH positive cells is observed in SN. Some studies have found this decrease to be transient (Yamada et al., 1996; Soriano et al., 1997), whereas others have seen it last for the duration of the experiment, and to be linked to loss of SN neurons (Zhao et al., 2001; 2002; Winter et al., 2005; Kronenberg et al., 2012; Rodriguez-Grande et al., 2013; Prinz et al., 2015). Two studies have linked the exofocal

degeneration to a decrease in striatal DA and to changes in behaviour (Winter et al., 2005; Kronenberg et al., 2012). One study also demonstrated degeneration of the ventral tegmental area (VTA) (Kronenberg et al., 2012).

Delayed SN lesions following striatal infarcts have also been observed in human case studies (Kinoshita et al., 2002), examinations of clinical populations (Nakane et al., 1992; Ogawa et al., 1997) and in post mortem human tissue analysis (Ogawa et al., 1997; Zhang et al., 2012). In two separate studies of clinical populations looking at 18 and 25 stroke patients, all patients who had striatal damage had the appearance of T2 weighted hyperintensity in the ipsilateral SN around 1-2 weeks after stroke. Theses hyperintensities were not present earlier after stroke, and became less intense over the ensuing months. No such hyperintensities were seen in patients with purely cortical stroke (Nakane et al., 1992; Ogawa et al., 1997). One of the patients with SN hyperintensity died of pneumonia during the study period. Post mortem analysis of this individual revealed degeneration of the SN ipsilateral to the side of stroke with marked neuronal loss, gliosis and a few macrophages, with no reactive neovascularization (Ogawa et al., 1997).

3.4 Stroke can impact the response of the DA system to DA modulating drugs

In a MCAO stroke model in rats 8 weeks or 5 months old at the time of stroke, ischemic animals showed significantly less of a catalepsy response to haloperidol (a low selectivity D2 antagonist) as compared to age matched sham animals. Young ischemic rats had the least catalepsy response at two hours after drug injection, however they returned to the level of sham animals sooner than aged rats. In a test of locomotor activity following AMPH administration at 5 weeks post stroke, ischemic animals in both age groups reacted with a significantly greater increase in locomotor activity in response to AMPH, despite all groups having similar pre-drug locomotor activity levels. Again, the young ischemic animals had a more dramatic reaction than the older ones, but

also returned to baseline levels more quickly, whereas the older ischemic animals were significantly more active than controls at the conclusion of the experiment, 2 hours after AMPH administration. The aged and young control animals reacted similarly to AMPH. The authors hypothesize that lower levels of DA receptors due to cell loss in the striatum likely drove the attenuated response to haloperidol-based antagonism, whereas a hyperresponsive mesolimbic system due to striatal damage, may have caused the enhanced reaction to AMPH (Borlongan et al., 1995). Interestingly, this study shows a differential response of the DA system to stroke based on age, much like the study by Seiber et al (2014) discussed above.

Most of the studies to date have used variations on MCAO in rodent models. This fact could be creating a misleading homogeneity in the results. Although strokes in the territory of the MCA are the most common in humans, many of these vary from the typical MCAO model stroke, and other stroke types do occur in humans. One study in a population of 48 stroke patients found that lesions in the brainstem correlated to decreases in DA and increases in HVA (indicating increased DA turnover), whereas cortical and striatal lesions were linked to increases in DA and decreases in DA turnover (Hama et al., 2017). These results must be interpreted with caution, as patients were tested at varying time points in the three months following stroke, while, as animal studies have shown, a given patient could have vastly different DA system landscape depending on timepoint. The levels of DA and HVA were determined based on a 24 hour urine sample, which does not necessarily perfectly represent brain levels. Additionally they had to exclude patients who were not capable of giving a 24 hour urine sample due to severity of stroke or urinary incontinence (Hama et al., 2017). Nonetheless it reinforces the idea that different strokes may affect the DA system differently. Therefore, future animal work on the subject using more mobile and adaptable stroke methods such as endothelin-1 (ET-1) or photothrombosis will be

required to test this. Lastly, more data regarding the state of the DA system following different strokes would help in deciding when and where DA system modulating therapies are best applied.

4. Animal Studies on the Effect of Dopamine-boosting Drugs in Post-Stroke Motor Recovery

Results of previous studies employing DA-augmenting drugs in poststroke motor recovery are summarized in appendix 4, Table 4.

4.1 Recovery Paradigms Showing Rapid Improvement of Motor Skills

Some studies have demonstrated a benefit to motor performance of DA-enhancing drugs, which begins during the period of intoxication by the drug. Many studies have been performed exploring the effects of AMPH on motor and sensorimotor recovery following brain injury since a beneficial effect of AMPH on righting-attempts in decerebrated cats was first observed in 1946 (Maling and Acheson, 1946).

4.1.1 Beam Walk Test

A large amount of work has been done using the beam walking test to evaluate AMPH facilitated recovery in rats and cats following brain damage/stroke. This finding has been robust, with accelerated recovery being seen in many studies, usually very quickly after AMPH administration (Feeney et al., 1982; Hovda and Fenney, 1984; Sutton et al., 1989; Goldstein and Davis, 1990a; b; Boyeson and Feeney, 1991; Schmanke et al., 1996). The 1982 paper by Feeney et al. demonstrated the importance of experience in conjunction with amphetamine, when they showed that the benefit of AMPH could be blocked by restraining the animals during the active period of the drug. This study also showed that the effect could be blocked by coadministration

of haloperidol, potentially indicating a role for catecholamines, although it should be noted that the effect of haloperidol alone in this study, as well as the effect of haloperidol on locomotor activity may, suggest the reduced performance on the beam walk test with haloperidol may be due to reduced locomotor activity (Feeney et al., 1982). Meanwhile, several studies did not show a benefit to AMPH treatment on performance on the beam walking task (Boyeson and Feeney, 1991; Brown et al., 2004; Auriat and Colbourne, 2008).

Further work in the rat beam walking paradigm suggest this rapid beam-walking effect may be mediated more through NE than DA (Boyeson and Feeney, 1990; Feeney et al., 1993). It seems likely that the role of the DA system becomes more obvious given multiple administrations, and a longer time course. In the rat beam walking paradigm, α1-adrenergic receptor (α1AR) antagonists can inhibit the AMPH mediated enhancement of motor recovery, although α1AR-agonists were not found to be sufficient to reproduce this recovery. The α2-adrenergic receptor (α2AR) agonists slowed recovery, while antagonists could facilitate it. These finding seem to contradict the theory that the effect of amphetamine is mediated solely through NE. Interestingly, desipramine, which blocks norepinephrine re-uptake but is not a stimulant, is able to facilitate recovery, while MK-801, which blocks NE reuptake but is also an NMDA receptor antagonist, cannot (Feeney et al., 1993).

4.1.2 Tactile Placing in Cats

Another classic paradigm for testing the effect of AMPH on recovery from stroke is testing tactile placing after stimulation of the dorsal paw surface in cats with sensorimotor cortex ablations. Recovery and reaction to AMPH seems to be determined by the lesion location. Large prefrontal cortex lesions never show recovery regardless of AMPH administration (Hovda and Fenney, 1984). Lesions affecting primarily the visual cortex, showed early and lasting complete

restoration after injection with AMPH. Like in beam walking this effect could be blocked with haloperidol (Hovda et al., 1987). A study affecting the motor cortex led to permanent deficits in paw placing in most animals, but exposure to AMPH could temporarily restore tactile placing. Animals tested at 4 days post lesion had very minimal and short-lived recovery of the response at 3 hours after the drug. Animals tested at 9 and 15 days after lesioning had much more robust responses that lasted about 12 hours after administration of AMPH. In partly recovered cats, but not unlesioned cats, this recovery could be blocked with haloperidol (Feeney and Hovda, 1983). Due to the early onset of recovery, and the ability for the animal to respond positively to AMPH at 4 days postop, a time thought to be too soon for many plasticity and sprouting processes, these results have been taken to indicate that a rapid response to the drug is involved. However, the weakness of the response at 4 days postop may suggest that while AMPH may be of some benefit that early, it becomes more powerful at later timepoints when slower acting recovery mechanisms may have come into play. Again, haloperidol was able to block the benefit of AMPH. Interestingly a weaker and more transient, but significant, increase in tactile placing was seen in cats given 0.25 and 0.5 mg/kg apomorphine (a mixed D1R/D2R agonist). Use of higher and lower doses showed no effect (Feeney and Hovda, 1983).

4.2 Paradigms Demonstrating Recovery after a Delay

In several motor behavioural tasks showing recovery mediated by AMPH, L-dopa or VTA stimulation, recovery was shown not during the first period of intoxication, but after a delay. In these studies, repeated administration was used.

4.2.1 Tactile Placing in Response to Vibrissae or Forelimb Stimulation in Rats

A paradigm similar to tactile placing in cats has been used in rats, this time with more lasting recovery. Placing recovery in response to vibrissae stimulation or forelimb stimulation can recover in rats with small electrolytic lesions of the motor cortex, given three administrations of AMPH. Rats recover sooner and more completely on placing in response to vibrissae stimulation, than they do on forelimb stimulus-forelimb placing (Schmanke et al., 1996; Schmanke and Barth, 1997). In both vibrissae>forelimb and forelimb>forelimb paradigms performance that is significantly greater than vehicle controls appears after a delay (10 days and 35 days respectively) (Schmanke et al., 1996; Schmanke and Barth, 1997).

4.2.2 The Foot Fault Test and The Horizontal Ladder

The foot fault test and the horizontal ladder test both involve an evaluation of the percentage of missteps an animal makes while traversing an elevated grid or ladder surface. While not a fine motor task, this task requires more refined motor control than the beam walking task (Barbay and Nudo, 2009). Among studies showing a benefit of AMPH on this test, all gave AMPH at least twice, indicating that more chronic stimulation may be more effective in this paradigm (Stroemer et al., 1998; Ramic et al., 2006; Papadopoulos et al., 2009; Wolf et al., 2014).

4.2.3 Skilled Reaching Tests

Many studies have also been done using skilled pellet reaching tasks in rats. Several studies have had success demonstrating accelerated or superior recovery on pellet reaching tasks when multiple administrations of AMPH are paired with either practice or focussed activity, although AMPH alone or paired with environmental enrichment also showed recovery above the level of controls in some studies (Adkins and Jones, 2005; Gilmour et al., 2005; Ramic et al., 2006; Papadopoulos et al., 2009; Wolf et al., 2014). In non-human primates following cauterization of

surface vasculature in the hand representation, one dose of 0.25mg/kg AMPH and 14 days of training initiated 10 days postop was able to significantly improve performance on a skilled reaching task as compared to controls on days 4, 5 and 8-13 of training. AMPH-treated monkeys were still significantly better than rehab only controls at 9 weeks post operation (Barbay et al., 2006). The efficacy of a single dose of AMPH in a non-human primate model, when multiple doses are typically required in rodent models may be due to the differences in lesion type.

Among the studies that did not demonstrate a benefit of AMPH on skilled reaching recovery there are several possible factors (Rasmussen et al., 2006; Alaverdashvili et al., 2007; Auriat and Colbourne, 2008; Rasmussen et al., 2011). Alaverdashvili et al. (2007) used an oral administration route, and used all female mice, which may have affected the bioavailability of the drug (Barbay and Nudo, 2009). Both the study by Auriat and Colbourne, which is mentioned above, and the 2011 study from Rasmussen et al., also showed no benefit to AMPH on skilled reaching. These are the only studies to look at skilled reaching recovery under AMPH in rats with striatal damage.

4.2.4 Other Tests

A single study, using long term 2mg/kg AMPH in rats with MCAO lesions saw reduced infarct size in the AMPH group and improvements in turning asymmetry and body posture in the body swing test (Liu et al., 2011).

Only one study in animals has demonstrated improved motor recovery on behavioural tests using L-Dopa treatment after stroke. In an MCAO stroke model, significantly better recovery was seen in rats on the rotating pole test (various speeds), the cylinder test and a neuroscore, when animals were given a high (20 mg/kg) dose of L-Dopa. On some tests improved recovery could be seen at

the 5 mg/kg dose (Ruscher et al., 2012). In lesion studies on rats, stimulation of the VTA led to recovery of pre-lesion performance levels on a lever pressing to stop aversive stimuli task within four days, versus no recovery seen in controls (Castro-Alamancos et al., 1992; Castro-Alamancos and Borrell, 1995). These studies, in which the mechanism of action is almost certainly the dopaminergic system, indicate that DA likely plays a role in the above AMPH triggered effects.

4.2.5 DA-boosting Pharmacotherapy and Forced Use of the Impaired Limb

Forced use of the impaired limb by immobilising the non-affected limb as part of training or during exposure to environmental enrichment, in conjunction with AMPH, showed improvement on the cylinder test, but not on the speed of beam crossing (Goldstein, 2009). In skilled reaching, animals were prevented from using their unimpaired limb during training and testing either by the setup of the apparatus (Barbay et al., 2006) with success or via application of a tape-bracelet to prevent reaching through narrow slots (Alaverdashvili et al., 2007) without seeing recovery and by bandaging the ipsilateral limb during testing (Gilmour et al., 2005) which lead to skilled reaching improvement but not improved recovery on the foot fault test. These results indicate that there may be some benefit in combining CIMT with DA-augmenting approaches.

5. Potential Mechanisms of Action

5.1 Reversal of Catecholamine Diaschisis

Due to the rapid time course of response to AMPH on the beam walking paradigm, and the tactile placing test in cats, the dominant theory regarding the mechanism of the benefit of AMPH on these tasks is a resolution of diaschisis in depressed regions remote to the site of the injury. In cats given bilateral lesions of varying sizes this improved recovery is still observed on the beam

walking task. This demonstrates that this rapid improvement on beam walking is not dependent on the corresponding contralateral region, nor on the adjacent regions (Sutton et al., 1989). The resolution of diaschisis theory is supported by the finding that cytochrome oxidase activity, which provides a readout of cellular energy production, is supressed in rats with cortical ablations in a number of regions that are important for motor performance, but that this suppression can be reversed with a single injection of 2 mg/kg AMPH 24 hours after ablation (Sutton et al., 2000). Additionally, it has been shown that AMPH can attenuate traumatic brain injury-triggered remote reductions in cerebral metabolic glucose utilization (Queen et al., 1997). It may be that this rapid effect is more strongly mediated by NE than by DA, which would fit well with the findings mentioned above showing NE is more effective in the beam walking paradigm, and the weaker effect of apomorphine on tactile placing in cats. It is possible that a DA-mediated augmentation of recovery requires a longer time course to work, or perhaps requires repeated stimulation of the dopaminergic system to be fully apparent.

5.2 Enhanced Synaptic Plasticity, Axonal Sprouting and Cortical Map Reorganization

The results implicating NMDA receptors in the early recovery response to AMPH indicate a role for long term potentiation processes in this rapid AMPH mediated recovery. AMPH has been shown to increase long-term potentiation (LTP) in the hippocampus and the prefrontal cortex in non-injured rodents (Delanoy et al., 1983; Xu et al., 2010). Interestingly, in the prefrontal cortex AMPH-driven augmentation of LTP was primarily mediated by D1R, except in the case of hyperdopaminergic mice, where AMPH reinstated LTP via a recruitment of β-adrenergic receptors. Both of these receptors are coupled to the cyclic AMP (cAMP) -protein kinase A (PKA) pathway (Xu et al., 2010). DA receptors, in particular D1-class subtypes, have been shown to be important modulators of LTP in many parts of the brain, including the prefrontal

cortex (PFC) (Gurden et al., 1999; Gurden et al., 2000; Huang et al., 2004; Wirkner et al., 2004; Hotte et al., 2005; Hotte et al., 2006; Ruan et al., 2014), the hippocampus (Otmakhova and Lisman, 1996; Lemon and Manahan-Vaughan, 2006) and the striatum (Calabresi et al., 1997; Calabresi et al., 2000; Centonze et al., 2001; Centonze et al., 2003; Dudman et al., 2003; Wolf et al., 2003; Calabresi et al., 2007; Shen et al., 2008). Most relevantly, D1-class subtypes, have been heavily implicated in motor learning processes in both the striatum and the primary motor cortex (Willuhn et al., 2003; Luft et al., 2004; Willuhn and Steiner, 2006; 2008; Molina-Luna et al., 2009; Hosp et al., 2011; Rioult-Pedotti et al., 2015). Evidence from human studies of patients with Korsakoff's (McEntee et al., 1987), and in healthy human patients using transcranial magnetic stimulation (TMS) (Floel et al., 2005; Breitenstein et al., 2006; Meintzschel and Ziemann, 2006; Nitsche et al., 2006; Floel et al., 2008; Nitsche et al., 2009; Kesar et al., 2017) suggest that DA receptors play an important role in these processes in humans.

In the motor-cortex ablation studies with intracranial ventral tegmental area stimulation, immunostaining for fos-like proteins, a marker of neuronal activity and plasticity, was more intense in the hind-limb region of the sensory motor cortex than the level observed in non-recovered, lesioned animals. Lesions to the hindlimb area in recovered animals reinstated the post-ablation deficit on the forelimb lever pressing task (Castro-Alamancos et al., 1992). In a follow-up study of similar design, the forelimb motor area of rats was mapped before lesion and only the forelimb area targeted was in the lesion operation. Again, better motor recovery was seen in the group receiving intracranial VTA stimulation. Post-injury mapping of this group showed the forelimb motor area had moved to an area caudal of the lesion. Little to no forelimb area reorganization was observed in animals not receiving the VTA stimulation (Castro-

Alamancos and Borrell, 1995). These results suggest a role for DA in cortical remapping processes.

In tests which showed delayed recovery, there is evidence implicating longer term processes. Several studies showing improvement on the foot fault and horizontal ladder test as well as skilled reaching tasks, saw improvement was associated with indicators of axonal sprouting and neurite growth. Stroemer et al (1998) saw significant increases in growth associated protein 43 (GAP-43), a protein used as a marker of synaptogenesis, at 3 and 7 days as compared to saline injected animals. From day 14 postop to month 2 postop, AMPH animals had increased distribution of synaptophysin, which is thought to indicate synaptogenesis, on the side contralateral to the infarct (Stroemer et al., 1998). Biotinylated dextran amine neuroanatomical tracing was used to demonstrate significant increases in fibers projecting across the midline from the uninjured cortex at the level of the basilar pontine nuclei, red nucleus and cervical spinal cord in AMPH treated animals (Ramic et al., 2006; Papadopoulos et al., 2009; Wolf et al., 2014).

Interestingly, a study on somatosensory recovery following photothrombotic stroke found that administration of haloperidol from day 2 post-stroke until day 5 post-stroke impaired the rate of spontaneous recovery as compared to controls. They linked this recovery to increased levels of DA in the contralateral hemisphere and increased activity of astrocytes there (Obi et al., 2018). While mice in this model regain sensation spontaneously, recovery processes also lead to maladaptive plasticity, in the form of a shift to single neurons receiving stimulation from more than one limb, visible as early as 1 week after stroke, and fully developed by four weeks post-stroke. Administration of partial D2-class agonist aripiprazole starting two weeks after stroke halted this process and resulted in significantly less neurons receiving input from all four limbs

(Obi et al., 2018). These findings suggest that D2R may play a role in the prevention of maladaptive cortical map reorganization.

5.3 Growth Factors

The study by Wolf et al (2014) also showed that the performance of the animals was associated with a transient increase in fibroblast growth factor 2 (FGF-2) expression in layer V sensorimotor neurons in the unlesioned cortex. In primary cultured neurons, media from FGF-2 conditioned astrocytes were able to stimulate neurite outgrowth (Wolf et al., 2014). Despite previous focus on FGF-2 as a neuroprotective agent in stroke, it has been found to be an angiogenic agent, to promote sprouting, and to promote neurogenesis processes in stroke models (Lin and Finklestein, 1997; Wada et al., 2003; Issa et al., 2005; Slevin et al., 2006; Paciaroni and Bogousslavsky, 2011). In the Wolf et al study this was found to be linked to β- and α1-adrenergic receptors, although the effects of DA receptors were not tested (Wolf et al., 2014). DA receptors have been shown to increase levels of FGF-2 in a number of studies, suggesting that they may be involved in FGF-2 levels following stroke as well (Roceri et al., 2001; Fumagalli et al., 2006; Li et al., 2006). The above studies demonstrate recovery mediated by the intact hemisphere, however, the role of the contralateral hemisphere in motor recovery is a controversial one. Although the assumption of lost motor function by the intact hemisphere has been shown to improve outcome, this recovery mechanism is correlated with worse outcomes than the resumption of motor control by the affected side (Boyd et al., 2017). It has been suggested that perhaps those who are more seriously afflicted may be more likely to undergo contralateral side-based recovery because their affected hemisphere is unable to recover. In such cases sprouting from the contralateral hemisphere may be the most powerful recovery mechanism available (Hallett, 2001; Jang, 2013).

The study by Liu et al. (2011) showed increased fiber growth and white matter growth as well as increased neurofilament protein (ipsilateral and contralateral side), synaptophysin (ipsilateral side), matrix metalloproteinase 2 activity (ipsilateral cortex) and an attenuation of the infarct-induced reduction in brain-derived neurotrophic factor (BDNF) in the affected cortex. This suggests an increase in sprouting processes in the infarcted cortex as well. BDNF has been linked to increased motor recovery following stroke via increased sprouting and plasticity and through increased neurogenesis, and has been implicated as a factor in the beneficial effects of rehabilitation (Kurozumi et al., 2004; Schabitz et al., 2007; Ploughman et al., 2009; Mang et al., 2013; Berretta et al., 2014; Cook et al., 2017). The AMPH-mediated increase in BDNF may be mediated via the dopaminergic system as evidence shows that DA receptors can increase levels of BDNF through D1R and/or D5R (Küppers and Beyer, 2001; Perreault et al., 2012; Xing et al., 2012), and can potentially transactivate tropomyosin receptor kinase B (TrkB) receptors via D1R activation (Iwakura et al., 2008). Additionally, DA-boosting drugs used in the treatment of Parkinson's disease are known to increase BDNF levels (Fumagalli et al., 2006).

In addition to FGF-2 and BDNF, another growth factor known to influence stroke recovery has been linked to therapies which boost the dopaminergic tone of the brain. Using the same paradigm which showed that L-Dopa had beneficial effects on stroke recovery, Kuric et al (2013) showed that L-dopa treatment caused an increase in GDNF in the infarct core and peri-infarct region. This increase in GDNF was attributed to D1R expressing reactive astrocytes which appear in the peri-infarct region around 7 days following stroke. Interestingly, in cell culture, this relationship was only seen following oxygen-glucose deprivation experiments (Ruscher et al., 2012; Kuric et al., 2013). GDNF has been shown to promote neurogenesis and

has been associated with the beneficial effects of exercise in stroke recovery (Kobayashi et al., 2006; Ohwatashi et al., 2013).

5.4 Modulation of Immune and Inflammatory Responses

L-Dopa treatment in the MCAO paradigm was also linked to modulation of the immune and inflammatory response to stroke. The immune response to stroke is complicated, and still poorly understood. It doubtlessly includes elements that are part of endogenous recovery processes, and elements that are maladaptive in overall recovery. Cytotoxic T-cells may be one of these detrimental immune elements, and could contribute to blood -brain barrier permeability, increase inflammation and release cytotoxins. L-Dopa treated animals had lower levels of cytotoxic T-cells ($CD3^+CD8^+$ T-cells), lower levels of pro-inflammatory cytokines IL-5, IFN-ϒ, IL-4 and TFN-α in the infarcted hemisphere and lower level of major histocompatibility complex class II (MHC II) cells in the infarct zone (Kuric and Ruscher, 2014a; b). These MHC II cells expressed D1Rs (Kuric and Ruscher, 2014a). Meanwhile, L-Dopa treatment was able to ameliorate ischemia-induced lymphocytopenia and increase levels of $CD3^+CD4^+$ T-helper cells in the blood (Kuric and Ruscher, 2014c). Similar to the findings of de novo L-dopa responsiveness in reactive astrocytes, de novo expression of D2R has been observed on microglia following stroke. These microglia were observed in the infarct area and were responsive to a D2R/D3R agonist (Huck et al., 2015). In an intracerebral hemorrhage (ICH) stroke model D2R expression in microglia, astrocytes and neurons increased in the perihemorrhage region around 24 hours after ICH and stayed high for 7 days post-stroke. Knockdown of D2R lead to increases in pro-inflammatory cytokines and chemokines in the infarct region. This D2R mediated effect was linked to levels of the anti-inflammatory protein αB- crystallin, which was expressed in a similar pattern as D2R following ICH (Zhang et al., 2015). A further study, in a mouse MCAO model, found that the

anti-inflammatory bioactive alkaloid sinomenine, was able to reduce inflammatory processes after the stroke, and that this was mediated through an increase in D2R expression on astrocytes, as well as an increase in αB-crystallin levels (Qiu et al., 2016).

5.5 Vascular Recovery

One region of stroke recovery which is often overlooked is vascular recovery. While endogenous recovery processes do promote angiogenesis, there is evidence that these processes can be modulated. In the wake of stroke it is important to re-establish blood flow, and appropriate blood flow can help support recovery processes (Arai et al., 2009; Liu et al., 2014). As mentioned above, DA has been linked to molecules which show angiogenic effects following stroke, such as FGF-2. DA receptors are also capable of increasing cerebral blood flow, possibly through a D1R mediated pathway, and D1R/D5R are found on the microvasculature (von Essen, 1974; Krimer et al., 1998; Choi et al., 2006; Tan, 2009; Ohlin et al., 2012). These findings suggest that DA may be able to modulate this aspect of recovery. Indeed, the D1-class agonist dihydrexidine has been shown to enhance cerebral perfusion in humans (Mu et al., 2007).

Given the diverse findings surrounding possible mechanisms of a dopaminergic system mediated enhancement of recovery following stroke, it seems likely that DA plays a pleiotropic role in stroke recovery processes. While DA may not be the driving force behind reversal of diaschisis, DA receptors are likely capable of modulating many aspects of stroke recovery (Figure 1). Recovery from stroke is an extremely complicated process, and a pharmacological treatment which is able to modulate multiple aspects of recovery in a beneficial manner may be able to markedly help with stroke.

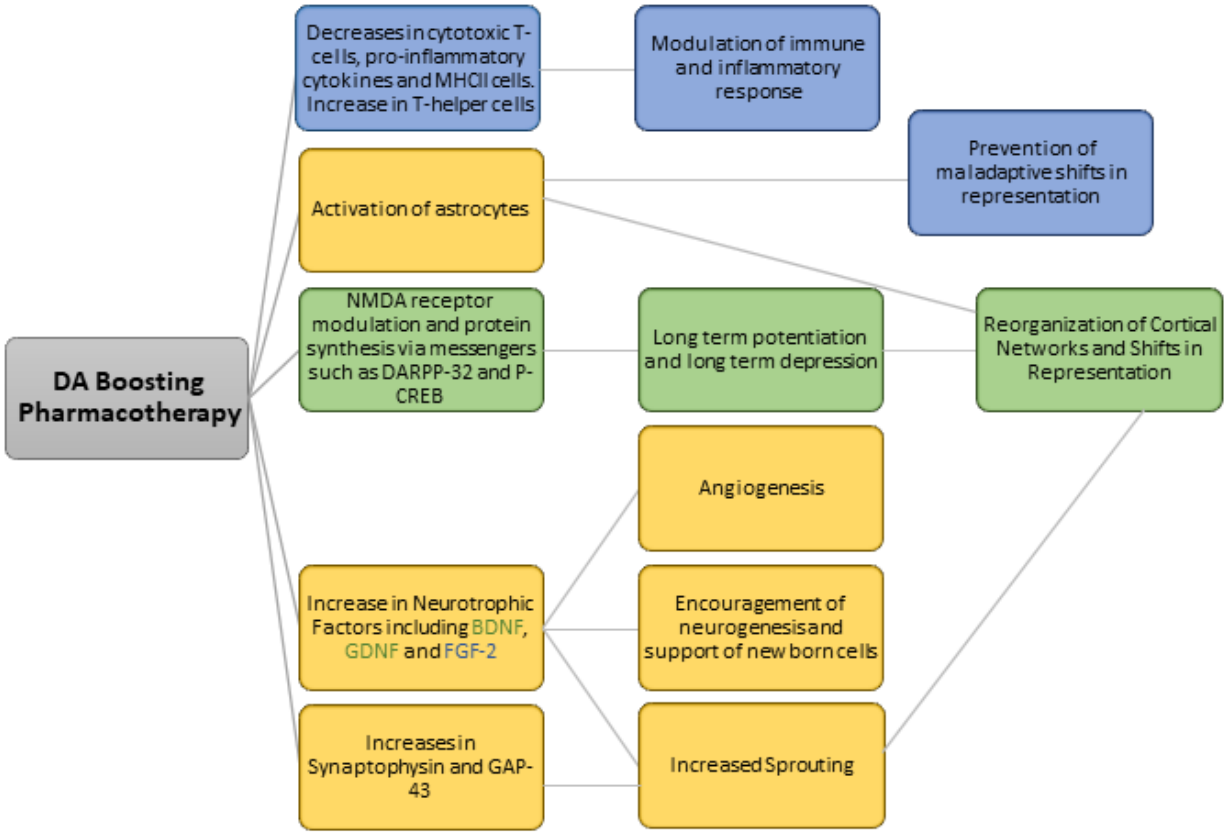

Figure 1: Concept Map of the Most Likely Mechanisms of Action for DA Boosting Pharmacotherapies in Stroke. Mechanisms which appear likely to be primarily mediated by D1-class receptors are in green, whereas mechanisms most likely to be mediated by D2-class receptors are in blue. In yellow are mechanisms which evidence suggests can be mediated by either D1 or D2-class receptors.

6. Clinical Evidence for the Use of DA-Augmenting Drugs in Motor Recovery from Stroke

Results of clinical studies employing DA-boosting drugs to enhance motor recovery following stroke are summarized in appendix 4, Table 5.

6.1 Amphetamine Studies

The successful transfer of the beneficial effect of AMPH observed in animal stroke models into medical practice has produced mixed results in clinical trials. Indeed, an early study with eight stroke patients showed that a single dose of AMPH followed by physical therapy could significantly increase motor function improvement above the level attained with placebo and physical therapy (Crisostomo et al., 1988). Meanwhile, another preliminary study in 25 patients found a single dose of AMPH was not sufficient to improve motor function recovery above the level of placebo treatment (Long and Young, 2003). Some studies employing repeated dosing and physiotherapy, usually at an interval of several days, have shown a significant benefit of AMPH on motor recovery and/or activities of daily living, particularly in the hemiparetic arm (Walker-Batson et al., 1995; Martinsson and Wahlgren, 2003; Schuster et al., 2011). Other trials using similar dosing patterns have found no benefit (Sonde et al., 2001; Treig et al., 2003; Platz et al., 2005; Gladstone et al., 2006; Sonde and Lökk, 2007). More aggressive dosing approaches, with daily AMPH, have yet to produce positive results (Long and Young, 2003). Unfortunately, studies supporting the use of AMPH for motor recovery often have low enrollment. Experimental group dissimilarity is also a concern, with many studies showing differences in mean patient age or in level of motor function after stroke, across both successful and unsuccessful trials. A 2007 meta-analysis of six studies (176 patients) that looked at motor recovery saw evidence of better relative change from post-stroke baseline in patients given

AMPH and a nearly significant effect of AMPH on activities of daily living score (Martinsson et al., 2007). A later meta-analysis did not observe a significant improvement of motor scores in patients given AMPH, although the trend for an increased motor benefit was still present (Sprigg and Bath, 2009).

6.2 L-Dopa Studies

Studies employing L-dopa for motor recovery from stroke have had somewhat more success, although results are still mixed. In the sub-acute phase several studies show L-dopa and physical therapy improved recovery above the level of placebo and physical therapy (Scheidtmann et al., 2001; Lokk et al., 2011; Masihuzzaman et al., 2011). One study was only able to find a trend towards improvement with L-dopa, although this group had only 4 patients in the L-dopa group, and 7 patients in a combination L-dopa and AMPH group, as well as a high degree of initial variability and an uneven distribution of deficits on the Fugl-Meyer arm portion (Sonde and Lökk, 2007). The study by Masihuzzaman et al (2011) reported that both ischemic and hemorrhagic strokes responded well to the L-dopa treatment. In the chronic phase a single dose of L-dopa was sufficient to significantly boost motor learning on a TMS-induced thumb movement paradigm (Floel et al., 2005), but a single dose could not boost performance on the Nine-hole Peg Test, the Action Research Arm Test or on grip strength (Restemeyer et al., 2007). The study by Floel et al (2005) trained participants on the test task, whereas, the Restemeyer et al (2007) study employed a physiotherapy session with an emphasis on dexterity. These studies possibly suggest that a single dose is not enough to lead to generalizable motor improvement in a chronic stroke population, although it should also be noted that there was substantial variability in scores for the Nine-hole Peg test, and that performance on the Action Research Arm test (a test which is prone to ceiling effects) was high before intervention (Restemeyer et al., 2007;

Salter, 2013). In support of multiple doses being required for enhancing stroke recovery, a group of patients received three doses of L-dopa over two days which led to increased procedural motor learning in the paretic hand without changes to motor arousal or response style (Rösser et al., 2008). Moreover, five weeks of L-dopa without physiotherapy ameliorated scores on the Nine-hole Peg test and augmented speed of 10-meter walking, as well as lengthening the cortical silent period, while no change was seen in placebo group (Acler et al., 2009). Although the study was not able to show improvement on the Rivermead Motor Assessment, these findings indicate that L-dopa alone could be sufficient to improve motor recovery and modulate cortical excitability (Acler et al., 2009). While the above findings do suggest that there is a beneficial role for L-dopa in post-stroke motor recovery, the trials performed thus far are not without flaws. Several of the trials have involved few patients or have used a crossover design, and one study only employed single blinding. The DARS trial (Bhakta et al., 2014) and "Effect of Serotonin and Levodopa in Ischemic Stroke" (SELEIS, ClinicalTrials.gov identifier: NCT02386475), two large scale clinical trials using L-dopa and physical therapy in stroke patients have been announced, and perhaps will be able to give further insight into the efficacy of this treatment approach.

There has also been interest in combining L-dopa pharmacotherapy with other emerging recovery strategies. One group tested an aggressive treatment plan involving a total of 7 weeks of daily L-dopa, and 15 days of inpatient care that involved 20 minutes of low frequency TMS applied to the contralateral hemisphere, one hour of one-on-one occupational therapy and one hour of self-exercise, repeated twice daily, on chronic stroke sufferers. All patients in the small cohort showed improved motor function in the paretic arm, most of which was maintained at the four week follow up, however the absence of any control groups makes drawing any conclusions

difficult (Kakuda et al., 2011). Lastly, the use of L-dopa to facilitate robot-assisted motor therapy following stroke has also been proposed (Tran et al., 2016).

6.3 Methylphenidate Studies

The use of methylphenidate (MPH) and physical therapy to enhance stroke recovery have also been tested in few clinical studies. One to three weeks of MPH combined with physical therapy significantly increased scores on the modified functional independence measure (FIM) and showed a trend of increased performance on the Fugl-Meyer scale in post-acute stroke patients (Grade et al., 1998). Moreover, a study by Lokk et al (2011) found both MPH and combined MPH plus L-dopa were able to improve scores on the Barthel Index and National Institute of Health Stroke Scale, but not on the Fugl-Meyer Scale, as compared to placebo treatment. Further, one study found a single dose of 20 mg of MPH was able to improve performance on a finger tapping task, though not performance of grip strength or a target pursuit task. The improvement in finger tapping was correlated to hyperactivity of the ipsilateral primary sensorimotor cortex and the contralateral premotor region, as measured by functional MRI during the drug's active period. A hypoactivity of the anterior cingulum was also observed under the MPH condition (Tardy et al., 2006). Interestingly, another study reported that the combination of MPH and transcranial magnetic stimulation led to significant motor benefits, which were above the level of either treatment alone on the Perdue Peg Board test (Wang et al., 2014). However, as no placebo group was included in this study, it is unclear if the modest improvement seen in peg board performance in the MPH alone group is of any potential clinical relevance (Wang et al., 2014). Likewise, while this study did not show changes in cortical excitability in any of their MPH or TMS groups, it is worth mentioning that MPH has been

shown to regulate cortical excitability during a motor task in healthy patients (Kratz et al., 2009; Wang et al., 2014).

6.4 Direct Dopamine Agonists

To date only one study tested a direct dopamine agonist, the D2-class drug ropinirole, along with physical therapy to facilitate motor recovery from stroke (Cramer et al., 2009). In spite of all groups showing significant improvement over the study period, there was no benefit of ropinirole treatment in either recent (before 3 months from onset) or later (between 3 and 12 months from onset) stroke cases. Importantly, ropinirole showed no serious adverse effects, and medication compliance was high, yet the majority of patients in the drug group never reached the planned dose (3 mg daily) for the study. Additionally, the drug group had a higher rate of comorbidities such as diabetes and depression, and the placebo group received a higher level of physiotherapy outside of the parameters of the study (Cramer et al., 2009).

Further studies in human stroke patients, examined drugs given to stroke patients for co-morbidities, classifying them as "potentially deleterious" or not, based on preclinical findings. The "potentially deleterious" group, which included dopamine antagonists, were found to worsen motor outcome (Goldstein et al., 1990; Goldstein, 1995; 1998).

7. Rationale and Statement of the Problems

7.1 Why have clinical results been so mixed?

There are many challenges inherent in translating results from animal studies into human patients. Animal studies are typically done in young, otherwise healthy animals without comorbidities or other medications. Animal models have the benefit of producing extremely similar strokes in animals with identical genetic backgrounds. In clinical stroke studies factors

such as age or stroke severity can have a greater impact on outcome than the expected benefit of a given treatment, so groups must be well balanced, or analysis should take co-variants into account (Bath et al., 2012). Furthermore, appropriately scaling factors such as dosage, timing and rehab intensity from animal to human models is likely to be of critical importance, but may not be straight forward (Adkins et al., 2009). Consider the issue of timing after stroke. While humans typically show spontaneous recovery over the six months following stroke, rodents see spontaneous recovery plateauing at about 4 weeks following stroke, however it is difficult to determine what the timing of the physiological recovery processes observed in animals would look like in humans (Chollet et al., 2014).

Aside from the intricacies of dose, timing and rehab type and intensity, one potential problem as to why studies have had such mixed results is that studies may not be targeting the correct patient populations (Chollet et al., 2014), nor are the studies large enough to allow for stratification of patient populations. In terms of characteristics of the stroke, responsiveness to pharmacotherapy could depend on time from onset of stroke, severity of deficit or stroke location. Stroke location should be considered carefully. For example, one study suggested that patients whose stroke occurs on their dominant side respond better to dopaminergic pharmacotherapy. This is believed to occur because the dominant hemisphere, equally boosted by the pharmacotherapy, may overpower and inhibit the infarcted, non-dominant hemisphere, which as has been mentioned before, can be an undesirable recovery strategy (Hallett, 2001; Rösser et al., 2008; Jang, 2013). That is not to say that such patients cannot benefit from pharmacotherapy, however, they may also need to engage other strategies, such a cortical inhibition of the unaffected hemisphere using TMS, or CIMT to ensure optimal recovery.

Another factor that may be a source of a lot of variation between studies is the genetics of the individuals involved. Genetic polymorphisms exist at varying rates in the population for COMT, the DAT, as well as D1R, D2R and D3R (Pearson-Fuhrhop et al., 2013). Taking into account the well-established inverted U-shaped dose-effect relationship to DA levels on a variety of behavioural outputs, the response of a patient to DA modulating treatments may differ greatly based on where their genetics place them on the curve (Cools and D'Esposito, 2011; Thirugnanasambandam et al., 2011; Floresco, 2013; Vaillancourt et al., 2013; Arnsten et al., 2015). Evidence linking variations in the gene for COMT to differences in motor skill outcome in stroke patients, given regular physical therapy treatment, suggests that baseline "dopaminergic tone" is one factor in recovery (Liepert et al., 2013; Kim et al., 2016). The genetics of the individual may also interact with DA-boosting treatments. Several studies in healthy adults have shown that use of drugs increasing DA levels are beneficial on motor learning, impulse control and working memory tasks in participants whose genetics make them prone to a lower dopaminergic tone (Mattay et al., 2003; Pearson-Fuhrhop et al., 2013; MacDonald et al., 2016). In contrast, participants with a higher dopaminergic tone perform better at baseline, but see their performance worsen under the influence of DA increasing drugs (Mattay et al., 2003; Pearson-Fuhrhop et al., 2013; MacDonald et al., 2016). Further complicating the picture, there is evidence that polymorphisms in the BDNF gene may interact with polymorphisms for dopaminergic system linked genes and effect plasticity processes (Witte et al., 2012). A great deal of work remains to be done on the pharmacogenetics of stroke recovery, particularly as it pertains to the dopaminergic system.

Finally, studies thus far have mostly used drugs that act upon a wide variety of targets. By virtue of a modulation of the DAT and norepinephrine transporter function, amphetamine and

methylphenidate can potentially activate numerous adrenergic (α1AR, α2AR, β1AR, β2AR) and dopaminergic (D1, D2short, D2long, D3, D4 and D5) receptor subtypes. Another confounding factor with respect to the usage of these drugs is the potential recruitment of the trace amine-associated receptor 1 (TAAR1), a Gs-linked GPCR widely expressed in the central nervous system (CNS). Notably, TAAR1, which chiefly responds to endogenous trace amines (β-phenylethylamine, tyramine and octopamine), can also bind to and be activated by dopamine, norepinephrine and amphetamine-like compounds (Pei et al., 2016). Likewise, administration of L-dopa, the precursor to DA and NE, possibly leads to a non-specific activation of a large number of CNS dopaminergic and adrenergic receptors. Consequently, the potential recruitment of a large spectrum of molecular targets by these drugs may ultimately mitigate their therapeutic effects. For instance, D1 and D2-class dopaminergic receptors act in opposing directions in their classical pathways. Previous pharmacological manipulations of the dopaminergic system, for example the use of D2-class receptor antagonists in the treatment of psychosis, have shown that drugs that preferentially target a given receptor or receptor-class are often better able to treat symptoms without causing unwanted side effects (Beaulieu et al., 2015). It is likely that the best dopaminergic facilitation of stroke recovery comes from some optimal balance between D1 and D2-class receptor activation, and it is possible that a non-specific increase in DA receptor activation is not able to attain that optimal balance. Several of the likely mechanisms of action for a DA-driven facilitation of stroke recovery could be particularly associated with D1-class dopaminergic receptors, including the modulation of motor learning, augmentation of BDNF and GDNF levels and increase in cerebral blood flow and neurovascular coupling (Luft et al., 2004; Choi et al., 2006; Nitsche et al., 2009; Tan, 2009; Perreault et al., 2012; Xing et al., 2012; Kuric et al., 2013). Specific activation of D1-class receptors would be unlikely to have great effects on

endogenous DA levels, as feedback inhibition is the domain of D2-class receptors, particularly presynaptic D2Rs (Boyar and Altar, 1987; Beaulieu et al., 2015). Furthermore, the only human study to date to use a specific DA agonist in motor recovery from stroke was the unsuccessful trial using the D2R agonist ropinirole (Cramer et al., 2009). Notwithstanding these findings, the intricate role of biogenic amines in the facilitation of post-stroke recovery, notably that of DA and its receptors, remains to be experimentally tested in a more systematic fashion. This is likely to improve our understanding of the mechanistic underpinnings that underlie stroke recovery, which in turn may lead to better treatment in the future.

7.2 Rehabilitation in Animal Models of Pharmacotherapy for Post-Stroke Motor Recovery

In most studies employing DA augmenting drugs for stroke recovery, rehabilitation has also been given. Conventional wisdom within the field indicates that training and rehabilitation are best applied during the active period of the drug, based on an early study that found restraint during the active period of AMPH was able to block its benefits on beam walking (Feeney et al., 1982). However, there is also evidence that allowing for basic movement during the active period of the drug is enough, and that task specific practice may not always be beneficial. Several studies which gave AMPH to rodents after stroke using the horizontal ladder test, the foot fault test or tests of skilled reaching had trends toward decreased performance of tasks on drug administration days, either throughout the study or in the earlier exposures to the drug (Adkins and Jones, 2005; Gilmour et al., 2005; Rasmussen et al., 2006; Alaverdashvili et al., 2007; Auriat and Colbourne, 2008). This is likely due to drug induced stereotypies and over activity (Barbay and Nudo, 2009). Meanwhile, studies employing non-task specific rehab methods, such as environmental enrichment and exposure to climbing tasks found positive outcomes (Ramic et al.,

2006; Papadopoulos et al., 2009; Wolf et al., 2014). It may be that recovery is best augmented by general use of the impaired limb under the influence of the drug, although it is possible that task-specific practice would be more effective if steps were taken to ensure the animal engages with the task. Furthermore, in a study employing L-dopa in a rat MCAO model, no motor training was used, and beneficial results were still observed on the rotating pole test, cylinder test and a neuroscore, indicating that motor training concurrent with drug delivery may not be a requirement for the effectiveness of DA-boosting therapies (Ruscher et al., 2012). There is one conceptualization of stroke recovery which may explain this. Stroke is followed by a period of spontaneous recovery, which is facilitated by the upregulation of growth factors. This is followed by a period wherein growth-inhibiting factors are upregulated, the so-called closing of the critical window (Murphy and Corbett, 2009). While this is generally seen as negative to the recovery process, this may be an opportunity for pruning of unnecessary new connections and refinement of networks (Wahl et al., 2014). Based on this hypothesis, growth promoting therapies and physical rehabilitation would be best applied sequentially rather than concurrently. Indeed, animal stroke and spinal cord injury studies using antibodies for the growth-inhibiting protein NogoA (anti-NogoA) found that rats given the anti-NogoA therapy at the same time as a complex motor rehabilitation program had less effective motor recovery and more aberrant sprouting relative to rats receiving placebo treatment. In contrast, animals given a period of the anti-NogoA therapy, and then later the physical rehab paradigm, had significantly better recovery than controls and had healthier, more organized axonal growth (Wahl et al., 2014; Chen et al., 2017). Similarly, sequential administration of growth factors, followed by enriched rehabilitation, was successfully used and shown to be better than either treatment alone in stroke

recovery, although no comparison was made to the two treatments occurring in parallel (Jeffers et al., 2014).

Aside from the issue of timing of rehabilitation, the method of rehabilitation must also be considered. One approach to rehabilitation is to use task-specific, focused motion and numerous repetitions (Schmidt et al., 2014; Caleo, 2015). This is probably the best approach for improvement on specific, fine motor tasks, but in some studies the skill declines after the cessation of practice, or the improvement is not generalizable to other motor tasks (Caleo, 2015; Livingston-Thomas et al., 2016). Another approach is through environmental enrichment and/or aerobic exercise. Animal models have shown improvement under both these non-specific paradigms, although the effects of each are not always easy to separate as environmental enrichment often includes equipment for voluntary exercise. These paradigms have been shown to be capable of improving somatosensory recovery, priming the animal for further motor learning and improving cognitive outcomes (Ploughman et al., 2015; Livingston-Thomas et al., 2016). These effects are mediated by growth factors including BDNF, insulin-like growth factor-1 and nerve growth factor, as well as promotion of dendritic branching and synaptogenesis (Ploughman et al., 2015; Livingston-Thomas et al., 2016). In humans, aerobic exercise has been shown to improve motor outcomes, most notably in gait (Langhorne et al., 2009; Globas et al., 2012; Pang et al., 2013).

When discussing the effects of aerobic exercise, a distinction should be made between forced or voluntary exercise. In studies comparing the effects of forced and voluntary exercise in non-ischemic animals, voluntary runners tended to reach higher speeds and to run in more brief bursts (although they still run very long distances), while forced runners ran at shorter speeds and had to run for much longer to match the distance of voluntary runners (Leasure and Jones, 2008).

Forced runners had higher levels of anxiety-like behaviours and have been observed to have higher levels of corticosterone as compared to voluntary runners. Although both running conditions had higher levels of neurogenesis than sedentary runners, forced runners were higher than voluntary runners (Leasure and Jones, 2008). In contrast, voluntary running has been found to be as effective as forced running in protecting against and reversing the effects of stress in the development of anxiety and depression-like phenotypes, and voluntary running appears to be better at integrating with reward systems, specifically D1R containing direct pathway neurons in the striatum (Greenwood et al., 2013; Herrera et al., 2016). Voluntary running has been shown to be able to increase levels of BDNF, TrkB, vascular endothelial growth factor A, and its receptors, insulin-like growth factor-1 and its receptor, cAMP response element binding protein (CREB), synapsin 1, and GAP-43. Some of these molecules are significantly upregulated above the level of sedentary controls within 3-7 days of wheel use (Gomez-Pinilla et al., 2002; Solvsten et al., 2017). Several studies in rodent stroke models comparing the benefits of forced exercise with the benefits of voluntary running have found improved behavioural outcomes and increases in levels of purported mediators of recovery such as increased neurogenesis, increased levels of phosphor-CREB and BDNF and less indications of a stress response in voluntary running animals (Luo et al., 2007; Ploughman et al., 2007; Ke et al., 2011). In contrast to these findings several reviews of behaviour-based therapeutics in animal stroke models classify forced exercise as more beneficial than voluntary exercise (Schmidt et al., 2014; Ploughman et al., 2015). Despite this, it is clear that voluntary exercise is capable of exerting beneficial effects in stroke recovery, making it a reasonable therapeutic tool as it provides aerobic exercise and adds novelty to the animals' environments.

7.3 D1-class Agonists and Antagonists

The level of activation of D1-class receptors can be manipulated using drugs which are specific to the class of receptor, including agonists dihydrexidine (DHX) and SKF81297 and antagonist SCH23390. There are currently no drugs which can specifically target individual DA receptor subtypes.

Of the currently existing D1-class agonists, DHX is one of the most attractive for *in vivo* work, with its high D1-agonist intrinsic activity. DHX does not have a propensity to cause seizures, as other classes of D1-agonists do, and DHX does not lead to rapid drug tolerance like other members of its class (Arnsten et al., 2017). Interestingly, recent data suggests DHX is a full agonist at D1 receptors coupled to G_s (which dominate D1 receptors in the cortex) and is a partial agonist at G_{olf}-coupled D1 receptors, which are the predominant D1-class subtypes in the striatum. DHX displayed no bias towards G-protein or β-arrestin effector pathways (Yano et al., 2018). An early clinical trial of DHX for Parkinson's disease found problems with hypotension during their intravenous administration, particularly when the drug was administered over a shorter period of time (Blanchet et al., 1998). Later work, examining the use of DHX for the negative symptoms of schizophrenia, found no severe adverse effects with subcutaneous delivery, or with intravenous delivery offset with concurrent delivery of normal saline as needed (George et al., 2007; Mu et al., 2007; Rosell et al., 2015; Girgis et al., 2016). One potential pitfall of DHX use is its short half-life (1-2 h in humans) (Salmi et al., 2004).

While DHX has been used in several different animal models, its use in C57BL6 mice has not been extensive, and little dosage information is available. Doses in the range of 3-10mg/kg have been shown to affect social reactivity and acoustic startle responses in C57BL6 mice (Lewis et al., 1994; Gendreau et al., 1997). In rats, the locomotor activity response to DHX has been mixed, with some studies showing increased locomotor activity (Darney et al., 1991) and others

showing a reduction in locomotor activity or no change, depending on the animals' acclimation levels (Deveney and Waddington, 1997; Isacson et al., 2004). Although the specifics of the acute locomotor response to DHX is more complicated than one might initially expect, nonetheless, DHX is able to modulate the level of locomotor activity and the expression of immediate early gene c-fos (Darney et al., 1991; Isacson et al., 2004; Salmi et al., 2004).

One concern with the chronic use of D1-class agonists is the potential development of tolerance to the drug (Arnsten et al., 2017), although the possibility of sensitization to the drug should also be considered. Little work has been done employing chronic DHX, but one study in rats found that 14 days of daily injections of 18 mg/kg (a higher end dose as compared to the ones used in the rat locomotor activity under DHX studies) resulted in no change to the baseline levels of acetylcholine in the hippocampus, but did result in tolerance to the pro-cholinergic effect of the 18 mg/kg dose of DHX in the hippocampus (Wade and Nomikos, 2005).

In contrast, to the mixed behavioural results of D1-class agonists, the D1-class antagonist SCH23390 consistently blocks locomotor activity and stereotypy (Bourne, 2001).

7.4 The Effect of Light-Dark Cycle on Functional Outcome in Rodent Stroke Models

In order to develop alternate therapies for stroke recovery, and to test efficacy and safety of those therapies before they are tested in humans, it is necessary to use rodent stroke models. As part of optimization of my stroke model, the photothrombosis mouse model, I sought to examine the effect of time of stroke in the diurnal light-dark cycle on stroke outcome. Mice and rats are nocturnal animals, yet research is often done during the light period of the day when the animals would typically be inactive (Kopp, 2001; Prager et al., 2011). This could possibly affect the outcome of the stroke, as many aspects of the central nervous system are altered throughout the

circadian rhythm (Peirson et al., 2018). It may also have a bearing on the sensitivity or accuracy of behavioural testing, as animal behaviour has been shown to be affected by time in the diurnal cycle in some specific cases (Hossain et al., 2004; Beeler et al., 2006; Roedel et al., 2006).

Previous work on the effect of time of stroke on outcomes in animal models have had mixed results. One study, using an MCAO model in rats found that there was a circadian rhythm to the size of the stroke produced, with the largest strokes occurring at 4:00AM (during the rat's active period) and the smallest strokes at 16:00PM, during the rat's inactive period. The largest strokes were 3-4 times larger than the smallest ones (Vinall et al., 2000). The change in stroke sizes correlated well with changes in the animals' body temperature, a circadian-controlled factor which was maintained in this study. The authors noted, however, that based on previous findings on the neuroprotective effect of hypothermia, the observed variation in stroke size (range of 2-8% of total brain volume) was larger than could be explained by the variations in body temperature (ranged from about 36.5-37.5^{o}C), and they hypothesised that other factors were involved (Vinall et al., 2000). Another study, which applied ET-1 to the cortex of awake rats, found no differences in behavioural outcome on skilled pellet reaching, the horizontal ladder or infarct sizes (Rakai and Antle, 2013). It should be noted, that this groups used 16 animals, with a total of four treatment groups, meaning that the study did not have much power, especially considering the variability of stroke behaviour (Rakai and Antle, 2013). As this question has never been addressed in the photothrombosis model, experiments were undertaken to evaluate this.

7.5 Objectives and Hypotheses

The objectives of my thesis are three-fold:

1. Establish the effect of time of stroke in the light-dark cycle on the outcome of motor function following a photothrombotic stroke.

2. Determine an optimal dose of D1-class agonist DHX, which is able to modulate locomotor activity (and thus reaches the brain), in C57BL6 mice.

3. Test the efficacy of D1-class receptor stimulation, followed by voluntary exercise, to enhance motor recovery from stroke in an asynchronous study design.

In response to these objectives, I hypothesised the following:

1. That strokes which occurred during a mouse's dark phase would result in a worse motor outcome, and a longer lasting motor deficit, as compared to mice whose strokes occurred during their light phase.
2. That the D1-class agonist DHX would be effective in improving motor recovery following stroke, above the level of recovery observed in saline treated animals. I also hypothesised that access to aerobic exercise would play an important role in the promotion of this motor recovery.

Methods

1. General Procedures

1.1 Animal Housing and Behavioural Protocols

All mice used were male C57BL6 mice ordered from Charles River (Kingston, NY, U.S.A.). Animals were single housed upon arrival in Greenline cages (Techniplast, Italy) with standard mouse cage bedding (Teklad 1/4" corncob bedding, Envigo), a nesting square and a cardboard house. Food (Teklad 2018 Rodent Diet, Envigo) and water were available ad libitum. Except for those mice in the Normal Light-Dark group of the Light-Dark Stroke study, all mice were housed in reverse light-dark housing, with lights off at 7:00 and on at 19:00. Normal Light-Dark mice had lights on at 7:00 and lights off at 19:00. Temperature and humidity in the vivarium and behavioural testing suites were maintained at 23.5^{o}C and 40% respectively.

All mice were acclimated to the facility for two weeks prior to any behavioural testing, surgical procedures or pharmaceutical administration. Three days after arrival mice were ear tagged (National Band and Tag Co., Newport, U.S.A.). Two days after this, handling acclimation began. Mice were typically handled once daily for seven non-consecutive days. Handling included lifting the mice onto the hopper of their cages, allowing them to explore the hopper with or without the tail being held, lifting briefly by the tail, allowing them to sniff the handlers, light touching of the back and occasionally being lifted onto the handler's labcoat sleeve. Handling escalated in intensity as the mice became more acclimated, with the first day consisting of the mouse exploring the hopper with tail held and being lifted about 3 times, and the final day involving exploring the hopper without restraint, lifting by the tail up to 8 times, and multiple touches on the mouse's back. In cases where individual mice or entire cohorts were not acclimating to handling as quickly as previous cohorts, extra handling was implemented, either

in the form of additional days, or in more intensive, longer lasting handling. When possible both of the personnel performing the adhesive removal test participated in the acclimation of animals. At least one day prior to all behavioural testing, cards were placed on the cages to prevent vivarium technicians from changing the cages, a possible confound to behavioural testing. Prior to all behavioural testing animals acclimated in their home cages to the testing room for at least 30 minutes, except for those tests where such acclimation would be counter-productive (cylinder test, locomotor activity test). Behavioural testing and surgical procedures were not performed in the hour preceding or following the light change in their housing room. All animal procedures were approved by the University of Ottawa Animal Care Committee.

1.2 Photothrombosis Stroke Induction

All strokes were induced by photothrombosis, an established method which utilizes a photochemical reaction to produce local thrombosis in a targeted region of the cortex (Watson et al., 1985). Strokes were induced at 0.7 mm anterior and 1.5 mm lateral to bregma, to target the left side forelimb representation area of the primary motor cortex (Paxinos and Franklin, 2013) (Paxinos and Franklin, 2013). 24 hours before photothrombosis surgery, animals were weighed in preparation of making rose bengal. Rose bengal (Tocris, Bristol, U.K.) was prepared the day of surgery by mixing rose bengal powder with 1X phosphate buffered saline (PBS) to a concentration of 10mg/ml. The solution was vortexed thoroughly and then sterilized using a 0.22 µm syringe filter. Filtered rose bengal was stored in amber tubes and every effort was made to prevent exposure to light during the preparation process.

Mice were weighed the morning of surgery and brought to the surgical chamber ahead of time by vivarium staff. Reverse light-dark mice were kept covered from light as much as possible. Anesthesia was administered with induction at 5% isoflurane in about 2 L/min oxygen, until the

animal was unresponsive to touch. The animal was then transferred from the induction box to a facemask with the same isoflurane concentration. The animals' heads were shaved and cleaned with chlorohexidane. The animals were given 1ml of saline subcutaneously on the back, as well as xylocaine jelly (2% lidocaine hydrochloride, AstraZeneca) in the ears and Tear Gel (Alcori, Mississauga, ON, Canada) over the eyes. Animals were then moved to the surgical table, with anesthesia maintained at the induction level. Mice were secured in place under the stereotaxic apparatus using lightly applied ear bars. Rose bengal was administered intraperitoneally at a dose of 10 μl/g of mouse, such that mice received 1 mg of rose bengal per g of body weight. At this time a 5-minute timer was started, and isoflurane was reduced to a maintenance dose of 1.5-3.5%, depending on visual inspection of the animal. In the ensuing five-minute period, chosen to allow the circulation of rose bengal throughout the body, an incision was made along the midline of the animal's head, ranging from between the eyes to about as far back as lamba, to expose the skull. The laser, mounted on a stereotaxic frame was centred such that the laser was above bregma on the exposed skull, and at a height of 3cm above the skull. The stereotaxic coordinates of bregma were recorded on a surgical record sheet and coordinates for the left-side primary motor area, located at 0.7 mm anterior and 1.5 mm lateral from bregma were calculated and the laser was moved to this location. At the end of the five-minute period the laser (power ~20mW; wavelength: 532 nm (Beta instruments)) was turned on and left on for 10 minutes. After the ten minutes the laser was turned off and the incision was closed using Vetbond glue (3M Company). Topical analgesia in the form of bupivicaine cream (2% Bupivicaine HCL, Chiron Compounding Pharmacy Inc. Guelph, ON, Canada) was administered along the site of the incision. Mice were freed from the ear bars and anesthesia was turned off.

Mindful of the effect of anesthesia on body temperature of such small animals and the neuroprotective effect of hypothermia in stroke, efforts were made to maintain body temperature throughout the procedure. During surgical prep animals were kept on a heating pad and under a heating lamp. Throughout surgery animal temperature was monitored by a rectal thermometer probe. The probe was attached to a self-regulating heating pad which was set to keep the animal's temperature at 37^{o}C, and animals were covered by surgical toweling. Careful arrangement of surgical towels was employed to prevent burn to the sensitive tail and paws of the animals. When this did occur, animals were kept in the study, as their performance on behavioural tasks was judged not to be impaired by this; however slight changes in handling were made to accommodate tail damage. Statistical analysis was performed excluding these animals to test if they influenced outcome.

Mice were moved to recover in an incubator at 37^{o}C until conscious and then were moved to clean standard cages, with the addition of a soft foam sheet covering the cage filling for increased comfort and 8-10 Cheerios (General Mills) to promote feeding and a bit of their old nest. Mice were returned to their housing room and were wellness-checked in the evening. Mice were given a second dose of bupivicaine to help with pain management at this time. In the morning mice were given another health assessment. Animals that were identified as unwell or not recovering from surgery as expected were given extra care as necessary, including placement on a heating pad, diet gel or H_2O recovery gel (Clear H_2O), moistened mouse chow, saline injections and removal of fecal blockages. During recovery, about 1-2 weeks after stroke, mice occasionally had their head wounds reopen. This was treated by erythromycin ointment (5mg/g, Pendopharm), a topical, non-steroidal, antibiotic, applied daily to the head wound until it healed.

The antibiotic was applied after the conclusion of behavioural testing on days when its administration overlapped with behavioural testing.

1.3 Magnetic Resonance Imaging (MRI) Procedure

After being accessed for wellness the morning after surgery, mice were transferred to the MRI suite in either light or dark conditions as needed. During the MRI procedure mice were anesthetized with isoflurane, similar to the procedure described above, and given 1 mL of saline subcutaneously, and had their ear tags removed. Mice were placed in the MRI machine. T2 weighted scans were taken, first in a 2-3 minute preliminary, whole brain scan to find the site of infarct, and then a longer, more detailed coronal scan of the infarct area in slices 0.5 mm apart, lasting about ten minutes. After the scan animals were given a new ear tag, returned to their home cage and left on a heating pad until they roused from the anesthesia. Animals were kept in the appropriate lighting conditions for their circadian rhythms, except for the brief periods before and after the anesthesia took effect.

1.4 Adhesive Removal Test

The adhesive removal test is a validated sensorimotor behaviour test (Bouet et al., 2009; Schonfeld et al., 2017). Mice were brought to the testing chamber a minimum of 30 minutes prior to the beginning of testing. To set up for the test, the mouse was placed on the hopper, which was then transferred to sit above an empty cage. The contents of the mouse's cage were then dumped into the empty cage and the mouse was placed into its empty home cage for a one-minute acclimation period. The mouse must be tested in an empty cage to allow for paw visibility and to keep the mouse from wiping the tape off on the bedding material. It is considered best practice to use the mouse's home cage to prevent the novelty of an unfamiliar

environment and the scent of unfamiliar mice, from distracting the mouse from removing the tape (Schonfeld et al., 2017). The minute of acclimation is meant to prevent the change in environment from distracting the mouse or increasing its anxiety.

After the minute of cage-acclimation, one handler restrained the mouse while the other placed a small piece of adhesive tape (Nexcare Durable Cloth First Aid Tape) on each of the mouse's forepaws. All efforts were made to ensure that the tape was the same size, was placed on the same part of the paw and was pressed down with the same force for each mouse. The same handler placed the tape for every mouse in a given cohort. To avoid any difference between paw performances caused by the order of the tape placement, we alternated paws. The next day the tape was applied first to the other paw for each mouse.

Once the tape was placed, the timers were started as the mouse was placed into the empty homecage. The handlers each stood on one side of the cage, with their own timer, and each recorded the time they saw the mouse contact and remove the tape from each paw. When the mouse removed both pieces of tape, or 2 minutes passed, the mouse was lifted out of the cage and the tape was removed from him if necessary. The cage was reassembled, and the process began again for the next mouse.

Prior to the test, the mice were given 2 pre-training sessions, usually in their housing room, wherein the handlers placed the tape on the mouse's paws and placed them back into their regular homecage. No time was recorded at the pre-training sessions. These sessions typically occurred on the Thursday and Friday before the formal training period began.

The training period consisted of 4-5 consecutive days of the animals performing the test once daily, in the morning. Data was recorded and monitored throughout this time to determine when

the animals' time to contact and remove the tape has reached a plateau. A mouse's score for a given day is the average of the scores from both handlers, although interrater agreement was typically very good. All the testing timepoints after the stroke consist of two consecutive days of the animals performing the test and the values reported are based on the average of both of these days. The animal's prestroke score is the average of its score on the last two days of training. In rare cases where a time was not able to be recorded for both paws on both days, the times from the day recorded was used in lieu of the average.

For a given cohort the handlers for this test were the same every time the test was done and the test was always done at the same time, in the same room. Normal light-dark cycle mice performed the test under moderate levels of white light (120-125 Lux). Reverse light-dark cycle mice performed the test under red light.

1.5 Horizontal Ladder Test

The second behavioural test used to assess motor function and recovery is the horizontal ladder test (Schaar et al., 2010; Schonfeld et al., 2017). Mice were placed on the hopper of an unfamiliar cage, at one end of a horizontal ladder. The ladder is composed of two plexiglass walls (69.5 cm × 15 cm) with a row of holes 2 cm from the bottom of the plexiglass. Rods (8 cm long, 1.5 mm in diameter) are placed in the holes in an irregular pattern, such that the space between the rods varies from 0.5-2.5 cm and the plexiglass walls are close enough to prevent easy turning in the ladder. At the other end of the ladder was the mouse's home cage. One day prior to pre-stroke testing the mice were trained on the ladder task. The goal of the training is to teach the mouse to walk across the ladder without stopping. The un-bristled end of a toothbrush was used to prod the mouse forward when needed to discourage pauses, rearing, turning, backing

up or grooming behaviours during the crossing. Training employed a minimum of 3 crosses and could continue for as long as it takes to teach the mouse to cross the ladder.

On testing days, the handler videotaped the mice as they crossed the ladder. The video camera was placed slightly below the level of the ladder and tilted up to allow for capture of all four paws. The goal was to get three crosses of the ladder that include minimal extraneous behaviours, so a minimum of three trials were performed, but again, the trials continued until this requirement was met. The toothbrush was still used to prod the mouse when necessary, but these trials were not used in scoring except as a last resort.

Two of the video trials for each mouse, on each testing day, were scored by watching the videos in slow motion. Trials to be scored were not chosen based off of the mouse's success in crossing the ladder, but rather based on whether the mouse displayed unwanted (non-walking) behaviours, as a means to make it easier to score and to standardize how trials to be scored were selected. When scoring a trial that does include extraneous behaviours, only those steps which moved the mouse forward were scored. Steps moving backwards, paw replacements or returning the paws to a rung after a rear or a slip were not counted. In the rare case of a trial where the mouse was nudged must be scored, the first step after the nudge was discounted. Steps were classified as successful or as an error step (the mouse's paw slips off a rung, resulting in a displacement of their weight or the mouse cheats and uses the wall to support its weight). Results were expressed as the percentage of steps (successful steps + error steps) that were error steps, for each limb, and are averaged across both trials scored, for each timepoint.

1.6 Cylinder Test

The final motor behaviour test used to assess stroke recovery was the cylinder test, which assesses paw use-asymmetry during voluntary motor function (Schaar et al., 2010; Schonfeld et al., 2017). In this test the mice were placed one at a time in a clear glass cylinder (diameter 10 cm, height 15 cm) with a video camera directly above it. The handler monitored the mice from outside of the room and noted each time the animal reared up on its hind legs to touch the wall of the cylinder. After 15-20 rears, the mouse was returned to its homecage. As the novelty of the cylinder wore off, mice tended to explore the cylinder walls less, so various strategies to encourage activity were employed, including: using cylinders with markings on the sides, making noise, opening and closing the door to the testing room, switching out the cylinder in use and placing a mouse-house outside of the cylinder. To encourage exploratory behaviour this test is always done in the dark under red light.

After testing was completed, a scorer re-watched the videos at 0.12 speed using VLC media player (videolan.org) and timed how long the mouse spent with each paw alone on the wall over the course of each of the 15-20 rears. A single rear was defined as any time the mouse rears up on his hind legs and touches the wall with one or both forepaws. A rear ends when both forepaws have been returned to the cylinder floor. This score was summed and divided by the sum of the time the mouse spends with either their left or their right forepaw alone on the cylinder wall. Time with both forepaws on the cylinder wall was disregarded as it would contribute to scores for both paws.

1.7 Drug Preparation and Administration

Over the course of my stroke recovery work and dosage determination work I administered dihydrexidine (DHX) hydrochloride, SCH23390 hydrochloride and SKF81297 hydrobromide (Tocris, Bristol, UK) by intraperitoneal injection (IP). All drugs were dissolved in sterile 0.9%

saline and vortexed well to ensure complete mixing. Drugs were then filter sterilized using a 0.22 µm syringe filter and were aliquoted into sterilized Eppendorf tubes. With the exception of weighing out the drugs and vortexing, all drug preparation was performed in a biological safety cabinet to keep the process as aseptic as possible. In studies where the drugs were being used as a treatment for stroke, I left the room after preparing the drugs and a collaborator would put the sterile drug and saline control solutions into their respective Eppendorf tubes, and label them with a code, so that I was blind to the animals' drug conditions.

Drugs were given intraperitoneally at a volume of 10 µl/g of mouse, with the exception of dosage experiments requiring multiple injections in one day. In this case mice were given 5 µl/g of body weight of a twice as concentrated solution. In experiments necessitating multiple injections over the course of several days, the side of injection was alternated each day.

1.8 Running Wheels

During the DHX stroke recovery studies, mice were given wireless running wheels in their homecages for 9 days. Running wheels (Med Associates, ENV-044-01) were taped to the cage bottom and the cage was filled with about half the usual amount of filling. Water bottles with shortened spouts were used to ensure the mice could still access the water bottles with the wheels in place. Running wheels connected wirelessly to a hub which recorded wheel rotations and was able to output data in discrete bins of various sizes (Med Associates, DIG-804).

1.9 Perfusion and Tissue Processing

To collect brain tissue for analysis, mice were injected with 0.1ml of sodium pentobarbitol (65 mg/ml, euthanyl, Biomeda-MTC Animal Health Inc., Cambridge, ON, Canada). Once mice were unconscious and non-responsive, incisions were made to open the chest cavity and expose the

heart. A needle connected to a pump (Dynamin Peristaltic Pump, Model RP-1) was inserted into the left ventricle, and a nick was created in the right atrium. The pump was then turned on, at a rate of 7 ml/min, to pump 1X PBS, for 5 minutes. At the conclusion of the 5 minutes of PBS the pump was turned off and, the intake was switched to 4% paraformaldehyde (Sigma) in 1X PBS (PFA), and the pump was turned back on for 10 minutes. Both perfusion solutions were made fresh the day before the procedure and were kept on ice throughout perfusion. Once the animal was fully perfused with PFA the brain was removed and stored in a falcon tube of fresh PFA, which was placed in a 4ºC refrigerator with agitation, overnight. After this the brains were transferred to a 30% sucrose and 0.1% sodium azide in 1X PBS solution and stored at 4ºC with agitation until the sucrose solution had fully permeated the brain and the brain sunk to the bottom of the tube. The tubes were then removed from agitation and stored at 4ºC until cutting. Perfusion and brain removal were performed under a fume hood.

Brains were cut on a microtome (Leica SM 2000R, SM 2010R). Briefly, brains were trimmed using a 1 mm brain matrix (Zivic Instruments, 5325) and mounted on a dry-ice cooled stage. Mounted brains were then covered in dry ice, and cut. Serial coronal sections were collected into 9 consecutive vials of 0.1% sodium azide in 1X PBS, such that the end result was 9 vials filled with representative slices throughout the brain. A tenth vial contained any remaining brain tissue in the sucrose solution. Sliced brains were returned to storage at 4ºC. For the light-dark stroke study, all brains were cut at a 40 µm thickness. For the drug dosage and the DHX stroke recovery studies, brains were cut at a 30 µm thickness.

1.10 Cresyl Violet Staining and Quantification of Infarct Size

Tissue from a single vial was taken and ordered. All slices containing visible infarct, as well as two slices to either side of the infarct were mounted on microscope slides to obtain a

representative sample of the complete infarct. Mounted slides were left to dry overnight and then were stained with 0.25% cresyl violet acetate (pH 4.0, Sigma, St. Louis, MO) in acetate buffer (0.96% acetic acid and 0.54% sodium acetate). Briefly, the staining protocol consisted of dehydration by consecutive dipping into 70% ethanol (EtOH), 95% EtOH, 100% EtOH, followed by rehydration by reversing the order. The slides were then left in milli-Q water for 1 minute, incubated in the cresyl violet solution for 2-5 minutes and submerged repeatedly into fresh milli-Q water, 70% EtOH and 95% EtOH. The slices were then differentiated in 0.25% glacial acetic acid in EtOH for 1-2 minutes, until white matter was visible, and dipped repeatedly into 95% EtOH and 100% EtOH. Lastly the slides were submersed in the clearing agent Citrisolv for a total of 4 minutes and were coverslipped using DPX Mountant for histology (Sigma, St Louis, MO). Coverslipped slides were left to dry overnight at which point the excess mountant was scraped off with a razor blade.

Slides were scanned using an Aperio Scanscope (Leica Biosystems). Whole slide scans were then examined and close-up, high definition pictures of the infarcts were taken with a scale bar in frame. These pictures were then analyzed in ImageJ (NIH), using the scale bar to set the scale and the polygon tool to estimate the area of the infarct. Infarct areas were multiplied by 9 (to account for the 9 representative vials taken) and by 0.03 (for slices cut at 30 μm) or 0.04 (slices cut at 40 μm) to account for the thickness of each slices. These volumes were summed to find the approximate volume of each infarct in mm^3.

1.11 MRI Quantification of Infarct Size

MRI infarct sizing was performed in a similar method to infarct sizing on cresyl violet stained slices. MRI images were opened on ImageJ and the area of the infarct in each image was

measured using the polygon tool and multiplied by 0.5 (the distance between the images in mm), then these volumes were summed to estimate the total volume of the infarct on the MRI.

1.12 Locomotor Activity Test

Locomotor activity was tested using a locomotor activity frame and a Micromax analyzer (Accuscan Instruments Inc., Columbus OH) running the Fusion 5.3 software (Omnitech Electronics Inc.). The activity frames are each able to fit two shoebox cages and use a grid of infrared light beams to monitor the mouse's activity. The frame detects when the mouse breaks one of the infrared beams and thus is able to calculate the distance the mouse travels, how much time the mouse spends in locomotor activity or in stillness and many other output measures.

Cages with a small amount of cage filling (to prevent inadvertent breaking of the beams), a hopper and a cage lid were set up on the frames. For tests lasting longer than a few hours hoppers contained standard mouse food and water bottles were provided. Bedding and houses were not provided, to prevent these items from breaking the infrared beams.

1.13 Immunohistochemistry

The brains were mounted onto slides and allowed to dry overnight. Slices were taken from the region of our strokes, approximately 0.7 mm from bregma according to a mouse brain atlas (Paxinos and Franklin, 2013). An antigen unmasking step, wherein the slides were submerged in hot 0.01M citric acid solution (pH 6) for 15 min was performed. Slides were rinsed quickly in a 1X Tris-buffered saline (TBS) solution and submerged in 0.1% trypsin in 0.1M Tris and 0.1% $CaCl_2$ solution for 10 min for permeabilization. After 3 more quick rinses in TBS, the slides were denatured in 2N HCl in TBS for 30 min. After 3 further rinses in TBS, the slides were dried and circled with a PAP pen (Sigma-Aldrich, Oakville, ON, Canada), to prevent runoff of solutions

placed on the slide. Next a blocking solution, consisting of 3% normal donkey serum (NDS) (Cedarlane, 017-000-121) and 0.3% Triton X100 in TBS, was applied for 1 hour. The blocking solution was removed and without washing, the primary antibody solution was applied. The primary polyclonal rabbit antibody, anti-c-fos (Ab190289, 1:4000 dilution, Abcam), in a 3% NDS, 0.3% Tween20 and TBS solution was applied and left to incubate in room temperature for 24 hours. After the incubation period 3 quick washes were applied. The secondary antibody, Biotinylated donkey anti-rabbit antibody (1:200 dilution, Abcam) in a 1.5% NDS and TBS solution, is then applied for an hour. After three quick rinses with TBS, a solution of 0.3% hydrogen peroxide in TBS was left on the slides for 30 minutes to quench the endogenous peroxidases. After that solution was rinsed, a solution of avidin-biotin complex (Vector Elite) in TBS, made at least 30 minutes ahead of time, was applied and left for 1.5 hours and then rinsed 3 times with TBS. To visualize the antibodies, a solution of 3,3'-diaminobenzidine (DAB, Pierce) in a 1:10 dilution in Pierce DAB buffer was applied under a fume hood for 3.5 minutes. The DAB was rinsed off 3 times with TBS and the slides were counter-stained by quickly dipping into Fast Red solution (Vector Laboratories Inc) and then running clean water through the slide-container. The slides were then dehydrated in escalating concentrations of EtOH and left in Citrisolv for clearing. Slides were coverslipped with DPX mountant and left to dry overnight. Once slides were dried any excess mountant is scraped off, and the slides were scanned on an Aperio Scanscope.

For analysis, images of whole slides were opened in FIJI (NIH), and the Physical Size X and Size X parameters were recorded. Images of the individual brains were saved as TIFFs. Individual TIFFs were opened and a scale was set for the lower resolution picture. Eight boxes, 300×300 pixels, taken from both sides of the striatum were manually selected. The background

was subtracted, using the subtract background tool at setting 5 pixels, and the sections were converted to RGB stack format. The red colour channel was the only one analyzed. A threshold was applied, which created a binary picture. The threshold level was 229 for the light-dark stroke c-fos analysis and 220 for the acute DHX injection c-fos analysis. The image was processed by applying a watershed and filling holes. The analyze particles menu was used to specify a size of 3-50 pixels^2 and a circularity of 0.25-1.00 to select only for those particles which are c-fos positive cells. Furthermore, the quantifier reviewed the images and was able to discount any particles they believed did not represent cells, based on the presence of artifacts in the original image at the same location. A particle count was generated, and this was averaged across the eight boxes taken for each brain. This average was then divided by the physical size of the 300×300 pixel box in μm to ensure an even comparison.

1.14 Statistical Analysis and Randomization

Statistical Analysis was performed by GraphPad Prism (version 7.03). Simple comparisons between two conditions at one timepoint were performed using an unpaired t-test, with Welch's correction applied when analysis indicated heteroscedasticity of variances in the groups tested. Locomotor activity following drug administration was evaluated using two-way analysis of variation (ANOVA) when comparing the effect over time. One-way ANOVA was used when examining the effect in a single time-bin. A Dunnet's post-test was employed to examine individual differences. When overly large differences in groups skewed testing such that a one-way ANOVA was not practical, multiple t-tests comparing a given drug condition to the saline group at different timepoints, and corrected for the multiple comparisons within the two groups at different timepoints, but not for comparisons with other groups, were employed instead. Stroke behavioural data was evaluated using repeated measures two-way ANOVA. This was

followed by a Sidak's post-test, comparing all timepoints to the poststroke timepoint. Lastly, the Spearman correlation was used to examine the relationship between two factors. All data is expressed as the mean ± standard error of the mean (SE), unless otherwise stated.

For the drug dosage studies mice were randomly assigned to a drug condition using a free, online, random group generator (https://www.randomlists.com/team-generator) or random list generator (https://www.random.org/lists/). When mice were reused they were re-randomized for each experiment. The injection history of mice in the dosage experiments can be seen in appendix 3, Tables 1,2 and 3.

For the DHX stroke recovery studies mice were separated into two groups of roughly equal deficit based upon their performance on the adhesive removal test at the poststroke timepoint. Mice were typically ordered by their deficit and assigned a group in alternating fashion (the most impaired animal in group 1, the second most impaired animal in group 2, the third most impaired animal in group 2, the fourth most impaired animal in group 1, etc.), and then further adjustments were made to try to maximize the agreement between the two groups in terms of mean and SE of the deficit, for both time to contact and time to remove the tape. Once the groups were made a collaborator with no knowledge of the degrees of deficit of each group, assigned the groups to either the DHX or saline condition, keeping the drug administrator blind.

2. Outlines of Specific Experiments

2.1 Light-Dark Stroke

Data for the Light-Dark stroke study was collected over the course of two cohorts, each consisting of 20 mice. Mice were brought into the facility at 6 weeks of age and single housed in either normal light-dark cycle or reverse-light dark cycle housing. Due to the use of reverse light-

dark housing the acclimation period (as detailed above) lasted two weeks for both groups. Mice were pretrained with 4-5 days of the adhesive removal test (8:00 AM-10:00 AM), two days of the horizontal ladder test (13:00 PM-17:00 PM), and one baseline test of the cylinder test (12:00 PM -17:00 PM). Mice underwent photothrombosis. All mice in cohort 1 underwent MRI, while only 6 mice (3 from each group) from cohort 2 underwent MRI. The first poststroke timepoint began 2 days after stroke. This consisted of the adhesive removal test on the first day, the adhesive removal test and the horizontal ladder test the second day and the cylinder test on the third day. Two weeks later tests were repeated at poststroke timepoint 2 (21-23 days from stroke). Finally, on day 25 after stroke, the animals were sacrificed, and brain tissue was collected, as outlined above. The timeline for these experiments can be seen in Figure 2.

For these cohorts analysis of each cylinder test video was performed by two independent scorers, one of which was blind to mouse group during analysis. Scorers were always within 10% error of one another. Data is presented as an average of both scorers. Analysis of the horizontal ladder was again done by two independent scorers, one blinded to experimental timepoint. Scorers were always within 10% of one another and the average of both scores was reported. Infarct sizing from cresyl violet stained slices was performed by two scorers, again agreement between scorers was high, and an average was reported.

As the variable tested in this experiment was the established housing conditions of the animals, randomization was done at the level of housing assignment when the animals first arrived at the facility. Outcomes of the mice in both cohorts of the light-dark stroke study can be seen in Figure 3.

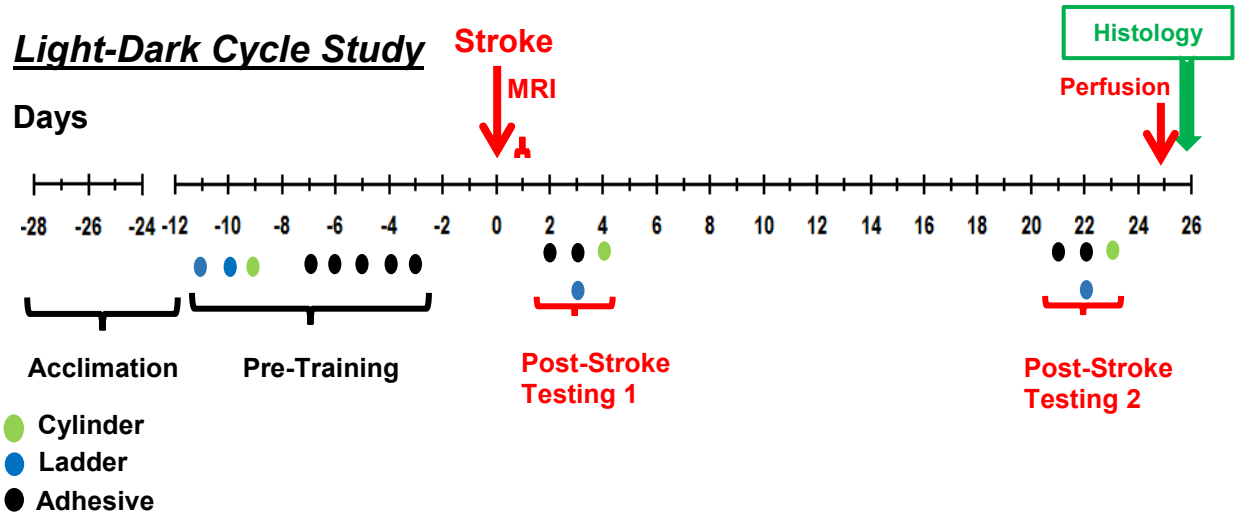

Figure 2: Outline of the Light-Dark Stroke Study

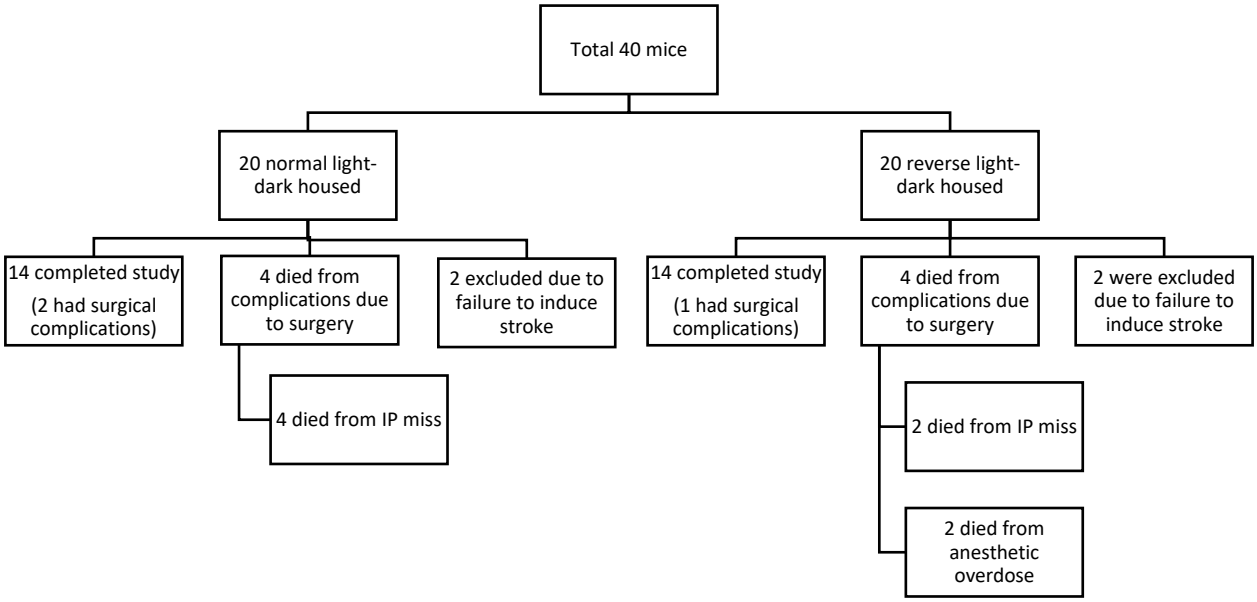

Figure 3: Mouse Outcomes during the Light-Dark Stroke Study. Of the 20 mice assigned to the normal light-dark housing condition (light group), 14 completed the study, two of which had mild complications due to surgery, 4 died as a result of an IP miss during the photothrombosis study and 2 were excluded as they did not sustain a stroke during the photothrombosis procedure. Of the 20 mice placed in reverse light-dark housing (dark group), 14 completed the study, one of which had mild complications from the photothombosis procedure. Additionally, 2 died due to an IP miss during surgery, and 2 died due to a presumed anesthetic overdose during photothrombosis. A further 2 mice were excluded as they did not develop strokes. The mortality and complication outcomes between the two groups were very similar and do not suggest a difference between groups whose strokes occur during their active or inactive cycle at this level.

2.2 Acute DHX Dosage

Eight-week-old mice were ordered and housed in reverse-light dark housing. After two weeks of acclimation, including standard handling, locomotor activity of the mice following an acute injection of either saline, 0.25 mg/kg DHX, 1 mg/kg DHX, 4 mg/kg DHX or 16 mg/kg DHX, was tested, over two consecutive days (each mouse was tested once, see appendix 2 Fig. 35 for results). After a 5 day washout period, 8 mice, from this experiment, were randomized to a single dose of DHX either 4 mg/kg DHX or saline, between 9:00 AM and 12:30 PM, and this time, underwent perfusion 1 hour after injection. Brain tissue was collected for immunohistochemical analysis of c-fos levels.

2.3 Chronic DHX Dosage

Another cohort of 8 weeks old mice were ordered to study chronic DHX administration. Again, animals were reverse light-dark housed and given two weeks of acclimation to the facility and to standard handling. Animals were placed in cages on the locomotor activity frame for two hours of acclimation prior to injection. Based upon the results of the acute DHX dosage experiment, doses of 1, 4 and 16 mg/kg of DHX and a saline control were used. Mice were given daily DHX injections (between 10:30 PM -12:20 PM) for seven days, and remained on the locomotor activity frame for the entirety of the time.

2.4 Acute Dosage Determination for SCH23390

Forty-eight 8-week old mice were ordered, and underwent acclimation as described. Over two consecutive days, mice were moved to cages on the locomotor activity frame between 9:30 AM-10:00 AM and allowed to acclimate for 1 hour. On day one mice were injected with either saline, 2.5 mg/kg SKF81297, 0.01 mg/kg SCH23390 or 0.5 mg/kg SCH23390. On day two, naive mice

were injected with either saline, 2.5 mg/kg SKF81297, 0.1 mg/kg SCH23390 or 1 mg/kg SCH23390. Animals acclimated to the locomotor activity frame for 1 hour prior to injection. After injection their locomotor activity was monitored for a further 1.5 hours.

2.5 Co-administration of SCH23390 and DHX

After a two-week washout, animals which had received the lowest doses during the chronic DHX dosage experiment, were returned to the beam break (around 9:00 AM) for two hours of acclimation and then injected first with either saline or 0.5 mg/kg SCH23390. After 20 minutes, the animals received a second injection of either saline or 4 mg/kg DHX, and then their locomotor activity was monitored for 1.5 hours. A total of 18 mice were used in this experiment. Mice were re-randomized to prevent undue carry-over effects from the previous experiment.

2.6 Seven-Days DHX Stroke Recovery

Three cohorts of mice (20, 22 and 24 mice) underwent the procedures for the 7-days DHX stroke recovery experiments. The mice arrived, at 6 weeks old, and were given normal acclimation procedures. After two weeks in the facility, mice began behaviour, with training and testing on the horizontal ladder (1:00 PM-5:00 PM) and testing on the cylinder test (12:00 PM-6:00 PM). The exact days of these initial tests varied somewhat between cohorts, but a sample timeline can be seen in Figure 4. After these tests was a week of adhesive removal training for 4-5 days (between 8:00 AM-12:00 PM), depending on how fast a given cohort reached a plateau in performance. The animals then underwent photothrombosis followed the next day by MRI. Mice were tested two days after stroke on the adhesive removal test for two consecutive days, the horizontal ladder test once, in the afternoon following the adhesive removal test, and the cylinder test, in what I have called the post-stroke timepoint. On day 5 after stroke, the 7-days drug

regimen, consisting of daily injections of 4 mg/kg DHX or saline between 9:30 AM-12:00 PM, began. Three days after the conclusion of the drug regimen (14 days after stroke), the three-day behavioural testing procedure was repeated for the post-drug timepoint. At 18 days after stroke, the mice had running wheels installed in their home cages, as described above. Two days after the wheels were removed, at 28 days post stroke, the final round of behavioural testing (post-wheel timepoint) began. At 31 days post stroke the mice were euthanized, and brain tissue was collected, as described above. Mouse outcomes throughout the study can be seen in Figure 5.

For this study and the DHX 2-days study, video analysis was performed by a single primary scorer. An independent secondary scorer performed spot checks of 20-25% of the videos to confirm accuracy. The same agreement between scorers' criteria as the Light-Dark stroke study was applied.

2.7 Two-Days DHX Stroke Recovery

Two cohorts of mice, (22 and 24 respectively) were used in this experiment. The procedures for the 2-days DHX stroke recovery experiment were exactly the same as those for the 7-days DHX experiment, with one exception: the drug was given for 2 days, starting 10 days after stroke. Drug administration was performed between 10:00 AM-10:30 AM. The timing of behavioural testing was kept consistent between the studies to allow for comparison between their outcomes. The timeline for the 2-day DHX experiment is shown in Figure 6 and the mouse outcomes in Figure 7.

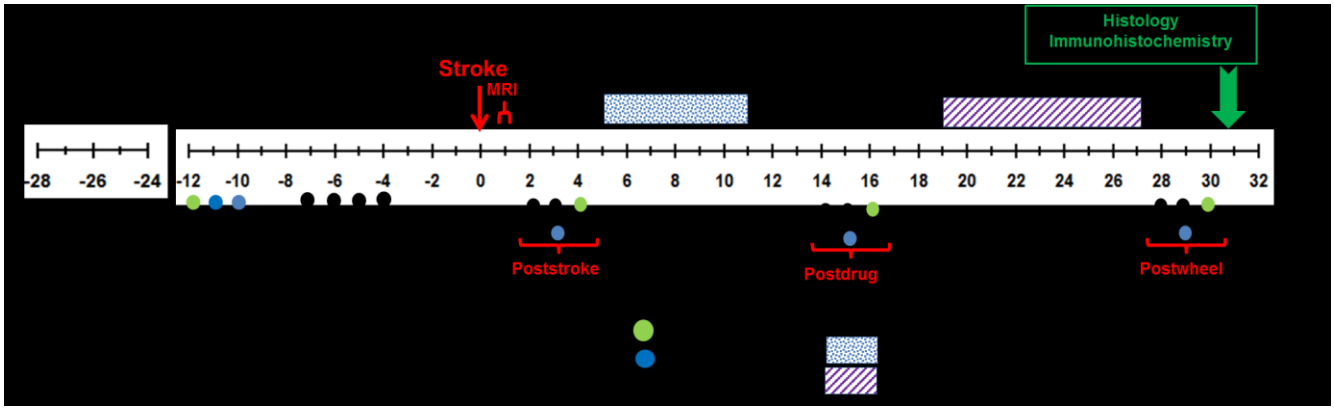

Figure 4: Timeline of the DHX 7-days Stroke Recovery Experiment

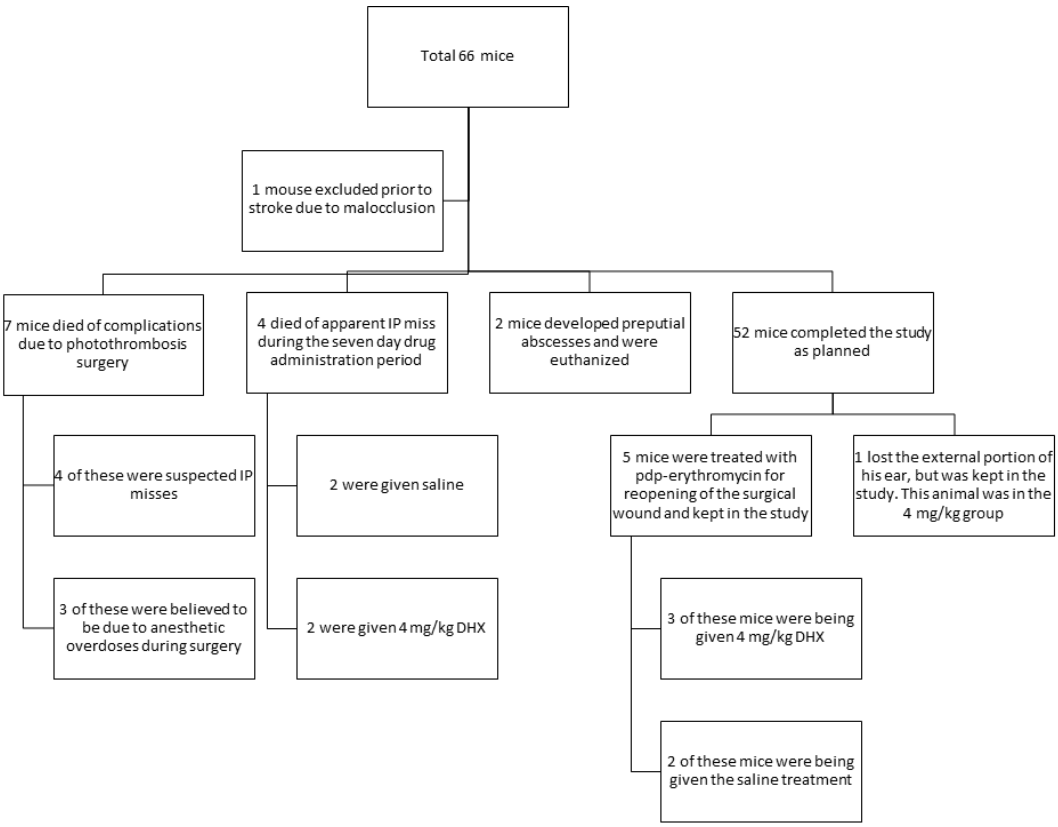

Figure 5: Mouse Outcomes and Complications from the 7-Days DHX Study. A total of 66 mice were ordered for the DHX 7 days studies. Of these, 10 died prior to the beginning of drug administration (1 to malocclusion, 7 to surgical complications, and 2 to preputial abscesses). Over the course of 3 cohorts, 4 mice died during the drug administration period, however these were equally split between saline and DHX treatment. Among the 52 mice that completed the study, 5 were treated for reopening of the surgical head wound. These were nearly equally split between the two treatment groups, indicating that the drug treatment did not increase the occurrence of this complication. 1 DHX mouse lost the external portion of his ears. While the cause of this is unclear, we consider it unlikely to be associated with the drug treatment was we have observed it in another mouse that was undergoing a different treatment regimen (data not shown).

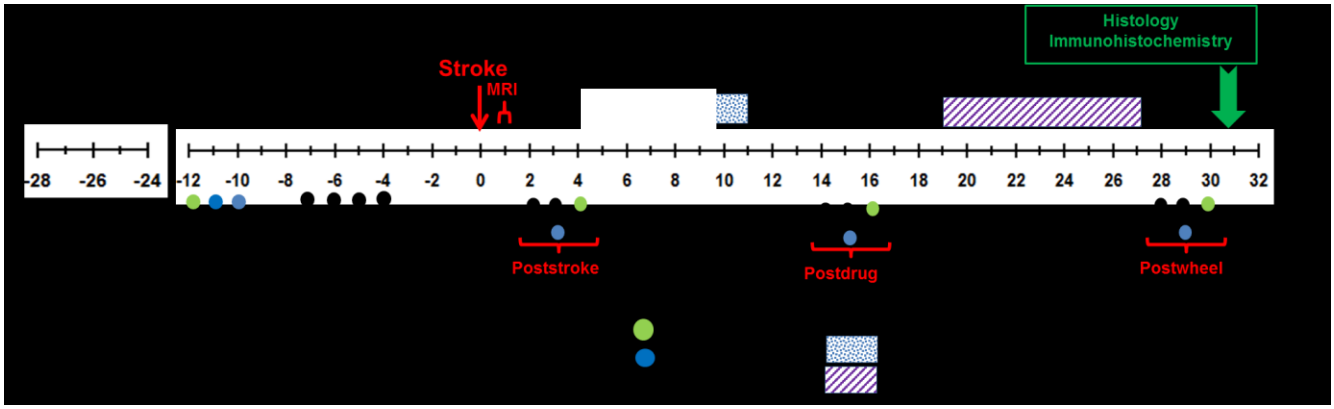

Figure 6: Timeline of the DHX 2-days Stroke Recovery Experiment

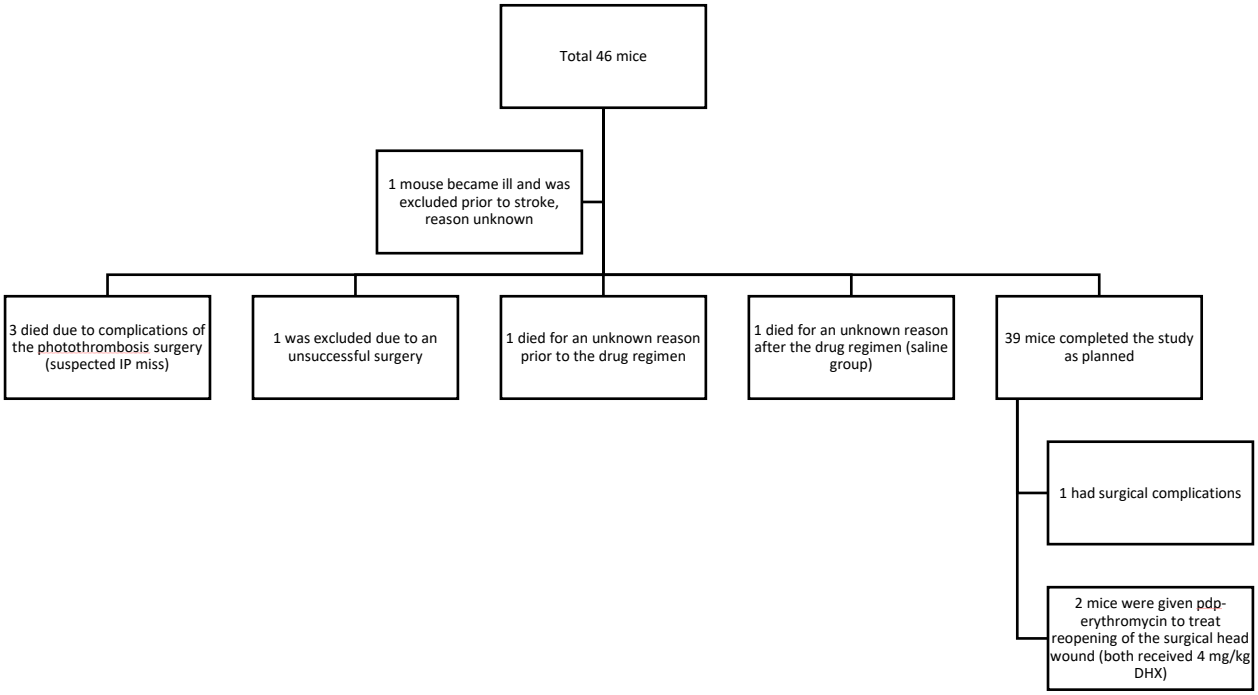

Figure 7: Two-Days DHX Study Mouse Outcomes and Complications. A total of 46 mice were used to examine the effect of 2 days of 4 mg/kg DHX treatment on stroke recovery in mice. 1 mouse was excluded due to illness before the stroke. 3 died due to suspected IP misses, sustained during the photothrombosis surgery. 1 died for unknown reasons days after the stroke and prior to the drug administration period. 1 died during the drug regimen, but belonged to the saline group. 39 mice completed the study as planned. 1 experienced complications due to surgery. As no sign of pain or altered behaviour was observed this mouse was kept in the study, however as a precaution this mouse was excluded from horizontal ladder analysis. 2 mice were given erythromycin due to reopening of the surgical head wound, but were kept in the study.

Results

1. Light-Dark Stroke Study

1.1 Sensorimotor Behaviour Following Stroke

In order to optimize the photothrombosis stroke model for use in stroke recovery studies, the light-dark stroke study was undertaken to determine if there is an effect of the time of stroke induction on sensorimotor and motor behavioural outcomes following stroke. To this end, half of each cohort was housed in a room under normal light-dark cycle conditions (light group) and half in reverse light-dark cycle housing (dark group), with stroke and behaviour occurring under condition appropriate lighting.

1.1.1 Cylinder Test

Results on the cylinder test (Fig. 8) revealed a significant preference for use of the left paw while exploring the cylinder wall after stroke as evidenced by the disuse of the right paw. This preference was stable, and did not show spontaneous recovery at 22 days poststroke. There were no statistically detectable differences between animals that had their strokes during their inactive (light) phase, versus during their active (dark) phase.

1.1.2 Horizontal Ladder Test

On the horizontal ladder test mice in both groups showed an increase in the percentage of missteps after the stroke with both their right forepaws and hindpaws (Fig. 9 B and D). Meanwhile both groups showed very little change in their ability to use their left (unaffected) fore and hind-limbs while crossing the ladder (Fig. 9A and C). In the right forepaw, both groups performed similarly, and in both groups the deficit was still apparent at 21 days poststroke. Surprisingly, there is an apparent increase in deficit between poststroke day 3 and poststroke day 21 on the right forepaw in the light group. This appears to be caused by uneven distribution of

animals which show no deficit on this test between the two groups (Four mice in the light group versus only 1 in the dark group). Change in performance of the right hind paw was similar between the two groups, with both groups showing apparent spontaneous recovery in the right hind paw.

1.1.3 Adhesive Removal Test

Training on the adhesive removal test demonstrated a trend towards faster learning of the task in the dark group than in the light group, with the dark group appearing to reach their fastest time earlier in the training period for several of the outcome measures (Fig. 10). Additionally, dark group animals proved to be more proficient at the task, as their average time to remove the tape before stroke (the average of the time to remove the left tape and the right, over the last two days of training) proved to be significantly faster (Fig. 10E). The training period lasted 4 days for Cohort 1 and 5 days for Cohort 2, so the training data was not collapsed. The time to contact and remove the tape with the left forepaw did not change in either group (Fig. 11A and C). After stroke both groups showed a statistically detectable deficit in the latency to remove the tape from the right forepaw. The light group approached a significant deficit on the latency to contact the tape, while the dark group did show a significant deficit on this outcome measure. There was no significant difference between the level of deficit between the light and dark groups (Fig. 11B and D).

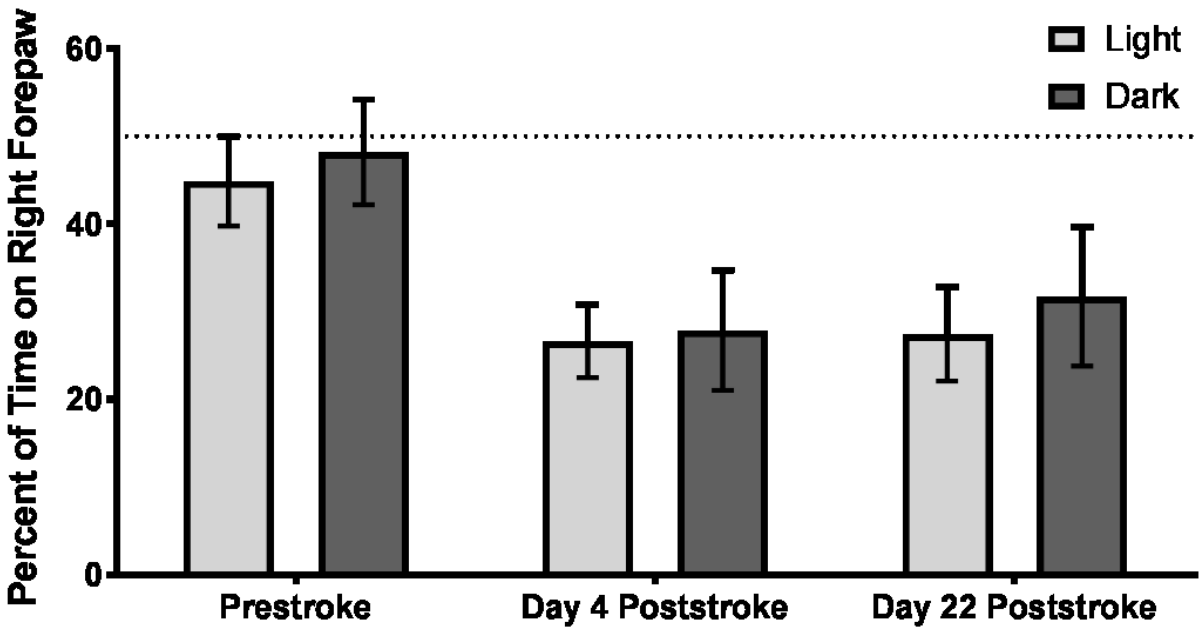

Figure 8: Light-Dark Stroke Study Percent Time Spent with the Right Forepaw on the Cylinder Wall during Rears. The mean percentage of time spent using the right forepaw to explore the wall versus total time spent exploring the wall (time spent on right paw / time spent on either the right or the left paw) is shown for each timepoint measured. The dotted line indicates 50% use of the right forepaw. A significant effect of time ($p<0.0001$) and subject matching ($p<0.0001$) was observed on a two-way repeated measures ANOVA. A Sidak's post-test indicates significant differences between the Prestroke and Day 4 Poststroke timepoints for the light group ($p=0.0041$) and the dark group ($p=0.0013$). n=14 for each group.

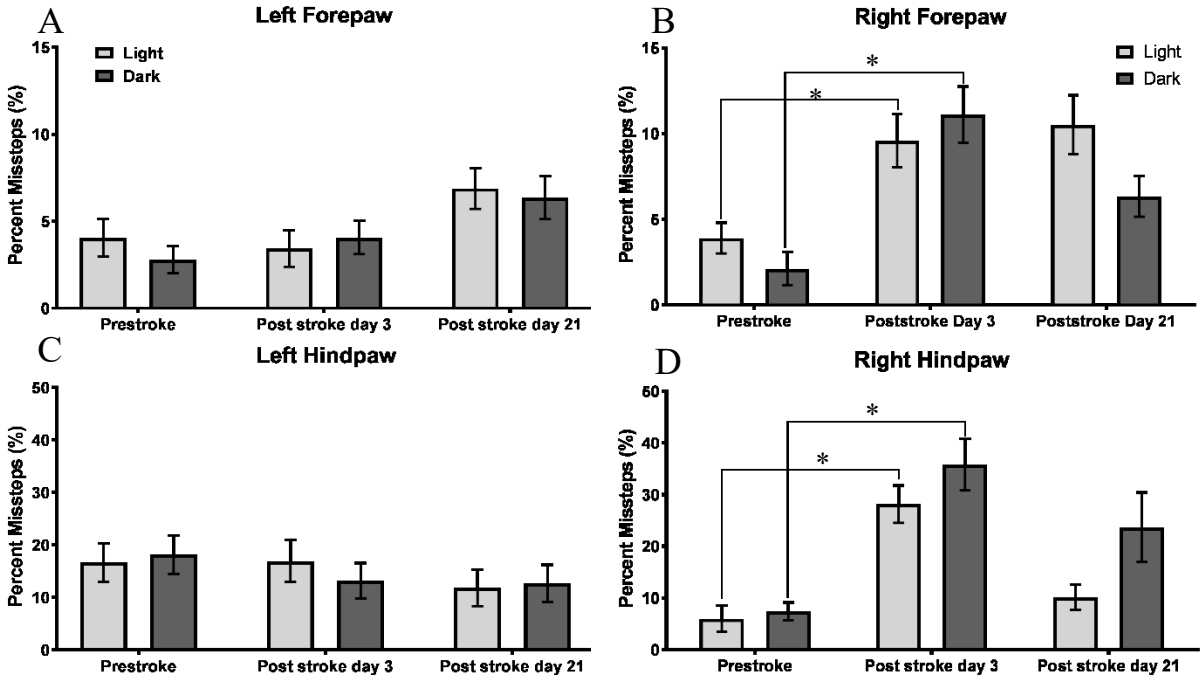

Figure 9: Light-Dark Stroke Study Percent Missteps on the Horizontal Ladder. Panels A and B show the results of both groups in use of the left and right forepaw respectively. A two-way repeated measures ANOVA shows a significant effect of time ($p<0.0001$), with a Sidak's post-test showing statistically detectable differences between the Prestroke and Poststroke Day 3 timepoints for both light ($p=0.0116$) and dark ($p<0.0001$). Panels C and D show the results of the left and right hindpaw during ladder crossing. Repeated measures two-way ANOVA shows a significant effect of time ($p<0.0001$) and subject matching ($p=0.0013$). The Sidak's post-test indicates a statistically detectable difference between the Prestroke and Poststroke Day 21 timepoints for both light ($p<0.0001$) and dark ($p<0.0001$). All data is shown as the percentage of missteps (total missteps with a given paw/total steps taken with a given paw)*100, n=14 for both groups.

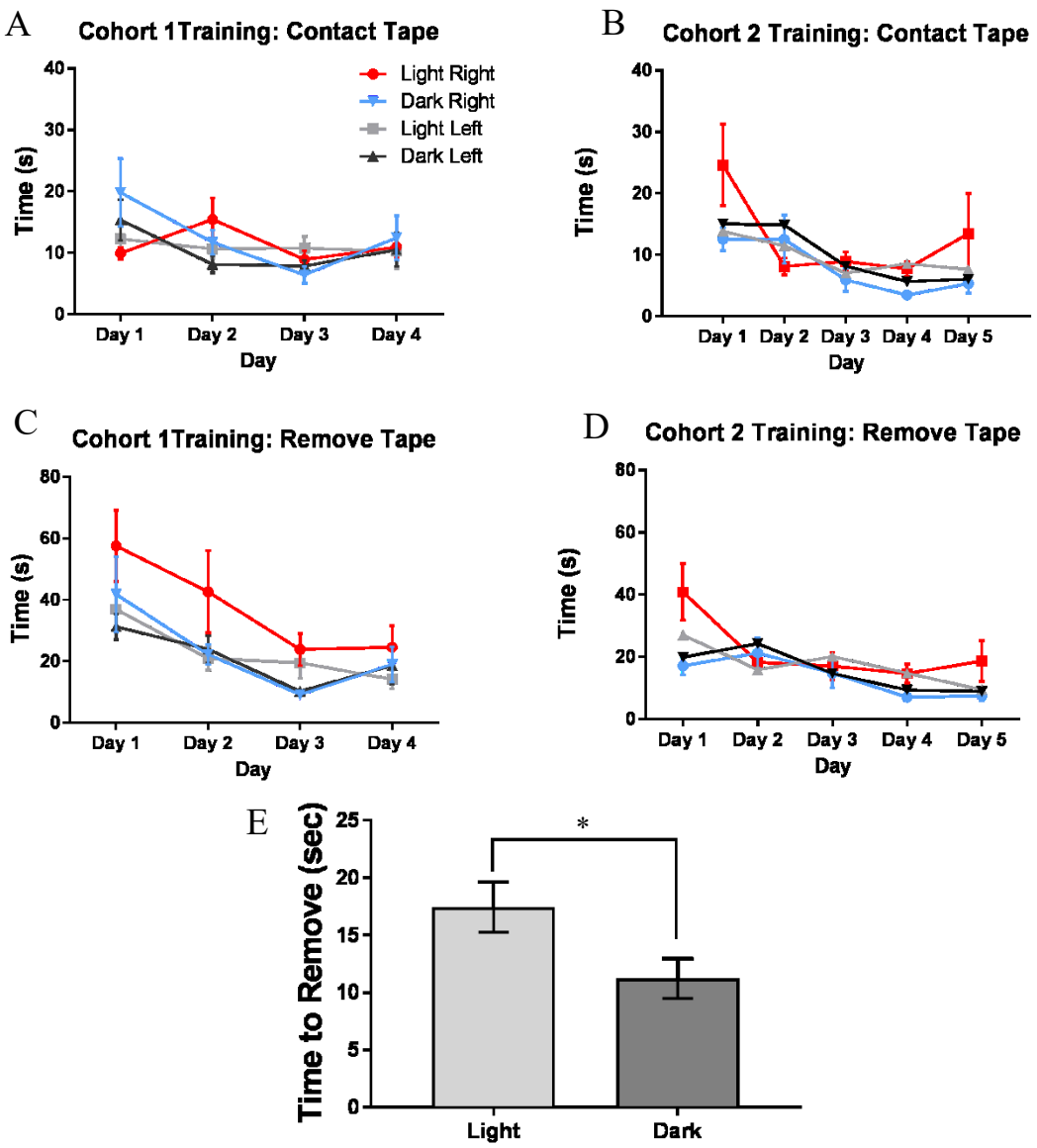

Figure 10: Light-Dark Stroke Study Adhesive Removal Test Training Panels A and C show Cohort 1's mean training times to contact (A) and remove (C) the tape. These mice were trained for 4 days, at which point they showed a tendency to overtraining, and so training was halted. Panels B and D show Cohort 2's mean training time to contact (B) and remove (D) the tape. These mice were given 5 days of training as they had not reached a plateau at 4 days. The sample size was identical (n = 10) for all cohort groups as training data was taken prior to stroke. Panel E shows the mean time to remove the tape (average of both left and right paw) on the last two days of training for animals included in the study (the prestroke score) for both groups. p=0.0310 on an unpaired t-test with Welch's correction. n=20 for both groups.

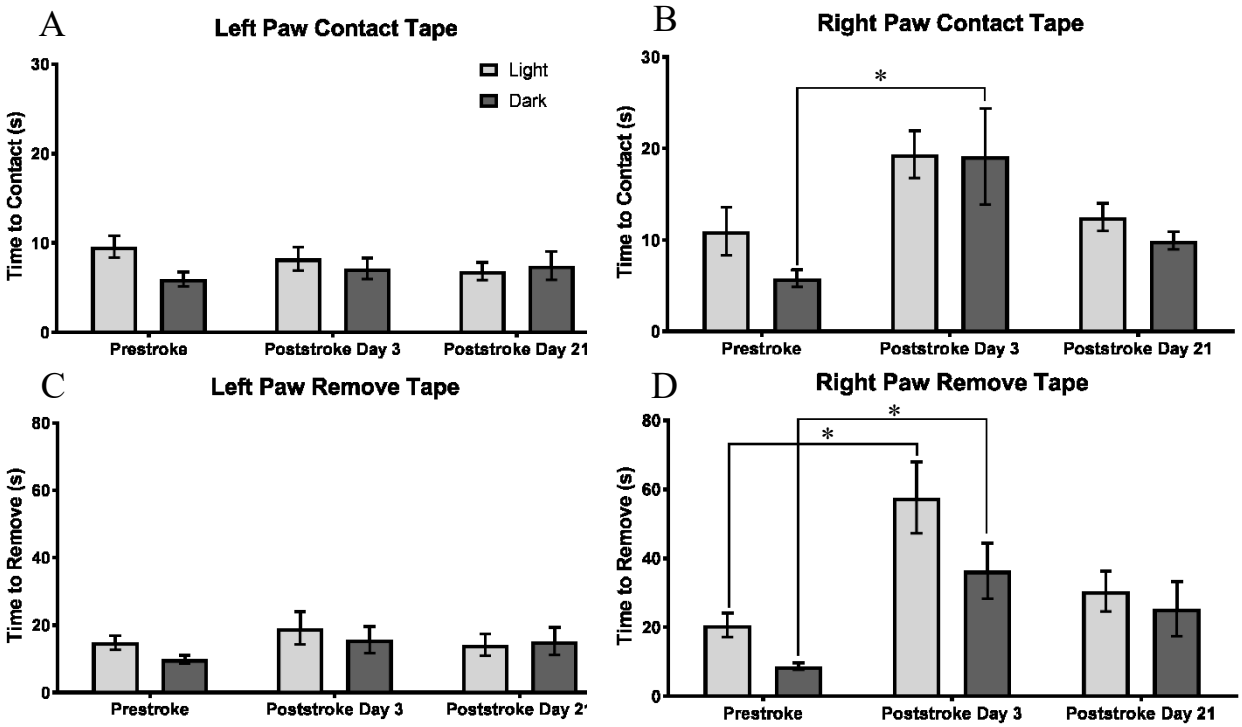

Figure 11: Light-Dark Stroke Study Adhesive Removal Test Results A and B show the time to contact the tape before stroke and at 3 and 21 days post stroke for the left forepaw and right forepaw. A significant effect of time ($p=0.0004$) for the right forepaw time to contact the tape was detected. The post-test indicates a significant difference between the Prestroke and Poststroke Day 3 timepoints in the dark group ($p=0.0015$), with the light group approaching a significant difference between the Prestroke and Poststroke Day 3 timepoints ($p=0.0562$). C and D show the time to remove the tape prior to stroke and 3 days and 21 days after stroke for the left and right forepaws. There is a significant effect of time ($p<0.0001$) and subject matching (0.0157) for the right forepaw time to remove the tape, and a statistically detectable difference between the Prestroke and Poststroke Day 3 timepoints for both the light ($p=0.0001$) and dark ($p=0.0036$) groups. The timepoints of Poststroke Day 3 and Poststroke Day 21 represents results calculated from the scores obtained on testing done on days 2 and 3, and days 20 and 21 poststroke respectively. Significance was determined by a repeated measures two-way ANOVA followed by a Sidak's post-test. n= 14 for all groups.

1.2 Infarct Size as Measured from Cresyl Violet Stained Slices

No statistically detectable differences were e observed in the infarct sizes at 24 days poststroke, between the light and dark groups, as shown in Fig. 12. MRI images, such as those depicted in Fig. 12A, show infarcts that appear larger than those measured from cresyl violet stained slices (Fig. 12B), both due to the earlier timepoint of these images and the method employed, which indicates edema as part of the infarct. MRI infarct sizes were not quantified for this study.

1.3 Amount of c-fos Positive Cells

As a rough measure of brain activity and plasticity during the behavioural testing hours, immunohistochemistry for immediate early gene c-fos, a putative marker of cell activity, was employed. No stimulus or drug treatment was applied to these animals, other than the euthanasia methods necessary for tissue preservation. As the timeframe of c-fos expression exceeds the timeframe for euthanyl to take effect, this is expected to represent differences in the baseline expression of c-fos during the light-dark cycle, and not as a response to stimulus. Examination of the average level of c-fos in the striatum of stroke mice sacrificed during their dark cycle (206 cells/μm^2) showed more than double the amount of c-fos^+ cells expressed in animals sacrificed in their light phase (94 cells/μm^2) (Fig. 13).

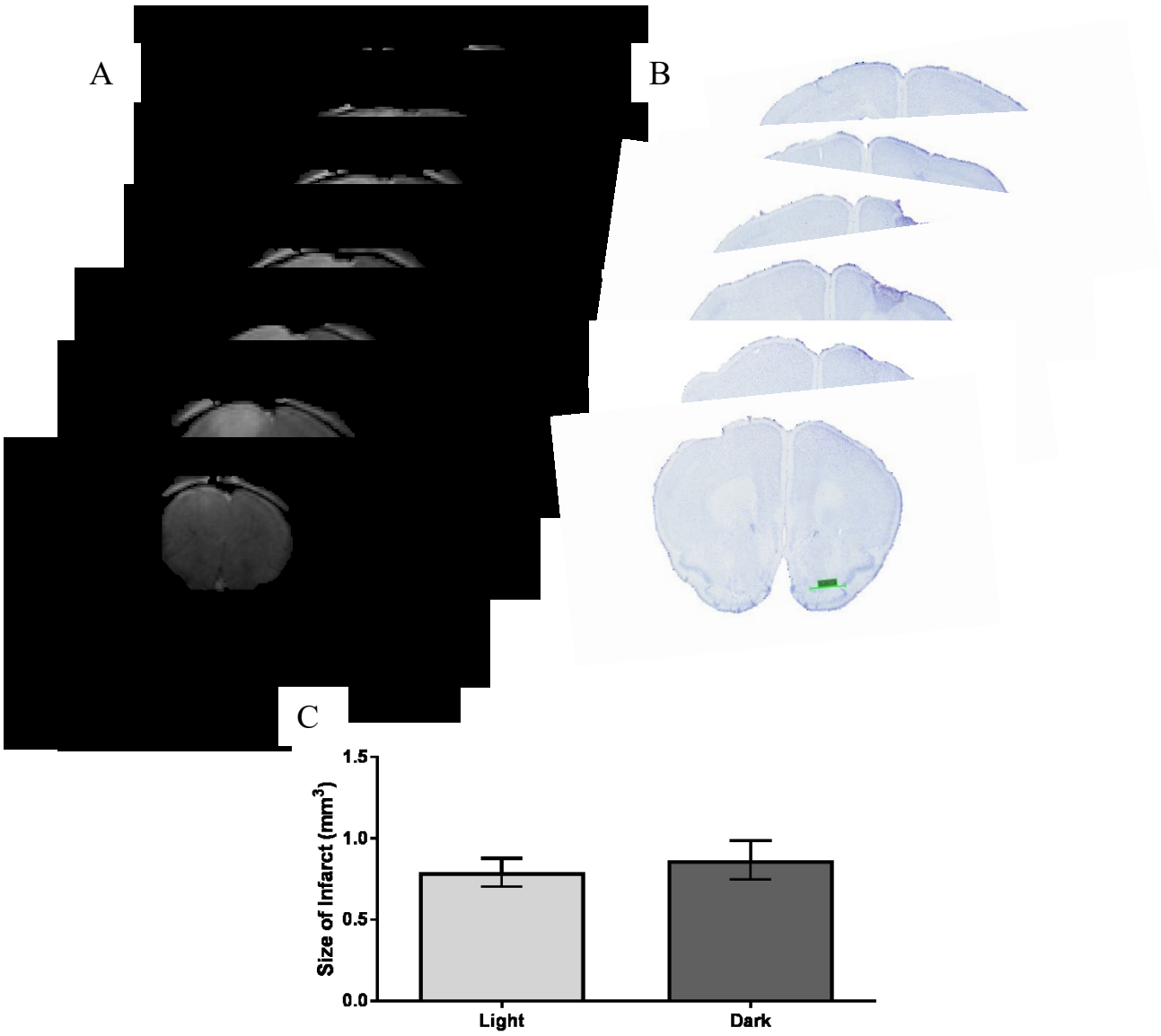

Figure 12: Light-Dark Stroke Study Infarct Size Results A and B show a representative sample of our MRI (24 hours poststroke) and cresyl violet stained (24 days poststroke) brains. This particular brain comes from a Light group mouse whose infarct at 24 days was 0.83mm^3. C shows the results of infarct sizing done from cresyl violet stained brains. n=14 in each group.

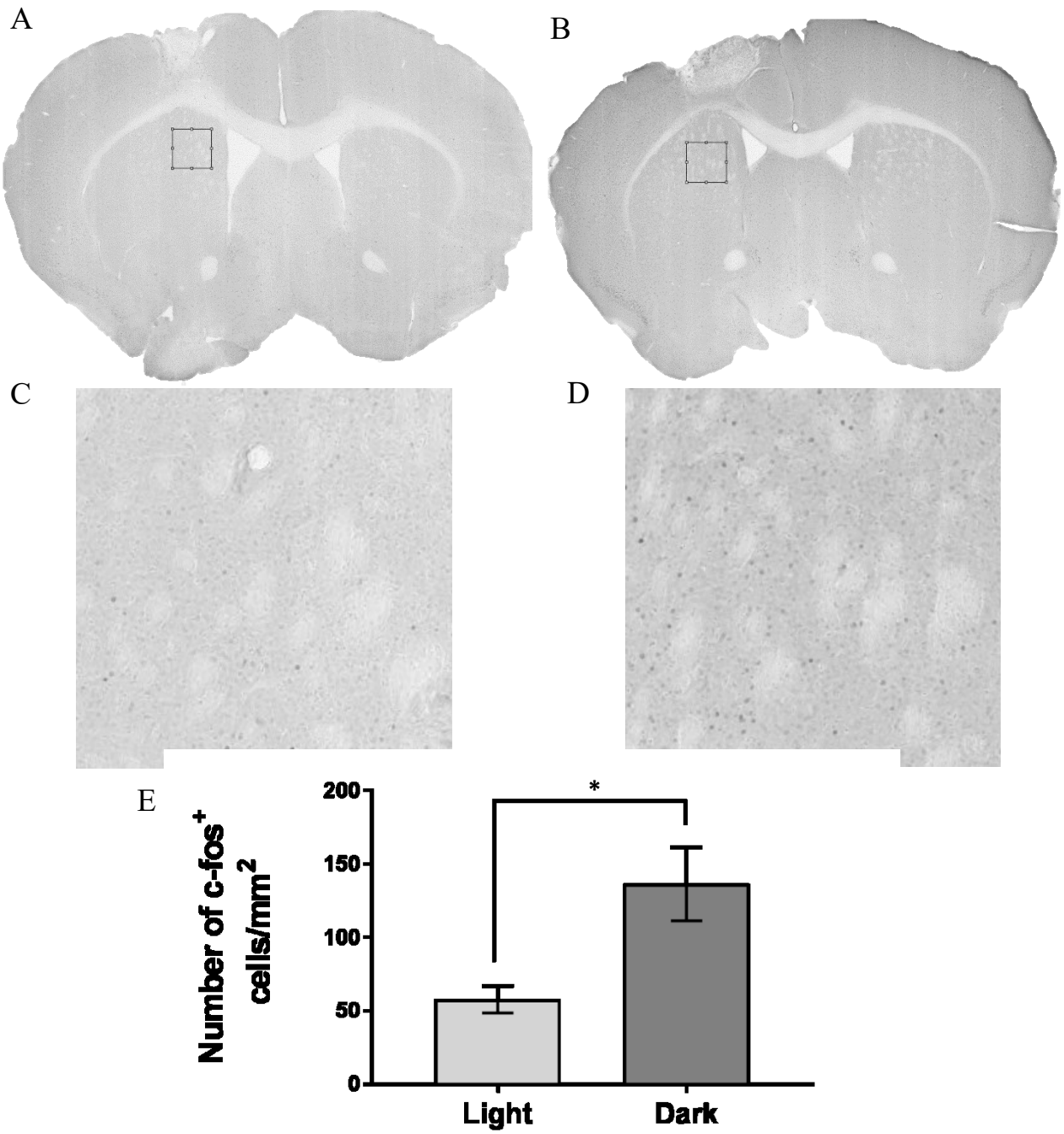

Figure 13: Levels of c-fos$^+$ Striatal Cells in Light and Dark group animals. Panel A and B shows a representative coronal mouse brain section from a light and a dark group animal, respectively. Panels C and D show 300×300 pixel boxes shows the c-fos$^+$ cells counted for the regions highlighted in panels A and B, respectively. Eight 300×300 pixel boxes were sampled for a single slice in the region of 0.7 mm anterior of bregma, for each brain sampled, and data was scaled to reflect the physical area sampled. The graph in panel D shows the group mean of the average number of c-fos containing cells in the striatum expressed as cells/μm^2. p= 0.0099 by an unpaired t-test with Welch's correction. n=11 for the light group and n= 13 for the dark group.

2.Dosage Determination for DHX and SCH23390 In Reverse Light-Dark Housed Mice

2.1 Twenty-Four Hour Locomotor Activity Over Seven Days of DHX Administration

In order to select an appropriate suboptimal, yet still bioactive dose, the effect of various doses of D1-class agonist DHX, on locomotor activity was evaluated. Twenty-four hour monitoring of mice receiving seven consecutive daily i.p. injections of DHX showed a clear and dramatic effect of 16 mg/kg DHX (Fig. 14). The 16 mg/kg animals showed large increases in locomotor activity after the injection and interestingly, around day 4 of injection we observed an increase in activity above the levels of the other groups, outside of the time surrounding the injection. This suggests a possible behavioural sensitization to this high dose of the drug. Anecdotally, it was noted that these animals became much harder to work with as they were jumpy, hyperactive and prone to darting or trying to jump out of the cage. Even two weeks after the conclusion of this experiment, when the mice had been returned to regular home cages, they remained noticeably jumpier as compared to mice who had received lower doses. Interestingly, two of the five mice in this group died shortly after the 7^{th} injection. It is unclear if this was a result of a drug overdose or an IP miss brought on because of squirming of the hyperactive mice. The differences in the behaviour of mice receiving the 16 mg/kg DHX dose chronically is further emphasized by the fact that the 16 mg/kg group had an average distance travelled over the seven days, that was statistically detectably greater than the saline group. Given the short active period of DHX in rodents, and the very long period of measurement, this truly indicates a change in behaviour outside of the active period of the drug.

2.2 Analysis of Short-Term Locomotor Response Following Chronic DHX Injection

As DHX is a relatively short acting drug, it is not expected to cause changes in motor behaviour outside the active period of the drug at most doses. As such, it was important to look at the level of locomotor activity shortly after injection. Figure 15 shows the level of locomotor activity 60 minutes after the injection for each of the seven days. DHX doses of 4 mg/kg and 16 mg/kg tended to increase locomotor activity above the level of saline injected animals. Both the 4 and 16 mg/kg doses produced statistically detectable increases in locomotor activity on day 1 of the chronic injection paradigm. The 16 mg/kg dose continued to display a strong trend towards increasing locomotor activity after injection, but this difference was not statistically detectable until day 4. The 4 mg/kg dose also continued to display a trend toward increasing locomotor activity as compared to saline treated animals, albeit to a much lesser degree than the 16 mg/kg dose. This trend did not return to statistically detectable levels until day 6 of the drug regimen. This furthers the evidence of sensitization to the behavioural effects of the drug, although the 4 mg/kg dose did not produce an increase in locomotor activity outside of the active period of the drug. As the 4 mg/kg dose produced a consistent trend of increased locomotor activity which was statistically detectable at several timepoints, while not having the same undesirable effects on the overall activity levels of the mice as the 16 mg/kg dose, the 4 mg/kg dose of DHX was selected for further testing.

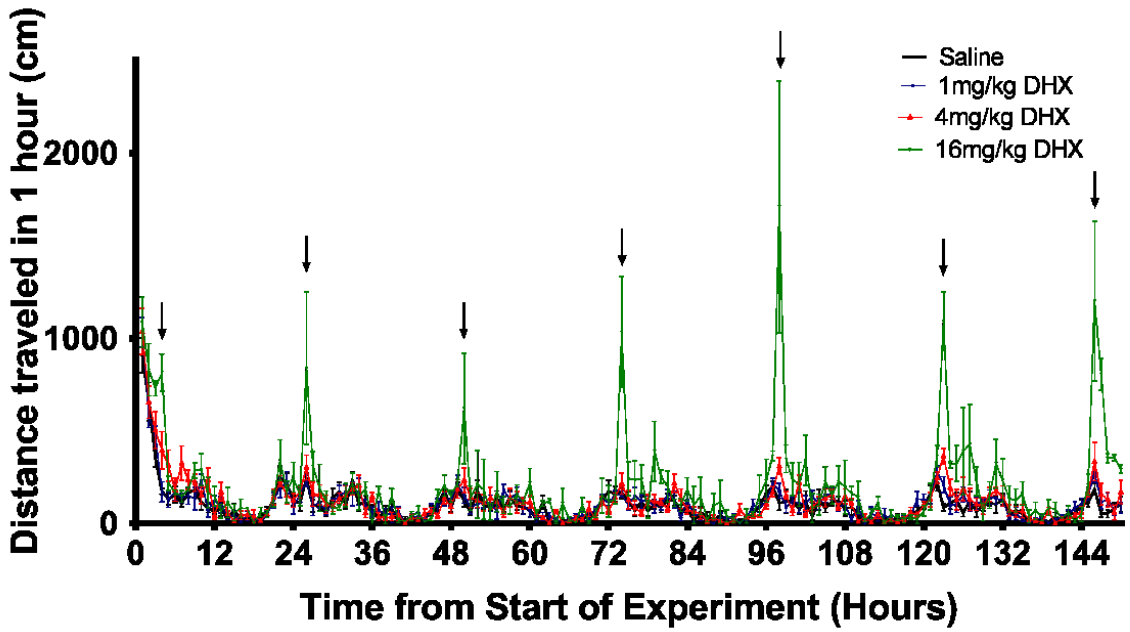

Figure 14: Locomotor Activity throughout 7 Days of DHX Administration. The figure shows the total distance travelled in cm in 1 hour bins for mice receiving daily injections of 1, 4 or 16 mg/kg DHX, or saline. The times of injection are indicated by the arrows. A two-way ANOVA used to examine the effect of time and drug groups found a significant contribution of subject matching ($p<0.0001$), time ($p< 0.0001$), drug group ($p=0.0001$), and an interaction between time and drug group ($p<0.0001$). A Dunnet's multiple comparisons test comparing the mean of each group across the duration of the experiment, found that only the 16 mg/kg group had a mean distance travelled that was significantly greater than the saline group (mean distance saline travelled= 96.08cm/hour, mean distance 16 mg/kg travelled= 202.5 cm/hour, $p=0.0001$).

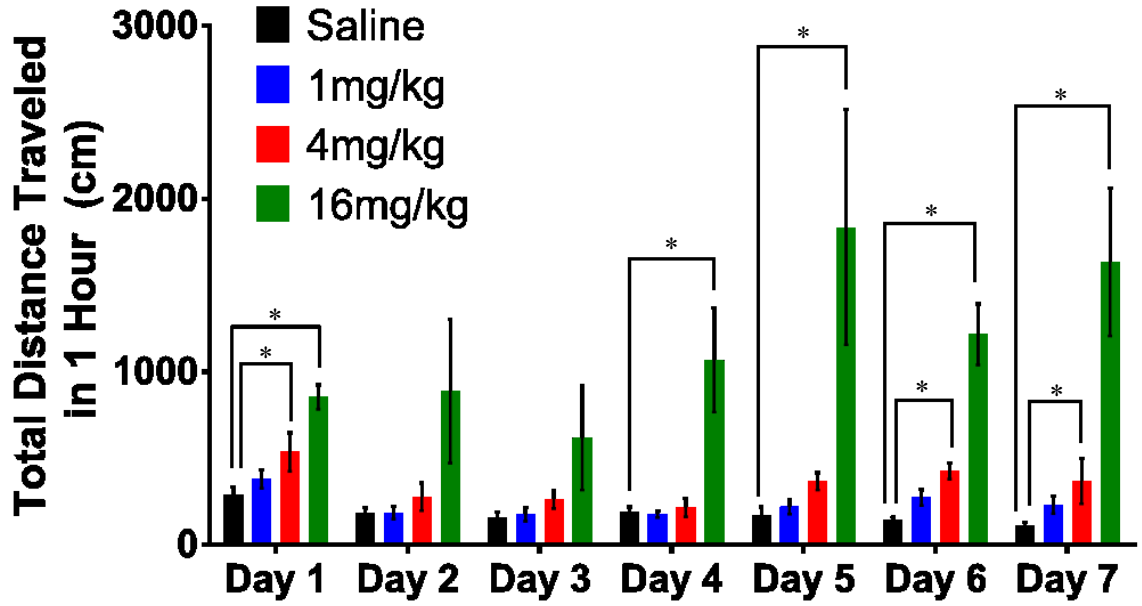

Figure 15: Total Distance Traveled in One Hour After Injection of DHX During A Seven Day Injection Regimen. Multiple t-tests indicate statistically detectable differences between saline and 4 mg/kg DHX on Day 1 (p=0.0203), Day 6 (p=0.0082) and Day 7 (p=0.0146). Statistically detectable differences were observed on Day 1 (p=0.0009), Day 4 (p=0.0136), Day 5 (p=0.0229), Day 6 (p=0.0003) and Day 7 (p=0.0048) between the saline and 16 mg/kg groups. n=6 for saline and 1 mg/kg DHX, n=5 for 4 mg/kg DHX, n=3 for 16 mg/kg DHX.

2.3 Acute Dosage Testing for SCH23390

In order to test that the locomotor effects of DHX were mediated by D1-class receptors, various doses of classical D1-class antagonist SCH23390 were tested to determine a dose which would inhibit locomotor activity in my model. Locomotor activity, as measured by the distance travelled across the bottom of the cage, was reduced by administration of SCH23390, as expected (Fig. 16A). Higher doses of 0.5 mg/kg SCH23390 and 1 mg/kg SCH23390 significantly reduced locomotor activity below the level of saline injected mice in the hour after injection, while lower doses (0.01 mg/kg and 0.1 mg/kg SCH23390) did not have a significant effect (Fig. 16B). This reduction was apparent as early as 5 minutes after injection. The lack of effect of the 0.1 mg/kg dose was a surprise in this experiment and was not recapitulated in later experiments using this dose (appendix 2, Fig. 37 and 38). This may have occurred due to the tendency of the animals in this group to be more active prior to the injection. As the 0.5 mg/kg dose was the lowest dose to clearly inhibit locomotor activity, this dose was selected for the subsequent experiment, blocking DHX with SCH23390.

2.4 Blocking the Locomotor Response Induced by 4 mg/kg DHX with SCH23390

To validate that locomotor response to DHX (4 mg/kg) was mediated through activation of D1-class receptors, mice received a single dose of the classical D1-class antagonist SCH23390 prior to DHX injection. The locomotor activity enhancing effect of a single dose of 4 mg/kg DHX was blocked by the 0.5 mg/kg dose of SCH23390 (Fig. 17). Animals receiving SCH23390 performed very little locomotor activity in the hour following their second injection, whereas animals receiving 4 mg/kg DHX engaged in locomotor activity. As the animals who had received the 16 mg/kg dose of DHX were excluded due to their irregular behaviour, even after the washout

period, and since a statistically detectable difference in locomotor activity following an acute injection of 4 mg/kg DHX had already been demonstrated, no saline-saline control group was used. Further, an increase in locomotor activity can be seen following the injection of DHX, which is not seen following the saline injection in the saline and DHX group, suggesting that the 4 mg/kg DHX dose did in fact increase the locomotor behaviour when not blocked by SCH23390.

2.5 c-fos Expression Levels in the Striatum after Acute DHX Injection

As a final measure to ensure the 4 mg/kg dose was interacting with D1-class dopamine receptors, levels of c-fos positive cells in the striatum around 0.7 mm anterior of bregma 1 hour after 4 mg/kg DHX or saline injection were evaluated (Fig. 18). The 4 mg/kg dose of DHX increased c-fos levels in the striatum, a major site of dopamine signalling, indicating that this dose was successfully engaging with D1-class dopamine receptors. Indeed, although not quantified here, the 4 mg/kg dose appears to have increased cortical levels of c-fos above the level induced by the saline injection as well.

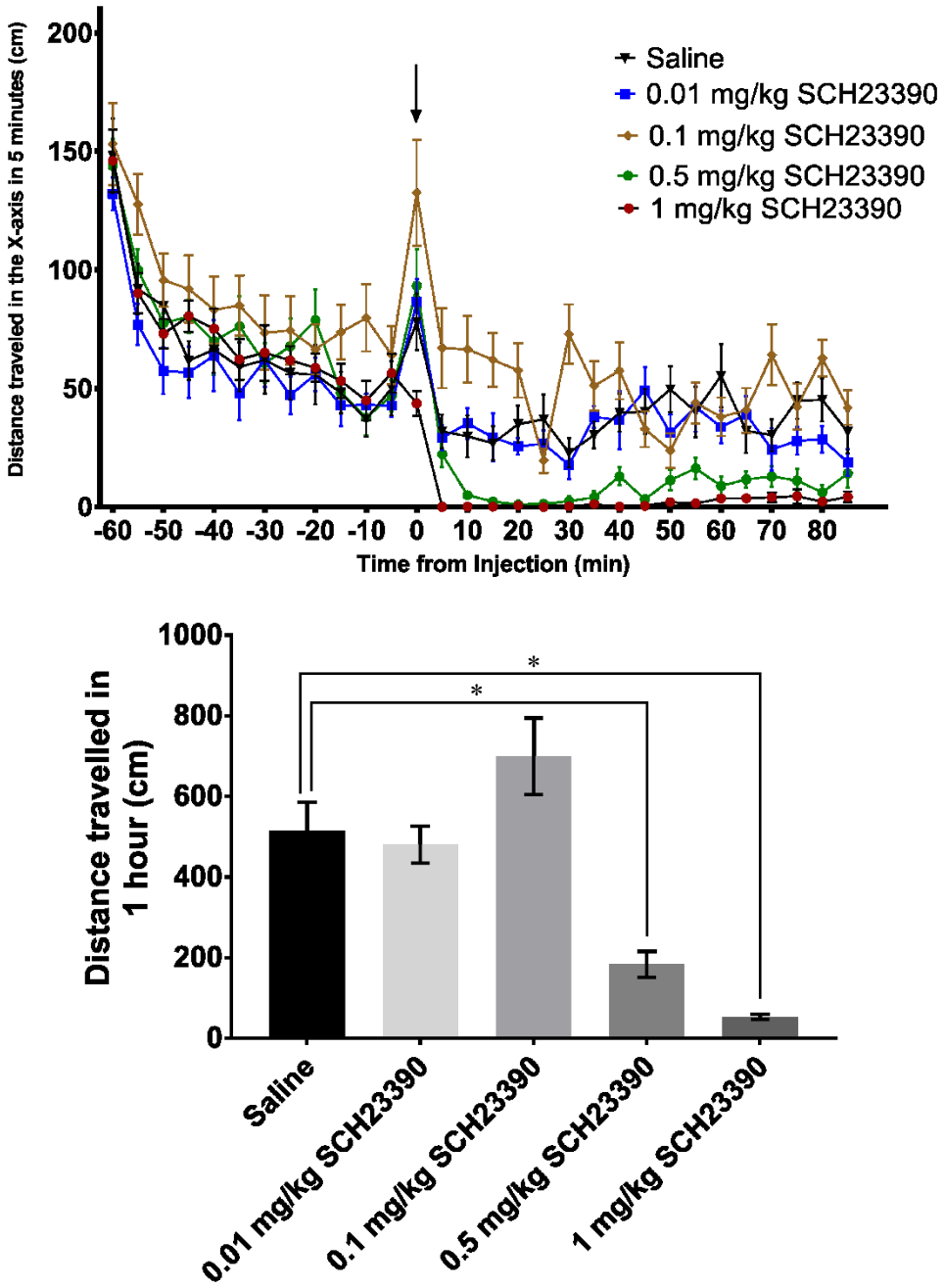

Figure 16: Locomotor Activity Following a Single Injection of SCH23390. Locomotor activity, as measured by the distance travelled across the bottom of the cage in five minute bins is shown (top panel). Data was collected over two consecutive days using two populations of naive mice. n=8 in each group. The total distance traveled in the x-axis in the hour after injection is also shown (bottom panel). A one-way ANOVA indicated detectable differences. Animals that received 0.5 mg/kg SCH23390 and 1 mg/kg SCH23390 demonstrated a significant decrease in locomotor activity compared to animals receiving saline ($p=0.0208$, $p=0.0008$ by a Dunnet's post-test).

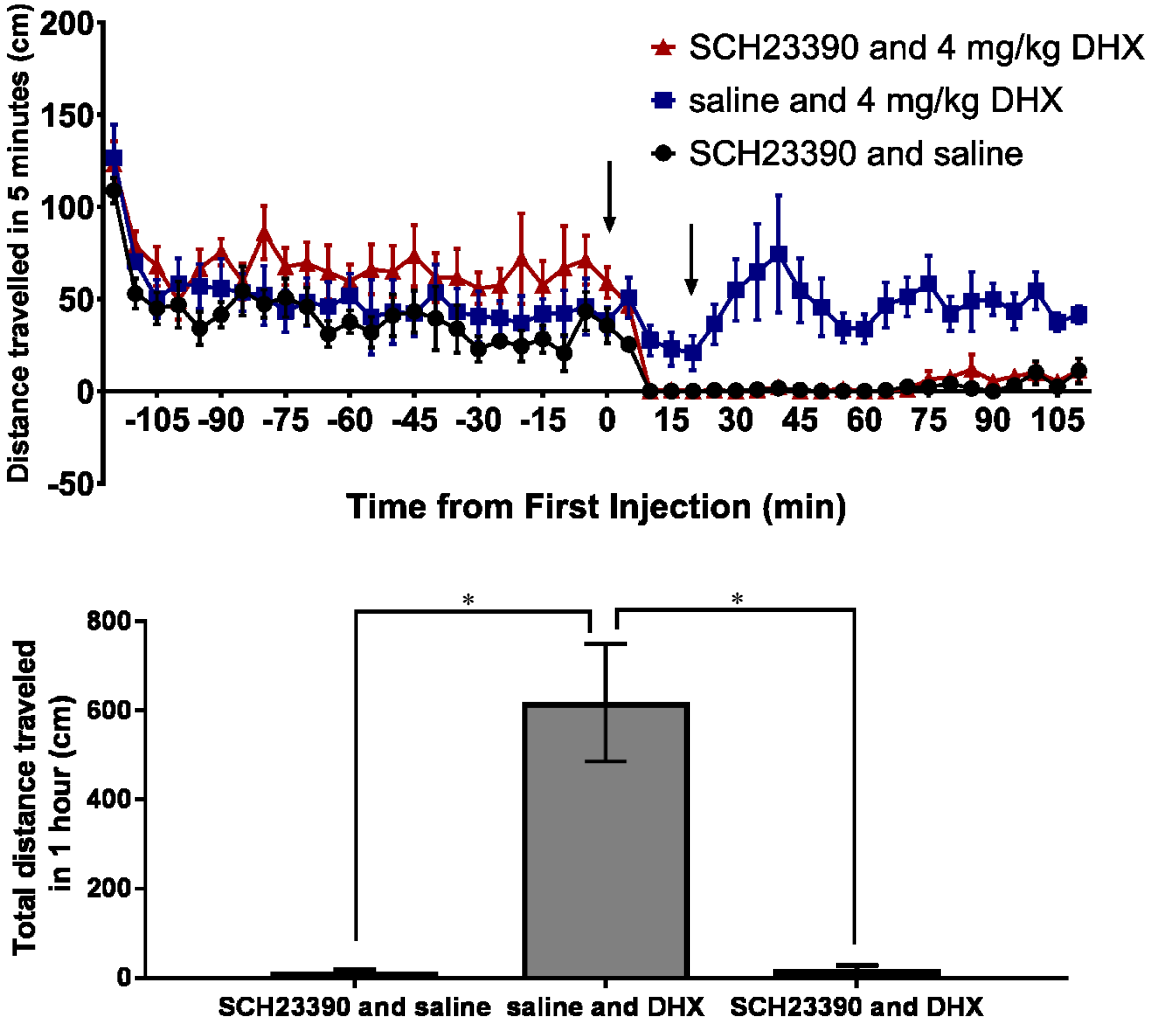

Figure 17: SCH23390 Blocked the Locomotor Activity Enhancing Effect of 4 mg/kg DHX. The top panel shows the locomotor activity for the duration of the experiment in 5 minute bins. Injection 1, at 120 minutes from the start of the experiment, and injection 2, 20 minutes later are indicated by the arrows. In the bottom panel the total distance travelled along the bottom of the cage in the hour following the second injection is shown. A one-way ANOVA indicated statistically detectable differences. The SCH23390 and saline group and the SCH23390 and DHX group were statistically different from the saline and DHX group as determined by a Dunnet's multiple comparisons test ($p=0.0001$ for both groups). $n=6$ in all groups.

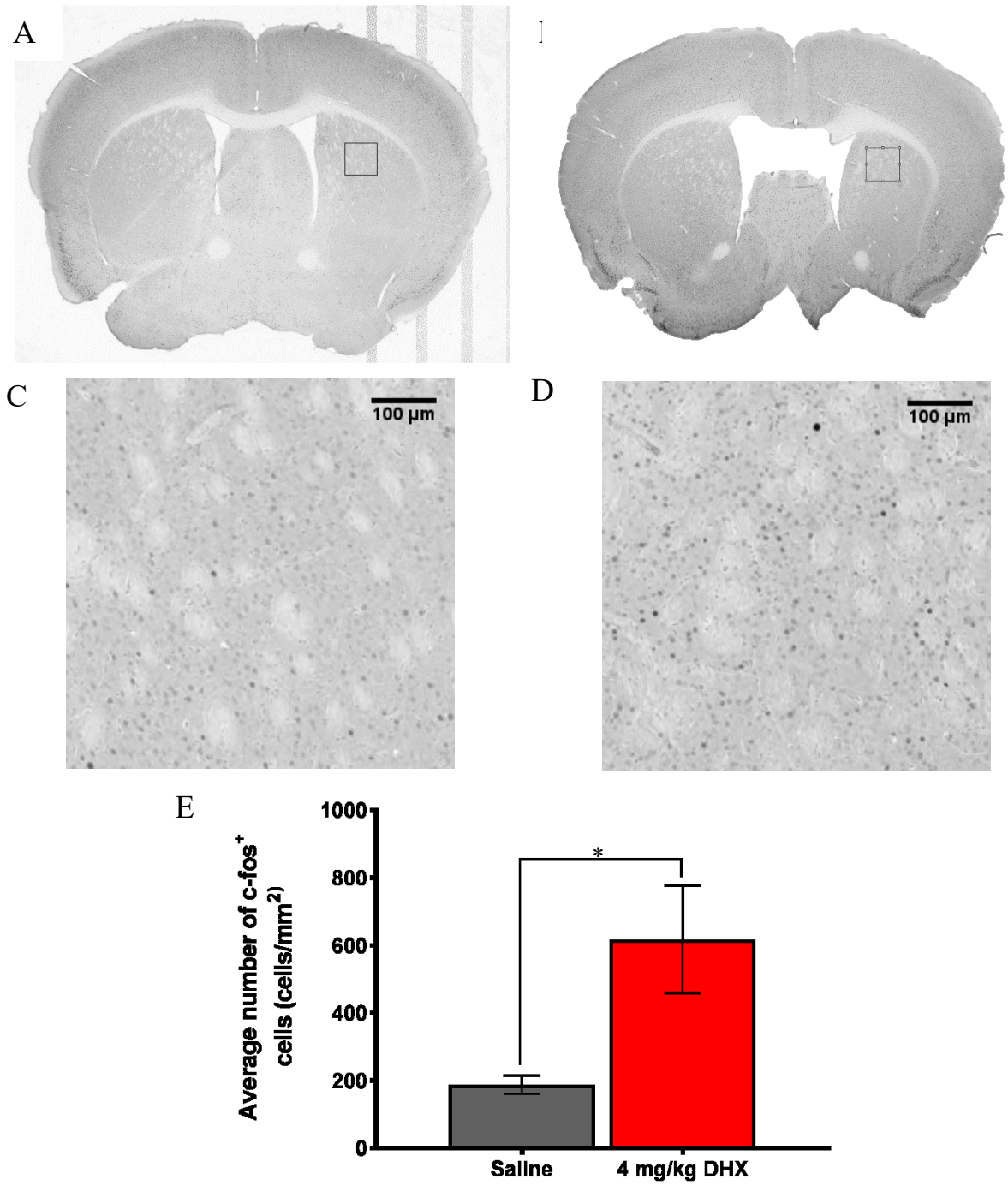

Figure 18: Amount of c-fos$^+$ Cells in the Striatum Following Injection of 4 mg/kg DHX. A and B show representative images of the saline and 4 mg/kg brains respectively. Panel C shows the highlighted region from panel A and D shows the highlighted regions from panel B. The average number of c-fos$^+$ cells/mm^2 from throughout the striatum is plotted for both saline and 4 mg/kg DHX conditions in panel E. n=4 in each group. A total of eight sample regions were evaluated bilaterally in the striatum for each brain, and averaged for each animal. An unpaired t-test comparing the level of c-fos positive cells in the saline group with that of the 4 mg/kg group found a statistically detectable difference between the 4 mg/kg dose and the saline dose (p=0.0376).

3. The Seven-Day DHX Stroke Recovery Study

3.1 Motor and Sensorimotor Recovery from Stroke

Having established a behaviourally active dose of DHX (4mg/kg) and having shown an effect of DHX in the brain, the next step was investigating the efficacy of this drug to boost recovery of motor function following stroke. Motor and sensorimotor recovery from photothrombotic stroke was assessed by the same motor test battery as the light-dark stroke study.

3.1.1 Cylinder Test

Results from the cylinder test (Fig. 19) show a significant deficit induced in both groups after the stroke. Mice show a marked preference for the left forepaw while exploring the wall of the cylinder, as evidenced by their decreased use of the right paw. Similar to the results of the light-dark stroke experiment we do not see any spontaneous recovery on this test. We also do not observe any benefit of the drug treatment on this test.

3.1.2 Horizontal Ladder Test

The horizontal ladder test showed a deficit in the right forepaw and hindpaw, while no deficit was observed in the left paws (see appendix 1 Fig. 33 for left paw data). In the right forepaw the deficit tended to be modest, although still significant, but also subject to less spontaneous recovery (Fig. 20A and B). The right hindpaw however, had a larger increase in errors, but also displayed high levels of spontaneous recovery (Fig. 20C and D). Both groups demonstrated a statistically detectable deficit, and no differences were detected in the extent of recovery on this test between the saline and DHX groups.

3.1.3 Adhesive Removal Test

Training on the adhesive removal test proceeded for four days for cohorts 1 and 2 (Fig. 21A) and for five days for cohort 3 (Fig. 21B). Results of the adhesive removal test indicate no change in the time to contact and remove the tape after stroke in the unaffected left paw (see appendix 1, Figure 34). In the right paw, time to contact (Fig. 22A) and time to remove (Fig. 22C) the tape demonstrate a significant increase following stroke. On these tasks in the right paw, the DHX groups reaches a significantly faster time than the saline group at the postdrug timepoint (Fig. 22B and D). In fact, the DHX groups show nearly complete recovery after the drug period. Due to the large sample size, stratification of the population by degree of deficit was possible. Figure 23 shows the data for the time to contact and to remove the tape separated into three groups (no deficit, between 1 and 100% increase from baseline, and >100% increase from baseline). An examination of the data under this lens makes it clear that the DHX treatment benefited mice with greater deficits (>100% increase from baseline) the most. In this group mice uniformly improved by the postdrug period, whereas mice in the saline group had a mix of the degree of spontaneous recovery and had animals that worsened in this period. The uniform improvement, to near full recovery of the DHX group on this test, when compared with the lingering deficit seen in the saline group, indicates that DHX is improving performance on this test.

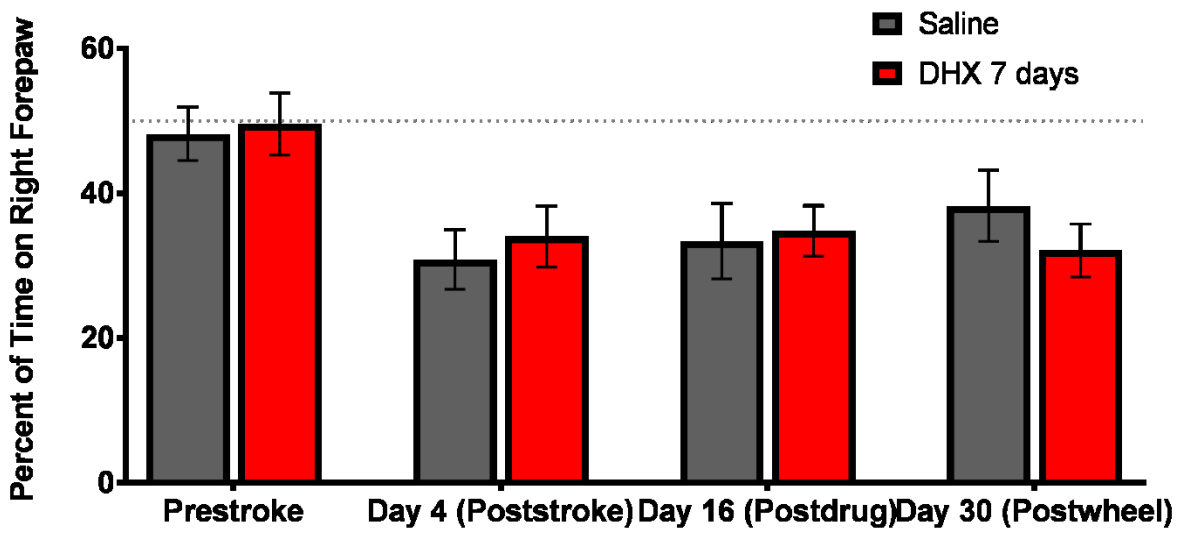

Figure 19: Use of the Right Forepaw during the Cylinder Test during the 7-Days DHX Study. Data is reported as the percentage of time spent on the right forepaw. The dotted line highlights the 50% chance use that would indicate no clear preference for paw use. Two-way repeated measures ANOVA indicated a significant effect of time ($p<0.0001$) and subject matching ($p<0.0001$). A Sidak's post-test indicates significant differences between the Prestroke and Day 4 (Poststroke) timepoints for both the saline ($p=0.0002$) and DHX ($p=0.0009$) groups. n=26 in both groups.

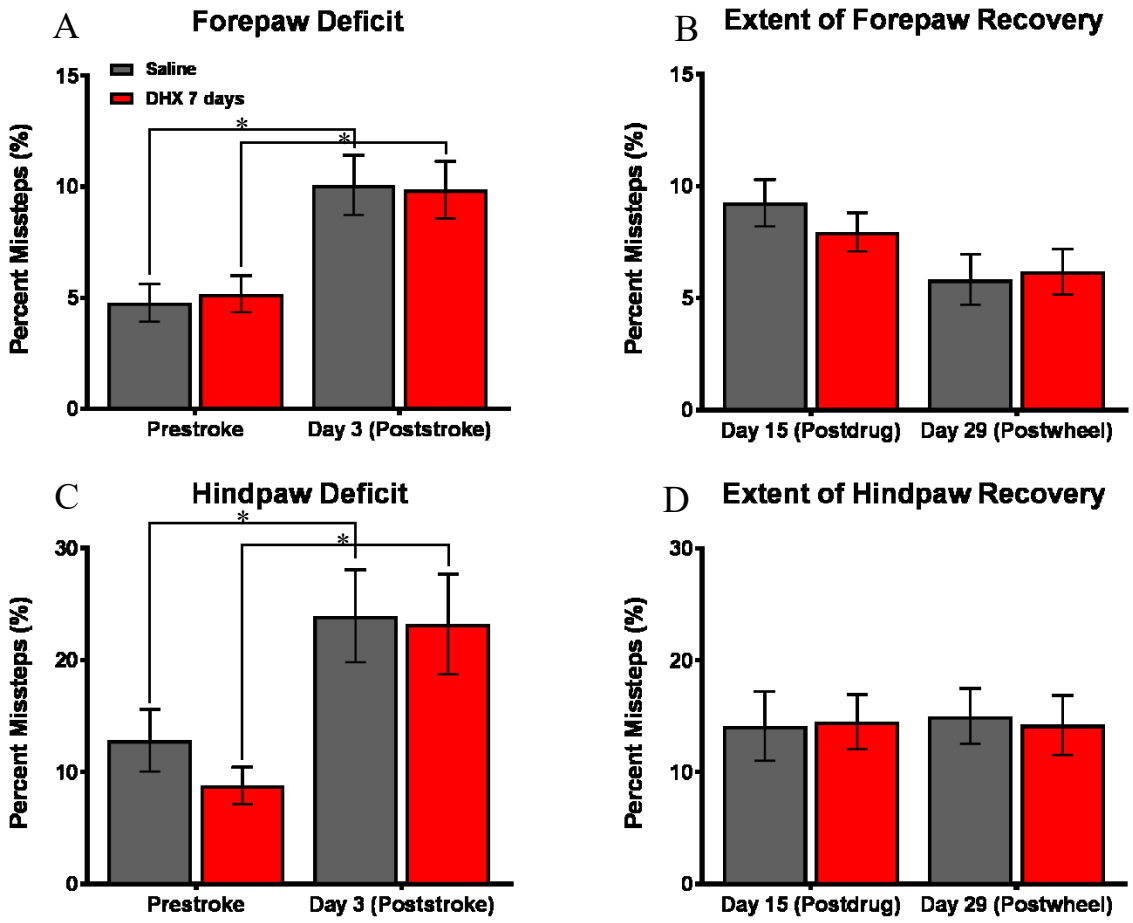

Figure 20: Right Paw Performance on the Horizontal Ladder, 7-Days DHX Study. Panel A shows the deficit that develops following stroke in the right forepaw. A significant effect of time ($p=0.0002$), and significant differences between the saline Prestroke and Day 3 (Poststroke) timepoints ($p=0.0072$) and the DHX Prestroke and Day 3(Poststroke) timepoints ($p=0.0187$) were observed. No significant differences were observed between the two groups at these timepoints. The results of the right forepaw after the drug treatment and voluntary running period are shown in panel B. Analysis revealed a significant effect of time ($p=0.0129$), but no differences between the saline and DHX groups were apparent. Panel C shows the right hindpaw deficit. A significant effect of time ($p<0.0001$) and subject matching ($p=0.0005$) was detected. A multiple comparisons test indicates significant differences between the Prestroke and Day 3 (Poststroke) timepoints for saline ($p=0.0071$) and DHX ($p=0.0005$). Panel D shows the extent of recovery of the right hindpaw. Two-way ANOVA with repeated measures indicated a significant effect of matching ($p<0.0001$). All data is expressed as the percentage of steps that were missteps. n=26 for all groups. Data was analysed by a repeated measures two-way ANOVA and a Sidak's post-test.

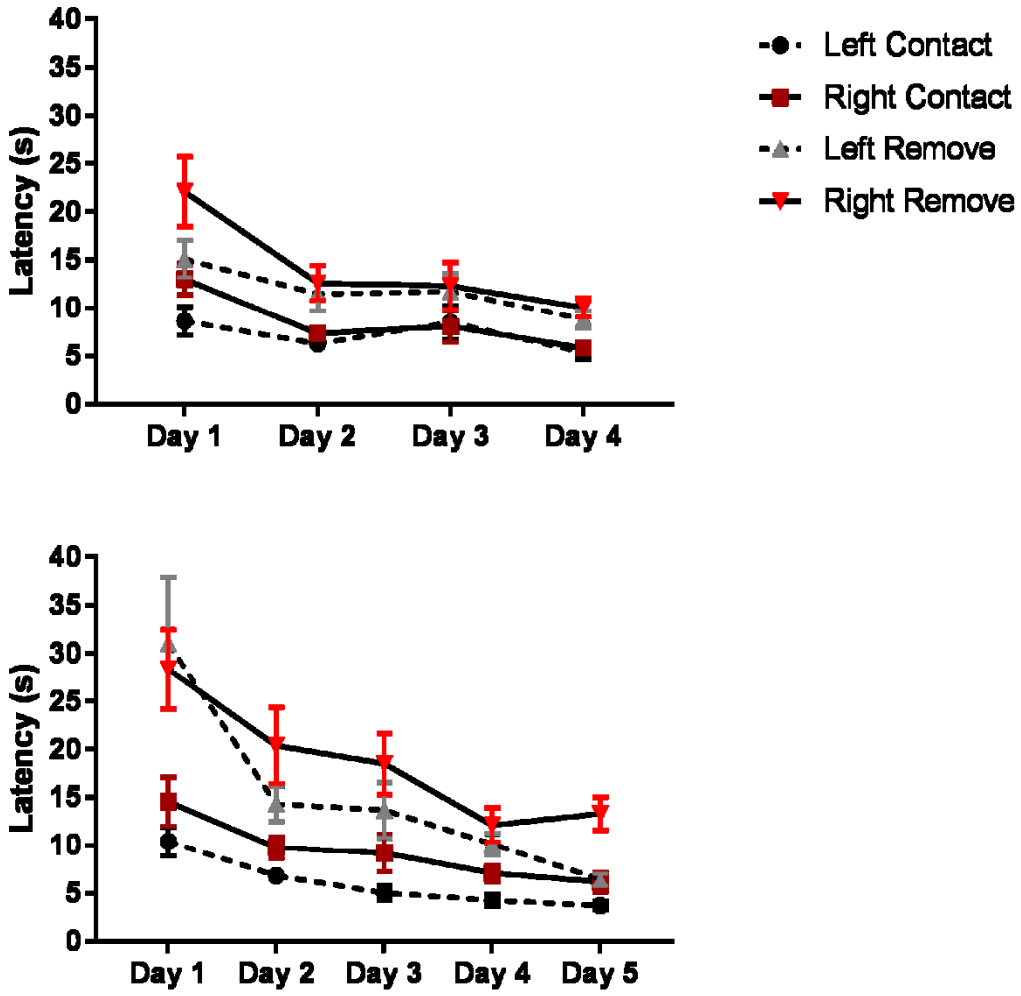

Figure 21: Training on the Adhesive Removal Test 7-Days DHX Study. The top panel shows the results of training for the first two cohorts of 7-days DHX stroke recovery, which proceeded for 4 days. n=41 as it includes all animals which completed training. The bottom panel shows the results of training for the third cohort of 7-days DHX stroke recovery, which lasted 5 days. n=24.

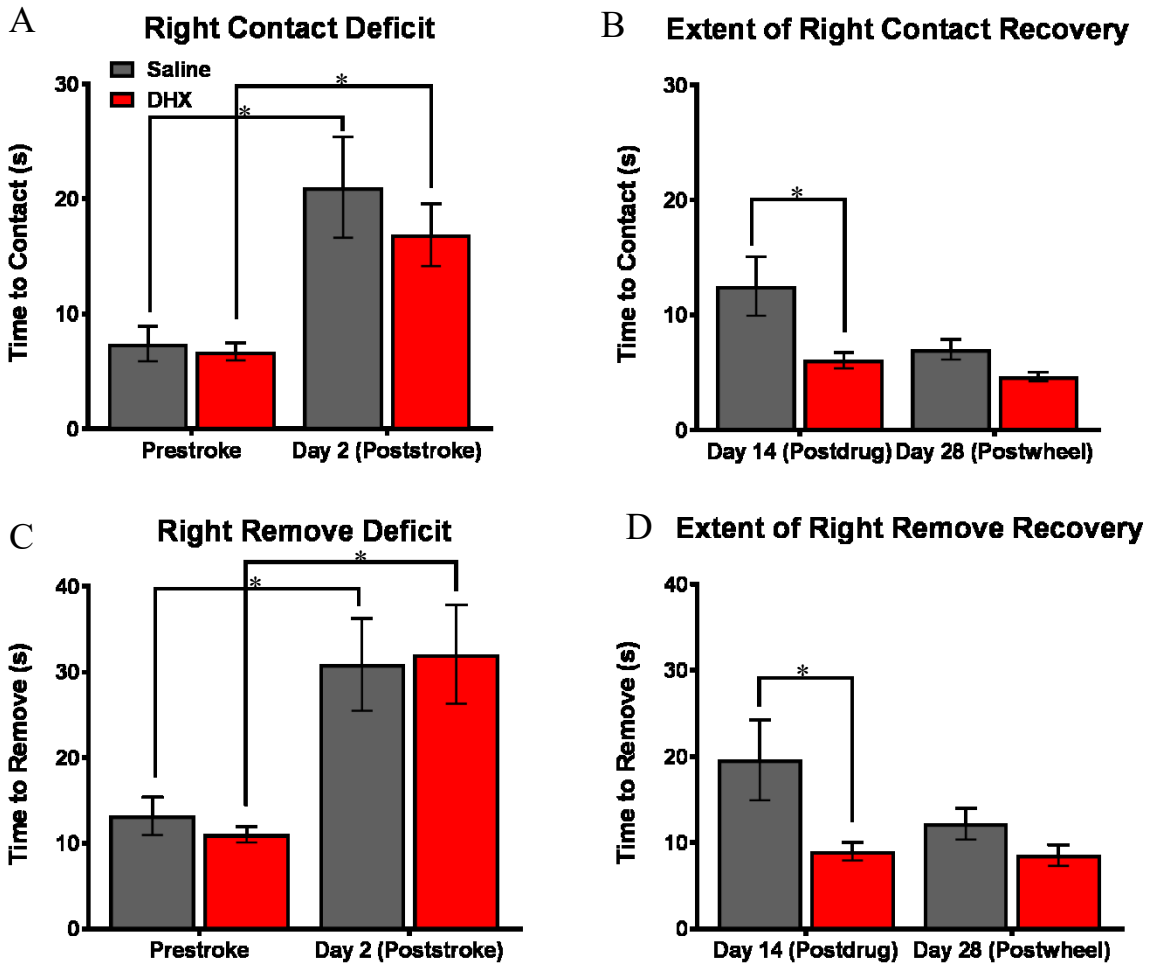

Figure 22: Seven-Days DHX Study Right Paw Adhesive Removal Test. Panel A shows the deficit in time to contact the tape. A significant effect of time is observed ($p<0.0001$), with statistically detectable differences in the time to contact the tape before and after stroke in both the saline ($p=0.0007$) and DHX ($p=0.0125$) groups. Panel B shows the extent of the recovery in the time to contact the tape. A significant effect of time ($p=0.014$), drug treatment ($p=0.0131$) and subject matching ($p=0.0002$) were detected. There was a statistically detectable difference between the saline and DHX groups at the Postdrug timepoint ($p=0.0033$). Panel C shows the deficit in the time to remove the tape with the right forepaw. A significant effect of time ($p<0.0001$) was observed, as well as statistically detectable differences between the Prestroke and Poststroke timepoints for both the saline ($p=0.0043$) and DHX ($p=0007$) groups. Panel D shows the Extent of Recovery for the removal of the tape from the affected paw. A significant effect of time ($p=0.0385$), drug treatment ($p=0.0324$) and subject matching ($p<0.0001$) was observed. A statistically detectable difference can be seen between the DHX and saline groups at the Postdrug period ($p=0.0131$). Significance was determined by a two-way ANOVA followed by a Sidak's post-test.

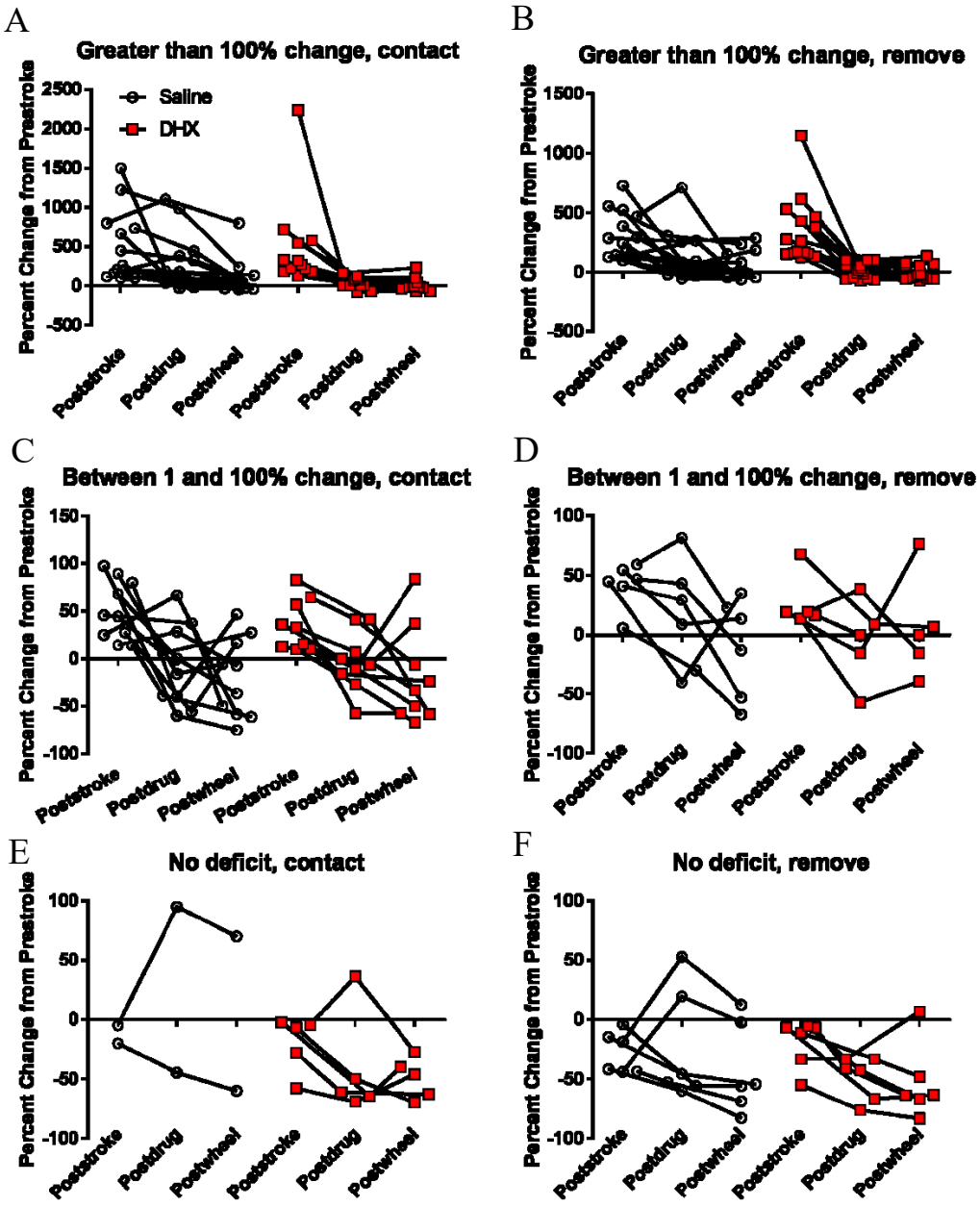

Figure 23: Results of the Adhesive Removal Test in Groups Stratified by Poststroke Deficit for the 7-Days DHX Study. Panel A shows the results for contacting the adhesive for the individual mice that had a deficit of greater than 100% change from baseline. n=14 for the saline group, n=12 for the DHX group. Panel B shows percent change for removing the tape for mice in the greater than 100% change groups. n=14 for saline, n=15 for DHX. Panel C is the percent change for mice between 1 and 100% change to contact the tape. n=10 for saline and n=9 for DHX. Panel D is the percent change for tape removal of mice in the 1-100% change category. n=6 for saline, n=5 for DHX. Panel E is the results for mice displaying no deficit on time to contact the tape. n=2 for saline and n=5 for DHX. Panel F shows the results for mice with no deficit on tape removal. n=6 for both groups. Data is expressed as the scaled increase from baseline ((prestroke time-poststroke time)/prestroke time).

3.2 Wheel Usage

After the drug administration period and the postdrug testing the mice were given running wheels in their home cages for 9 days. The running wheels recorded the number of wheel rotations over the course of the running wheel period. Figure 24 shows wheel use between the second and eighth days of wheel exposure. Both the saline and DHX groups used the running wheels in very similar patterns and to a very similar degree, indicating no changes in the circadian rhythms of the DHX treated mice, and no lasting increase in locomotor activity. This suggests that increased performance on the behavioural tasks at the postwheel timepoint did not result from increased engagement with the aerobic exercise treatment.

3.3 Infarct Sizes

Infarcts were sized first at 24 hours post-stroke by T2-weighted MRI and later by cresyl violet stained slices, taken from animals perfused at 31 days poststroke (Fig. 25). There were no significant differences between the DHX and saline groups in the infarct sizes measured by either MRI or by cresyl violet. While the MRI infarct sizes are larger, the two measurement methods correlate very well for both groups (DHX group $r=0.9314$, $p<0.0001$, Saline group $r=0.9412$, $p<0.0001$) (Fig. 25E). Additionally, the slopes of the correlations are not statistically different between the two groups. This suggests that the drug treatment had no effect on the progression of the infarcted tissue during the recovery period.

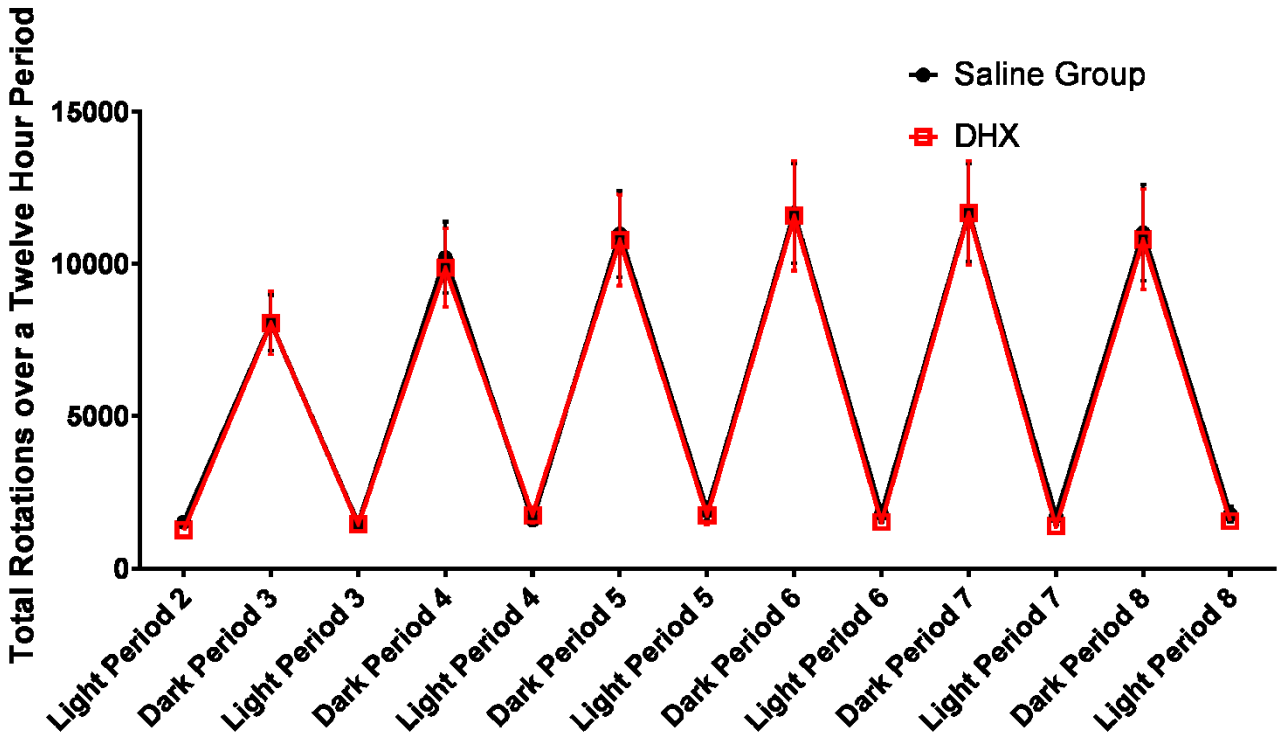

Figure 24: Wheel Usage between Day 2 and 8 of Wheel Access, 7-Days DHX Study. Data is binned by total wheel rotations in 12 hour periods of light or dark. Days 2-8 are shown as Dark Period 1 and Dark Period 9 are not complete 12 hour periods. n=26 for both groups.

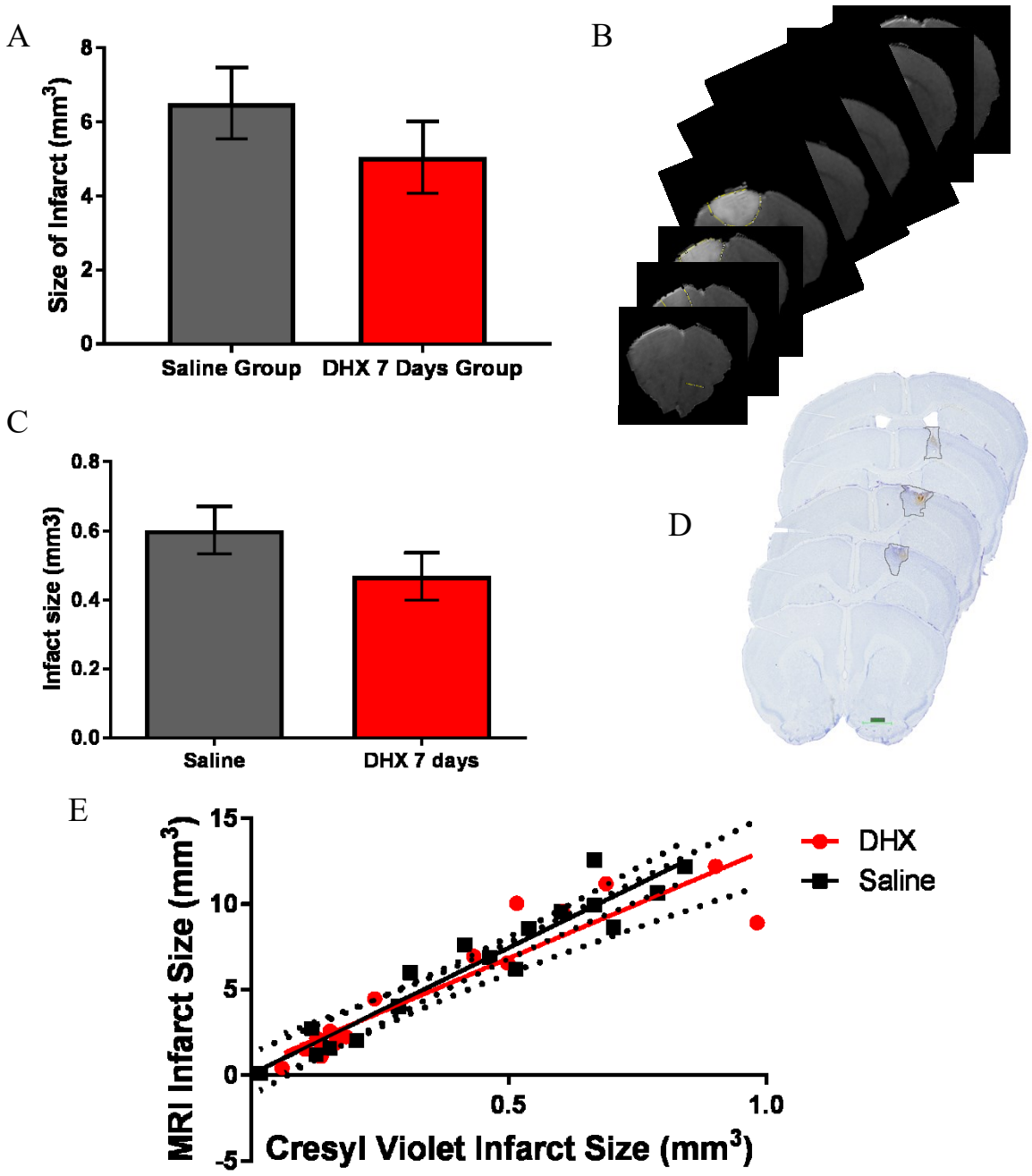

Figure 25: Infarct Sizes, 7-Days DHX Study Panel A shows infarct sizes as estimated from MRIs taken 24 hours after stroke (prior to drug treatment). n=17 for both groups, as the third cohort did not undergo MRI. Panel B is a representative MRI from one of our stroke mice. This mouse's infarct was estimated as 9.91mm^3. Panel C illustrates the infarct sizes as estimated from cresyl violet stained slices collected at 31 days poststroke. n=26 for both groups. Panel D shows a representative cresyl violet stained stroke brain. This brain is taken from the same mouse depicted in panel B. By the cresyl violet staining method the infarct is 0.668mm^3. Panel E shows the correlation between MRI and cresyl violet stained infarct sizes for both the DHX and saline groups. As the slope of the lines are not statistically different, the slope may be considered for both lines. The pooled slope is 13.5731. n=17 for both groups.

4. Two-Days DHX Stroke Recovery Study

4.1 Motor Recovery from Stroke

Given the success of the 7-days DHX stroke recovery study, the next step was to probe the limits of the treatment, in terms of days that treatment was administered, and how long after stroke the treatment might be effective. To that end, the 2-days DHX study, which gave 2 days of DHX treatment starting ten days after stroke and used the same behavioural battery at the same timepoints from stroke, as the 7-days DHX study, was performed.

4.1.1 Cylinder Test

Like the previous Light-Dark stroke study and the 7-days DHX study, a significant reduction in the time spent on the right paw while exploring the cylinder wall after stroke was observed. The results show that deficits in the use of the right forepaw after stroke were not subject to spontaneous recovery, nor were the deficits reduced by the 2-day DHX treatment (Fig. 26).

4.1.2 The Horizontal Ladder Test

Both groups showed similar deficits in the right paws following stroke (for left paw data see appendix 1, Figure 35). In the right forepaw and hindpaw (Fig. 27A, C), we again observed a greater initial deficit in the hindpaw for both groups, but also a greater tendency toward spontaneous recovery in the hindpaw as compared to the forepaw (Fig. 27B, D). Results of the horizontal ladder test suggest no benefit of the 2-day DHX treatment on this test.

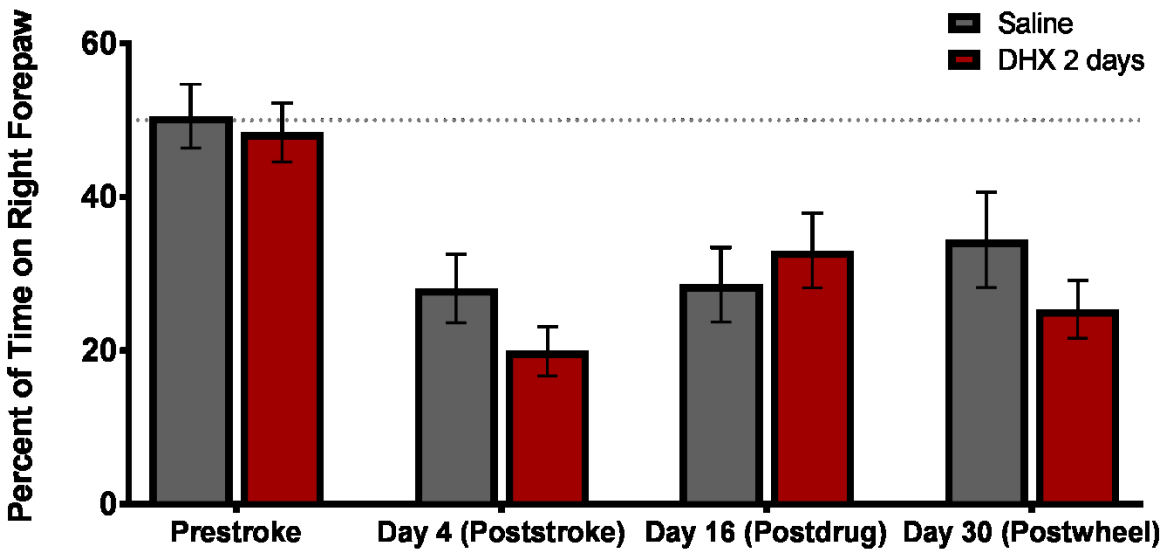

Figure 26: Time Spent Using the Right Paw during the Cylinder Test, 2-Days DHX Study. Data is expressed as the percent of the time spent exploring the cylinder wall that was spent on the right paw. A significant effect of time ($p<0.0001$) and subject matching ($p<0.0001$) were observed. Post-testing revealed a significant difference between the Prestroke and Poststroke time points for both saline ($p<0.0001$) and DHX ($p<0.0001$). The dotted line indicates the 50% mark, which would be expected with chance use of both paws during the cylinder test. Statistics were performed using repeated measures two-way ANOVA and a Sidak's post-test. n= 19 for the saline group, n=20 for the DHX group.

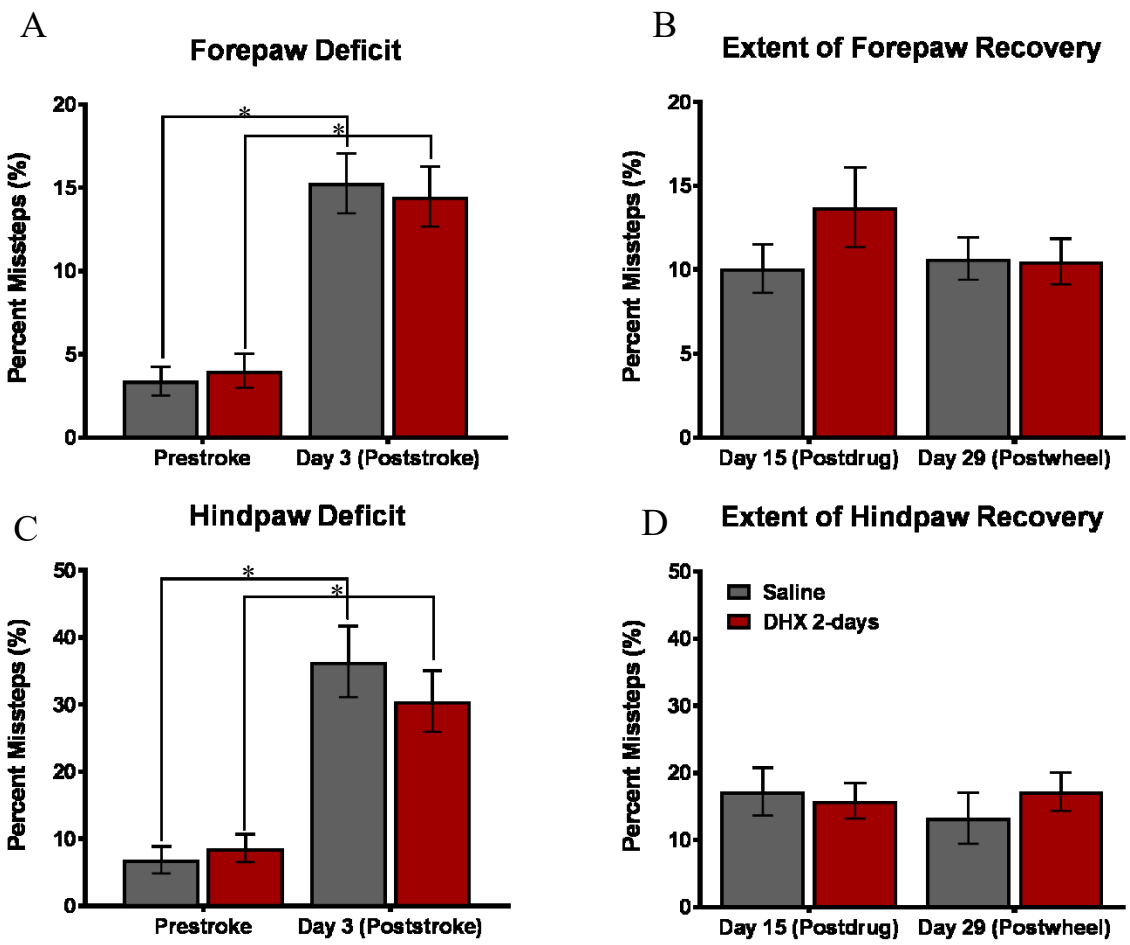

Figure 27: Results of the Horizontal Ladder Task, 2-Days DHX Study Panel A shows the deficit that develops in the right forepaw after stroke. A significant effect of time is seen ($p<0.0001$). Post-testing revealed both the saline ($p<0.0001$) and DHX ($p<0.0001$) groups showed a statistically detectable difference between the Prestroke and Poststroke timepoints. Panel B shows the extent of recovery in the right forepaw. Panel C shows the deficit on the right hindpaw. A significant effect of time ($p<0.0001$) and subject matching ($p=0.0169$), along with significant differences between the Prestroke and Poststroke timepoints in both the saline ($p<0.0001$) and DHX ($p<0.0001$) groups, are observed. Panel D shows the extent of the hindpaw recovery as measured after the drug and running wheel periods. Analysis was performed using two-way ANOVA with repeated measures and a Sidak's multiple comparisons test. n =19 for both groups in all graphs.

4.1.3 Adhesive Removal Test

Training on the adhesive removal test occurred over four days for cohort 1 and over five days for cohort 2 (Fig. 28). Results of the adhesive removal test are shown in Fig. 29. While both the time to contact and remove the tape show similar and significant deficits in the saline and DHX groups, there is no indication of improved recovery in the DHX groups as compared to the saline group. As expected the results of contacting and removing the tape on the left paw (appendix 1, Fig. 36) does not demonstrate much change throughout the study.

4.2 Wheel Usage

Data from the running wheels suggests that both groups used the running wheels to a very similar degree and in very similar patterns, in agreement with the results from the 7-days DHX study (Fig. 30).

4.3 Infarct Sizes

Infarct sizes between the two groups, as measured from cresyl violet stained slices at 31 days poststroke, were not statistically detectably different, as was expected based on the results of the 7-days DHX study. (Fig. 31).

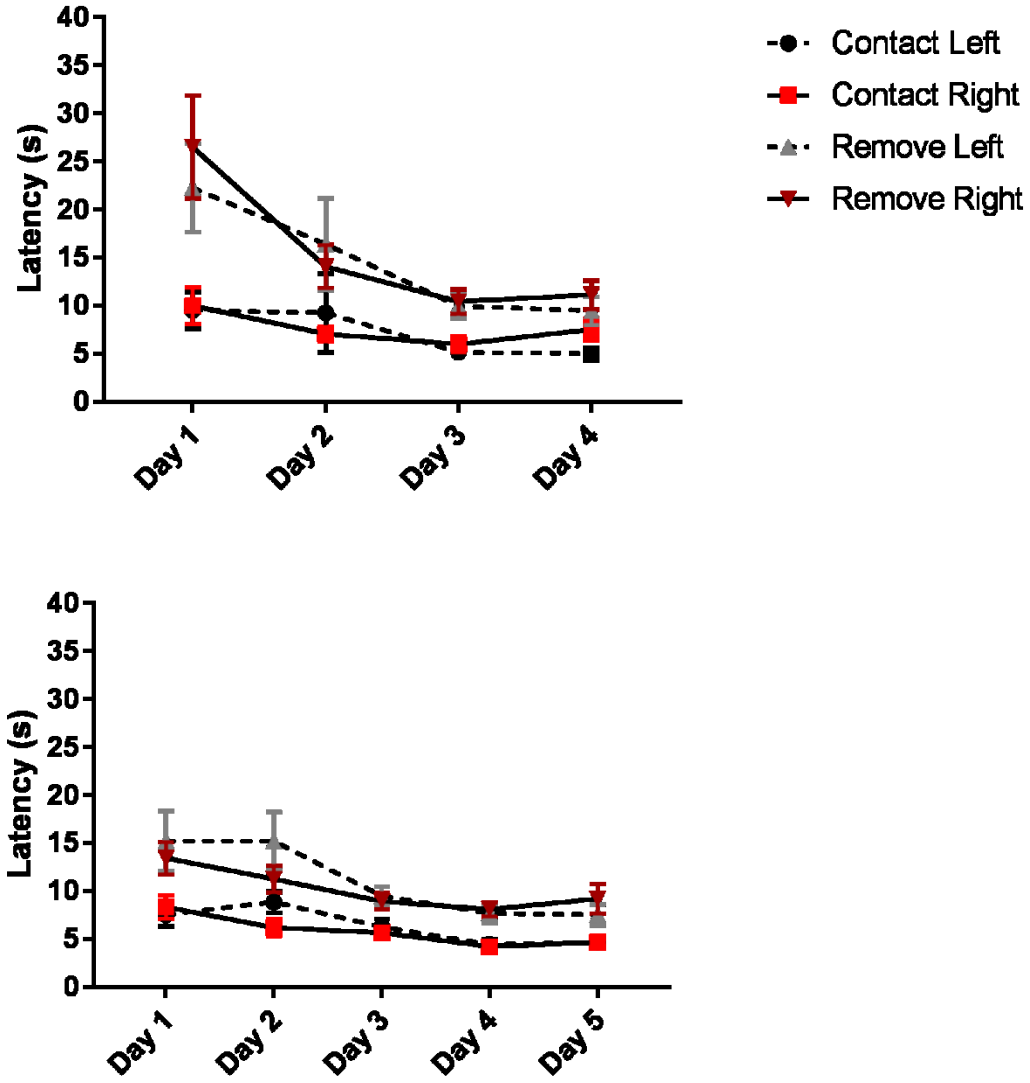

Figure 28: Adhesive Removal Training for the 2-Days DHX Treatment Cohorts. The top panel shows the training results of the first cohort of DHX 2-days mice. Training lasted 4 days for this cohort. n= 21, as this was prior to exclusions from the study. Bottom panel shows the training results for the second cohort, where training lasted 5 days. n=22 for this cohort.

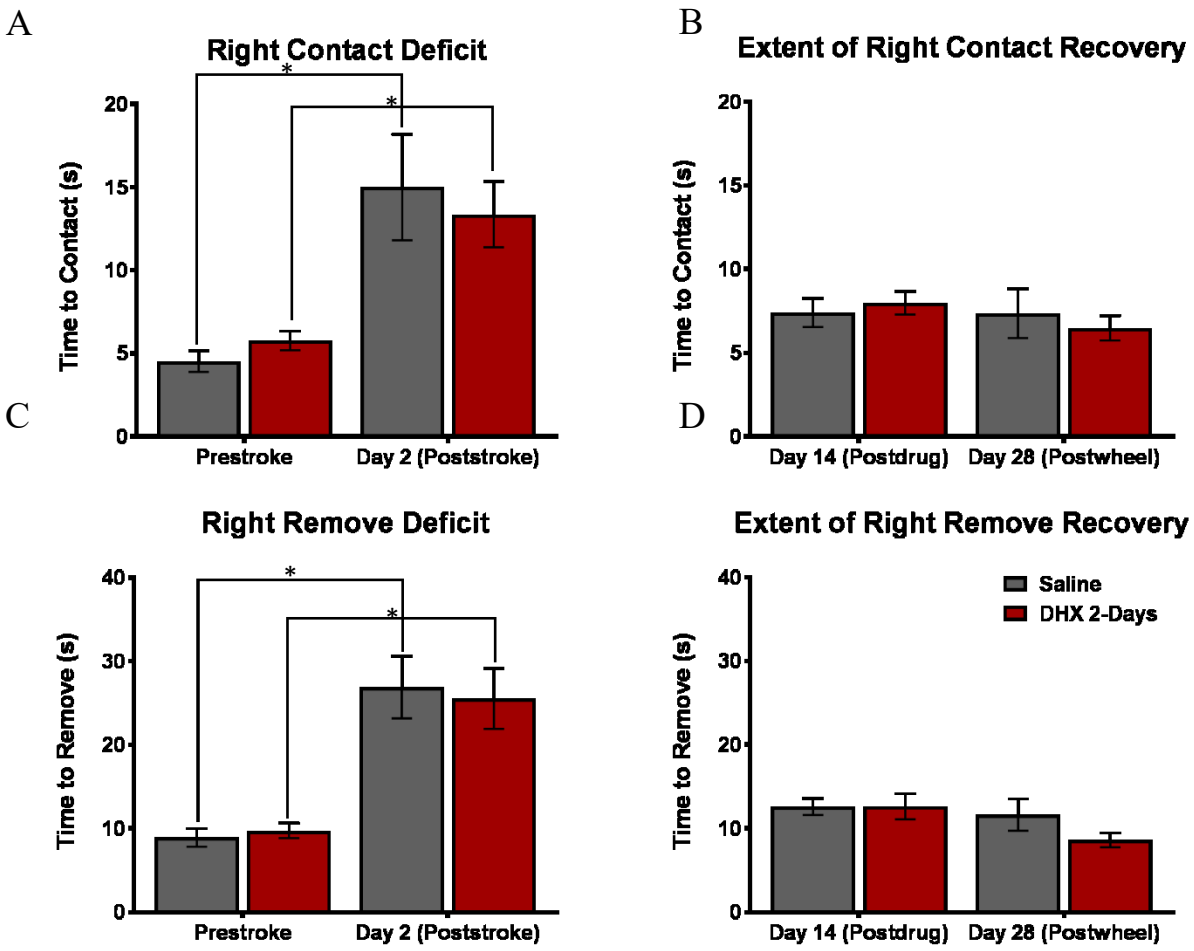

Figure 29: Adhesive Removal Test for the 2-Days DHX Study Panel A shows the deficit in the time to remove the tape. A significant effect of time can be detected ($p<0.0001$), and a post-test shows statistically detectable differences between the Prestroke and Poststroke time points at a level of $p=0.0007$ for saline and $p=0.0118$ for DHX. Panel B shows the extent of recovery in the time to contact the tape. Panel C shows the deficit in the time to remove the tape after stroke. A significant effect of time ($p<0.0001$) was observed, as well as significantly detectable differences between performance at the Prestroke and Poststroke timepoints in the saline ($p<0.0001$) and DHX ($p<0.003$) groups. Panel D shows the extent of recovery in the time to remove the tape. Statistical analysis was performed using repeated measures and a Sidak's post-test. $n=20$ for the DHX group and $n=19$ for the saline group.

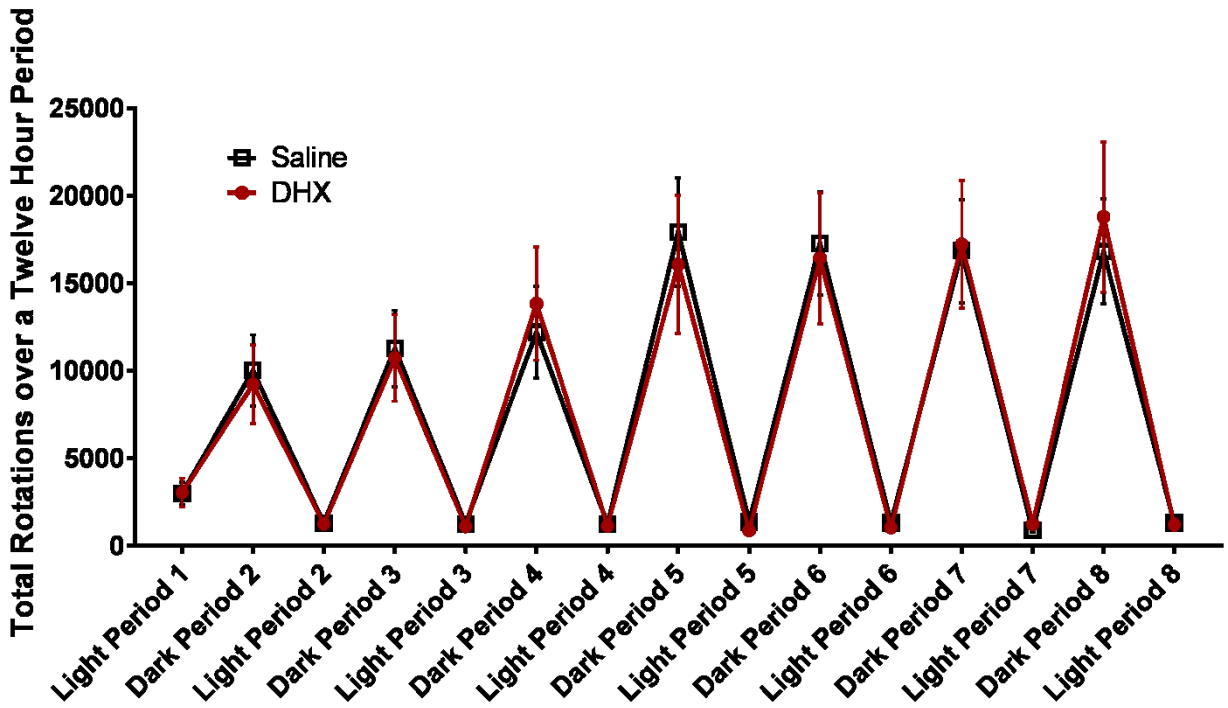

Figure 30: Wheel Usage during the 2-Days DHX Study. Data shown is the wheel usage for the second 2-days DHX treatment cohort. n=11 for the saline group, n= 12 for the DHX group.

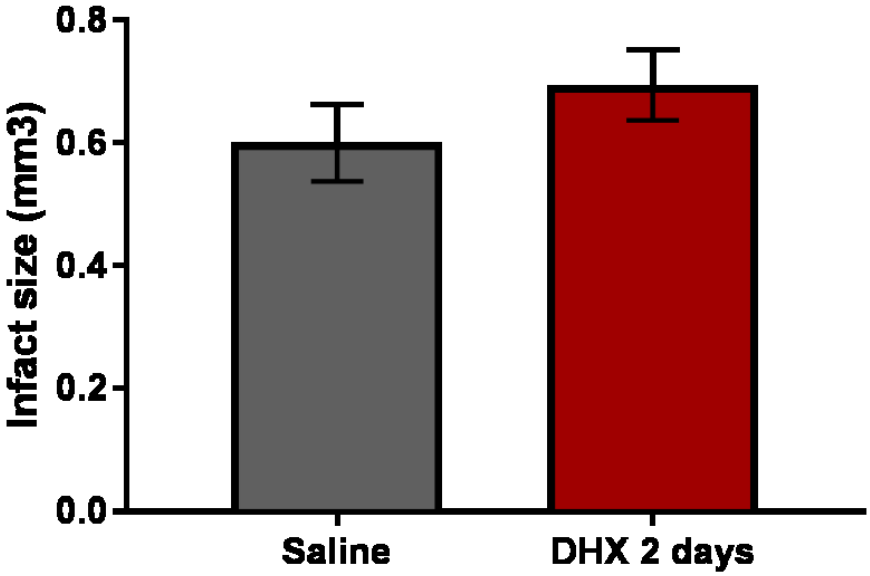

Figure 31: Cresyl Violet Infarct Sizes for the 2-Days DHX Study. Infarct size analysis was performed on tissue collected 31 days poststroke**.** n=19 for the saline group, n= 20 for the DHX group.

Discussion

1. Light-Dark Stroke

Results of the Light-Dark Stroke study did not show statistically detectable differences in behavioural outcomes for strokes occurring in the light period or dark period of a mouse's circadian rhythm. An examination of experimental outcomes suggested that there are not issues with greater mortality or adverse outcomes in either lighting condition (Fig. 3). Results of the horizontal ladder, cylinder test and adhesive removal test revealed no statistically detectable differences, nor even a consistent trend, towards one group or another demonstrating worse, or more lasting deficits. Although this is contrary to my initial hypothesis, it is in agreement with the only other study to examine this issue in rodent stroke models with behaviour (Rakai and Antle, 2013). Additionally, no difference between the lesion sizes generated in the two groups was found. This again agrees with the Rakai and Antle (2013) study but disagrees with the previous findings of Vinall et al. (2000). The discrepancy between the Vinall findings and my findings may stem from a difference in methodology. While the Vinall study chose to maintain the animals' internal body temperature at the level it was prior to the onset of surgery, thus preserving temperature differences generated by the circadian rhythm, my work chose to maintain a steady body temperature of 37^{o}C during the surgery for all animals. Given the well documented neuroprotective effect of hypothermia on stroke, and the close correlation between an animal's temperature and their stroke size in the Vinall study, this seems to be a contributing factor. Interestingly, the Rakai and Antle study also preserved body temperature by inducing the stroke in conscious animals via a previously implanted cannula, and this study found no effect of time of induction on stroke size. It may be that this neuroprotective effect of temperature is most obvious when also combined with potentially neuroprotective anesthesia. Indeed, evidence exists for a neuroprotective effect of ketamine (Kohrs and Durieux, 1998; Hudetz and Pagel, 2010) and

isoflurane, particularly in short term recovery (Kawaguchi et al., 2000; Kawaguchi et al., 2005) during ischemia. Another contributing factor is the use of an MCAO stroke model in the Vinall study. This model results in much larger strokes than either a photothrombosis or surface ET-1 model, likely making it easier to detect differences in lesion size. It should also be noted that the use of TTC staining, which is thought to be vulnerable to overestimation of infarct size, could have contributed to the results (Liu and McCullough, 2011).

Despite not observing a difference in behavioural outcome, I still chose to use reverse light-dark housing on the remainder of my work. This was done for a number of reasons. Firstly, results of the training for the adhesive removal test indicates that dark group mice learn more readily and ultimately perform better on the task. From this result it seems likely that active versus inactive cycle can have a bearing on behavioural task, including motor and sensorimotor tasks, and it may be that the most accurate accounting of an animal's behavioural ability would be found during the dark cycle. Further, this greater prestroke proficiency is beneficial as the better an animal performs before stroke, the easier it is to detect deficits in that performance after stroke. Interestingly, an effect of light and dark cycle on behaviour has been shown on tasks requiring cognitive engagement (Roedel et al., 2006), although contradictory evidence does exist (Peirson et al., 2018). This agrees with our findings that the task which required the most skill learning, was able to show a difference between light and dark groups in mouse skill acquisition. Secondly, the results of the c-fos staining indicate that, as would be expected, the level of important transcription factors and the level of brain activity changes throughout the circadian rhythm. c-fos is known to be downstream of D1-class receptors, but has been classically used as a marker of cell activation and to suggest plasticity (Kovacs, 2008). This finding should not be taken to indicate changes in the DA system specifically, though they appear to agree with known

circadian changes in levels of DA system components (Feenstra et al., 2000; Castaneda et al., 2004). While the variation in c-fos levels and in task learning does not appear to have a significant bearing on the outcome of experimental stroke, there is no evidence to exclude the possibility of changes in levels of this transcription factor, or circadian-controlled components of the DA system, having a bearing on the effectiveness of medications or rehabilitation methods. Therefore, in order to maximize translatability, as most human patients receive rehabilitation and medication during their active periods, it seemed prudent to continue to work within the active period in my mouse model.

Ultimately, this work indicates that both normal light-dark housing and reverse light-dark housing is valid in stroke studies evaluating motor behaviour. It remains to be seen if time of stroke would be a factor in studies examining other domains of function (for example post-stroke cognition or depression). This work also suggests that results (although likely not raw data) between work performed in reverse light-dark or normal light-dark models are readily comparable.

2. DHX and SCH23390 Dosage

Previous work on the effect of DHX on locomotor activity levels has produced mixed results. The results herein, with two hours of acclimation to the new environment, produced an increase in locomotor activity following administration of DHX. One study using rats also found an increase in locomotor activity compared to control injection, with higher doses (3-30 mg/kg) of DHX given subcutaneously. This increase in locomotor activity could be blocked by both D1- and D2-class antagonists (Darney et al., 1991). In contrast, another study using doses ranging from 0.25 mg/kg-16 mg/kg DHX subcutaneously and did not find any significant changes in locomotor activity (Deveney and Waddington, 1997). Both studies used Sprague-Dawley rats of

similar ages, in their inactive phase, and both expressed data as the number of occurrences during discrete sampling periods over the course of 45 minutes-1 hour. The studies did differ on the length of the sampling periods and on the length of the habituation periods employed before injection (Darney et al., 1991; Deveney and Waddington, 1997). A third study, also in Sprague-Dawley rats, in the active phase of the animals found that DHX (1-8 mg/kg) reduced locomotor activity in unhabituated rats and had no change in locomotor activity in habituated (45 minutes in testing environment) animals, and that this could be blocked by the D1-class antagonist SCH23390. They also demonstrated a DHX mediated increase in c-fos mRNA in the medial PFC, nucleus accumbens (NAc) and caudate putamen, which could be blocked by SCH23390. Lastly they found that DHX decreased locomotor activity in a hyperdopaminergic state caused by AMPH co-administration (Isacson et al., 2004).

It has been proposed that the increase in locomotor activity observed in the study by Darney and colleagues may be due to activation of D2-class DA receptors occurring at the higher doses used, however this seems unlikely, as significant increases in locomotor activity were seen as low as 3 mg/kg DHX, which overlaps with doses used in the other studies (Salmi et al., 2004). Additionally, while DHX has only a ten-fold greater affinity for D1-class over D2-class receptors, its functional affinity for D2-class receptors is said to be very low (Arnsten et al., 2017).

Another potential explanation can be found in examining the effects of other D1-class agonists. These too have had mixed results with D1-class agonists such as SKF38393, and SKF81297 generally being thought to stimulate motor activity, while findings with D1-class agonist A68930 found a locomotor inhibitory effect (Salmi and Ahlenius, 2000; Heijtz et al., 2007). In contrast, other studies using SKF81297 in rats and SKF38393 in C57BL6 mice found a biphasic effect on

locomotion, with early inhibition and a later, more pronounced, stimulatory phase in non-habituated animals or in habituated animals at higher doses (10 mg/kg SKF81297, 100-300 mg/kg SKF38393) (Tirelli and Terry, 1993; Heijtz et al., 2002; Diaz Heijtz et al., 2004; Diaz Heijtz and Castellanos, 2006). Interestingly, an acute dosage study of DHX (appendix 2, Fig. 35) with only one hour of acclimation, in our reverse light-dark housed C57BL6 mice, led to a different pattern of locomotor activity, with lower doses (0.25 mg/kg, 1 mg/kg DHX) producing a reduction in locomotor activity and higher doses (4 mg/kg, 16 mg/kg DHX) producing first a reduction and then an increase in locomotor activity, as compared to saline.

In SKF81297, previous studies variation of level of baseline locomotor activity and dose, age (Diaz Heijtz et al., 2004; Scott et al., 2005), sex (Heijtz et al., 2002) and strain (Ralph and Caine, 2005; Diaz Heijtz and Castellanos, 2006) have been shown to modulate the outcome of the locomotor behaviour response of D1-class agonists, particularly the magnitude of the response. It has been proposed that D1-class agonists may be able to inhibit locomotor activity, stimulate it or both depending on the time from injection measured, by stimulating two distinct populations of D1-class receptors (Heijtz et al., 2007). In a study observing the biphasic response to SKF81297, levels of c-fos were found to be higher in the PFC during the inhibitory phase and higher in the NAc during the stimulatory phase (Diaz Heijtz et al., 2004). Additionally, lesions of the orbital frontal cortex (OFC) or PFC abolished or attenuated the inhibitory phase seen with SKF81297 respectively. This led to the proposal of an early, inhibitory output from the PFC and OFC via PFC DA terminals which synapse onto pyramidal neurons that project to the NAc. A possible locomotor inhibitory role of D5R and locomotor stimulatory role for D1R has also been proposed (Heijtz et al., 2007). Lastly, it must be noted that the motor activity inhibitory phase was not found to be DARPP-32 dependent, whereas the locomotor activity stimulation was

DARPP-32 dependent (Scott et al., 2005). It is tempting to suggest the two populations of cells may be the $G_{\alpha s}$-coupled cortical D1R expressing cells and $G_{\alpha olf}$-coupled striatal D1R expressing cells, however given that the inhibitory phase is not DARPP-32 dependent, the cortical cells would need to be biased towards non-canonical pathways (Yano et al., 2018). As this effect has been observed across several D1-class agonists with different functional selectivities (Yano et al., 2018), this effect is probably not solely due to biased agonism, however, biased agonism adds another level of complication to the picture, especially as it is likely to interact with the cellular context of the agonist-receptor interaction.

Under the theory of separate populations of cells in the PFC/OFC and striatum mediating this effect, it follows that the conflicting results seen using DHX and other D1-class agonists may be explained by differences in the sampling method, sampling timing or the level of the animals' baseline activity, preventing detection of certain aspects of the response, or intrinsic characteristics of the animals (age, sex, strain) changing the response.

Despite the complicated background of the locomotor response to DHX and other D1-class agonists, the present study was able to differentiate between different doses of DHX by an increase in locomotor activity after administration, when a long acclimation period was employed. Ultimately, the 4 mg/kg dose was chosen for the DHX stroke recovery studies. While the 16 mg/kg dose was the most consistent in producing a significant change in locomotor activity following injection, it also changed the baseline level of locomotor activity and led to notable and lasting changes in mouse behaviour, to the point of making it difficult to handle the mice. The data from the chronic administration of this dose strongly suggests sensitization to the drug, as the response to the drug tends to increase over time. By contrast, the 1 mg/kg dose did not display significant differences or even a trend towards increased locomotor activity

compared to saline. While the 4 mg/kg dose did not always produce a significant locomotor response to the drug, there was a consistent trend towards increased locomotor activity as compared to saline, under this dose. To further evaluate this dose, we tested to ensure that this dose could be blocked by co-administration of 0.5 mg/kg of D1-class antagonist SCH23390. SCH23390 was able to completely block the locomotor effects of DHX, indicating that we are likely only reaching D1-class receptors with this drug, as is intended. Finally, to increase the evidence that the 4 mg/kg dose of DHX is active in the brain, mice were sacrificed 1 hour after saline or DHX injections, and the levels of c-fos positive cells in the striatum were examined. c-fos has been shown to be upregulated downstream of DHX stimulation of D1-class receptors (Isacson et al., 2004). There was significantly more c-fos positive cells in the striatum of animals who had received 4 mg/kg DHX than saline. This should not be considered an absolute count of cells, as analysis necessitated the use of a threshold of intensity to qualify as a c-fos positive cell. Nonetheless, as the same parameters were used to evaluate both conditions the comparison is reasonable and shows that the 4 mg/kg dose of DHX reaches the brain and exerts effects there, as compared to saline. Given the results of the baseline level of c-fos expression in dark-cycle animals, and that the injections can be considered a stimulus which would likely increase c-fos levels further, the level of c-fos seen in the saline brain may be higher than that seen in other studies.

Very little data is available on the effects of chronic DHX administration, although what data is available suggests development of tolerance (Wade and Nomikos, 2005). Our data instead indicated development of behavioural sensitization, detrimentally in the case of the DHX 16 mg/kg animals. In our 4 mg/kg animals we also saw a pattern of increasing response to the drug

on the later days of the chronic dosage. The mechanism of a possible behavioural sensitization to chronic DHX remains to be elucidated by further studies.

Dosage was also tested for the D1-class antagonist SCH23390, with a view towards its use to block DHX, and for future experiments to examine the effect of the blockade of D1-class receptors on stroke recovery. Acute injection experiments revealed an effect of the 0.5 mg/kg and 1 mg/kg SCH23390 doses. Additionally, an alternate D1-class agonist, SKF81297 was tested for use in experiments with SCH23390, and was found to increase locomotor activity at a dose of 2.5 mg/kg, under conditions employed here (see appendix 2 Fig. 36). Co-administration experiments, where 0.1 mg/kg or 0.5 mg/kg SCH23390 was followed by either saline or SKF81297 revealed that both the 0.1 and 0.5 mg/kg doses were able to block locomotor activity whether SKF81297 was given or not (appendix 2 Fig. 37). This proves that SCH23390 is working at the D1-class receptor as it is able to block induction of locomotor activity by both DHX and SKF81297. Interestingly, in this experiment the 0.1 mg/kg dose of SCH23390 blocked locomotor activity, whereas it did not in the initial acute dosage experiment. Although the reason for this remains unclear, it may have something to do with the abnormally high pre-injection activity of this group, combined with the moderate nature of the dose. Certainly, when the 0.1 mg/kg and 0.5 mg/kg SCH23390 doses were given chronically (appendix 2, Fig. 38), both doses produced a significant reduction in locomotor activity following injection as compared to saline, on all seven days. The 0.5 mg/kg dose showed signs of sensitization, as mice who had received it performed less and less locomotor activity in the 40 minutes following injection as the week progressed, although this change is difficult to read, as the saline group also showed a reduction in locomotor activity following injection, likely due to acclimation to the daily injections. At the end of the seven days of injection with SCH23390 or saline, all animals were given a challenge

injection of 2.5 mg/kg SFK81297. Although it did not reach significance, the reaction of the 0.5 mg/kg SCH23390 mice was higher than that of the saline mice, further suggesting behavioural sensitization to this higher-end dose. SCH23390 consistently inhibits locomotor activity in rodent models (Bourne, 2001). Sensitization to D1-class agonists following chronic administration of SCH23390 has been reported in the literature before, in agreement with the results presented here (Hess et al., 1986; Hess et al., 1988).

3. DHX Stroke Recovery Studies

In both the 2-days DHX and the 7-days DHX studies the data does not suggest an increased risk of mortality or severe complications under the 4 mg/kg drug treatment (Fig. 5 and 7). The choice of this dose seems appropriate based on locomotor behaviour results and given the drastic increase in locomotor activity in the 16 mg/kg chronic drug group during the dosage experiment and the noted presence of stereotypies in animals undergoing D1-receptor modulating treatments. We did not observe any increased stereotypies in our 7 or 2-days DHX stroke animals, although a thorough evaluation of this was not undertaken.

The 2-days DHX stroke recovery study does not suggest efficacy of a two day course of DHX treatment starting ten days after stroke, in this stroke recovery model. The results of the cylinder test do not show recovery in either the DHX or saline groups. Results of the horizontal ladder and adhesive removal test do not show any benefit of the 2-days DHX treatment above the level of spontaneous recovery observed in the saline animals. Additionally, both groups are quite similar in terms of their infarct sizes.

In the 7-days DHX study, like the 2-days DHX study, we saw neither spontaneous, nor drug mediated recovery on the cylinder test. Results of the horizontal ladder test shows a high degree

of spontaneous recovery in both groups and no benefit of the 2-days and 7-days DHX treatments. In contrast, the adhesive removal test shows accelerated recovery only in the 7-days DHX group. On the time to contact the tape on the right paw, the 7-days DHX group shows nearly complete recovery by the postdrug time point and performs significantly better than the saline group at this timepoint. On the removal of the tape from the right paw, the results are similar, with the 7-days DHX group showing complete recovery by the postdrug timepoint, and a significantly faster mean time to remove the tape as compared to the saline group. There were no differences between the two groups in terms of infarct size as measured either by MRI at 24 hours after stroke or by cresyl violet at 31 days after stroke. The MRI and cresyl violet infarct sizes were very highly correlated with one another.

The positive results of the adhesive removal test in the 7-days DHX study raises the question of why the 2-days DHX regimen did not work. In considering this question it must be remembered that the 2-days cohorts represent a different group of animals, and that these two paradigms are not directly comparable, as there is greater impairment in the 2-days DHX group, particularly on the horizontal ladder test. These issues aside, there are to be two additional possible reasons for the inefficacy of the 2-days DHX treatment. Firstly, it may be that the 2-days course of treatment is not long enough to show a benefit on motor function following stroke. This agrees somewhat with the results of the dosage experiment, which found the 4 mg/kg dose of DHX had a significant effect on locomotor activity on the first exposure but did not have a statistically detectable effect again until day 6. Secondly, that the time from the stroke until the start of the drug period is 10 days in the 2-days DHX study, compared to only 5 days in the 7-days DHX study, may be an important factor. In designing these two studies the decision was made to keep all the testing time points, as well as the running wheel timepoint at the same time from the

stroke in both cohorts to allow for better comparison. Consequently, to be consistent with the drug administration paradigm of the 7-days DHX study, the 2-days DHX study needed to have five extra days, either between the poststroke testing and the start of the drug delivery or between the end of the drug delivery and the start of the running wheels. As it was hypothesised that the running wheels may play a more critical role in recovery in the 2-day treatment, and as it would be more informative regarding how long after stroke the treatment might be effective, the decision was made to give the drug on the last two days of what would be seven days of drug administration in the 7-days DHX cohorts. Stroke recovery may be optimal during a critical window and it is possible that the later onset of treatment fell within a period where the brain is less capable of recovery. However, given that trials using AMPH have seen success starting as late as 10-14 days after stroke (Adkins and Jones, 2005), it seems likely that both drug conditions began within the critical window for rodent stroke models. It is possible that by waiting longer until treatment, more spontaneous recovery occurred in both groups before the 2-days DHX mice had a chance to benefit from the drug. The 7-days DHX group experienced faster recovery on the adhesive removal test, as compared to the 7-days saline group, based upon the failure of the 7-days saline group to recover significantly as early as the 7-days DHX group. By contrast, the 2-days saline group recovers similarly to the 2-days DHX groups on the adhesive removal test. One potential reason for this is that it is possible that spontaneous recovery occurs more readily when the mice are not undergoing the stress of injection. While this would explain the discrepancy between the saline groups in the 2-days and 7-days studies, it would be unlikely to be an issue at the clinical level, as human patients are likely to be more amenable to an offered treatment.

Another question which naturally follows from the results here is why the 7-days DHX group only shows accelerated recovery on one of the tests and not on others. In the data presented here it is common for an animal to demonstrate a deficit on some of the outcome measures and not others (Figure 32). Additionally, findings of efficacy on some test(s) but not others are not uncommon in the literature when multiple tests are employed (Schmanke et al., 1996; Schmanke and Barth, 1997; Stroemer et al., 1998; Gilmour et al., 2005; Goldstein, 2009). These points of evidence demonstrate that although the tests employed are all tests of motor and sensorimotor behaviour, they measure different aspects of motor capability and thus a given treatment should not necessarily be expected to lead to improvements on all outcome measures. That there was no effect of the drug on infarct size does not come as much of a surprise, as comparable treatments (AMPH, MPH, L-dopa) have only rarely been suggested to improve infarct size and most studies employing these treatments have not shown this, with one exception (Liu et al., 2011). The results of the horizontal ladder are difficult to interpret as they feature a large amount of variation and small deficits. The lack of effect on the cylinder test is perhaps more informative. The cylinder test can give an indication of how much an animal chooses to use the impaired forelimb and can be an indicator of learned non-use. It may be that the 7-days DHX treatment was able to improve the limb's capability but did not increase the animals' comfort in using the limb. As this test is one upon which there was no suggestion of spontaneous recovery in the current model, it is possible that the DHX treatment works by augmenting or speeding spontaneous recovery processes, and in the absence of spontaneous recovery, would be ineffective. Another possibility is that the dose employed, while effective on the adhesive removal test, is not effective on the cylinder test. Given the inverted-U shape of effectiveness of D1-class receptor activation in particular, and that different skills can require different optimal levels of activation (consider

cognitive and working memory processes), the 4 mg/kg dose could be sub-optimal for the cylinder test and horizontal ladder test, while still facilitating recovery on the adhesive removal test (Floresco, 2013; Vaillancourt et al., 2013). Another possibility is that the cylinder test and horizontal ladder test requires activation of another relevant receptor. As a beneficial effect of AMPH and L-dopa has been demonstrated on the cylinder test, a likely contender is D2-class receptors (Goldstein, 2009; Ruscher et al., 2012).

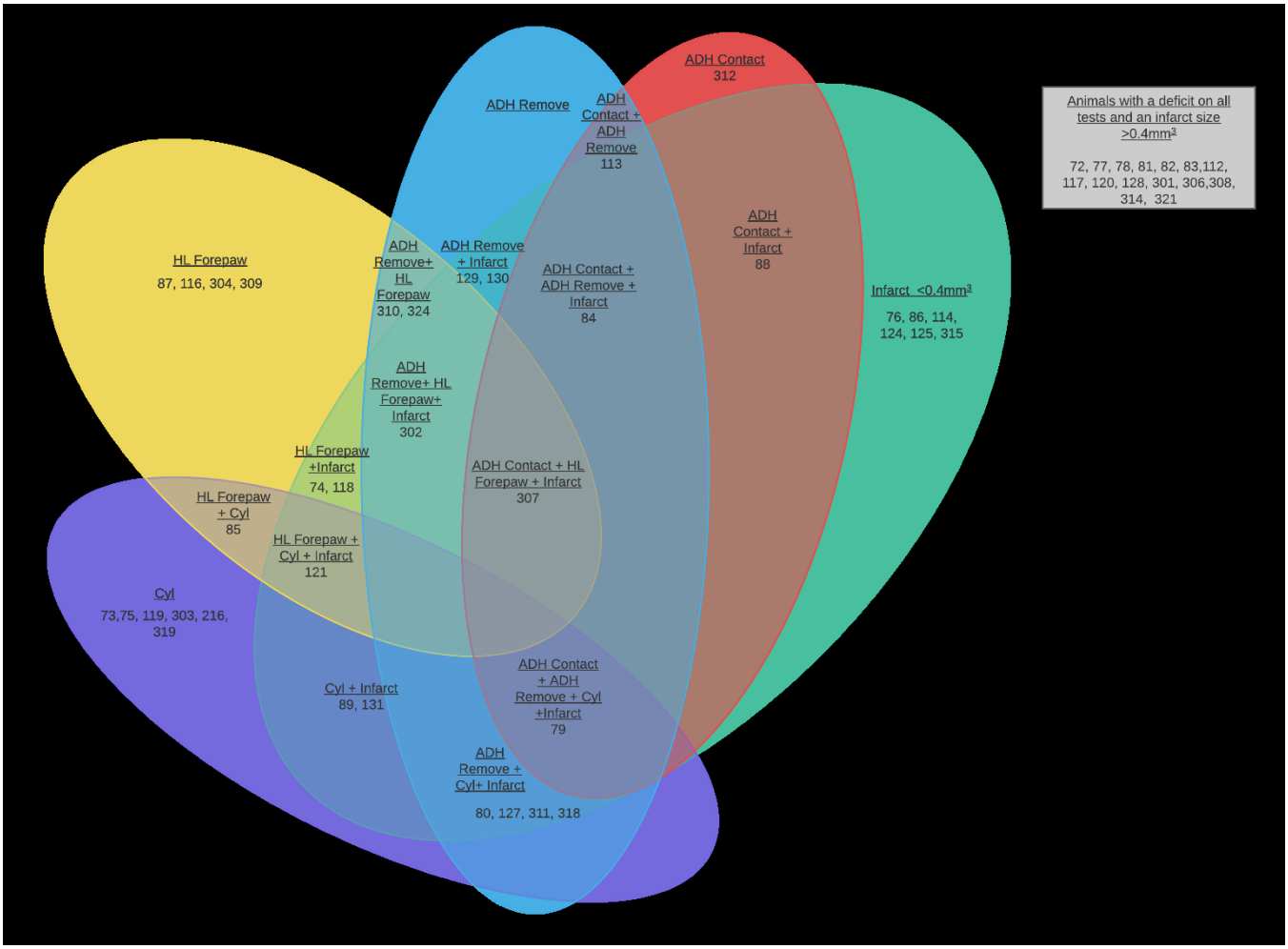

Figure 32: Mice with No Deficit on One or More Outcome Measure. The figure illustrates the various combinations of deficits that were observed across three cohorts of the 7-days DHX study. Mice, listed by their tag numbers, in the coloured Venn diagram have no deficit on a given outcome measure, or have an infarct <0.4mm^3 (arbitrarily cut-off for a "small" infarct in this stroke model). Mice listed in the grey box adjacent to the Venn diagram have a deficit on every outcome measure and a "large" (>0.4mm^3) infarct. On the cylinder test an increase in percent time spent on the right paw was counted as no deficit. On the adhesive removal test a decrease in time to contact or time to remove, after the stroke was considered no deficit on those outcome measures. On the horizontal ladder a decrease in the percentage of missteps on the right forepaw was considered no deficit. The results of the right hindpaw on the horizontal ladder were excluded for the sake of clarity. Infarct sizes as determined at 31 days poststroke are used to categorize animals in this diagram. A total of 52 mice are included.

All strokes in the study were performed on the left hemisphere, to result in right-paw deficits. Interestingly, on the horizontal ladder test, there was a trend towards the appearance of a modest left (unaffected) paw deficit in the 2-days DHX study. This is likely due to the large number of infarcts which impacted the corpus callosum, thus leading to left side deficits as well. In the 2-days DHX study 85% of DHX mice and 84% of saline mice had a stroke which contacted or infringed upon the corpus callosum. Furthermore, 35% of DHX mice and 31% of control mice had strokes in which the damage extended all the way through the corpus callosum on at least one coronal slice of the cresyl violet infarct sizing samples. Human patients with agenesis of the corpus callosum demonstrate deficiencies in bimodal motor skills and in gait (Mueller et al., 2009). Furthermore, corpus callosum damage has been correlated with worsened motor outcome, particularly in less severe strokes (Stewart et al., 2017). Similarly, a deficit developed in the left forepaw of the saline group of the 7-days DHX mice. Again, this seems tied to the incidence of stroke affecting the corpus callosum, as in the 7-days DHX study 79% of saline animals and only 54% of DHX animals had strokes that contacted the corpus callosum. This discrepancy between the groups likely originated because randomization into drug and control groups was done on the basis of the severity on the poststroke adhesive removal test. The differences in the percentage of strokes impacting the corpus callosum between the two groups is unlikely to have been the cause of the DHX group's accelerated recovery on the horizontal ladder, as in both groups 21% of mice had a "severely impacted" corpus callosum, and because the uneven weighting originated mostly from the third cohort, while similar behavioural outcomes were seen across all three cohorts.

Examination of the adhesive removal data expressed as the percent change from prestroke and stratified by the degree of deficit (Figure 23), indicates that the 7-days DHX treatment is most

beneficial to those animals who are most severely affected. In looking at animals whose poststroke scores were 100% more than their baseline score or greater, it is quite striking that all the animals in the DHX groups respond to the treatment and display improved scores at the postdrug period, going from a variable level of deficits, to a tightly clustered group close to full recovery, which sustains until the postwheel period. By contrast the saline animals in this deficit category respond variably. While many display spontaneous recovery, others stay similar or worsen at the postdrug timepoint. This group maintains a higher degree of variability, even later, at the postwheel period. Results of the groups in the lower deficit categories are less easy to decipher as spontaneous recovery appears to be a strong force at lower levels of impairment, and lower scores are more vulnerable to noise caused by chance fluctuations in individual scores. The fact that the DHX treatment seems most effective in severe cases of motor impairment, and that it seems to do so without focused rehabilitation or aerobic exercise, gives hope that it may be able to help patients who are unable to engage in rehabilitation, a population which is severely underserved by today's treatment options.

While it seems clear that DHX is able accelerate recovery on the adhesive removal test, the role of the running wheels remains less clear. At the outset of the project it was hypothesised that the DHX would be able to prime the animals for better recovery, but that it would require the running wheels to complete or to refine this recovery. This has not proved to be the case. The complete recovery of the 7-days DHX mice on the adhesive removal test before the introduction of the running wheels demonstrates that DHX is capable of augmenting recovery on its own. This agrees with the results of a study which employed L-dopa in a rodent stroke model, with no physical rehabilitation, and saw improved recovery with the drug (Ruscher et al., 2012). Where some deficit remains at the poststroke period, a role for wheels is possible. Unfortunately, the

setup of the experiment, with all the animals getting operational running wheels for the running wheel period, makes it impossible to determine what degree of recovery occurring after the postdrug timepoint is due to the running wheels and what can be attributed to spontaneous recovery and the continued passing of time. The decision to exclude a condition in which the animals did not have a voluntary running period was made to increase the power of our analysis, particularly in light of the full recovery made on the adhesive removal test by the postdrug period in the 7-days DHX animals. If the running wheels are exerting an effect on recovery, it may actually involve mechanisms in common with the DHX treatment as voluntary running and aerobic exercise has been shown to increase levels of DA (Herrera et al., 2016) and BDNF (Solvsten et al., 2017).

In stroke recovery, the division between true recovery of function and compensation is an ongoing issue. Unfortunately, the behavioural tests employed here are somewhat vulnerable to compensation mediated improvement. Further work, employing kinematic analysis can clarify the degree of improvement that is mediated by true recovery, and the degree mediated by compensation (Corbett et al., 2017). Although true recovery is more desirable in stroke recovery, compensation can still improve quality of life for human patients and improve their proficiency on activities of daily living. Another concern with the data presented herein is that the demonstrated improvements are not due to better motor ability but rather to D1-class receptor mediated changes in motivation. Given the intimate link between the DA system and the reward processing in the brain this is a natural question (Beaulieu and Gainetdinov, 2011). While some studies do suggest that DA-modulating drugs can have beneficial effects on post-stroke depression this seems unlikely to be the primary mechanism at play here (Kohno et al., 2010; Delbari et al., 2011; Adam et al., 2013; Stanfill et al., 2016). The DHX-treated animals are not

more motivated to use the running wheels during the voluntary running period. Further, the continued high performance of the left paw on the adhesive removal test suggest that the mice remain highly motivated to perform this task to the best of their ability throughout the study, regardless of their drug condition.

Human stroke recovery is a process that lasts months, and typically plateaus at a level where significant impairment is still present. Unfortunately, the currently available stroke models do not precisely mimic the pattern of human stroke recovery, as complete recovery, even complete spontaneous recovery over time, is possible in some models (Corbett et al., 2017). This means that instead of being able to demonstrate superior recovery from stroke, preclinical studies are often demonstrating more rapid recovery from stroke. This has been the case in many of the previous AMPH studies when longer follow-up evaluations were employed (Hovda and Fenney, 1984; Schmanke et al., 1996; Adkins and Jones, 2005; Papadopoulos et al., 2009). This is also the case in the data presented here. The data indicates accelerated recovery; however, the experiment set-up is not ideal for this type of analysis. A better setup might include more testing timepoints for at least some of the tests, allowing for better charting of the altered time course of recovery under the treatment. This is difficult with the tests used here, as the level of deficit produced, and the current level of spontaneous recovery does not leave much room for improvement from practice on the tests, yet further exposure to the tests could act as rehabilitation for simple tests like the adhesive removal test and the horizontal ladder. A more challenging fine motor test, with a longer learning curve, such as single pellet reaching, might better serve for this purpose.

Another improvement on this work would be to sacrifice mice at each major testing timepoint (poststroke, postdrug and postwheel), instead of just at the end of the experiment, to allow for

analysis of protein levels at every step along the process. Analysis of the tissue samples collected is planned but has not yet yielded any results, so presently, there are no hints toward the mechanism of the 7-days DHX mediated acceleration of recovery. From the literature, several mechanisms demonstrated in AMPH, MPH and L-dopa studies can be linked to D1-class receptors. Reorganization of cortical maps, via LTP and LTD, has been linked to stroke recovery (Castro-Alamancos and Borrell, 1995; Nudo, 2013) and may be modulated by D1-class receptors (Luft et al., 2004; Molina-Luna et al., 2009; Hosp et al., 2011; Rioult-Pedotti et al., 2015). Further, D1-class receptors are particularly associated with growth factors BDNF and GDNF (Küppers and Beyer, 2001; Perreault et al., 2012; Ruscher et al., 2012; Xing et al., 2012; Kuric et al., 2013), which have both been shown to play important roles in stroke recovery, (Kurozumi et al., 2004; Kobayashi et al., 2006; Ohwatashi et al., 2013; Berretta et al., 2014; Cook et al., 2017). Of particular interest is the findings from L-dopa studies in animal stroke models, which linked L-dopa mediated recovery to GDNF levels mediated by reactive astrocytes which were newly expressing D1-class receptors in the peri-infarct zone (Ruscher et al., 2012; Kuric et al., 2013).

The work presented here represents the first evidence for the efficacy of D1-class receptor activation as a tool to promote motor recovery from stroke. As was the initial goal, this work provides proof of principle for the use of DHX in stroke recovery. One day, a D1-class receptor agonist-based treatment may join the arsenal of tools for recovery from stroke and may help patients regain their motor function and their quality of life. Before that point, much work remains to be done. Future work must focus on clarifying the mechanisms involved and if this effect is specific to DHX or can be mediated by other D1-class agonists. Additionally, the results should be verified in other stroke models, in aged animals and in female animals (Corbett et al., 2017). Further work to define the optimal timing of application for this treatment, whether it

works better in sequence or in parallel to physical rehabilitation, and how it might interact with other treatments under current development (eg stem cells, TMS) should be determined. Although the path of translation of stroke recovery treatments has been notoriously rocky, refinements at the preclinical level can help smooth the road. More specific activation of relevant receptors could be considered one such refinement, and can help the field to build on large, positive bodies of work, such as that suggesting the efficacy of AMPH and L-dopa in poststroke recovery, rather than discarding such hopeful evidence in the face of mixed clinical results.

References

Abe, K., Kashiwagi, Y., Tokumura, M., Hosoi, R., Hatazawa, J., and Inoue, O. (2004). Discrepancy between cell injury and benzodiazepine receptor binding after transient middle cerebral artery occlusion in rats. *Synapse* 53(4)**,** 234-239. doi: 10.1002/syn.20057.

Acler, M., Fiaschi, A., and Manganotti, P. (2009). Long-term levodopa administration in chronic stroke patients. A clinical and neurophysiologic single-blind placebo-controlled cross-over pilot study. *Restor Neurol Neurosci* 27(4)**,** 277-283. doi: 10.3233/RNN-2009-0477.

Acler, M., and Manganotti, P. (2013). Role, indications and controversies of levodopa administration in chronic stroke patients. *Eur J Phys Rehabil Med* 49(2)**,** 243-249.

Adam, R., Leff, A., Sinha, N., Turner, C., Bays, P., Draganski, B., et al. (2013). Dopamine reverses reward insensitivity in apathy following globus pallidus lesions. *Cortex* 49(5)**,** 1292-1303. doi: 10.1016/j.cortex.2012.04.013.

Adeoye, O., Hornung, R., Khatri, P., and Kleindorfer, D. (2011). Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: a doubling of treatment rates over the course of 5 years. *Stroke* 42(7)**,** 1952-1955. doi: 10.1161/STROKEAHA.110.612358.

Adkins, D.L., and Jones, T.A. (2005). D-Amphetamine enhances skilled reaching after ischemic cortical lesions in rats. *Neurosci Lett* 380(3)**,** 214-218. doi: 10.1016/j.neulet.2005.01.036.

Adkins, D.L., Schallert, T., and Goldstein, L.B. (2009). Poststroke treatment: lost in translation. *Stroke* 40(1)**,** 8-9. doi: 10.1161/STROKEAHA.108.534248.

Ahagon, A., Ishikawa, M., and Handa, H. (1980). Histochemical changes of brain dopamine in an acute stage of cerebral ischemia in gerbils. *Stroke* 11(6)**,** 622-628.

Akiyama, Y., Ito, A., Koshimura, K., Ohue, T., Yamagata, S., Miwa, S., et al. (1991). Effects of transient forebrain ischemia and reperfusion on function of dopaminergic neurons and dopamine reuptake in vivo in rat striatum. *Brain Res* 561(1)**,** 120-127.

Alaverdashvili, M., Lim, D.H., and Whishaw, I.Q. (2007). No improvement by amphetamine on learned non-use, attempts, success or movement in skilled reaching by the rat after motor cortex stroke. *Eur J Neurosci* 25(11)**,** 3442-3452. doi: 10.1111/j.1460-9568.2007.05594.x.

Arai, K., Jin, G., Navaratna, D., and Lo, E.H. (2009). Brain angiogenesis in developmental and pathological processes: neurovascular injury and angiogenic recovery after stroke. *FEBS J* 276(17)**,** 4644-4652. doi: 10.1111/j.1742-4658.2009.07176.x.

Arnsten, A.F., Girgis, R.R., Gray, D.L., and Mailman, R.B. (2017). Novel Dopamine Therapeutics for Cognitive Deficits in Schizophrenia. *Biol Psychiatry* 81(1)**,** 67-77. doi: 10.1016/j.biopsych.2015.12.028.

Arnsten, A.F., Wang, M., and Paspalas, C.D. (2015). Dopamine's Actions in Primate Prefrontal Cortex: Challenges for Treating Cognitive Disorders. *Pharmacol Rev* 67(3)**,** 681-696. doi: 10.1124/pr.115.010512.

Auriat, A.M., and Colbourne, F. (2008). Influence of amphetamine on recovery after intracerebral hemorrhage in rats. *Behav Brain Res* 186(2)**,** 222-229. doi: 10.1016/j.bbr.2007.08.010.

Barbay, S., and Nudo, R.J. (2009). The effects of amphetamine on recovery of function in animal models of cerebral injury: a critical appraisal. *NeuroRehabilitation* 25(1)**,** 5-17. doi: 10.3233/NRE-2009-0495.

Barbay, S., Zoubina, E.V., Dancause, N., Frost, S.B., Eisner-Janowicz, I., Stowe, A.M., et al. (2006). A single injection of D-amphetamine facilitates improvements in motor training following a focal cortical infarct in squirrel monkeys. *Neurorehabil Neural Repair* 20(4)**,** 455-458. doi: 10.1177/1545968306290773.

Barbeau, A., Mars, H., Botez, M.I., and Joubert, M. (1972). Levodopa combined with peripheral decarboxylase inhibition in Parkinson's disease. *Can Med Assoc J* 106(11)**,** 1169-1174.

Bath, P.M., Lees, K.R., Schellinger, P.D., Altman, H., Bland, M., Hogg, C., et al. (2012). Statistical analysis of the primary outcome in acute stroke trials. *Stroke* 43(4)**,** 1171-1178. doi: 10.1161/STROKEAHA.111.641456.

Beaulieu, J.M., Espinoza, S., and Gainetdinov, R.R. (2015). Dopamine receptors - IUPHAR Review 13. *Br J Pharmacol* 172(1)**,** 1-23. doi: 10.1111/bph.12906.

Beaulieu, J.M., and Gainetdinov, R.R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 63(1)**,** 182-217. doi: 10.1124/pr.110.002642.

Beeler, J.A., Prendergast, B., and Zhuang, X. (2006). Low amplitude entrainment of mice and the impact of circadian phase on behavior tests. *Physiol Behav* 87(5)**,** 870-880. doi: 10.1016/j.physbeh.2006.01.037.

Berretta, A., Tzeng, Y.C., and Clarkson, A.N. (2014). Post-stroke recovery: the role of activity-dependent release of brain-derived neurotrophic factor. *Expert Rev Neurother* 14(11)**,** 1335-1344. doi: 10.1586/14737175.2014.969242.

Bhakta, B.B., Hartley, S., Holloway, I., Couzens, J.A., Ford, G.A., Meads, D., et al. (2014). The DARS (Dopamine Augmented Rehabilitation in Stroke) trial: protocol for a randomised controlled trial of Co-careldopa treatment in addition to routine NHS occupational and physical therapy after stroke. *Trials* 15**,** 316. doi: 10.1186/1745-6215-15-316.

Blanchet, P.J., Fang, J., Gillespie, M., Sabounjian, L., Locke, K.W., Gammans, R., et al. (1998). Effects of the full dopamine D1 receptor agonist dihydrexidine in Parkinson's disease. *Clin Neuropharmacol* 21(6)**,** 339-343.

Borlongan, C.V., Martinez, R., Shytle, R.D., Freeman, T.B., Cahill, D.W., and Sanberg, P.R. (1995). Striatal dopamine-mediated motor behavior is altered following occlusion of the middle cerebral artery. *Pharmacol Biochem Behav* 52(1)**,** 225-229.

Bouet, V., Boulouard, M., Toutain, J., Divoux, D., Bernaudin, M., Schumann-Bard, P., et al. (2009). The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. *Nat Protoc* 4(10)**,** 1560-1564. doi: 10.1038/nprot.2009.125.

Bourne, J.A. (2001). SCH 23390: the first selective dopamine D1-like receptor antagonist. *CNS Drug Rev* 7(4)**,** 399-414.

Boyar, W.C., and Altar, C.A. (1987). Modulation of in vivo dopamine release by D2 but not D1 receptor agonists and antagonists. *J Neurochem* 48(3)**,** 824-831.

Boyd, L.A., Hayward, K.S., Ward, N.S., Stinear, C.M., Rosso, C., Fisher, R.J., et al. (2017). Biomarkers of stroke recovery: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable. *Int J Stroke* 12(5)**,** 480-493. doi: 10.1177/1747493017714176.

Boyeson, M.G., and Feeney, D.M. (1990). Intraventricular norepinephrine facilitates motor recovery following sensorimotor cortex injury. *Pharmacol Biochem Behav* 35(3)**,** 497-501.

Boyeson, M.G., and Feeney, D.M. (1991). Adverse effects of catecholaminergic drugs following unilateral cerebellar ablations. *Restor Neurol Neurosci* 3(5)**,** 227-233. doi: 10.3233/RNN-1991-3501.

Brannan, T., Weinberger, J., Knott, P., Taff, I., Kaufmann, H., Togasaki, D., et al. (1987). Direct evidence of acute, massive striatal dopamine release in gerbils with unilateral strokes. *Stroke* 18(1)**,** 108-110.

Breitenstein, C., Flöel, A., Korsukewitz, C., Wailke, S., Bushuven, S., and Knecht, S. (2006). A shift of paradigm: from noradrenergic to dopaminergic modulation of learning? *J Neurol Sci* 248(1-2)**,** 42-47. doi: 10.1016/j.jns.2006.05.012.

Brown, A.W., Bjelke, B., and Fuxe, K. (2004). Motor response to amphetamine treatment, task-specific training, and limited motor experience in a postacute animal stroke model. *Exp Neurol* 190(1)**,** 102-108. doi: 10.1016/j.expneurol.2004.07.005.

Buisson, A., Callebert, J., Mathieu, E., Plotkine, M., and Boulu, R.G. (1992). Striatal protection induced by lesioning the substantia nigra of rats subjected to focal ischemia. *J Neurochem* 59(3)**,** 1153-1157.

Calabresi, P., Gubellini, P., Centonze, D., Picconi, B., Bernardi, G., Chergui, K., et al. (2000). Dopamine and cAMP-regulated phosphoprotein 32 kDa controls both striatal long-term depression and long-term potentiation, opposing forms of synaptic plasticity. *J Neurosci* 20(22)**,** 8443-8451.

Calabresi, P., Picconi, B., Tozzi, A., and Di Filippo, M. (2007). Dopamine-mediated regulation of corticostriatal synaptic plasticity. *Trends Neurosci* 30(5)**,** 211-219. doi: 10.1016/j.tins.2007.03.001.

Calabresi, P., Saiardi, A., Pisani, A., Baik, J.H., Centonze, D., Mercuri, N.B., et al. (1997). Abnormal synaptic plasticity in the striatum of mice lacking dopamine D2 receptors. *J Neurosci* 17(12)**,** 4536-4544.

Caleo, M. (2015). Rehabilitation and plasticity following stroke: Insights from rodent models. *Neuroscience* 311**,** 180-194. doi: 10.1016/j.neuroscience.2015.10.029.

Castaneda, T.R., de Prado, B.M., Prieto, D., and Mora, F. (2004). Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. *J Pineal Res* 36(3)**,** 177-185.

Castro-Alamancos, M.A., and Borrell, J. (1995). Functional recovery of forelimb response capacity after forelimb primary motor cortex damage in the rat is due to the reorganization of adjacent areas of cortex. *Neuroscience* 68(3)**,** 793-805.

Castro-Alamancos, M.A., Garcia-Segura, L.M., and Borrell, J. (1992). Transfer of Function to a Specific Area of the Cortex After Induced Recovery from Brain Damage. *Eur J Neurosci* 4(9)**,** 853-863.

Centonze, D., Grande, C., Saulle, E., Martin, A.B., Gubellini, P., Pavon, N., et al. (2003). Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. *J Neurosci* 23(24)**,** 8506-8512.

Centonze, D., Picconi, B., Gubellini, P., Bernardi, G., and Calabresi, P. (2001). Dopaminergic control of synaptic plasticity in the dorsal striatum. *Eur J Neurosci* 13(6)**,** 1071-1077.

Challman, T.D., and Lipsky, J.J. (2000). Methylphenidate: its pharmacology and uses. *Mayo Clin Proc* 75(7)**,** 711-721. doi: 10.4065/75.7.711.

Chen, K., Marsh, B.C., Cowan, M., Al'Joboori, Y.D., Gigout, S., Smith, C.C., et al. (2017). Sequential therapy of anti-Nogo-A antibody treatment and treadmill training leads to cumulative improvements after spinal cord injury in rats. *Exp Neurol* 292**,** 135-144. doi: 10.1016/j.expneurol.2017.03.012.

Choi, J.K., Chen, Y.I., Hamel, E., and Jenkins, B.G. (2006). Brain hemodynamic changes mediated by dopamine receptors: Role of the cerebral microvasculature in dopamine-mediated neurovascular coupling. *Neuroimage* 30(3)**,** 700-712. doi: 10.1016/j.neuroimage.2005.10.029.

Chollet, F., Cramer, S.C., Stinear, C., Kappelle, L.J., Baron, J.C., Weiller, C., et al. (2014). Pharmacological therapies in post stroke recovery: recommendations for future clinical trials. *J Neurol* 261(8)**,** 1461-1468. doi: 10.1007/s00415-013-7172-z.

Cook, D.J., Nguyen, C., Chun, H.N., I, L.L., Chiu, A.S., Machnicki, M., et al. (2017). Hydrogel-delivered brain-derived neurotrophic factor promotes tissue repair and recovery after stroke. *J Cereb Blood Flow Metab* 37(3)**,** 1030-1045. doi: 10.1177/0271678X16649964.

Cools, R., and D'Esposito, M. (2011). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 69(12)**,** e113-125. doi: 10.1016/j.biopsych.2011.03.028.

Corbett, D., Carmichael, S.T., Murphy, T.H., Jones, T.A., Schwab, M.E., Jolkkonen, J., et al. (2017). Enhancing the Alignment of the Preclinical and Clinical Stroke Recovery Research Pipeline: Consensus-Based Core Recommendations From the Stroke Recovery and Rehabilitation Roundtable Translational Working Group. *Neurorehabil Neural Repair* 31(8)**,** 699-707. doi: 10.1177/1545968317724285.

Cramer, S.C., Dobkin, B.H., Noser, E.A., Rodriguez, R.W., and Enney, L.A. (2009). Randomized, placebo-controlled, double-blind study of ropinirole in chronic stroke. *Stroke* 40(9)**,** 3034-3038. doi: 10.1161/STROKEAHA.109.552075.

Crisostomo, E.A., Duncan, P.W., Propst, M., Dawson, D.V., and Davis, J.N. (1988). Evidence that amphetamine with physical therapy promotes recovery of motor function in stroke patients. *Ann Neurol* 23(1)**,** 94-97. doi: 10.1002/ana.410230117.

Cvejic, V., Micic, D.V., Djuricic, B.M., Mrsulja, B.J., and Mrsulja, B.B. (1980). Monoamines and related enzymes in cerebral cortex and basal ganglia following transient ischemia in gerbils. *Acta Neuropathol* 51(1)**,** 71-77.

Darney, K.J., Jr., Lewis, M.H., Brewster, W.K., Nichols, D.E., and Mailman, R.B. (1991). Behavioral effects in the rat of dihydrexidine, a high-potency, full-efficacy D1 dopamine receptor agonist. *Neuropsychopharmacology* 5(3)**,** 187-195.

Dawson, V.L., Hsu, C.Y., Liu, T.H., Dawson, T.M., and Wamsley, J.K. (1994). Receptor alterations in subcortical structures after bilateral middle cerebral artery infarction of the cerebral cortex. *Exp Neurol* 128(1)**,** 88-96. doi: 10.1006/exnr.1994.1115.

Delanoy, R.L., Tucci, D.L., and Gold, P.E. (1983). Amphetamine effects on long term potentiation in dentate granule cells. *Pharmacol Biochem Behav* 18(1)**,** 137-139.

Delbari, A., Salman-Roghani, R., and Lokk, J. (2011). Effect of methylphenidate and/or levodopa combined with physiotherapy on mood and cognition after stroke: a randomized, double-blind, placebo-controlled trial. *Eur Neurol* 66(1)**,** 7-13. doi: 10.1159/000329275.

Delbarre, B., Delbarre, G., and Calinon, F. (1992). Free radicals and neurotransmitters in gerbil brain. Influence of age and ischemia reperfusion insult. *EXS* 62**,** 199-212.

Deveney, A.M., and Waddington, J.L. (1997). Psychopharmacological distinction between novel full-efficacy "D1-like" dopamine receptor agonists. *Pharmacol Biochem Behav* 58(2)**,** 551-558.

Diaz Heijtz, R., and Castellanos, F.X. (2006). Differential effects of a selective dopamine D1-like receptor agonist on motor activity and c-fos expression in the frontal-striatal circuitry of SHR and Wistar-Kyoto rats. *Behav Brain Funct* 2**,** 18. doi: 10.1186/1744-9081-2-18.

Diaz Heijtz, R., Scott, L., and Forssberg, H. (2004). Alteration of dopamine D1 receptor-mediated motor inhibition and stimulation during development in rats is associated with distinct patterns of c-fos mRNA expression in the frontal-striatal circuitry. *Eur J Neurosci* 19(4)**,** 945-956.

Dudman, J.T., Eaton, M.E., Rajadhyaksha, A., Macias, W., Taher, M., Barczak, A., et al. (2003). Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. *J Neurochem* 87(4)**,** 922-934.

Feeney, D.M., Gonzalez, A., and Law, W.A. (1982). Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. *Science* 217(4562)**,** 855-857.

Feeney, D.M., and Hovda, D.A. (1983). Amphetamine and apomorphine restore tactile placing after motor cortex injury in the cat. *Psychopharmacology (Berl)* 79(1)**,** 67-71.

Feeney, D.M., Weisend, M.P., and Kline, A.E. (1993). Noradrenergic pharmacotherapy, intracerebral infusion and adrenal transplantation promote functional recovery after cortical damage. *J Neural Transplant Plast* 4(3)**,** 199-213. doi: 10.1155/NP.1993.199.

Feenstra, M.G., Botterblom, M.H., and Mastenbroek, S. (2000). Dopamine and noradrenaline efflux in the prefrontal cortex in the light and dark period: effects of novelty and handling and comparison to the nucleus accumbens. *Neuroscience* 100(4)**,** 741-748.

Floel, A., Breitenstein, C., Hummel, F., Celnik, P., Gingert, C., Sawaki, L., et al. (2005). Dopaminergic influences on formation of a motor memory. *Ann Neurol* 58(1)**,** 121-130. doi: 10.1002/ana.20536.

Floel, A., Vomhof, P., Lorenzen, A., Roesser, N., Breitenstein, C., and Knecht, S. (2008). Levodopa improves skilled hand functions in the elderly. *Eur J Neurosci* 27(5)**,** 1301-1307. doi: 10.1111/j.1460-9568.2008.06079.x.

Floresco, S.B. (2013). Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions. *Front Neurosci* 7**,** 62. doi: 10.3389/fnins.2013.00062.

Fumagalli, F., Racagni, G., and Riva, M.A. (2006). Shedding light into the role of BDNF in the pharmacotherapy of Parkinson's disease. *Pharmacogenomics J* 6(2)**,** 95-104. doi: 10.1038/sj.tpj.6500360.

Gendreau, P.L., Gariepy, J.L., Petitto, J.M., and Lewis, M.H. (1997). D1 dopamine receptor mediation of social and nonsocial emotional reactivity in mice: effects of housing and strain difference in motor activity. *Behav Neurosci* 111(2)**,** 424-434.

George, M.S., Molnar, C.E., Grenesko, E.L., Anderson, B., Mu, Q.W., Johnson, K., et al. (2007). A single 20 mg dose of dihydrexidine (DAR-0100), a full dopamine D-1 agonist, is safe and tolerated in patients with schizophrenia. *Schizophrenia Research* 93(1-3)**,** 42-50. doi: 10.1016/j.schres.2007.03.011.

Gilmour, G., Iversen, S.D., O'Neill, M.F., O'Neill, M.J., Ward, M.A., and Bannerman, D.M. (2005). Amphetamine promotes task-dependent recovery following focal cortical ischaemic lesions in the rat. *Behav Brain Res* 165(1)**,** 98-109. doi: 10.1016/j.bbr.2005.06.027.

Girgis, R.R., Van Snellenberg, J.X., Glass, A., Kegeles, L.S., Thompson, J.L., Wall, M., et al. (2016). A proof-of-concept, randomized controlled trial of DAR-0100A, a dopamine-1 receptor agonist, for cognitive enhancement in schizophrenia. *Journal of Psychopharmacology* 30(5)**,** 428-435. doi: 10.1177/0269881116636120.

Gladstone, D.J., Danells, C.J., Armesto, A., McIlroy, W.E., Staines, W.R., Graham, S.J., et al. (2006). Physiotherapy coupled with dextroamphetamine for rehabilitation after hemiparetic stroke: a randomized, double-blind, placebo-controlled trial. *Stroke* 37(1)**,** 179-185. doi: 10.1161/01.STR.0000195169.42447.78.

Globas, C., Becker, C., Cerny, J., Lam, J.M., Lindemann, U., Forrester, L.W., et al. (2012). Chronic stroke survivors benefit from high-intensity aerobic treadmill exercise: a randomized control trial. *Neurorehabil Neural Repair* 26(1)**,** 85-95. doi: 10.1177/1545968311418675.

Globus, M.Y., Busto, R., Dietrich, W.D., Martinez, E., Valdes, I., and Ginsberg, M.D. (1988). Effect of ischemia on the in vivo release of striatal dopamine, glutamate, and gamma-aminobutyric acid studied by intracerebral microdialysis. *J Neurochem* 51(5)**,** 1455-1464.

Globus, M.Y., Ginsberg, M.D., Dietrich, W.D., Busto, R., and Scheinberg, P. (1987). Substantia nigra lesion protects against ischemic damage in the striatum. *Neurosci Lett* 80(3)**,** 251-256.

Goldstein, L.B. (1995). Common drugs may influence motor recovery after stroke. The Sygen In Acute Stroke Study Investigators. *Neurology* 45(5)**,** 865-871.

Goldstein, L.B. (1998). Potential effects of common drugs on stroke recovery. *Arch Neurol* 55(4)**,** 454-456.

Goldstein, L.B. (2009). Amphetamine trials and tribulations. *Stroke* 40(3 Suppl)**,** S133-135. doi: 10.1161/STROKEAHA.108.533703.

Goldstein, L.B., and Davis, J.N. (1990a). Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury. *J Neurosci Methods* 31(2)**,** 101-107.

Goldstein, L.B., and Davis, J.N. (1990b). Influence of lesion size and location on amphetamine-facilitated recovery of beam-walking in rats. *Behav Neurosci* 104(2)**,** 320-327.

Goldstein, L.B., Matchar, D.B., Morgenlander, J.C., and Davis, J.N. (1990). Influence of Drugs on the Recovery of Sensorimotor Function After Stroke. *J Neurol Rehabil* 4(3)**,** 137-144. doi: 10.1177/136140969000400303.

Gomez-Pinilla, F., Ying, Z., Roy, R.R., Molteni, R., and Edgerton, V.R. (2002). Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol* 88(5)**,** 2187-2195. doi: 10.1152/jn.00152.2002.

Grade, C., Redford, B., Chrostowski, J., Toussaint, L., and Blackwell, B. (1998). Methylphenidate in early poststroke recovery: a double-blind, placebo-controlled study. *Arch Phys Med Rehabil* 79(9)**,** 1047-1050.

Greenwood, B.N., Spence, K.G., Crevling, D.M., Clark, P.J., Craig, W.C., and Fleshner, M. (2013). Exercise-induced stress resistance is independent of exercise controllability and the medial prefrontal cortex. *Eur J Neurosci* 37(3)**,** 469-478. doi: 10.1111/ejn.12044.

Gurden, H., Takita, M., and Jay, T.M. (2000). Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. *J Neurosci* 20(22)**,** RC106.

Gurden, H., Tassin, J.P., and Jay, T.M. (1999). Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal-prefrontal cortex long-term potentiation. *Neuroscience* 94(4)**,** 1019-1027.

Hallett, M. (2001). Plasticity of the human motor cortex and recovery from stroke. *Brain Res Brain Res Rev* 36(2-3)**,** 169-174.

Hama, S., Murakami, T., Yamashita, H., Onoda, K., Yamawaki, S., and Kurisu, K. (2017). Neuroanatomic pathways associated with monoaminergic dysregulation after stroke. *Int J Geriatr Psychiatry* 32(6)**,** 633-642. doi: 10.1002/gps.4503.

Harrison, M.J., Marsden, C.D., and Jenner, P. (1979). Effect of experimental ischemia on neurotransmitter amines in the gerbil brain. *Stroke* 10(2)**,** 165-168.

Hashimoto, N., Matsumoto, T., Mabe, H., Hashitani, T., and Nishino, H. (1994). Dopamine has inhibitory and accelerating effects on ischemia-induced neuronal cell damage in the rat striatum. *Brain Res Bull* 33(3)**,** 281-288.

Heijtz, R.D., Beraki, S., Scott, L., Aperia, A., and Forssberg, H. (2002). Sex differences in the motor inhibitory and stimulatory role of dopamine D1 receptors in rats. *Eur J Pharmacol* 445(1-2)**,** 97-104.

Heijtz, R.D., Kolb, B., and Forssberg, H. (2007). Motor inhibitory role of dopamine D1 receptors: implications for ADHD. *Physiol Behav* 92(1-2)**,** 155-160. doi: 10.1016/j.physbeh.2007.05.024.

Herrera, J.J., Fedynska, S., Ghasem, P.R., Wieman, T., Clark, P.J., Gray, N., et al. (2016). Neurochemical and behavioural indices of exercise reward are independent of exercise controllability. *Eur J Neurosci* 43(9)**,** 1190-1202. doi: 10.1111/ejn.13193.

Hess, E.J., Albers, L.J., Le, H., and Creese, I. (1986). Effects of chronic SCH23390 treatment on the biochemical and behavioral properties of D1 and D2 dopamine receptors: potentiated behavioral responses to a D2 dopamine agonist after selective D1 dopamine receptor upregulation. *J Pharmacol Exp Ther* 238(3)**,** 846-854.

Hess, E.J., Norman, A.B., and Creese, I. (1988). Chronic treatment with dopamine receptor antagonists: behavioral and pharmacologic effects on D1 and D2 dopamine receptors. *J Neurosci* 8(7)**,** 2361-2370.

Hosp, J.A., Pekanovic, A., Rioult-Pedotti, M.S., and Luft, A.R. (2011). Dopaminergic projections from midbrain to primary motor cortex mediate motor skill learning. *J Neurosci* 31(7)**,** 2481-2487. doi: 10.1523/JNEUROSCI.5411-10.2011.

Hossain, S.M., Wong, B.K., and Simpson, E.M. (2004). The dark phase improves genetic discrimination for some high throughput mouse behavioral phenotyping. *Genes Brain Behav* 3(3)**,** 167-177. doi: 10.1111/j.1601-183x.2004.00069.x.

Hotte, M., Naudon, L., and Jay, T.M. (2005). Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. *Neurobiol Learn Mem* 84(2)**,** 85-92. doi: 10.1016/j.nlm.2005.04.002.

Hotte, M., Thuault, S., Lachaise, F., Dineley, K.T., Hemmings, H.C., Nairn, A.C., et al. (2006). D1 receptor modulation of memory retrieval performance is associated with changes in pCREB and pDARPP-32 in rat prefrontal cortex. *Behav Brain Res* 171(1)**,** 127-133. doi: 10.1016/j.bbr.2006.03.026.

Hovda, D.A., and Fenney, D.M. (1984). Amphetamine with experience promotes recovery of locomotor function after unilateral frontal cortex injury in the cat. *Brain Res* 298(2)**,** 358-361.

Hovda, D.A., Sutton, R.L., and Feeney, D.M. (1987). Recovery of tactile placing after visual cortex ablation in cat: a behavioral and metabolic study of diaschisis. *Exp Neurol* 97(2)**,** 391-402.

Huang, Y.Y., Simpson, E., Kellendonk, C., and Kandel, E.R. (2004). Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proc Natl Acad Sci U S A* 101(9)**,** 3236-3241. doi: 10.1073/pnas.0308280101.

Huck, J.H., Freyer, D., Bottcher, C., Mladinov, M., Muselmann-Genschow, C., Thielke, M., et al. (2015). De novo expression of dopamine D2 receptors on microglia after stroke. *J Cereb Blood Flow Metab* 35(11)**,** 1804-1811. doi: 10.1038/jcbfm.2015.128.

Hudetz, J.A., and Pagel, P.S. (2010). Neuroprotection by ketamine: a review of the experimental and clinical evidence. *J Cardiothorac Vasc Anesth* 24(1)**,** 131-142. doi: 10.1053/j.jvca.2009.05.008.

Isacson, R., Kull, B., Wahlestedt, C., and Salmi, P. (2004). A 68930 and dihydrexidine inhibit locomotor activity and d-amphetamine-induced hyperactivity in rats: A role of inhibitory dopamine D-1/5 receptors in the prefrontal cortex? *Neuroscience* 124(1)**,** 33-42. doi: 10.1016/j.neuroscience.2003.11.016.

Ishihara, N., Welch, K.M., Meyer, J.S., Chabi, E., Naritomi, H., Wang, T.P., et al. (1979). Influence of cerebral embolism on brain monoamines. *J Neurol Neurosurg Psychiatry* 42(9)**,** 847-853.

Issa, R., AlQteishat, A., Mitsios, N., Saka, M., Krupinski, J., Tarkowski, E., et al. (2005). Expression of basic fibroblast growth factor mRNA and protein in the human brain following ischaemic stroke. *Angiogenesis* 8(1)**,** 53-62. doi: 10.1007/s10456-005-5613-8.

Iwakura, Y., Nawa, H., Sora, I., and Chao, M.V. (2008). Dopamine D1 receptor-induced signaling through TrkB receptors in striatal neurons. *J Biol Chem* 283(23)**,** 15799-15806. doi: 10.1074/jbc.M801553200.

Jang, S.H. (2013). Motor function-related maladaptive plasticity in stroke: a review. *NeuroRehabilitation* 32(2)**,** 311-316. doi: 10.3233/NRE-130849.

Jeffers, M.S., Hoyles, A., Morshead, C., and Corbett, D. (2014). Epidermal growth factor and erythropoietin infusion accelerate functional recovery in combination with rehabilitation. *Stroke* 45(6)**,** 1856-1858. doi: 10.1161/STROKEAHA.114.005464.

Kakuda, W., Abo, M., Kobayashi, K., Momosaki, R., Yokoi, A., Fukuda, A., et al. (2011). Combination treatment of low-frequency rTMS and occupational therapy with levodopa administration: an intensive neurorehabilitative approach for upper limb hemiparesis after stroke. *Int J Neurosci* 121(7)**,** 373-378. doi: 10.3109/00207454.2011.560314.

Kawaguchi, M., Furuya, H., and Patel, P.M. (2005). Neuroprotective effects of anesthetic agents. *J Anesth* 19(2)**,** 150-156. doi: 10.1007/s00540-005-0305-5.

Kawaguchi, M., Kimbro, J.R., Drummond, J.C., Cole, D.J., Kelly, P.J., and Patel, P.M. (2000). Isoflurane delays but does not prevent cerebral infarction in rats subjected to focal ischemia. *Anesthesiology* 92(5)**,** 1335-1342.

Kawano, T., Tsutsumi, K., Miyake, H., and Mori, K. (1988). Striatal dopamine in acute cerebral ischemia of stroke-resistant rats. *Stroke* 19(12)**,** 1540-1543.

Ke, Z., Yip, S.P., Li, L., Zheng, X.X., and Tong, K.Y. (2011). The effects of voluntary, involuntary, and forced exercises on brain-derived neurotrophic factor and motor function recovery: a rat brain ischemia model. *PLoS One* 6(2)**,** e16643. doi: 10.1371/journal.pone.0016643.

Kesar, T.M., Belagaje, S.R., Pergami, P., Haut, M.W., Hobbs, G., and Buetefisch, C.M. (2017). Effects of monoaminergic drugs on training-induced motor cortex plasticity in older adults. *Brain Res* 1670**,** 106-117. doi: 10.1016/j.brainres.2017.06.015.

Kim, B.R., Kim, H.Y., Chun, Y.I., Yun, Y.M., Kim, H., Choi, D.H., et al. (2016). Association between genetic variation in the dopamine system and motor recovery after stroke. *Restor Neurol Neurosci* 34(6)**,** 925-934. doi: 10.3233/RNN-160667.

Kinoshita, T., Moritani, T., Shrier, D.A., Wang, H.Z., Hiwatashi, A., Numaguchi, Y., et al. (2002). Secondary degeneration of the substantia nigra and corticospinal tract after hemorrhagic middle cerebral artery infarction: diffusion-weighted MR findings. *Magn Reson Med Sci* 1(3)**,** 175-178.

Kobayashi, T., Ahlenius, H., Thored, P., Kobayashi, R., Kokaia, Z., and Lindvall, O. (2006). Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. *Stroke* 37(9)**,** 2361-2367. doi: 10.1161/01.STR.0000236025.44089.e1.

Kogure, K., Scheinberg, P., Matsumoto, A., Busto, R., and Reinmuth, O.M. (1975). Catecholamines in experimental brain ischemia. *Arch Neurol* 32(1)**,** 21-24.

Kohno, N., Abe, S., Toyoda, G., Oguro, H., Bokura, H., and Yamaguchi, S. (2010). Successful treatment of post-stroke apathy by the dopamine receptor agonist ropinirole. *J Clin Neurosci* 17(6)**,** 804-806. doi: 10.1016/j.jocn.2009.09.043.

Kohrs, R., and Durieux, M.E. (1998). Ketamine: teaching an old drug new tricks. *Anesth Analg* 87(5)**,** 1186-1193.

Kopp, C. (2001). Locomotor activity rhythm in inbred strains of mice: implications for behavioural studies. *Behav Brain Res* 125(1-2)**,** 93-96.

Kovacs, K.J. (2008). Measurement of immediate-early gene activation- c-fos and beyond. *J Neuroendocrinol* 20(6)**,** 665-672. doi: 10.1111/j.1365-2826.2008.01734.x.

Kratz, O., Diruf, M.S., Studer, P., Gierow, W., Buchmann, J., Moll, G.H., et al. (2009). Effects of methylphenidate on motor system excitability in a response inhibition task. *Behav Brain Funct* 5**,** 12. doi: 10.1186/1744-9081-5-12.

Krimer, L.S., Muly, E.C., 3rd, Williams, G.V., and Goldman-Rakic, P.S. (1998). Dopaminergic regulation of cerebral cortical microcirculation. *Nat Neurosci* 1(4)**,** 286-289. doi: 10.1038/1099.

Kronenberg, G., Balkaya, M., Prinz, V., Gertz, K., Ji, S., Kirste, I., et al. (2012). Exofocal dopaminergic degeneration as antidepressant target in mouse model of poststroke depression. *Biol Psychiatry* 72(4)**,** 273-281. doi: 10.1016/j.biopsych.2012.02.026.

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

Küppers, E., and Beyer, C. (2001). Dopamine regulates brain-derived neurotrophic factor (BDNF) expression in cultured embryonic mouse striatal cells. *Neuroreport* 12(6)**,** 1175-1179.

Kuric, E., and Ruscher, K. (2014a). Dynamics of major histocompatibility complex class II-positive cells in the postischemic brain--influence of levodopa treatment. *J Neuroinflammation* 11**,** 145. doi: 10.1186/s12974-014-0145-z.

Kuric, E., and Ruscher, K. (2014b). Reduction of rat brain CD8+ T-cells by levodopa/benserazide treatment after experimental stroke. *Eur J Neurosci* 40(2)**,** 2463-2470. doi: 10.1111/ejn.12598.

Kuric, E., and Ruscher, K. (2014c). Reversal of stroke induced lymphocytopenia by levodopa/benserazide treatment. *J Neuroimmunol* 269(1-2)**,** 94-97. doi: 10.1016/j.jneuroim.2014.02.009.

Kuric, E., Wieloch, T., and Ruscher, K. (2013). Dopamine receptor activation increases glial cell line-derived neurotrophic factor in experimental stroke. *Exp Neurol* 247**,** 202-208. doi: 10.1016/j.expneurol.2013.04.016.

Kurozumi, K., Nakamura, K., Tamiya, T., Kawano, Y., Kobune, M., Hirai, S., et al. (2004). BDNF gene-modified mesenchymal stem cells promote functional recovery and reduce infarct size in the rat middle cerebral artery occlusion model. *Mol Ther* 9(2)**,** 189-197. doi: 10.1016/j.ymthe.2003.10.012.

Langhorne, P., Coupar, F., and Pollock, A. (2009). Motor recovery after stroke: a systematic review. *Lancet Neurol* 8(8)**,** 741-754. doi: 10.1016/S1474-4422(09)70150-4.

Leasure, J.L., and Jones, M. (2008). Forced and voluntary exercise differentially affect brain and behavior. *Neuroscience* 156(3)**,** 456-465. doi: 10.1016/j.neuroscience.2008.07.041.

Lemon, N., and Manahan-Vaughan, D. (2006). Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J Neurosci* 26(29)**,** 7723-7729. doi: 10.1523/JNEUROSCI.1454-06.2006.

Lewis, M.H., Gariepy, J.L., Gendreau, P., Nichols, D.E., and Mailman, R.B. (1994). Social reactivity and D1 dopamine receptors: studies in mice selectively bred for high and low levels of aggression. *Neuropsychopharmacology* 10(2)**,** 115-122. doi: 10.1038/npp.1994.13.

Li, A., Guo, H., Luo, X., Sheng, J., Yang, S., Yin, Y., et al. (2006). Apomorphine-induced activation of dopamine receptors modulates FGF-2 expression in astrocytic cultures and promotes survival of dopaminergic neurons. *FASEB J* 20(8)**,** 1263-1265. doi: 10.1096/fj.05-5510fje.

Liepert, J., Heller, A., Behnisch, G., and Schoenfeld, A. (2013). Catechol-O-methyltransferase polymorphism influences outcome after ischemic stroke: a prospective double-blind study. *Neurorehabil Neural Repair* 27(6)**,** 491-496. doi: 10.1177/1545968313481282.

Lin, D.A., and Finklestein, S.P. (1997). REVIEW : Basic Fibroblast Growth Factor: A Treatment for stroke? *The Neuroscientist* 3(4)**,** 247-250. doi: 10.1177/107385849700300412.

Liu, F., and McCullough, L.D. (2011). Middle cerebral artery occlusion model in rodents: methods and potential pitfalls. *J Biomed Biotechnol* 2011**,** 464701. doi: 10.1155/2011/464701.

Liu, H.S., Shen, H., Harvey, B.K., Castillo, P., Lu, H., Yang, Y., et al. (2011). Post-treatment with amphetamine enhances reinnervation of the ipsilateral side cortex in stroke rats. *Neuroimage* 56(1)**,** 280-289. doi: 10.1016/j.neuroimage.2011.02.049.

Liu, J., Wang, Y., Akamatsu, Y., Lee, C.C., Stetler, R.A., Lawton, M.T., et al. (2014). Vascular remodeling after ischemic stroke: mechanisms and therapeutic potentials. *Prog Neurobiol* 115**,** 138-156. doi: 10.1016/j.pneurobio.2013.11.004.

Livingston-Thomas, J., Nelson, P., Karthikeyan, S., Antonescu, S., Jeffers, M.S., Marzolini, S., et al. (2016). Exercise and Environmental Enrichment as Enablers of Task-Specific Neuroplasticity and Stroke Recovery. *Neurotherapeutics* 13(2)**,** 395-402. doi: 10.1007/s13311-016-0423-9.

Lokk, J., Salman Roghani, R., and Delbari, A. (2011). Effect of methylphenidate and/or levodopa coupled with physiotherapy on functional and motor recovery after stroke--a randomized, double-blind, placebo-controlled trial. *Acta Neurol Scand* 123(4)**,** 266-273. doi: 10.1111/j.1600-0404.2010.01395.x.

Long, D., and Young, J. (2003). Dexamphetamine treatment in stroke. *QJM* 96(9)**,** 673-685.

Luft, A.R., Buitrago, M.M., Ringer, T., Dichgans, J., and Schulz, J.B. (2004). Motor skill learning depends on protein synthesis in motor cortex after training. *J Neurosci* 24(29)**,** 6515-6520. doi: 10.1523/JNEUROSCI.1034-04.2004.

Luo, C.X., Jiang, J., Zhou, Q.G., Zhu, X.J., Wang, W., Zhang, Z.J., et al. (2007). Voluntary exercise-induced neurogenesis in the postischemic dentate gyrus is associated with spatial memory recovery from stroke. *J Neurosci Res* 85(8)**,** 1637-1646. doi: 10.1002/jnr.21317.

MacDonald, H.J., Stinear, C.M., Ren, A., Coxon, J.P., Kao, J., Macdonald, L., et al. (2016). Dopamine Gene Profiling to Predict Impulse Control and Effects of Dopamine Agonist Ropinirole. *J Cogn Neurosci* 28(7)**,** 909-919. doi: 10.1162/jocn_a_00946.

Maling, H.M., and Acheson, G.H. (1946). Righting and other postural activity in low-decerebrate and in spinal cats after d-amphetamine. *J Neurophysiol* 9**,** 379-386. doi: 10.1152/jn.1946.9.5.379.

Mang, C.S., Campbell, K.L., Ross, C.J., and Boyd, L.A. (2013). Promoting neuroplasticity for motor rehabilitation after stroke: considering the effects of aerobic exercise and genetic variation on brain-derived neurotrophic factor. *Phys Ther* 93(12)**,** 1707-1716. doi: 10.2522/ptj.20130053.

Martin, A., Gomez-Vallejo, V., San Sebastian, E., Padro, D., Markuerkiaga, I., Llarena, I., et al. (2013). In vivo imaging of dopaminergic neurotransmission after transient focal ischemia in rats. *J Cereb Blood Flow Metab* 33(2)**,** 244-252. doi: 10.1038/jcbfm.2012.162.

Martinsson, L., Hardemark, H., and Eksborg, S. (2007). Amphetamines for improving recovery after stroke. *Cochrane Database Syst Rev* (1)**,** CD002090. doi: 10.1002/14651858.CD002090.pub2.

Martinsson, L., and Wahlgren, N.G. (2003). Safety of dexamphetamine in acute ischemic stroke: a randomized, double-blind, controlled dose-escalation trial. *Stroke* 34(2)**,** 475-481.

Masihuzzaman, A.M., Uddin, M.J., Majumder, S., Barman, K.K., and Ullah, M.A. (2011). Effect of low dose levodopa on motor outcome of different types of stroke. *Mymensingh Med J* 20(4)**,** 689-693.

Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., et al. (2003). Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Natl Acad Sci U S A* 100(10)**,** 6186-6191. doi: 10.1073/pnas.0931309100.

McEntee, W.J., Mair, R.G., and Langlais, P.J. (1987). Neurochemical specificity of learning: dopamine and motor learning. *Yale J Biol Med* 60(2)**,** 187-193.

Meintzschel, F., and Ziemann, U. (2006). Modification of practice-dependent plasticity in human motor cortex by neuromodulators. *Cereb Cortex* 16(8)**,** 1106-1115. doi: 10.1093/cercor/bhj052.

Molina-Luna, K., Pekanovic, A., Rohrich, S., Hertler, B., Schubring-Giese, M., Rioult-Pedotti, M.S., et al. (2009). Dopamine in motor cortex is necessary for skill learning and synaptic plasticity. *PLoS One* 4(9)**,** e7082. doi: 10.1371/journal.pone.0007082.

Mu, Q., Johnson, K., Morgan, P.S., Grenesko, E.L., Molnar, C.E., Anderson, B., et al. (2007). A single 20 mg dose of the full D1 dopamine agonist dihydrexidine (DAR-0100) increases prefrontal perfusion in schizophrenia. *Schizophr Res* 94(1-3)**,** 332-341. doi: 10.1016/j.schres.2007.03.033.

Mueller, K.L., Marion, S.D., Paul, L.K., and Brown, W.S. (2009). Bimanual motor coordination in agenesis of the corpus callosum. *Behav Neurosci* 123(5)**,** 1000-1011. doi: 10.1037/a0016868.

Murphy, T.H., and Corbett, D. (2009). Plasticity during stroke recovery: from synapse to behaviour. *Nat Rev Neurosci* 10(12)**,** 861-872. doi: 10.1038/nrn2735.

Nakane, M., Teraoka, A., Asato, R., and Tamura, A. (1992). Degeneration of the ipsilateral substantia nigra following cerebral infarction in the striatum. *Stroke* 23(3)**,** 328-332.
Nitsche, M.A., Kuo, M.F., Grosch, J., Bergner, C., Monte-Silva, K., and Paulus, W. (2009). D1-receptor impact on neuroplasticity in humans. *J Neurosci* 29(8)**,** 2648-2653. doi: 10.1523/JNEUROSCI.5366-08.2009.
Nitsche, M.A., Lampe, C., Antal, A., Liebetanz, D., Lang, N., Tergau, F., et al. (2006). Dopaminergic modulation of long-lasting direct current-induced cortical excitability changes in the human motor cortex. *Eur J Neurosci* 23(6)**,** 1651-1657. doi: 10.1111/j.1460-9568.2006.04676.x.
Nudo, R.J. (2013). Recovery after brain injury: mechanisms and principles. *Front Hum Neurosci* 7**,** 887. doi: 10.3389/fnhum.2013.00887.
Nutt, J.G., and Fellman, J.H. (1984). Pharmacokinetics of levodopa. *Clin Neuropharmacol* 7(1)**,** 35-49.
Obi, K., Amano, I., and Takatsuru, Y. (2018). Role of dopamine on functional recovery in the contralateral hemisphere after focal stroke in the somatosensory cortex. *Brain Res* 1678**,** 146-152. doi: 10.1016/j.brainres.2017.10.022.
Oczkowski, W. (2013). Pharmacological therapies to enhance motor recovery and walking after stroke: emerging strategies. *Expert Rev Neurother* 13(8)**,** 903-909. doi: 10.1586/14737175.2013.814940.
Ogawa, T., Okudera, T., Inugami, A., Noguchi, K., Kado, H., Yoshida, Y., et al. (1997). Degeneration of the ipsilateral substantia nigra after striatal infarction: evaluation with MR imaging. *Radiology* 204(3)**,** 847-851. doi: 10.1148/radiology.204.3.9280270.
Ohlin, K.E., Sebastianutto, I., Adkins, C.E., Lundblad, C., Lockman, P.R., and Cenci, M.A. (2012). Impact of L-DOPA treatment on regional cerebral blood flow and metabolism in the basal ganglia in a rat model of Parkinson's disease. *Neuroimage* 61(1)**,** 228-239. doi: 10.1016/j.neuroimage.2012.02.066.
Ohwatashi, A., Ikeda, S., Harada, K., Kamikawa, Y., and Yoshida, A. (2013). Exercise enhanced functional recovery and expression of GDNF after photochemically induced cerebral infarction in the rat. *EXCLI J* 12**,** 693-700.
Okada, Y., Sakai, H., Kohiki, E., Suga, E., Yanagisawa, Y., Tanaka, K., et al. (2005). A dopamine D4 receptor antagonist attenuates ischemia-induced neuronal cell damage via upregulation of neuronal apoptosis inhibitory protein. *J Cereb Blood Flow Metab* 25(7)**,** 794-806. doi: 10.1038/sj.jcbfm.9600078.
Otmakhova, N.A., and Lisman, J.E. (1996). D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *J Neurosci* 16(23)**,** 7478-7486.
Paciaroni, M., and Bogousslavsky, J. (2011). Trafermin for stroke recovery: is it time for another randomized clinical trial? *Expert Opin Biol Ther* 11(11)**,** 1533-1541. doi: 10.1517/14712598.2011.616888.
Pang, M.Y.C., Charlesworth, S.A., Lau, R.W.K., and Chung, R.C.K. (2013). Using Aerobic Exercise to Improve Health Outcomes and Quality of Life in Stroke: Evidence-Based Exercise Prescription Recommendations. *Cerebrovascular Diseases* 35(1)**,** 7-22. doi: 10.1159/000346075.
Papadopoulos, C.M., Tsai, S.Y., Guillen, V., Ortega, J., Kartje, G.L., and Wolf, W.A. (2009). Motor recovery and axonal plasticity with short-term amphetamine after stroke. *Stroke* 40(1)**,** 294-302. doi: 10.1161/STROKEAHA.108.519769.

Paxinos, G., and Franklin, K. (2013). *The Mouse Brain in Stereotaxic Coordinates Fourth Edition.* London: Academic Press.

Pearson-Fuhrhop, K.M., Minton, B., Acevedo, D., Shahbaba, B., and Cramer, S.C. (2013). Genetic variation in the human brain dopamine system influences motor learning and its modulation by L-Dopa. *PLoS One* 8(4)**,** e61197. doi: 10.1371/journal.pone.0061197.

Pei, Y., Asif-Malik, A., and Canales, J.J. (2016). Trace Amines and the Trace Amine-Associated Receptor 1: Pharmacology, Neurochemistry, and Clinical Implications. *Front Neurosci* 10**,** 148. doi: 10.3389/fnins.2016.00148.

Peirson, S.N., Brown, L.A., Pothecary, C.A., Benson, L.A., and Fisk, A.S. (2018). Light and the laboratory mouse. *J Neurosci Methods* 300**,** 26-36. doi: 10.1016/j.jneumeth.2017.04.007.

Perreault, M.L., Fan, T., Alijaniaram, M., O'Dowd, B.F., and George, S.R. (2012). Dopamine D1-D2 receptor heteromer in dual phenotype GABA/glutamate-coexpressing striatal medium spiny neurons: regulation of BDNF, GAD67 and VGLUT1/2. *PLoS One* 7(3)**,** e33348. doi: 10.1371/journal.pone.0033348.

Platz, T., Kim, I.H., Engel, U., Pinkowski, C., Eickhof, C., and Kutzner, M. (2005). Amphetamine fails to facilitate motor performance and to enhance motor recovery among stroke patients with mild arm paresis: interim analysis and termination of a double blind, randomised, placebo-controlled trial. *Restor Neurol Neurosci* 23(5-6)**,** 271-280.

Ploughman, M., Austin, M.W., Glynn, L., and Corbett, D. (2015). The effects of poststroke aerobic exercise on neuroplasticity: a systematic review of animal and clinical studies. *Transl Stroke Res* 6(1)**,** 13-28. doi: 10.1007/s12975-014-0357-7.

Ploughman, M., Granter-Button, S., Chernenko, G., Attwood, Z., Tucker, B.A., Mearow, K.M., et al. (2007). Exercise intensity influences the temporal profile of growth factors involved in neuronal plasticity following focal ischemia. *Brain Res* 1150**,** 207-216. doi: 10.1016/j.brainres.2007.02.065.

Ploughman, M., Windle, V., MacLellan, C.L., White, N., Dore, J.J., and Corbett, D. (2009). Brain-derived neurotrophic factor contributes to recovery of skilled reaching after focal ischemia in rats. *Stroke* 40(4)**,** 1490-1495. doi: 10.1161/STROKEAHA.108.531806.

Prager, E.M., Bergstrom, H.C., Grunberg, N.E., and Johnson, L.R. (2011). The importance of reporting housing and husbandry in rat research. *Front Behav Neurosci* 5**,** 38. doi: 10.3389/fnbeh.2011.00038.

Prinz, V., Hetzer, A.M., Muller, S., Balkaya, M., Leithner, C., Kronenberg, G., et al. (2015). MRI heralds secondary nigral lesion after brain ischemia in mice: a secondary time window for neuroprotection. *J Cereb Blood Flow Metab* 35(12)**,** 1903-1909. doi: 10.1038/jcbfm.2015.153.

Qiu, J., Yan, Z., Tao, K., Li, Y., Li, Y., Li, J., et al. (2016). Sinomenine activates astrocytic dopamine D2 receptors and alleviates neuroinflammatory injury via the CRYAB/STAT3 pathway after ischemic stroke in mice. *J Neuroinflammation* 13(1)**,** 263. doi: 10.1186/s12974-016-0739-8.

Queen, S.A., Chen, M.J., and Feeney, D.M. (1997). d-Amphetamine attenuates decreased cerebral glucose utilization after unilateral sensorimotor cortex contusion in rats. *Brain Res* 777(1-2)**,** 42-50.

Rakai, B.D., and Antle, M.C. (2013). Lesion size and behavioral deficits after endothelin-1-induced ischemia are not dependent on time of day. *J Stroke Cerebrovasc Dis* 22(4)**,** 397-405. doi: 10.1016/j.jstrokecerebrovasdis.2011.10.001.

Ralph, R.J., and Caine, S.B. (2005). Dopamine D1 and D2 agonist effects on prepulse inhibition and locomotion: comparison of Sprague-Dawley rats to Swiss-Webster, 129X1/SvJ, C57BL/6J, and DBA/2J mice. *J Pharmacol Exp Ther* 312(2)**,** 733-741. doi: 10.1124/jpet.104.074468.

Ramic, M., Emerick, A.J., Bollnow, M.R., O'Brien, T.E., Tsai, S.Y., and Kartje, G.L. (2006). Axonal plasticity is associated with motor recovery following amphetamine treatment combined with rehabilitation after brain injury in the adult rat. *Brain Res* 1111(1)**,** 176-186. doi: 10.1016/j.brainres.2006.06.063.

Rasmussen, R.S., Overgaard, K., Hildebrandt-Eriksen, E.S., and Boysen, G. (2006). D-amphetamine improves cognitive deficits and physical therapy promotes fine motor rehabilitation in a rat embolic stroke model. *Acta Neurol Scand* 113(3)**,** 189-198. doi: 10.1111/j.1600-0404.2005.00547.x.

Rasmussen, R.S., Overgaard, K., Kristiansen, U., and Johansen, F.F. (2011). Acute but not delayed amphetamine treatment improves behavioral outcome in a rat embolic stroke model. *Neurol Res* 33(7)**,** 774-782. doi: 10.1179/1743132811Y.0000000009.

Restemeyer, C., Weiller, C., and Liepert, J. (2007). No effect of a levodopa single dose on motor performance and motor excitability in chronic stroke. A double-blind placebo-controlled cross-over pilot study. *Restor Neurol Neurosci* 25(2)**,** 143-150.

Richards, D.A., Obrenovitch, T.P., Symon, L., and Curzon, G. (1993). Extracellular dopamine and serotonin in the rat striatum during transient ischaemia of different severities: a microdialysis study. *J Neurochem* 60(1)**,** 128-136.

Rioult-Pedotti, M.S., Pekanovic, A., Atiemo, C.O., Marshall, J., and Luft, A.R. (2015). Dopamine Promotes Motor Cortex Plasticity and Motor Skill Learning via PLC Activation. *PLoS One* 10(5)**,** e0124986. doi: 10.1371/journal.pone.0124986.

Roceri, M., Molteni, R., Fumagalli, F., Racagni, G., Gennarelli, M., Corsini, G., et al. (2001). Stimulatory role of dopamine on fibroblast growth factor-2 expression in rat striatum. *J Neurochem* 76(4)**,** 990-997.

Rodriguez-Grande, B., Blackabey, V., Gittens, B., Pinteaux, E., and Denes, A. (2013). Loss of substance P and inflammation precede delayed neurodegeneration in the substantia nigra after cerebral ischemia. *Brain Behav Immun* 29**,** 51-61. doi: 10.1016/j.bbi.2012.11.017.

Roedel, A., Storch, C., Holsboer, F., and Ohl, F. (2006). Effects of light or dark phase testing on behavioural and cognitive performance in DBA mice. *Lab Anim* 40(4)**,** 371-381. doi: 10.1258/002367706778476343.

Rogozinska, K., and Skangiel-Kramska, J. (2010). Effect of focal cerebral ischaemia on modulatory neurotransmitter receptors in the rat brain: an autoradiographic study. *J Chem Neuroanat* 40(3)**,** 232-238. doi: 10.1016/j.jchemneu.2010.06.004.

Rosell, D.R., Zaluda, L.C., McClure, M.M., Perez-Rodriguez, M.M., Strike, K.S., Barch, D.M., et al. (2015). Effects of the D1 dopamine receptor agonist dihydrexidine (DAR-0100A) on working memory in schizotypal personality disorder. *Neuropsychopharmacology* 40(2)**,** 446-453. doi: 10.1038/npp.2014.192.

Rösser, N., and Flöel, A. (2008). Pharmacological enhancement of motor recovery in subacute and chronic stroke. *NeuroRehabilitation* 23(1)**,** 95-103.

Rösser, N., Heuschmann, P., Wersching, H., Breitenstein, C., Knecht, S., and Flöel, A. (2008). Levodopa improves procedural motor learning in chronic stroke patients. *Arch Phys Med Rehabil* 89(9)**,** 1633-1641. doi: 10.1016/j.apmr.2008.02.030.

Ruan, H., Saur, T., and Yao, W.D. (2014). Dopamine-enabled anti-Hebbian timing-dependent plasticity in prefrontal circuitry. *Front Neural Circuits* 8**,** 38. doi: 10.3389/fncir.2014.00038.

Ruscher, K., Kuric, E., and Wieloch, T. (2012). Levodopa treatment improves functional recovery after experimental stroke. *Stroke* 43(2)**,** 507-513. doi: 10.1161/STROKEAHA.111.638767.

Salmi, P., and Ahlenius, S. (2000). Sedative effects of the dopamine D(1) receptor agonist A 68930 on rat open-field behavior. *Neuroreport* 11(6)**,** 1269-1272. doi: Doi 10.1097/00001756-200004270-00025.

Salmi, P., Isacson, R., and Kull, B. (2004). Dihydrexidine - The first full dopamine D-1 receptor agonist. *Cns Drug Reviews* 10(3)**,** 230-242.

Salter, K.C., Nerissa; Richardson, Marina; Mehta, Swati; Jutai, Jeffrey; Zettler, Laura; Moses, Matthew; McClure, Andrew; Mays, Rachel; Foley, Norine; Teasell, Robert (2013). "Outcome Measures in Stroke Rehabilitation", in: *Evidence-Based Reviews of Stroke Rehabilitation.* (London, ON, Canada: Evidence-Based Reviews of Stroke Rehabilitation).

Schaar, K.L., Brenneman, M.M., and Savitz, S.I. (2010). Functional assessments in the rodent stroke model. *Exp Transl Stroke Med* 2(1)**,** 13. doi: 10.1186/2040-7378-2-13.

Schabitz, W.R., Steigleder, T., Cooper-Kuhn, C.M., Schwab, S., Sommer, C., Schneider, A., et al. (2007). Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. *Stroke* 38(7)**,** 2165-2172. doi: 10.1161/STROKEAHA.106.477331.

Scheidtmann, K., Fries, W., Muller, F., and Koenig, E. (2001). Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomised, double-blind study. *Lancet* 358(9284)**,** 787-790. doi: 10.1016/S0140-6736(01)05966-9.

Schmanke, T., and Barth, T.M. (1997). Amphetamine and task-specific practice augment recovery of vibrissae-evoked forelimb placing after unilateral sensorimotor cortical injury in the rat. *J Neurotrauma* 14(7)**,** 459-468. doi: 10.1089/neu.1997.14.459.

Schmanke, T.D., Avery, R.A., and Barth, T.M. (1996). The effects of amphetamine on recovery of function after cortical damage in the rat depend on the behavioral requirements of the task. *J Neurotrauma* 13(6)**,** 293-307. doi: 10.1089/neu.1996.13.293.

Schmidt, A., Wellmann, J., Schilling, M., Strecker, J.K., Sommer, C., Schabitz, W.R., et al. (2014). Meta-analysis of the efficacy of different training strategies in animal models of ischemic stroke. *Stroke* 45(1)**,** 239-247. doi: 10.1161/STROKEAHA.113.002048.

Schonfeld, L.M., Dooley, D., Jahanshahi, A., Temel, Y., and Hendrix, S. (2017). Evaluating rodent motor functions: Which tests to choose? *Neurosci Biobehav Rev* 83**,** 298-312. doi: 10.1016/j.neubiorev.2017.10.021.

Schuster, C., Maunz, G., Lutz, K., Kischka, U., Sturzenegger, R., and Ettlin, T. (2011). Dexamphetamine improves upper extremity outcome during rehabilitation after stroke: a pilot randomized controlled trial. *Neurorehabil Neural Repair* 25(8)**,** 749-755. doi: 10.1177/1545968311405674.

Scott, L., Forssberg, H., Aperia, A., and Diaz-Heijtz, R. (2005). Locomotor effects of a D1R agonist are DARPP-32 dependent in adult but not weanling mice. *Pediatr Res* 58(4)**,** 779-783. doi: 10.1203/01.PDR.0000180553.23507.31.

Shen, W., Flajolet, M., Greengard, P., and Surmeier, D.J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science* 321(5890)**,** 848-851. doi: 10.1126/science.1160575.
Sieber, M.W., Guenther, M., Jaenisch, N., Albrecht-Eckardt, D., Kohl, M., Witte, O.W., et al. (2014). Age-specific transcriptional response to stroke. *Neurobiol Aging* 35(7)**,** 1744-1754. doi: 10.1016/j.neurobiolaging.2014.01.012.
Slevin, M., Kumar, P., Gaffney, J., Kumar, S., and Krupinski, J. (2006). Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potential. *Clin Sci (Lond)* 111(3)**,** 171-183. doi: 10.1042/CS20060049.
Slivka, A., Brannan, T.S., Weinberger, J., Knott, P.J., and Cohen, G. (1988). Increase in extracellular dopamine in the striatum during cerebral ischemia: a study utilizing cerebral microdialysis. *J Neurochem* 50(6)**,** 1714-1718.
Solvsten, C.A.E., Daugaard, T.F., Luo, Y., de Paoli, F., Christensen, J.H., and Nielsen, A.L. (2017). The Effects of Voluntary Physical Exercise-Activated Neurotrophic Signaling in Rat Hippocampus on mRNA Levels of Downstream Signaling Molecules. *J Mol Neurosci* 62(2)**,** 142-153. doi: 10.1007/s12031-017-0918-9.
Sonde, L., and Lökk, J. (2007). Effects of amphetamine and/or L-dopa and physiotherapy after stroke - a blinded randomized study. *Acta Neurol Scand* 115(1)**,** 55-59. doi: 10.1111/j.1600-0404.2006.00728.x.
Sonde, L., Nordstrom, M., Nilsson, C.G., Lökk, J., and Viitanen, M. (2001). A double-blind placebo-controlled study of the effects of amphetamine and physiotherapy after stroke. *Cerebrovasc Dis* 12(3)**,** 253-257. doi: 10.1159/000047712.
Soriano, M.A., Justicia, C., Ferrer, I., Rodriguez-Farre, E., and Planas, A.M. (1997). Striatal infarction in the rat causes a transient reduction of tyrosine hydroxylase immunoreactivity in the ipsilateral substantia nigra. *Neurobiol Dis* 4(5)**,** 376-385. doi: 10.1006/nbdi.1997.0166.
Sprigg, N., and Bath, P.M. (2009). Speeding stroke recovery? A systematic review of amphetamine after stroke. *J Neurol Sci* 285(1-2)**,** 3-9. doi: 10.1016/j.jns.2009.04.040.
Stanfill, A., Elijovich, L., Baughman, B., and Conley, Y. (2016). A Review and Conceptual Model of Dopaminergic Contributions to Poststroke Depression. *J Neurosci Nurs* 48(5)**,** 242-246. doi: 10.1097/JNN.0000000000000240.
Stewart, J.C., Dewanjee, P., Tran, G., Quinlan, E.B., Dodakian, L., McKenzie, A., et al. (2017). Role of corpus callosum integrity in arm function differs based on motor severity after stroke. *Neuroimage Clin* 14**,** 641-647. doi: 10.1016/j.nicl.2017.02.023.
Stroemer, R.P., Kent, T.A., and Hulsebosch, C.E. (1998). Enhanced neocortical neural sprouting, synaptogenesis, and behavioral recovery with D-amphetamine therapy after neocortical infarction in rats. *Stroke* 29(11)**,** 2381-2393; discussion 2393-2385.
Sutton, R.L., Hovda, D.A., Chen, M.J., and Feeney, D.M. (2000). Alleviation of brain injury-induced cerebral metabolic depression by amphetamine: a cytochrome oxidase histochemistry study. *Neural Plast* 7(1-2)**,** 109-125. doi: 10.1155/NP.2000.109.
Sutton, R.L., Hovda, D.A., and Feeney, D.M. (1989). Amphetamine accelerates recovery of locomotor function following bilateral frontal cortex ablation in cats. *Behav Neurosci* 103(4)**,** 837-841.
Tan, C.O. (2009). Anticipatory changes in regional cerebral hemodynamics: a new role for dopamine? *J Neurophysiol* 101(6)**,** 2738-2740. doi: 10.1152/jn.00141.2009.

Tardy, J., Pariente, J., Leger, A., Dechaumont-Palacin, S., Gerdelat, A., Guiraud, V., et al. (2006). Methylphenidate modulates cerebral post-stroke reorganization. *Neuroimage* 33(3)**,** 913-922. doi: 10.1016/j.neuroimage.2006.07.014.

Thirugnanasambandam, N., Grundey, J., Paulus, W., and Nitsche, M.A. (2011). Dose-dependent nonlinear effect of L-DOPA on paired associative stimulation-induced neuroplasticity in humans. *J Neurosci* 31(14)**,** 5294-5299. doi: 10.1523/JNEUROSCI.6258-10.2011.

Tirelli, E., and Terry, P. (1993). Biphasic locomotor effects of the dopamine D1 agonist SKF 38393 and their attenuation in non-habituated mice. *Psychopharmacology (Berl)* 110(1-2)**,** 69-75.

Toner, C.C., and Stamford, J.A. (1996). 'Real time' measurement of dopamine release in an in vitro model of neostriatal ischaemia. *J Neurosci Methods* 67(2)**,** 133-140.

Tran, D.A., Pajaro-Blazquez, M., Daneault, J.F., Gallegos, J.G., Pons, J., Fregni, F., et al. (2016). Combining Dopaminergic Facilitation with Robot-Assisted Upper Limb Therapy in Stroke Survivors: A Focused Review. *Am J Phys Med Rehabil* 95(6)**,** 459-474. doi: 10.1097/PHM.0000000000000438.

Treig, T., Werner, C., Sachse, M., and Hesse, S. (2003). No benefit from D-amphetamine when added to physiotherapy after stroke: a randomized, placebo-controlled study. *Clin Rehabil* 17(6)**,** 590-599. doi: 10.1191/0269215503cr653oa.

Uzdensky, A., Demyanenko, S., Fedorenko, G., Lapteva, T., and Fedorenko, A. (2017). Protein Profile and Morphological Alterations in Penumbra after Focal Photothrombotic Infarction in the Rat Cerebral Cortex. *Mol Neurobiol* 54(6)**,** 4172-4188. doi: 10.1007/s12035-016-9964-5.

Vaillancourt, D.E., Schonfeld, D., Kwak, Y., Bohnen, N.I., and Seidler, R. (2013). Dopamine overdose hypothesis: evidence and clinical implications. *Mov Disord* 28(14)**,** 1920-1929. doi: 10.1002/mds.25687.

Viale, L., Catoira, N.P., Di Girolamo, G., and Gonzalez, C.D. (2018). Pharmacotherapy and motor recovery after stroke. *Expert Rev Neurother* 18(1)**,** 65-82. doi: 10.1080/14737175.2018.1400910.

Vinall, P.E., Kramer, M.S., Heinel, L.A., and Rosenwasser, R.H. (2000). Temporal changes in sensitivity of rats to cerebral ischemic insult. *J Neurosurg* 93(1)**,** 82-89. doi: 10.3171/jns.2000.93.1.0082.

von Essen, C. (1974). Effects of dopamine on the cerebral blood flow in the dog. *Acta Neurol Scand* 50(1)**,** 39-52.

Wada, K., Sugimori, H., Bhide, P.G., Moskowitz, M.A., and Finklestein, S.P. (2003). Effect of basic fibroblast growth factor treatment on brain progenitor cells after permanent focal ischemia in rats. *Stroke* 34(11)**,** 2722-2728. doi: 10.1161/01.STR.0000094421.61917.71.

Wade, M.R., and Nomikos, G.G. (2005). Tolerance to the procholinergic action of the D1 receptor full agonist dihydrexidine. *Psychopharmacology (Berl)* 182(3)**,** 393-399. doi: 10.1007/s00213-005-0106-4.

Wahl, A.S., Omlor, W., Rubio, J.C., Chen, J.L., Zheng, H., Schroter, A., et al. (2014). Neuronal repair. Asynchronous therapy restores motor control by rewiring of the rat corticospinal tract after stroke. *Science* 344(6189)**,** 1250-1255. doi: 10.1126/science.1253050.

Walker-Batson, D., Smith, P., Curtis, S., Unwin, H., and Greenlee, R. (1995). Amphetamine paired with physical therapy accelerates motor recovery after stroke. Further evidence. *Stroke* 26(12)**,** 2254-2259.

Wang, Q.M., Cui, H., Han, S.J., Black-Schaffer, R., Volz, M.S., Lee, Y.T., et al. (2014). Combination of transcranial direct current stimulation and methylphenidate in subacute stroke. *Neurosci Lett* 569**,** 6-11. doi: 10.1016/j.neulet.2014.03.011.

Watson, B.D., Dietrich, W.D., Busto, R., Wachtel, M.S., and Ginsberg, M.D. (1985). Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol* 17(5)**,** 497-504. doi: 10.1002/ana.410170513.

Weinberger, J. (2002). The role of dopamine in cerebral ischemic damage: a review of studies with Gerald Cohen. *Parkinsonism Relat Disord* 8(6)**,** 413-416.

Weinberger, J., Cohen, G., and Nieves-Rosa, J. (1983). Nerve terminal damage in cerebral ischemia: greater susceptibility of catecholamine nerve terminals relative to serotonin nerve terminals. *Stroke* 14(6)**,** 986-989.

Willuhn, I., and Steiner, H. (2006). Motor-skill learning-associated gene regulation in the striatum: effects of cocaine. *Neuropsychopharmacology* 31(12)**,** 2669-2682. doi: 10.1038/sj.npp.1300995.

Willuhn, I., and Steiner, H. (2008). Motor-skill learning in a novel running-wheel task is dependent on D1 dopamine receptors in the striatum. *Neuroscience* 153(1)**,** 249-258. doi: 10.1016/j.neuroscience.2008.01.041.

Willuhn, I., Sun, W., and Steiner, H. (2003). Topography of cocaine-induced gene regulation in the rat striatum: relationship to cortical inputs and role of behavioural context. *Eur J Neurosci* 17(5)**,** 1053-1066.

Winter, B., Juckel, G., Viktorov, I., Katchanov, J., Gietz, A., Sohr, R., et al. (2005). Anxious and hyperactive phenotype following brief ischemic episodes in mice. *Biol Psychiatry* 57(10)**,** 1166-1175. doi: 10.1016/j.biopsych.2005.02.010.

Wirkner, K., Krause, T., Koles, L., Thummler, S., Al-Khrasani, M., and Illes, P. (2004). D1 but not D2 dopamine receptors or adrenoceptors mediate dopamine-induced potentiation of N-methyl-d-aspartate currents in the rat prefrontal cortex. *Neurosci Lett* 372(1-2)**,** 89-93. doi: 10.1016/j.neulet.2004.09.015.

Witte, A.V., Kurten, J., Jansen, S., Schirmacher, A., Brand, E., Sommer, J., et al. (2012). Interaction of BDNF and COMT polymorphisms on paired-associative stimulation-induced cortical plasticity. *J Neurosci* 32(13)**,** 4553-4561. doi: 10.1523/JNEUROSCI.6010-11.2012.

Wolf, M.E., Mangiavacchi, S., and Sun, X. (2003). Mechanisms by which dopamine receptors may influence synaptic plasticity. *Ann N Y Acad Sci* 1003**,** 241-249.

Wolf, W.A., Martin, J.L., Kartje, G.L., and Farrer, R.G. (2014). Evidence for fibroblast growth factor-2 as a mediator of amphetamine-enhanced motor improvement following stroke. *PLoS One* 9(9)**,** e108031. doi: 10.1371/journal.pone.0108031.

Xing, B., Guo, J., Meng, X., Wei, S.G., and Li, S.B. (2012). The dopamine D1 but not D3 receptor plays a fundamental role in spatial working memory and BDNF expression in prefrontal cortex of mice. *Behav Brain Res* 235(1)**,** 36-41. doi: 10.1016/j.bbr.2012.06.035.

Xu, T.X., Ma, Q., Spealman, R.D., and Yao, W.D. (2010). Amphetamine modulation of long-term potentiation in the prefrontal cortex: dose dependency, monoaminergic contributions, and paradoxical rescue in hyperdopaminergic mutant. *J Neurochem* 115(6)**,** 1643-1654. doi: 10.1111/j.1471-4159.2010.07073.x.

Yamada, K., Goto, S., Yoshikawa, M., and Ushio, Y. (1996). Gabaergic transmission and tyrosine hydroxylase expression in the nigral dopaminergic neurons: an in vivo study using a reversible ischemia model of rats. *Neuroscience* 73(3)**,** 783-789.

Yano, H., Cai, N.S., Xu, M., Verma, R.K., Rea, W., Hoffman, A.F., et al. (2018). Gs- versus Golf-dependent functional selectivity mediated by the dopamine D1 receptor. *Nat Commun* 9(1)**,** 486. doi: 10.1038/s41467-017-02606-w.

Yulug, B., Yildiz, A., Guzel, O., Kilic, E., Schabitz, W.R., and Kilic, E. (2006a). Risperidone attenuates brain damage after focal cerebral ischemia in vivo. *Brain Res Bull* 69(6)**,** 656-659. doi: 10.1016/j.brainresbull.2006.03.017.

Yulug, B., Yildiz, A., Hudaoglu, O., Kilic, E., Cam, E., and Schabitz, W.R. (2006b). Olanzapine attenuates brain damage after focal cerebral ischemia in vivo. *Brain Res Bull* 71(1-3)**,** 296-300. doi: 10.1016/j.brainresbull.2006.09.018.

Zervas, N.T., Hori, H., Negora, M., Wurtman, R.J., Larin, F., and Lavyne, M.H. (1974). Reduction in brain dopamine following experimental cerebral ischaemia. *Nature* 247(5439)**,** 283-284.

Zhang, J., Zhang, Y., Xing, S., Liang, Z., and Zeng, J. (2012). Secondary neurodegeneration in remote regions after focal cerebral infarction: a new target for stroke management? *Stroke* 43(6)**,** 1700-1705. doi: 10.1161/STROKEAHA.111.632448.

Zhang, Y., Chen, Y., Wu, J., Manaenko, A., Yang, P., Tang, J., et al. (2015). Activation of Dopamine D2 Receptor Suppresses Neuroinflammation Through alphaB-Crystalline by Inhibition of NF-kappaB Nuclear Translocation in Experimental ICH Mice Model. *Stroke* 46(9)**,** 2637-2646. doi: 10.1161/STROKEAHA.115.009792.

Zhao, F., Kuroiwa, T., Miyasaka, N., Nagaoka, T., Nakane, M., Tamura, A., et al. (2001). Characteristic changes in T(2)-value, apparent diffusion coefficient, and ultrastructure of substantia nigra evolving exofocal postischemic neuronal death in rats. *Brain Res* 895(1-2)**,** 238-244.

Zhao, F., Kuroiwa, T., Miyasaka, N., Nagaoka, T., Nakane, M., Tamura, A., et al. (2002). Ultrastructural and MRI study of the substantia nigra evolving exofocal post-ischemic neuronal death in the rat. *Neuropathology* 22(3)**,** 91-105.

Appendices

Appendix 1: Results of the Left Paws during the Seven-Days and Two-Days DHX Studies

The percentage of missteps on the horizontal ladder test made with the left paw throughout the experiment for the 7-days DHX animals is shown below in Figure 33. Figure 34 shows the results of the adhesive removal test for the left forepaw in the 7-days DHX study. Figure 35 shows the results of the left fore- and hind-paws on the horizontal ladder test for the 2-days DHX study. Unexpectedly, in these cohorts, there was a tendency towards development of a modest left-paw deficit, particularly at the poststroke timepoint. This was likely due to the large number of strokes that infringed on the corpus callosum in these cohorts (17/20 in the DHX groups, 16/19 in the saline group). Figure 36 shows the time to contact and remove the tape from the left forepaw for the 2-days DHX study.

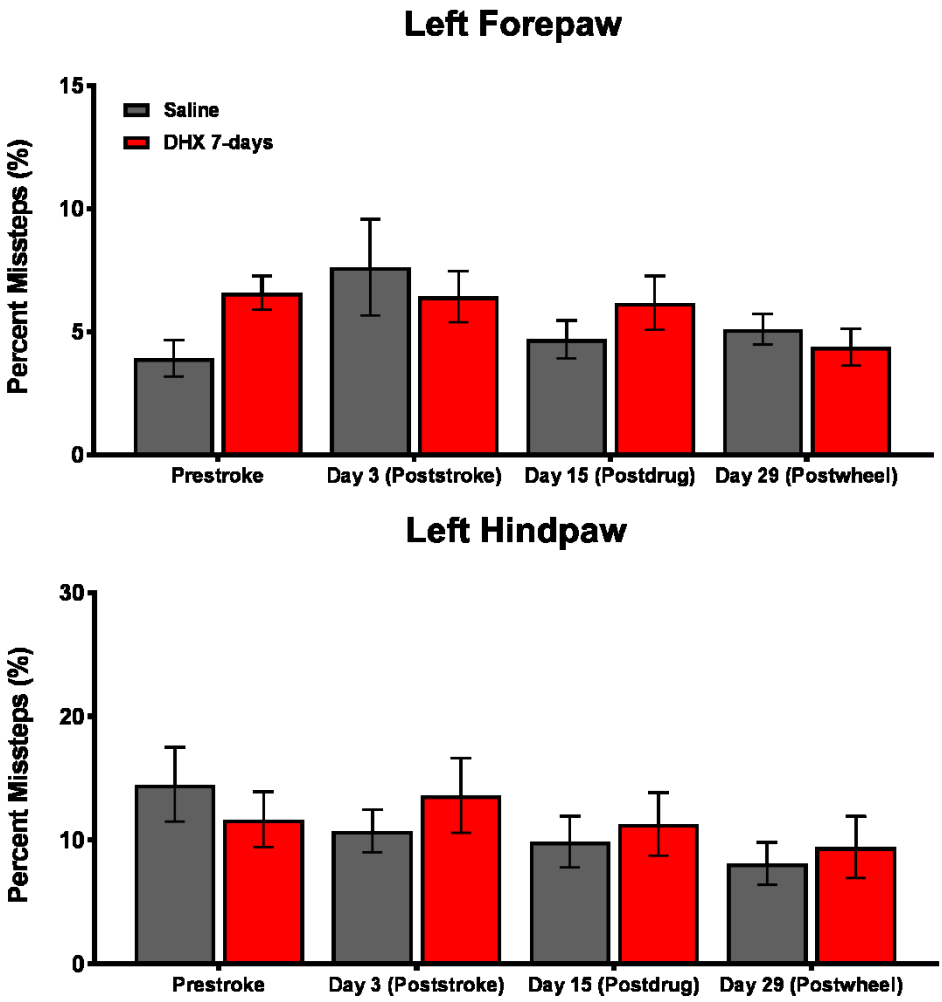

Figure 33: Results of the Horizontal Ladder Test for the Left Fore- and Hind-paws during the Seven-Days DHX Study. The top panel shows the results of the left forepaw while the bottom panel shows results for the left hindpaw. Results are expressed as the percent missteps and shown on the same scale as the right fore and hind-paw data. n=26 for both groups.

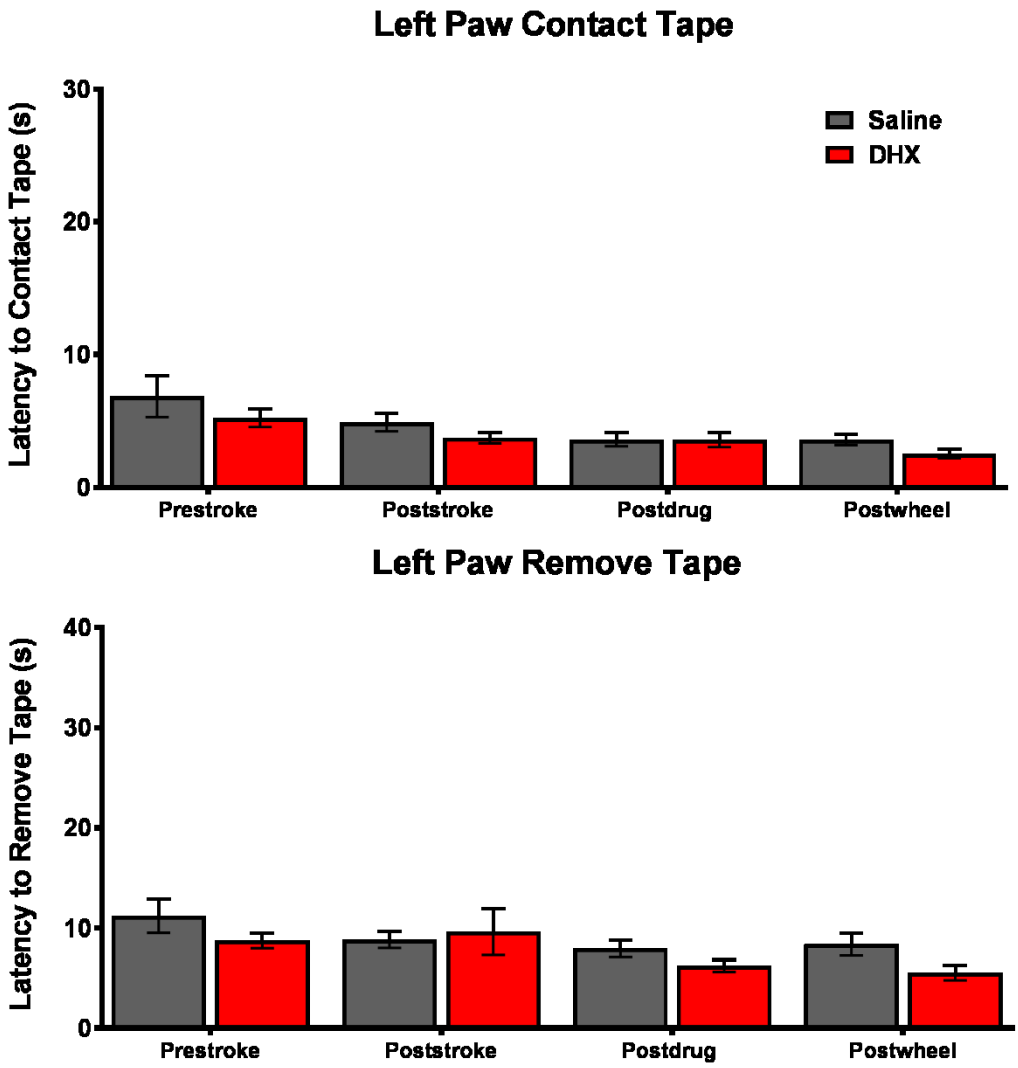

Figure 34: Results of the Adhesive Removal Test for the Left Paw throughout the Seven-Days DHX Study. The top panel shows the time to contact the tape. The bottom panel shows the results for the time to remove the tape. Results are expressed as seconds to contact or remove the tape and shown on the same scale as the right paw data. n=26 for both groups.

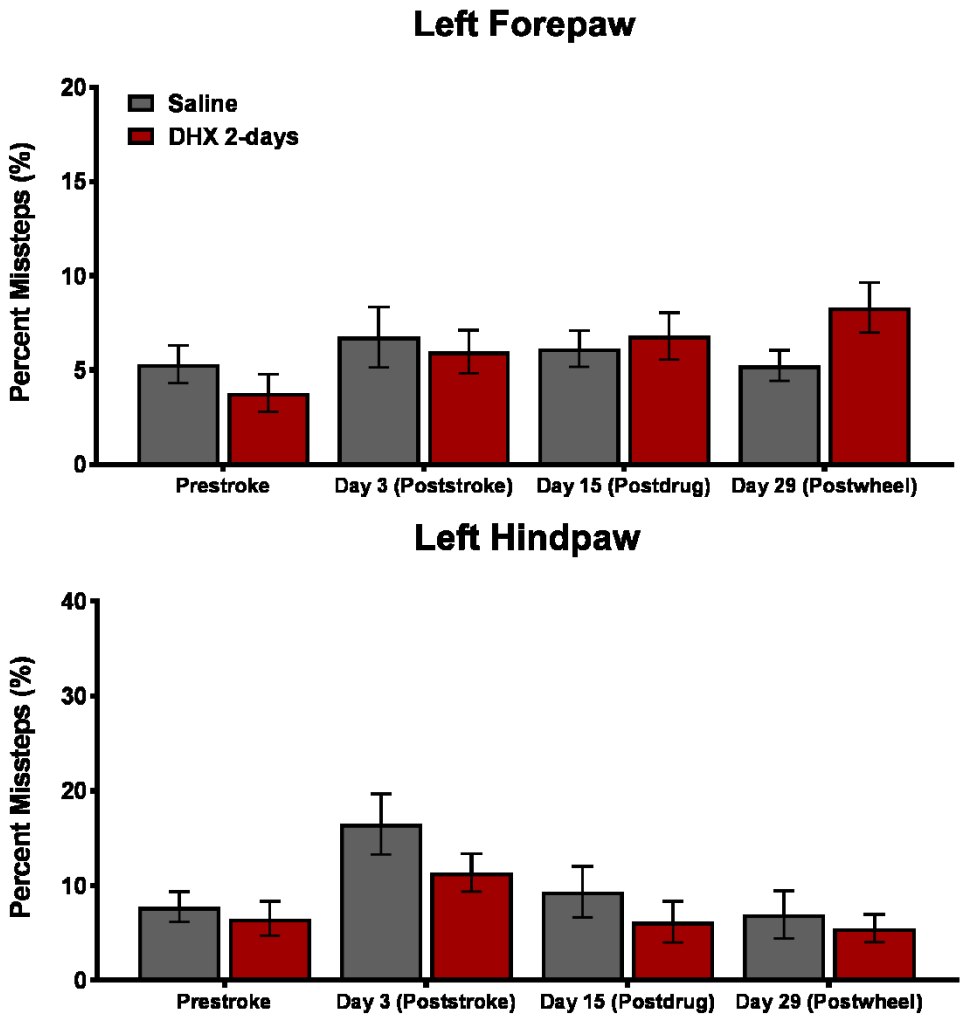

Figure 35: Results of the Left Paws on the Horizontal Ladder Test throughout the Two-Days DHX Study. The top panel shows the results of the left forepaw on the horizontal ladder test. The bottom panel shows the results for the left hindpaw. Data is expressed as the percent missteps, on the same scale as the right paws data. n=19 for both groups.

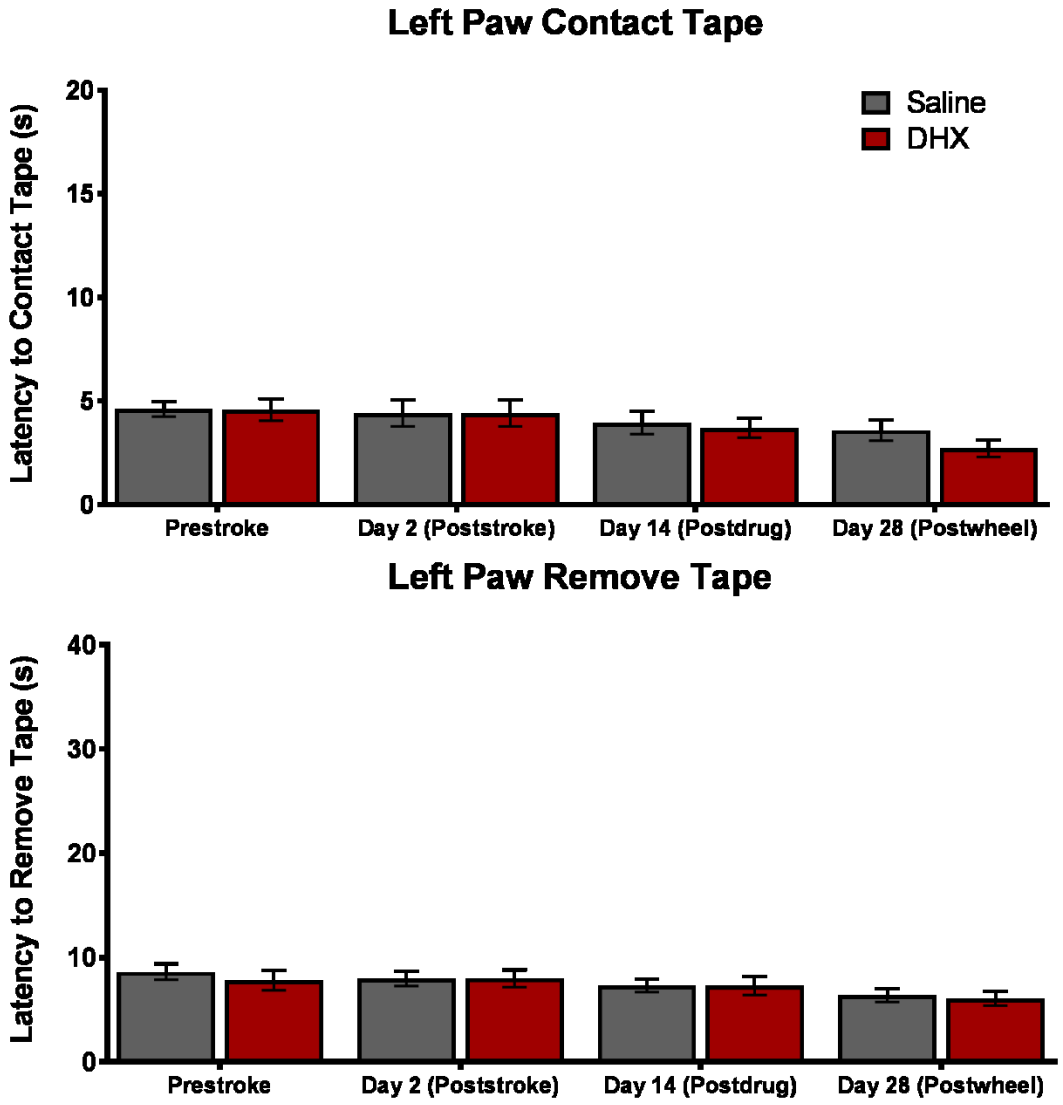

Figure 36: Results of the Left Paw on the Adhesive Removal Test during the Two-Days DHX Study. The top panel shows the time to contact the tape, the bottom panel shows the time to remove the tape. Data is expressed as seconds to contact or remove the tape, on the same scale as the right paw adhesive data for the 2-days DHX experiment. n=19 for the saline group, n=20 for the DHX group.

Appendix 2: Additional Dosage Experiments

Although not crucial to determining the sub-optimal, biologically active dose of DHX for the DHX Stroke Recovery experiments, further dosage experiments were performed. Figure 35 shows results of acute DHX dosage work in reverse light-dark housing with a shorter (one hour) period of acclimation. The results of testing of the efficacy of 2.5mg/kg of additional D1-class agonist SKF81297 in stimulating locomotor activity is shown in Figure 36. Having determined the efficacy of this dose in enhancing locomotor activity under the testing conditions used here, it was used to test for the specificity of the inhibition of locomotor activity induced by SCH23390. SCH23390 was able to block the locomotor enhancing effect of SKF81297 at doses of both 0.1 mg/kg and 0.5 mg/kg (Fig. 37). These doses were then tested in a chronic, 7-days dosage experiment, in preparation for future work which will evaluate the effect of specific inhibition of D1-class receptors on stroke recovery (Fig. 38). After 7-days of SCH23390 or saline, the animals were challenged with SKF81297, as a measure of possible sensitization to the drug.

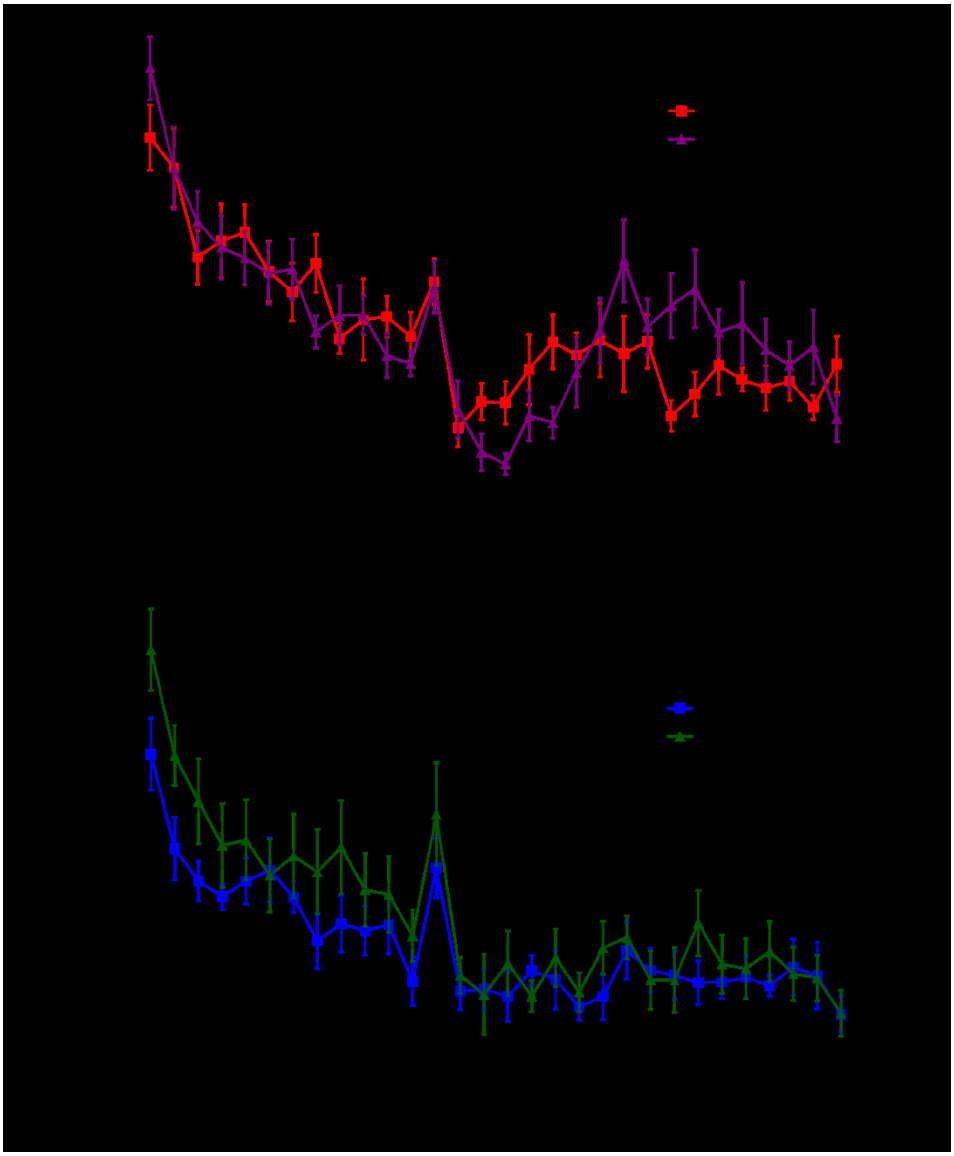

Figure 35: Locomotor Activity after a Single DHX Injection with Short Acclimation. Eight-week-old mice were ordered and housed in reverse-light dark housing. After two weeks of acclimation, including standard handling, the mice were moved to cages on the locomotor activity frame around 10:45AM, and after 1 hour of acclimation, they were injected, and their locomotor activity was monitored for another 1.5 hours. Over two consecutive days, mice received either saline or DHX (mg/kg: 0.25, 1, 4 or 16). The top panel shows results of the two higher DHX doses used (4 and 16 mg/kg) in comparison with saline. With 4 and 16 mg/kg DHX, a dose-dependent biphasic effect of the drug was observed with an early inhibition of locomotor activity followed by a later enhancement of locomotor activity above the level of saline. In the bottom panel the results of the lower DHX doses used (0.25 and 1 mg/kg) are shown. With 0.25 and 1 mg/kg DHX, an apparent early inhibition of locomotor activity, followed by a return to the level of activity exhibited by saline treated mice, was observed. The arrow indicates the time of injection. n=8 in each group.

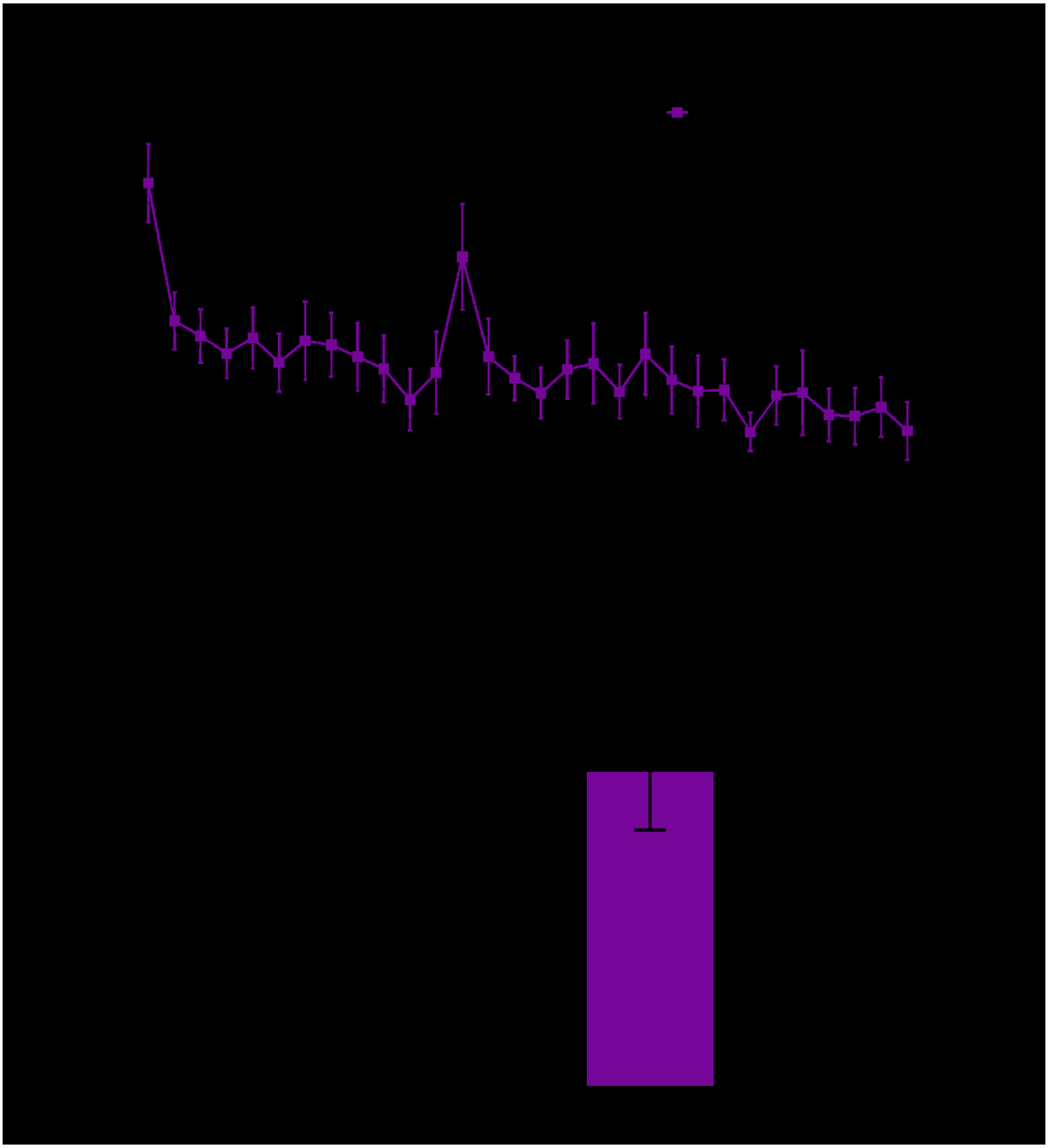

Figure 36: Acute Dosage Determination for SKF81297. Concurrent with the acute dosage experiment for SCH23390 (Figure 16), the efficacy of 2.5 mg/kg SKF81297 in enhancing locomotor activity, was tested. The 2.5 mg/kg dose was selected to be a moderate dose, based on previously used doses in the literature. Reverse light-dark housed animals, ten weeks of age, were given 1 hour of acclimation to the locomotor testing apparatus, and then were injected (black arrow) with either saline or 2.5 mg/kg SCH23390, and monitored for a further 85 minutes (top panel). This was performed over the course of two days. The bottom panel shows the total distance travelled across the bottom of the cage in the 40 minutes following injection. Animals that received SKF81297 performed more locomotor activity than saline animals. This difference was statistically detectable by an unpaired t-test ($p=0.0373$).

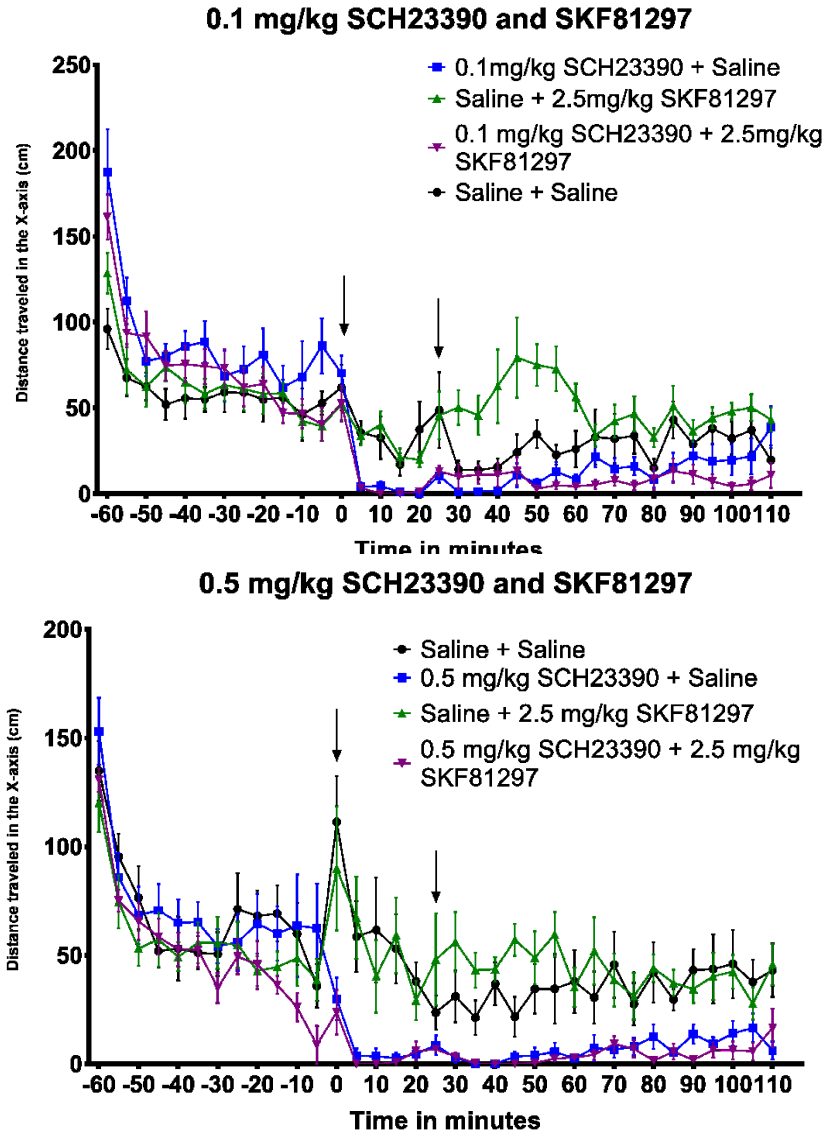

Figure 37: Both 0.1 mg/kg and 0.5 mg/kg SCH23390 Block the Locomotor Stimulating Effect of SKF81297. After a 7-day washout period, mice from the acute SCH23390 dosage experiments (Fig. 16) were re-randomized and reused. Mice acclimated to cages on the locomotor activity frame for 1-1.5 hours, starting at 10:00AM. On day one, mice were injected with either saline or 0.1 mg/kg SCH23390, followed, 25 minutes later by either saline or 2.5 mg/kg SKF81297. Mice were then monitored for 2 hours. On day 2 the procedure was repeated (again with mice who had only received a single injection a week ago), this time using 0.5 mg/kg SCH23390. Interestingly, both 0.1 mg/kg (top panel) and 0.5 mg/kg (bottom panel) effectively blocked locomotor activity, even when followed by D1-class agonist SKF 81297. This is contrary to the previous finding that the 0.1 mg/kg dose of SCH 23390 was not able to reduce locomotor activity in an acute dosage experiment (Fig. 16). The experiments were done on consecutive days, each with their own control groups. For panel (A) n=6 for all groups. For panel (B) n=7 for the 0.5 mg/kg SCH 23390 plus saline, n=6 for 0.5 mg/kg SCH 23390 plus 2.5 mg/kg SKF 81297 and saline plus saline, and n=5 for saline plus 2.5 mg/kg SKF 81297. Injection times are indicated by the arrows.

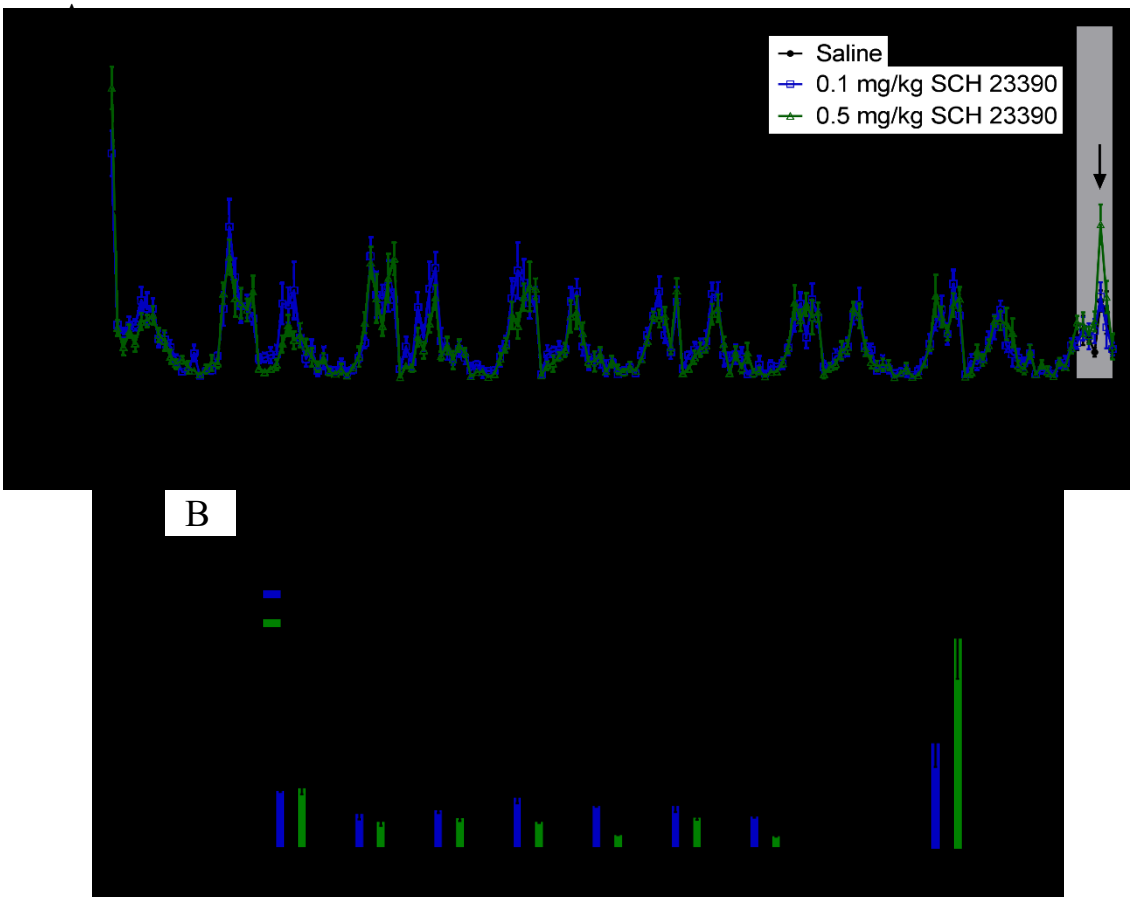

Figure 38: Chronic SCH23390 Administration followed by an SKF81297 Challenge. Eight days after the dual SCH23390 and SKF81297 injection experiments concluded, mice were again assigned random drug conditions and returned to the locomotor activity frame. This time the mice were given seven daily injections (beginning between 11:00AM-11:30AM) of either saline, 0.1 mg/kg SCH23390 or 0.5 mg/kg SCH23390. On the eighth day, 24 hours after the last injection of either saline or SCH23390 all mice were challenged with 2.5 mg/kg SKF81297 to assess locomotor sensitization to D1-class agonists. Mice remained on the locomotor activity frame for the entirety of the experiment. Both doses of SCH23390 severely attenuated locomotor activity following administration (panel B), however neither dose had an obvious effect on the overall locomotor activity or on the circadian rhythm of locomotor activity (panel A). The SKF81297 challenge is shown in the shaded area in panel A. Times of injection are indicated by the black arrows. A two-way ANOVA of the locomotor activity in the 40 minutes following injection for the seven days of SCH23990 administration, revealed a significant effect of subject matching ($p<0.0001$), time ($p<0.0001$), drug condition ($p<0.0001$) and an interaction between time and drug condition ($p<0.0001$). On days 1-5 $p=0.0001$ for both doses, $p= 0.009$ and 0.001 for the 0.1 mg/kg dose and $p= 0.0007$ and 0.0001 for the 0.5 mg/kg dose on days 6 and 7 respectively, by a Dunnet's post-test. Panel C shows the results of the SKF81297 challenge for mice who had received seven days of 0.1, 0.5 mg/kg SCH23390 or saline. Statistically detectable differences were not observed in the response to SKF81297 by animals who had received 7 days of 0.5 mg/kg SCH23390 or saline.

Appendix 3: Injection Histories of Mice That Underwent Multiple Drug Dosage Experiments.

In order to use as few animals as possible, mice in the dosage experiments were used in multiple experiments after re-randomization and washout periods. The following tables detail the injection histories of mice that were used in multiple drug dosage experiments.

INJECTION DATE	Injection 1	Injection 2
MOUSE #	June 15 or 16 2016	June 20 or 21, 2016
8	0.25 mg/kg DHX	Saline
11	Saline	4 mg/kg DHX
14	0.25 mg/kg DHX	Saline
22	1 mg/kg DHX	Saline
23	1 mg/kg DHX	4 mg/kg DHX
28	0.25 mg/kg DHX	4 mg/kg DHX
41	Saline	4 mg/kg DHX
42	0.25 mg/kg DHX	Saline

Table 1: Injection History of Mice Used in the Acute DHX with Short Acclimation Experiment and In the Acute DHX and Perfusion Experiment. Mice from the acute DHX with short acclimation experiment (Fig. 35) were reused after 5 days of washout for the acute DHX and perfusion experiment (Fig. 18). Mice are listed by tag number.

INJECTION DATE MOUSE #	Injection 1-7 April 26-May 2 2017	Injection 8 May 15 2017	Injection 9 May 15 2017
245	Saline	0.5 mg/kg SCH23390	Saline
246	1 mg/kg DHX	0.5 mg/kg SCH23390	Saline
247	16 mg/kg DHX	N/A	1 mg/kg DHX
248	Saline	Saline	4 mg/kg DHX
249	4 mg/kg DHX	0.5 mg/kg SCH23390	Saline
250	Saline	Saline	4 mg/kg DHX
262	1 mg/kg DHX	0.5 mg/kg SCH23390	Saline
263	16 mg/kg DHX	†	†
264	Saline	0.5 mg/kg SCH23390	4 mg/kg DHX
265	4 mg/kg DHX	Saline	4 mg/kg DHX
266	1 mg/kg DHX	Saline	4 mg/kg DHX
267	1 mg/kg DHX	0.5 mg/kg SCH23390	4 mg/kg DHX
268	16 mg/kg DHX	N/A	1 mg/kg DHX
269	4 mg/kg DHX	0.5 mg/kg SCH23390	4 mg/kg DHX
270	4 mg/kg DHX	Saline	4 mg/kg DHX
271	1 mg/kg DHX	0.5 mg/kg SCH23390	Saline
272	4 mg/kg DHX	Saline	4 mg/kg DHX
273	Saline	0.5 mg/kg SCH23390	4 mg/kg DHX
274	16 mg/kg DHX	†	†
275	4 mg/kg DHX	0.5 mg/kg SCH23390	4 mg/kg DHX
297	16 mg/kg DHX	N/A	1 mg/kg DHX
298	Saline	0.5 mg/kg SCH23390	Saline
299	16 mg/kg DHX	N/A	1 mg/kg DHX
300	1 mg/kg DHX	0.5 mg/kg SCH23390	4 mg/kg DHX

Table 2: Injection History of Mice in the Chronic DHX experiment and the Blocking DHX with SCH23390 Experiment. Mice in the chronic DHX experiment (Fig. 14, 15) were reused in the experiment which used SCH23390 to block DHX (Fig. 17) after a washout period. The table shows all the injections that each mouse received (mice are listed by tag number). The crosses indicate mice that died during the chronic DHX experiment.

INJECTION DATE / MOUSE #	Injection 1	Injection 2	Injection 3
	Jan. 25 or 26 2017	Feb. 1 or 2 2017	Feb. 1 or 2 2017
173	Saline	Saline	Saline
174	Saline	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
175	Saline	0.5 mg/kg SCH23390	Saline
176	Saline	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
177	2.5 mg/kg SKF81297	Saline	2.5 mg/kg SKF81297
178	2.5 mg/kg SKF81297	Saline	Saline
179	2.5 mg/kg SKF81297	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
180	2.5 mg/kg SKF81297	Saline	2.5 mg/kg SKF81297
181	0.01 mg/kg SCH23390	0.5 mg/kg SCH 23390	Saline
182	0.01 mg/kg SCH23390	Saline	Saline
183	0.01 mg/kg SCH23390	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
184	0.01 mg/kg SCH23390	Saline	Saline
185	0.01 mg/kg SCH23390	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
186	0.01 mg/kg SCH23390	0.1 mg/kg SCH23390	Saline
187	0.01 mg/kg SCH23990	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
188	0.01 mg/kg SCH23390	Saline	Saline
189	0.5 mg/kg SCH23390	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
190	0.5 mg/kg SCH23390	Saline	Saline
191	0.5 mg/kg SCH23390	0.1 mg/kg SCH23390	Saline
192	0.5 mg/kg SCH23390	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
194	0.5 mg/kg SCH23390	0.1 mg/kg SCH23390	Saline
195	0.5 mg/kg SCH23390	Saline	Saline
196	0.5 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
197	0.5 mg/kg SCH23390	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
198	1.0 mg/kg SCH23390	0.5 mg/kg SCH23390	2.5 mg/kg SKF81297
199	Saline	Saline	2.5 mg/kg SKF81297
200	Saline	Saline	2.5 mg/kg SKF81297
201	Saline	Saline	Saline
202	2.5 mg/kg SKF81297	0.5 mg/kg SCH23390	Saline
203	2.5 mg/kg SKF82297	0.5 mg/kg SCH23390	Saline
204	2.5 mg/kg SKF81297	0.5 mg/kg SCH23390	Saline

205	1 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
206	0.1 mg/kg SCH23990	0.1 mg/kg SCH23390	Saline
207	0.1 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
208	0.1 mg/kg SCH23390	Saline	Saline
209	0.1 mg/kg SCH23390	0.1 mg/kg SCH23390	Saline
210	0.1 mg/kg SCH23390	0.1 mg/kg SCH23390	Saline
211	1 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
212	0.1 mg/kg SCH23390	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
213	0.1 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
214	1 mg/kg SCH23390	Saline	Saline
215	1 mg/kg SCH23390	0.5 mg/kg SCH23390	Saline
217	1 mg/kg SCH23390	Saline	2.5 mg/kg SKF81297
218	1 mg/kg SCH23390	0.1 mg/kg SCH23390	2.5 mg/kg SKF81297
219	1 mg/kg SCH23390	0.5 mg/kg SCH23390	Saline
220	2.5 mg/kg SKF81297	Saline	Saline
221	Saline	Saline	2.5 mg/kg SKF81297
222	0.1 mg/kg SCH23390	Saline	Saline

Table 3: Injection History of Mice Used in The Acute SCH23390 and SKF81297 Dosage Experiment and the Blocking SKF81297 with SCH23390 Experiment. Mice (listed by tag number) from the acute SCH23390 and SKF81297 experiments (Fig. 16 and X) were reused for the experiment that examined blocking of the locomotor effect of SKF81297 by SCH23390 (Fig. X). Injections 2 and 3 were given on the same day (dual injection experiment)

Appendix 4: Summary Tables of the Literature of Animal and Clinical Studies Employing Dopamine-Augmenting Drugs in Motor Recovery from Stroke

Tables summarizing the methodology and results of studies employing DA-augmenting drugs L-dopa, MPH, AMPH and ropinirole in animals (Table 4) and humans (Table 5) are shown below.

Study	Model	Dosing/Rehab	Behaviour	Outcome
(Adkins and Jones, 2005)	Cortical infarct caused by application of ET-1 to the surface of the rat cortex	1mg/kg AMPH every third day starting 10-14 days postop, training given daily, 1-2 hours after injection on injection days, on the single pellet reaching test	Single pellet reaching	AMPH rats were robustly better than saline mice while training continued, by 2 months after the conclusion of training the AMPH mice had declined and the saline mice improved enough that the two were very similar
(Alaverdashvili et al., 2007)	Female rats, craniotomy followed by removal of pia mater and surface blood vessels	Oral d-amphetamine 1mg/kg beginning 24 hours after stroke given every 3rd day for 8 doses, half hour before training on their specific reaching task	Either single pellet reaching with trained trip to back of the box between reaches, modified single pellet or tray reaching	AMPH and controls were similar by the end of the experiment on all tasks, controls recovered faster than AMPH animals, having significantly better performance on some days, AMPH animals typically more qualitatively impaired than controls
(Auriat and Colbourne, 2008)	Collagenase intracerebral hemorrhage model, primarily striatal damage	2 mg/kg AMPH on days 7, 9 and 11, housed in an enriched environment and training on the tray reaching and beam walking tasks, 30 minutes after injection	Tested beam walking (non-aversive), a Neural deficit score, the Montoya staircase, and the tray task, horizontal ladder	Saw an effect of rehab but not drug on the beam walking and horizontal ladder, no effect on the Montoya staircase or the non-beam portion of the neural deficit score, tray task was not analyzed due to high number of animals which had to be excluded
(Barbay et al., 2006)	Adult squirrel monkeys, cauterization of surface blood vessels of hand representation and ~500um into arm representation	Single injection of 0.25 mg/kg AMPH 1 hour before hand dexterity training on day 10 postop, training continued for 14 days	Kluver board (skilled reaching)	AMPH and rehab animals were significantly better than rehab alone animals on days 13,14, 17, 18, 19,20, 21 and 22 poststroke, at 9 weeks postop AMPH was still significantly better than rehab controls

(Boyeson and Feeney, 1991)	Suction ablation of anterior and neocortical cerebellar cortex in rats	Starting 24 hours after ablation 2 mg/kg AMPH, 0.4 mg/kg haloperidol, both or saline injections every 4 days for a total of 6 injections	Beam walking task	Recovery in cerebellum-lesioned rats not as complete as in motor cortex lesioned rats, Saline group recovered the most, haloperidol group recovered the worst, with a marked dip in performance after drug
(Brown et al., 2004)	Photothrombotic lesion in rats, selecting for highly impaired animals	2 mg/kg d-AMPH or saline given 1 day after stroke, with or without training on the beam and daily testing, or without daily testing	Beam walking without aversive stimuli	Training and daily testing both improved recovery, while AMPH slowed recovery, although AMPH and experience or training did recover fully by 10 days poststroke
(Feeney and Hovda, 1983)	Motor cortex ablation followed by packing the wound, in cats	5 or 8 mg/kg d,l-AMPH on days 4, 9 and 15	Tactile placing in response to paw stimulation	Placing response is very weakly augmented about 3 hours after intoxication on day 4 postop, at day 9 and day 15 saw a much larger restoration of tactile placing which lasts 12-24 hours
(Feeney and Hovda, 1983)	Motor cortex ablation followed by packing the wound, in cats	5mg/kg of d-AMPH, l-AMPH, d,l-AMPH or d,l-AMPH followed by haloperidol	Tactile placing in response to paw stimulation	In cats with ablations and no recovery racemic mixture was the most effective, d-AMPH had some effectiveness and l-AMPH had almost no effectiveness, haloperidol at 0.2 mg/kg was able to supress d,l-AMPH, and at 0.4mg/kg was able to block it . In partially recovered cats but not unlesioned cats haloperidol was able to block tactile reaching
(Gilmour et al., 2005)	ET-1 stroke in rats	2mg/kg AMPH, staring on day 2 postop and continuing every 3rd day until day 26 (8 days administration total)	Paw reach and foot fault, ipsilateral limb was bandaged to prevent use	No benefit of AMPH on the foot fault test, saw a benefit of AMPH on paw reaching 24hr after drug, recovery of AMPH animals still present 6 days after last injection

(Goldstein, 2009)	Ablation of fore and hindlimb sensorimotor cortex in rats	d-AMPH, haloperidol or saline for 5 days, rats are housed in an enriched environment and some are fitted with casts that prevent use of unimpaired forelimb 24 hours before first drug dose	Cylinder test and beam walking (evaluating time to cross the beam)	On the cylinder test, among non-restricted animals AMPH animals performed best and haloperidol animals performed very poorly, restrictive casts offset the deficit in haloperidol animals and increased saline animal's performance to the level of AMPH+ restraint animals, no differences were seen between groups in the beam walking test
(Goldstein and Davis, 1990b)	Suction lesion to the level of the white matter	2.6 mg/kg d-AMPH	Beam walking with aversive stimulus, began 24 hours after surgery and continuing until 48 hours postop	Overall faster improvement of AMPH animals in one dose, however see AMPH non-responders and saline recoverers
(Goldstein and Davis, 1990c)	Suction ablation of the cortex to the level of white matter in rats	2.6 mg/kg +-AMPH-HCL, practice is 6 trials on the beam walking task at 1 hour intervals beginning 1 hour after injection	Beam walking	At 24 hours after injection AMPH+ practice had significantly better performance on the beam walking task as compared to all groups, the practice alone and AMPH alone groups were better than the saline group, but not significantly
(Goldstein and Davis, 1990a)	Rat, suction ablation of grey matter	2 mg/kg AMPH	Beam walking with aversive stimulus, given as massed or spaced trials	AMPH helped both massed and spaced trial rats, helped massed trials more early on
(Hovda and Fenney, 1984)	Cat motor cortex ablation	5mg/kg AMPH with experience, single dose or multiple doses days 10,14,18 and 22 after surgery	Beam walking and tactile placing	Fastest recovery with AMPH and experience, slower but complete recovery with AMPH alone (at 60 days post op) Saline group had incomplete recovery, no improvement on tactile placing test
(Hovda et al., 1987)	Cat, primary visual cortex ablations	5mg/kg AMPH on days 10, 14, 18 and 22 postop	Tactile placing (eyes covered, stimulate hairs of dorsal surface)	AMPH cats show recovery within 3h of first dose, recovery lasts to end of experiment (30 days)
(Liu et al., 2011)	Transient MCAO, lesions mainly in temporoparietal cortex	2mg/kg AMPH every 3rd day for four weeks	Turning asymmetry during body swing test	AMPH animals had significantly less asymmetry on body swing, AMPH group had significantly better body posture while suspended on day 12

(Papadopoulos et al., 2008)	MCAO in rats, only cortical damage	2 mg/kg AMPH given on days 2, 5 and 8 postop with or without enriched environment (EE) or focussed activity (starting on day two and continuing twice a day for 3 weeks and then once a day for a further 5 weeks)	Forelimb reaching task performed daily, Monday-Friday for 8 weeks, and horizontal ladder performed weekly, behaviour testing not done under the drug	AMPH combined with environmental enrichment and focussed activity was significantly better than all other groups on both skilled reaching and the horizontal ladder, show complete recovery on skilled reaching, AMPH and EE and AMPH with EE and focussed activity recovered completely
(Ramic et al., 2006)	Aspiration lesion of grey matter and some white matter damage	2mg/kg d-AMPH on days 2 and 5 postlesion, rehab under drug's effect, rehab involved a variety of forelimb involving climbing tasks and EE	Ladder walking, pellet reaching	AMPH + rehab group showed significant improvement at 1 week postop, AMPH only at 2 weeks postop on pellet reaching, see significant recovery at 1 week in combo group and 6 weeks in AMPH only on ladder walking, no significant benefit of rehab only
(Rasmussen et al., 2006)	Injection of autologous macro-clot to the middle cerebral artery in rats	3.5 mg/kg d-AMPH sulfate given on days 1, 3, 5 and 7 postop, physical therapy consisted of Montoya staircase for 15 minutes and T-maze for 20 runs on drug days	Montoya staircase and T-maze (not discussed here)	Animals given only therapy performed significantly better than controls and had no asymmetry, AMPH and AMPH + therapy animals still had asymmetry, AMPH + therapy was better than AMPH alone and AMPH alone was better than controls but not significantly so
(Rasmussen et al., 2011)	Embolic strokes in rats	No AMPH, early AMPH (3.5mg/kg 10 minutes after embolization) Late AMPH with training (days 2, 5, 8 and 11 1mg/kg with Montoya staircase) or both early and late AMPH	Montoya staircase, recovery assessed from days 14-25 poststroke	Acute AMPH group performed better than the control group on the Montoya staircase, the late AMPH and combination AMPH group both performed much worse than the controls
(Ruscher et al., 2011)	Transient MCAO in male rats	5 days of either 1, 5 or 20 mg/kg L-dopa (with benserazide) or placebo starting on day 2 after stroke.	Rotating pole test (various speeds), the cylinder test, a composite neuroscore	Significantly better improvement of the 20 mg/kg L-dopa group as compared to controls on all speeds of the rotating pole test at 7 days post-infarct, the neuroscore at 7 and 14 days poststroke and the cylinder test 14 days post stroke. Significantly better improvement above the controls on the 5rpm speed of the rotating pole at 7 days poststroke and on the cylinder test at 14 days poststroke with 5 mg/kg L-dopa

(Schmanke et al., 1996)	Male rats with large unilateral electrolytic lesions of the sensorimotor cortex	24 hours after surgery given either 2 mg/kg d-AMPH or saline	Beam walking, foot fault test, bilateral tactile stimulation (adhesive removal) test with neutralisation, vibrissae>forelimb placing, forelimb>forelimb placing	Early beam walking recovery improved with AMPH, from 1-6 hours post injection. On foot fault test, bilateral tactile stimulation test and vibrissae>forelimb placing and forelimb>forelimb placing there was no difference between the groups
(Schmanke et al., 1996)	Male rats with small unilateral electrolytic lesions in forelimb sensorimotor area	2mg/kg d-AMPH at 1, 3 and 5 days postop	Beam walking, foot fault test, bilateral tactile stimulation (adhesive removal) test with neutralisation, vibrissae>forelimb placing, forelimb>forelimb placing	AMPH mice showed recovery sooner on the beam walk and were significantly better on days 7,9 and 15 postop. AMPH mice showed improvement sooner on the vibrissae>forelimb placing and forelimb>forelimb placing, no differences in foot fault test or bilateral tactile stimulation test
(Schmanke and Barth, 1997)	Male rats with electrolytic lesions of the caudal forelimb representation area.	2mg/kg of d-AMPH 1, 3 and 5 days postop with or without forelimb placing training	Vibrissae> forelimb placing and Forelimb> forelimb placing	On vibrissae>forelimb placing AMPH + practice group performed the best starting about 10 days postop, AMPH alone and practice alone both performed better than saline, Forelimb>forelimb placing benefits from AMPH but not significantly, no improvement is observed until 35 days postop
(Stroemer et al., 1998)	Permanent MCAO in spontaneous hypertensive rats	2 mg/kg d-AMPH given on day 3, 6 and 13 poststroke and then every 3rd day until day 30, testing done 1 hour after injection and again 24 hours after injection	Foot fault test with large (6cm) openings or small (3cm) openings, Morris water maze (not discussed here), postural reflex test, somatosensory disengage behaviour, rearing behaviour and grip strength	No differences in postural reflex, somatosensory disengage behaviour, rearing behaviour or grip strength. AMPH rats did significantly better than saline rats on the foot fault test from 2 days after surgery onwards on large foot fault test, saw a similar pattern on the small foot fault test
(Sutton et al., 1989)	Bilateral cortical ablation in cats, varying size of lesions	5mg/kg AMPH on days 12, 16 and 20 after surgery	Beam walking with a 10-point scale, started 6 days after lesion	AMPH animals performed better than saline animals, although some AMPH animals were completely non-responsive to treatment and some saline animals recovered spontaneously

(Wolf et al., 2014)	Distal MCAO, affecting primarily the cortex, in rats	2mg/kg d-AMPH on 2, 5 and 8 days poststroke, focused activity where animals are placed on climbing apparatuses for 20 minutes beginning 15 minutes after injection and EE	Skilled reaching test performed daily, horizontal ladder performed once a week	AMPH + rehab performed significantly better than all groups and no longer had a significant difference from the baseline performance on skilled reaching and the horizontal ladder at 8 weeks post infarct

Table 4: Summary of Studies Using AMPH or L-dopa in Animal Stroke Models. The above table summarizes the study parameters and the outcomes of the studies quoted in the main text which involves administration of AMPH or L-dopa. For a summary of studies involving other drugs, or intraventricular infusion approaches, please see Feeney et al., 1993.

Study	Number of Patients	Time from Stroke	Drug dosage	Additional Rehabilitation	Outcome
(Acler et al., 2009)	10	10-48 months after stroke	Crossover study design, 100 mg L-dopa daily or placebo daily for 5 weeks, followed by 2 months of washout and the other condition (single blind)	No physiotherapy	L-dopa improved walking speeds (10 m walking test), an improved manual dexterity with the paretic hand (9 hole peg test), no change on the RMA, Increase in cortical silent period as detected by TMS, no changes were seen in the placebo group
(Cramer et al., 2009)	33	1-12 months poststroke	Daily doses of ropinirole or placebo for 9 weeks, goal to work up to 3 mg/kg with doses adjusted weekly	Physiotherapy given twice a week about 1 hour after drug intake from weeks 6-9, patients expected to complete 30 min/day of physiotherapy at home after taking medication	No differences were apparent between treatment groups on gait velocity, gait endurance, arm or leg FM score or BI
(Crisostomo et al., 1988)	8	Within 10 days after stroke	A single 10 mg dose of AMPH	45 minutes of physiotherapy within 3 hours of drug	Statistically significant improvement of the AMPH group above the level of the placebo group by the FM scale
(Floel et al., 2005)	9	Greater than 1 year from stroke	Crosssover trial design, with a single dose of 100 mg L-dopa (with carbidopa) and placebo separated by 24 hours	Task specific training 60 minutes following drug or placebo administration	Improved motor-learning on a TMS stimulated thumb movement task

(Gladstone et al., 2006)	71, stratified by hemiparesis severity	5-10 days after stroke	10 mg AMPH or placebo	10 1 hour sessions of physiotherapy given after drug administration	No significant difference in improvement above the level of placebo on the FM scale
(Grade et al., 1998)	21	Sub-acute stage, specifics unclear	3 weeks of daily MPH, starting at 5 mg and increasing to 30 mg or placebo	Physical therapy	Significant improvement of the MPH group on the motor portion of the FIM
(Kakuda et al., 2011)	5	18-143 months after stroke	1 week of prior 100 mg L-dopa, 15 days of inpatient protocol with continuing L-dopa and 4 weeks L-dopa after inpatient protocol, no placebo group	2 daily session of 30 minutes low frequency TMS applied to the contralateral hemisphere, 1 hour of intensive occupational therapy and 1 hour of self exercise during the inpatient protocol	All patients showed improvement in motor function as measured by the FM scale and the Wolf Motor Scale
(Lokk et al., 2011)	78	15-180 days after onset of stroke	125 mg L-dopa, 20 mg MPH, 125 mg L-dopa and 10 mg MPH or placebo, 5 days a week for 3 weeks	Physiotherapy 1 hour after drug administration	Significantly better improvement on BI and NIHSS for all drug groups as compared to placebo, but not on the FM scale, at the 6 month follow-up
(Martinsson and Wahlgren, 2003)	45	Within 72 hours after stroke	2.5, 5, or 10 mg dose of d-AMPH given orally twice a day, or placebo for 5 days	No additional physical therapy	Significantly better improvement on LMAC motor function score and AI motor score and SSS-68 at day 7 follow-up in AMPH group, no difference at 1 or 3 months

(Masihuzzaman et al., 2011)(abstract only)	97	Unspecified	125 mg L-dopa or placebo, frequency unspecified	Physiotherapy with drug administration	L-dopa group had significantly greater increase in RMA as compared to placebo
Mazagri et al 1995 (abstract only, reference (Long and Young, 2003))	25	Within 72 hours of ischemic stroke	A single 10 mg dose of d-AMPH or placebo	Physiotherapy	No improvement of AMPH group above the level of placebo group at 48 h or 3 months after treatment in FM scale, BI and CNS
(Platz et al., 2005)	26	An average of 5.6 weeks after stroke	10 mg d-AMPH or placebo twice a week for 3 weeks	Arm training 2 hours after drug administration	d-AMPH group did not improve above the level of placebo group during or after training, or at 1 year follow-up in TEMPA task (an ADL measure), an aiming task, a finger tapping task and time to walk 10 m
Reding et al 1995 (abstract only, reference (Long and Young, 2003))	21	Less than 1 month after stroke	10 mg d-AMPH for 14 days followed by 5 mg d-AMPH for 3 days or placebo	No therapy provided by the study	No benefit of AMPH group as compared to placebo on the FM scale or the BI
(Restemeyer et al., 2007)	10	More than 6 months after stroke	Crossover trial design, single dose of 100 mg L-dopa (with carbidopa and domperidone) and placebo	1 hour of physiotherapy after drug administration	No benefit of L-dopa on the 9 hole peg test, grip strength, Action Research Arm Test, or excitability as measured by TMS
(Rösser et al., 2008)	18	Patients in chronic stage, a mean of 3.3 years after stroke	Crossover study design, comparing 3 doses of 100 mg L-dopa (with carbidopa) or placebo	Drug administration followed by 1 session of procedural motor learning	Better procedural motor learning in the L-dopa group on a serial reaction time task with a probabilistic sequence in the paretic hand

(Scheidtmann et al., 2001)	53	Between 3-6 months from stroke	100 mg L-dopa (with carbidopa) or placebo daily for 3 weeks	Physiotherapy daily (with drug) for 3 weeks followed by 3 weeks of only physiotherapy Monday to Friday	Significant improvement of the L-dopa group as compared to the placebo group as measured by the RMA
(Schuster et al., 2011)	16	14-60 days before intervention	10 mg d-AMPH or placebo orally twice a week for 5 weeks	Physiotherapy given after each drug administration	Significantly better improvement on ADL and the arm subscale of the CMSA compared to placebo
(Sonde and Lökk, 2007)	25	Within 5-10 days of stroke	10 doses 20 mg of d-AMPH over 2 weeks or 10 mg d-AMPH and 50 mg L-dopa or 100 mg L-dopa or placebo	Physiotherapy after drug intake	Did not see benefit above the level of placebo on the FM scale or BI for any drug condition
(Sonde et al., 2001)	39	5-10 days after stroke	10 mg d,l-AMPH or placebo given twice a week for 5 weeks	Physiotherapy given 1 hour after each drug administration	AMPH group did not show improvement above the level of placebo on the FM or BI
(Treig et al., 2003)	24	Within 6 weeks of stroke	10 sessions of 10 mg d-AMPH or placebo every 4th day	Physical therapy within 1 hour of drug intake	No benefit of AMPH above placebo on the BI or the RMA over course of treatment or at 90 day follow-up
Vachalathiti et al. 2001 (abstract only, reference (Long and Young, 2003))	27	An average of 5.7 days after stroke onset	10 mg d-AMPH daily for 7 days	Not stated	No difference between AMPH and control groups on the FM scale and the BI

(Walker-Batson et al., 1995)	10	Between 16-30 days after stroke	10 mg d-AMPH orally every 4th day for 10 sessions (single blind)	Physiotherapy after drug administration	Drug group had significant benefit as compared to placebo at 1 week and 1 year from treatment conclusion as measured by the FM scale
(Wang et al., 2014)	9	7-30 days post stroke	One time dose of 20 mg liquid MPH, orally or placebo	transcranial direct current stimulation (tDCS) or sham tDCS	All groups showed improvement on the Perdue Pegboard test, combination MPH and tDCS showed improvement above the level of either treatment alone

Table 5: Summary of Clinical Trials Using Dopamine-Receptor Stimulating Drugs to Improve Motor Recovery from Stroke. The above table summarizes the studies discussed in the text using amphetamine (AMPH), levodopa (L-dopa), methylphenidate (MPH) and ropinirole. The following short forms have been used to indicate outcome measures: RMA (Rivermead Motor Assessment), FIM (Functional Independence Measure), BI (Barthel Index), FM (Fugl-Meyer), NIHSS (National Institute of Health Stroke Scale), ADL (Activities of Daily Living), CMSA (Chedoke-McMaster Stroke Assessment), CNS (Canadian Neurological Scale), AI (Activity Index), SSS68 (Scandinavian Stroke Scale 68), LMAC (Lindmark Motor Assessment Chart) and the TEMPA task (Test Evaluant les Membres superieurs des Personnes Agees, a test of upper extremity function in the elderly).