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Hypoxically preconditioned-bone marrow stromal cells attenuate post-stroke depression

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B.S., Georgetown University, 2013

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Abstract

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Ischemic stroke is the leading cause of long-term disability in the U.S., in part because of the debilitating post-stroke depression (PSD) that affects approximately one-third of stroke survivors. PSD leaves stroke survivors at a greater chance of increased disability, delayed functional recovery, decreased cognitive function, morbidity, and mortality. Due to the detrimental effects associated with PSD, it is necessary that new and innovative treatments for PSD are investigated in order to improve long-term patient outcomes. Here we will explore the use of hypoxically preconditioned-bone marrow stromal cells (HP-BMSCs) as a novel treatment for PSD. BMSCs are known to confer a number of benefits including enhanced cell migration to the site of injury, improved functional outcomes, and enhanced neurotrophic support, and we have previously shown that hypoxic-preconditioning increases oxytocin and oxytocin receptor expression *in vivo*. Oxytocin, a hormone secreted by the posterior lobe of the pituitary gland, is known to induce and facilitate social behaviors and is believed to be a psychosocial mediator of stroke outcome. Thus, treatment with HP-BMSCs may be especially beneficial for stroke survivors who commonly experience social impairment following stroke. The results of this investigation confirm our predictions, that treatment with HP-BMSCs, as a result of their influence on oxytocin levels, attenuate the depressive-like phenotype found in our stroke animals post-stroke, and restore sociability to levels similar to controls.

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List of Abbreviations:

AMPA: d-amino-3-hyroxy-5-methyl-4-isoxazolepropionic acid

ATP: adenosine triphosphate

BDNF: brain-derived neurotrophic factor

BHS: Beck Hopelessness Scale

BDI: Beck Depression Inventory

CES-D: Center for Epidemiologic Studies Depression Scale

CNS: central nervous system

CSF: cerebral spinal fluid

CT: computed tomography

DAI: diffuse axonal injury

DES: depression-executive dysfunction syndrome

DSM-III: Diagnostic and Statistical Manual of Mental Disorders-Third Edition

DSM-IV: Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition

DSM-V: Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition

DTI: diffusion tensor imaging

EEAs: excitatory amino acids

EPO: erythropoietin

GDS: Geriatric Depression Scale

HADS: Hospital Anxiety and Depression Scale

HDRS: Hamilton Depression Rating Scale

hESC: human embryonic stem cell

HIF-1α: hypoxia-inducible factor-1α

IGF-1: insulin-like growth factor-1

MADRS: Montgomery-Asberg Depression Rating Scale

MAG: myelin-associated glycoprotein

MAPK: mitogen-activated protein kinase

MCAO: middle cerebral artery occlusion

MDD: major depressive disorder

MMSE: Mini-Mental State Examination

MRI: magnetic resonance imaging

MSC: marrow stromal cell

NF-68: neurofilament-68

NGF: nerve growth factor

NMDA: n-methyl D-aspartate

NSC: neural stem cell

NSE: neuron specific enolase

NSPCs: neural stem/progenitor cells

OPCs: oligodendrocyte progenitor cells

PHQ: Patient Health Questionnaire-2

PSD: post-stroke depression

PSDS: Post-stroke Depression Scale

PSE: Present State Examination

ROCK: rho-associated coil kinase

SCI: spinal cord injury

SDF-1: stromal-derived factor-1

SERT: serotonin transporter

SDS: Zung Self-rating Depression Scale

SNAP-25: synaptic protein-25

SVU: subventricular zone

SWI: susceptibility-weighted imaging

TBI: traumatic brain injury

β-APP: beta-amyloid precursor protein

CHAPTER 1: Background: Post-stroke Depression

Stroke is the third most common cause of death worldwide and a leading cause of long-term disability[1]. On average, someone in the U.S. has a stroke every 40 seconds[2], and this year alone over 795,000 people in the U.S. will experience a stroke[1]. Stroke occurs as a result of obstructed blood flow to the brain or blood vessel rupture, both of which lead to neural oxygen deprivation and cell death. One person dies as a result of stroke every four minutes[1], and those that are lucky enough to survive are often left with a number of neuropsychiatric manifestations including depressive mood, irritability, appetite changes, agitation, apathy, anxiety, sleep disturbances, aberrant behavior, delusions, and hallucinations[3]. The most common of these neuropsychiatric manifestations is depression, commonly referred to as post-stroke depression (PSD), which is found to occur in approximately one-third of stroke survivors[4-6]. According to the Diagnostic and Statistical Manual of Mental Disorders-Fifth Edition (DSM-V) PSD is defined as a mood disorder due to a general medical condition within the specifiers of depressive features, including major depressive-like episodes, manic features, and/or mixed features[7].

To date little PSD research has been conducted, especially considering that it affects over 2 million stroke survivors in the US[4-6] and approximately 15 million worldwide[8]. What we do know has largely been extrapolated from current major depressive disorder (MDD) findings. Thus, It is imperative that the etiology of PSD, in addition to prevention and treatment strategies, be more thoroughly investigated as PSD has a considerable impact on the biological, psychological, and social aspects of stroke survivors and their families' lives.

A. Symptoms: Identifying the symptoms of depression is often difficult, especially in stroke patients who may not present the typical symptoms. Further complicating

diagnosis is the fact that the symptoms associated with PSD may vary in frequency, severity, and duration depending on the patient. The most common symptoms associated with PSD include: persistent sadness or anxiousness; sleep disturbances; increased or decreased appetite; feelings of helplessness, hopelessness, and/or worthlessness; social withdrawal; loss of interest in activities or hobbies; irritability; fatigue: difficulty concentrating and/or remembering details; suicidal thoughts; and/or aches, pains, headaches and digestive problems that do not ease with treatment[9]. It is important to note that individuals with PSD and caretakers may not perceive or report the same symptoms. A 2013 review by Klinedinst *et al.* reported that stroke survivors are more likely to recognize symptoms including 'feeling like everything was an effort', 'difficulty concentrating', 'being bothered by things that don't usually bother him/her', and 'talking less than usual', while caregivers identified those with PSD as 'feeling like everything was an effort', 'inability to shake off the blues', 'not enjoying life', and 'not feeling as good as other people' [10].

B. Prevalence: One of the primary problems with determining PSD prevalence is that reported incidence varies largely in the literature, anywhere from 12%[11] to 72%[12]. Determining the incidence is especially complicated because of underreporting, underdiagnosis, and lack of diagnostic refinement. The best estimate to date was published in a 2005 meta-analysis by Hackett *et al.*, in which the overall incidence of PSD was estimated to be 33% (95% confidence interval, 29%-36%)[13]. An updated 2014 meta-analysis by the same group revealed a estimated pooled frequency of 31% (95% confidence interval 28%-35%)[14] which was decreased, but not significantly decreased from their findings in 2005. Furthermore, the likelihood of PSD is known to fluctuate depending on the time-point of assessment post-stroke such that prevalence rates of PSD peak at 3-6 months post-stroke, with the greatest increased risk of prevalence during the

first month post-stroke[15]. Additionally, the likelihood of PSD remains elevated for the first 1-3 years following stroke[16].

C. Risk Factors: Due to the detrimental influence of PSD on recovery and quality of life, identifying risk factors is critical. To date many studies have examined the risk factors that increase the likelihood of a stroke survivor developing PSD; however, inconsistent results have prevented the identification of a confirmed set of risk factors to aid in the diagnosis and/or prevention of PSD[17]. In terms of demographic factors, sex and age are often regarded as legitimate PSD risk factors, as associations with a higher incidence of PSD have been found in females[18-20] and in older individuals[18]; however, contradictory findings have been found in other studies[19,21], precluding any definitive conclusions. Other risk factors found to positively correlate with PSD incidence include a previous history of depression and/or psychiatric illness[19,22], living alone,[19,22] extent of functional impairment[13,21-24], and social isolation[22]. Biological risk factors have also been theorized and studied with controversial results. The affect of the location of a lesion imposed by a stroke has long been disputed[25]. The current predominate view is that lesions to the left hemisphere, particularly those affecting the frontal lobe and basal ganglia, are associated with a greater risk of PSD[20,25]. However, these results have been difficult to reproduce, and thus may be viewed skeptically[17]. Additionally, a genetic polymorphism at the promoter sequence for a serotonin transporter gene (5-HTTPR) has been found to be associated with a higher incidence of PSD[26]. Studies also indicate that particular features of stroke, such as its severity (assessed according to the Scandinavian Stroke scale, the national institutes of health stroke scale, or the European stroke scale)[21-24,26], may also put individuals at increased risk for PSD.

D. Early Predictors: A study by Carola *et al*., pinpointed three factors that are strong early predictors of PSD in first-time stroke survivors including a low Barthel Index Score, being older than 68, and crying within the first few days post-stroke[21]. Carola *et al.* found that those with a severe functional disability, defined as a Barthel Index score of 60 or less, have significantly increased likelihood of acquiring PSD[21]. Crying within the first few days post-stroke was also found to be an early predictor. In the study crying was broken into three different subtypes: pathological crying, emotionalism, and catastrophic crying. Pathological crying, in which crying is relatively uncontrollable and occurs without an apparent trigger or following a stimulus that would not normally elicit crying, was found to have no association with PSD development. Emotionalism, which is crying with little or no warning in response to a meaningful stimulus, was found to be associated with a 41% chance of PSD development. Lastly, catastrophic crying, which is crying triggered by a task made difficult or impossible by a neurologic deficit, is associated with a 63% chance of PSD[21].

E. Assessment and Diagnosis: PSD may be assessed in a variety of ways including: clinical interview and history; collateral information from family and caregivers; observational standardized screening measures; and self-report standardized screening. Currently, the golden standard for PSD diagnostic criteria is the Diagnostic and Statistical Manual of Mental Disorders[9,27,28]. The DSM-V characterizes PSD as a mood disorder due to a general medical condition, but does not have a specific set of diagnostic criteria for PSD, so the metrics used to diagnose major depressive disorder (MDD) are frequently utilized. According to the DSM-V, MDD is defined as a medical illness that affects how you feel, think, and behave, causing persistent feelings of sadness or loss of interest in previously enjoyed activities. MDD is characterized by nine primary symptoms including depressed mood or irritability, decreased interest or pleasure in most activities,

significant weight change (exceeding 5% when not dieting) and/or a change in appetite, fatigue or loss of energy, change in sleep (insomnia or hypersomnia), change in activity level, feelings of guilt/worthlessness, diminished ability to think/concentration, and/or increased thoughts of death/suicide. Positive diagnosis requires the consistent presence of three or more symptoms for at least 2 weeks, with at least one of these symptoms being depressed mood or loss of interest/pleasure[29,30]. For a positive diagnosis PSD must also cause clinically significant distress or impairment in social, occupational, or other important functional areas. It is important to also highlight that prior to diagnosis, clinicians should confirm that the observed symptoms are not due to the effects of a substance or medical condition, the symptoms cannot be better explained by another psychiatric disorders (e.g. mania, hypomania, bipolar, schizophrenic, etc.), and/or that the patient has no medical history of manic or hypomanic episodes[9].

Many clinicians have called for diagnostic criteria customized to PSD, but this is difficult due to PSD's inconsistent clinical presentation. Some studies have claimed that there is a sub-set of depression symptoms that are more strongly associated with PSD that could be used to specialize the diagnostic criteria of MDD for PSD. Gainotti *et al.* highlights catastrophic reactions, hypermotility, and diurnal mood variations as depressive symptoms frequently found in patients with PSD, while suicidal thoughts and anhedonia are more often associated with MDD[31]. Paradiso *et al.* has provided evidence suggesting that PSD symptoms may also fluctuate with the time since PSD onset, such that early onset is more frequently characterized by feelings of anxiety, guilt, and loss of libido while late onset is more often associated with diurnal variations in mood and social isolation[32]. The fact that symptom presence is not homogenous amongst MDD and PSD suggests that the use of the DSM-V's MDD diagnostic criteria is an insufficient diagnostic tool. Other concerns regarding the use of the DSM-V's diagnostic criteria

include the fact that vegetative symptoms such as psychomotor retardation, fatigue, sleep, and appetite disturbances are assessed, all of which can occur as a direct consequence of stroke, and may not necessarily indicate the presence of PSD[33].

Other means of diagnosing PSD beyond the criteria outlined in the DSM include diagnostic scales such as the Beck Depressive Inventory[34-37], Barthel Index Score, Zung Self-Rating Depression Scale[38], Centre for Epidemiologic Studies of Depression Scale[39,40], Post-Stroke Depression Scale[41], Geriatric Depression Scale[42-44], and the Hospital Anxiety and Depression Scale[45,46]. The use of so many diagnostic scales leads to poor concordance amongst published studies and often under- or over-diagnosis of PSD due in part to the fact that diagnostic scales are intended for those with primary depression, not for those with depression as a secondary outcome[47]. Establishing diagnostic consistency is further complicated by the fact that diagnostic cut-off points for each scale often vary amongst studies. Below the primary PSD diagnostic scales are described and their most common diagnostic cut-offs defined:

1. Beck Hopelessness Scale (BHS): The BHS is an assessment designed by Aaron T. Beck to measure three major aspects of hopelessness: feelings about the future, loss of motivation, and expectations. The BHS consists of 20 items in a true-false format, reflecting pessimism or negative expectancies concerning oneself and one's future life[48]. Scores from 0 to 3 are considered within the normal range, scores of 4 to 8 identify mild hopelessness, scores from 9 to 14 identify moderate hopelessness, and scores greater than 14 identify severe hopelessness[49]. Scores of 10 or more on this scale have been associated with a significant suicide risk[50].

2. Beck Depression Inventory (BDI): The BDI is a 21 multi-choice question self-report inventory that is used in individuals 13 years and older to assess the presence of and measure the severity of depression. Since its initial publication in 1961[51], it has been revised twice to create the BDI-1A (published in 1979) and revised once again to correspond to the DSM-IV criteria for diagnosing depressive disorders (BDI-II; published 1996). Most items are scored from 0 (not at all) to 3 (extreme form of each symptom), and severity of depression is determined from the summed scores. Scores of 16 or above on the BDI-II indicative that the individual may require treatment[51].

3. Barthel Index Score: The Barthel Index Score is an ordinal measure of functional outcome used to measure performance in daily living[52]. The index assesses 10 activities of daily living including bowel control, bladder control, toilet use, grooming, feeding, chair transfers, walking, dressing, climbing stairs, and bathing. Zero to ten points are allotted for each activity, and the number of points allotted to each activity is dependent on the time/assistance required to complete each activity[52]. Scores range from 0-100, with lower scores indicating increased disability such that a scores of 0–20, 21–60, 61–90, and 91–99 indicate total dependence, severe dependence, moderate dependence, and slight dependence, respectively[53].

4. Center for Epidemiologic Studies Depression Scale (CES-D): The CES-D is a 20-item questionnaire originally developed as a screening tool for depression in the general population, which assesses four factors: depressed affect, positive affect, somatic problems and retarded activity, and interpersonal relationships[54]. There have been a number of CES-D reiterations, including a shortened 10-item questionnaire for older adults[55], a 5-item version[56], and a variation which reflects the DSM-IV diagnostic criteria[57]. Each item is scored from 0 (not at all) to 3 (extreme form of each

symptom/item), except for items 4, 8, 12, and 16, which are phrased in a positive direction and reverse coded to avoid response bias[54]. As a result of the scoring methodology utilized, a higher summed score is associated with greater symptoms of depression. Traditionally a score of 16 or greater is the cutoff for clinical depression[58].

5. Geriatric Depression Scale (GDS): The GDS was developed by Yesavage *et al,* and initially contained 30-items[59], but since its origination two reiterations, a 15-item[60] and 5-item[61] have been developed to better accommodate elderly patients. The scale asks questions in regards to how elderly patients have felt during the last week, and to ease comprehension, all questions have a yes/no answer[59,62]. In the 5-item GDS, a score of 2 or more is considered a positive screen for depression[63].

6. Hamilton Depression Rating Scale (HDRS): The HDRS is the most widely used clinician-administered depression assessment scale. It was first developed in 1960 to measure the severity of depression in the outpatient population[64], and has since been revised four times[65-68]. The scale consists of 21-items, the first seventeen of which are scored, while the remaining four questions serve to provide additional qualifying information about depression. The items are scored on either a 3-point or 5-point scale, which when summed are used to determine the presence of and/or the severity of depression. The HDRS assesses somatic symptoms, but few cognitive and/or affective symptoms[68]. Although once considered the gold standard of clinical depression diagnosis, more recently the efficacy of the HDRS has been called into question due to the fact that it places more emphasis on depressive symptoms such as insomnia than suicidal ideas.

7. Hospital Anxiety and Depression Scale (HADS): The HADS is a self-administered scale designed by Zigmond and Snaith given to patients between the ages of 16-65 in an outpatient setting[69]. The HADS originally consisted of 16-items, but was later revised to include 14-items in total, of which 7 are linked to anxiety and 7 are linked to depression. Items are scored from 0 (not at all) to 3 (extreme form of each item), leading to a range of scores from 0-21 on each subscale[70]. Consequently, scores of 0-7, 8-10, 11-14, and 15-21, indicate no depression, mild, moderate, and severe depression, respectively[69].

8. Mini-Mental State Examination (MMSE): The MMSE was originally developed in 1975 as a brief screening tool to allow for quantitative evaluation of cognitive impairment and to allow cognitive changes to be tracked[71]. Since then, the validity of the MMSE has been found to be reduced by client retesting, and thus it is no longer used to track cognitive changes between short time intervals[72]. The MMSE measures constructs such as time and place, immediate recall, short-term verbal memory, calculation, language, and construct ability[71]. Scores range from 0 to 30, with score of 24-30, 18-23, 0-17, being indicative of no, mild, and severe impairment, respesctively[73].

9. Montgomery-Asberg Depression Rating Scale (MADRS): The MADRS is a 10-item clinician-rate diagnostic questionnaire designed to measure the severity of depression and the changes in symptom severity associated with treatment[74]. Items are scored on a 0-6 scale, with an overall score range of 0-60. Summed totals from all items are indicative of depression severity, such that a summed score of 0-6 is considered normal, while scores of 7-19, 20-34, and 34< are indicative of mild, moderate, and severe depression, respectively. Lastly, it is important to note that due to a lack of somatic symptom assessment in the MADRS, it is especially well-suited for assessing depression in individuals with a physical illness, such as stroke.

10. Patient-Health Questionnaire (PHQ): Components of the PHQ are commonly used to screen for and/or diagnose PSD, one of which is the PHQ-9. The PHQ-9 is based on the 9-items utilized by the DSM-IV for the diagnosis of depression. Each item is scored on a 4-point scale, from 0 (not at all) to 3 (extreme form of each symptom). Diagnosis of depression occurs when an individual has 5 or more of the 9 items present at least "more than half of the days" in the past two weeks, with at least one of the symptoms being depressed mood or anhedonia. Additionally severity, whether mild, moderate, or severe, is determined by the summed score of the 9-items (5-9, 10-14, 20-27, respectively)[75,76] The PHQ-9 has been shown to have 90% sensitivity and 87% specificity in those with PSD when assessed one month post-stroke[77]. Additionally, the PHQ-2 may be utilized as a depression-screening tool. The PHQ-2 consists of the first 2 items on the PHQ-9 and assesses the degree to which individuals have experienced depressed mood and/or anhedonia over the past two weeks. Items are scored on a scale from 0 to 6, and three is thought to be the optimal cut-off point for depression screening purposes[75].

11. Present State Examination (PSE): The PSE is a semi-structure interview, which originated in an attempt to standardize psychiatric case identification and improve psychiatric classifications. The PSE tests a patient's present mental state, and contains a total of 140 items, each scored on either a 3- or 4-point scale[78].

12. Post-stroke Depression Scale (PSDS): A scale specifically developed to assess depressed in depressed stroke patients based on the presence of 10 symptoms: depressed mood, feelings of guilt, thoughts of death/suicide, vegetative symptoms, apathy and loss of interest, catastrophic reactions, hyperemotionalism, anhedonia, and diurnal mood variations. Scores associated with all symptoms except for diurnal mood variations range from 0 (normal) to 5 (severe disorder), while the patient's diurnal mood variations are

measured from -2 (unmotivated, clear presence of depression in early morning) to +2 (motivated, prevalence of depression during situations stressing handicaps and disabilities). No global scores are provided by the PSDS, as it was not designed to provide a global assessment of PSD severity[41].

13. Zung Self-rating Depression Scale (SDS): The SDS is a 20-item scale first published in 1965[79]. The self-administered scale requires the patient to indicate, on a 4-point scale, how frequently certain affective, psychomotor, physiological and cognitive symptoms manifest themselves[79,80]. Typically a score of 50< is indicative of mild depression, while scores of 60< and 70< indicate moderate and severe depression, respectively[79,81].

F. Underdiagnosis: PSD incidence is believed to be largely underdiagnosed, in particular because there is low concordance between studies[82]. Much of the estimated underdiagnosis is due in part to the failure of caregivers and patients to recognize symptoms of PSD and report them to healthcare providers, but this may be due in part to the fact that approximately 75% of patients do not receive literature regarding PSD in the hospital or in a rehabilitation setting[10]. It has also been reported that non-psychiatric physicians fail to diagnose up to 80% of stroke survivors with PSD[12]. Another explanation for PSD underdiagnosis is the recent trend of decreased hospital stays. Currently the average hospital stay is less than 14 days[83], which makes screening for PSD increasingly more difficult due to the DSM's requirement that depression symptoms be present for 14 or more days. In order to identify hospitalized stroke patients at risk for PSD, a clinical prediction model called the post-stroke depression prediction scale (DePreS) has been developed which has been shown to have acceptable discrimination and calibration. DePreS utilizes predictors of PSD available to clinicians during the first week post-stroke. A sampling of predictors utilized include gender, age, type and

location of stroke, functional status post-stroke as indicated by the Barthel Index and the modified Rankin Scale, patient's perceived social support prior to stroke as measured by the Social Support List-6, and medical history of vascular diseases, depression, or other psychiatric disorders[84]. Other means of reducing underdiagnosis that have yet to be explore and/or implemented include distribution of diagnostic tests to caregivers so that they may sporadically assess patients for the presence of PSD and/or more comprehensive doctoral education about PSD, its symptoms, and proper treatment.

G. Treatment: Early intervention following stroke to prevent PSD is ideal, particularly because approximately 20% of stroke survivors require institutional care for 3 months after stroke, which contributes to social isolation, increases healthcare burden, and increases the likelihood of PSD[85]. For those that are diagnosed with PSD, healthcare providers do not always choose to provide treatment, despite the fact that a recent study by Husseini *et al*., found that less than 2% of patients who took antidepressants after being diagnosed with PSD continued to experience depressive symptoms[86]. Doctors frequently do not treat PSD because it is often considered an inevitable and reasonable emotional reaction to the physical impairment induced by stroke[19], that is believed to improve as the patient's physical condition improves[87]. Even when healthcare professional do advise treatment, they are hesitant to prescribe anti-depressants because of the risk of drug-drug interactions, especially in patients with comorbidities[82]. Here we will briefly review the treatment options most commonly utilized.

1. Psychotherapy: In general, the use of psychotherapy is reserved for patients who do not respond to or do not tolerate treatment with anti-depressants. Unfortunately there have been few studies completed to assess the efficacy of psychotherapy in the treatment of PSD, and in those that have their results have been inconclusive[88,89]. One such study

conducted by Lincoln *et al*., examined the effectiveness of cognitive behavioral therapy. The study's results showed that no significant difference in patient's mood, independence in activities of daily living, handicap, or satisfaction of care were noted following cognitive behavioral therapy when compared to placebo treatment[90].

2. Pharmaceuticals: Despite the fact that antidepressants have existed since the 1950's, the first study assessing the efficacy of antidepressants on PSD was not conducted until 1984[91]. Currently, selective serotonin reuptake inhibitors (SSRIs) are the recommended pharmacotherapy for PSD because of their favorable tolerability. Despite the structural heterogeneity of many SSRI's, each may have different effects due to their unique pharmokinetics, half-lives, protein binding, metabolism, selectivity, and receptor affinity[92,93]; thus, a wide variety of antidepressants exist to treat the symptoms associated with depression.

2.1 Nortriptyline, a tricyclic antidepressant, was the first antidepressant whose efficacy was assessed in PSD. The results of two randomized, double-blind, placebo-controlled studies suggest that treatment with nortriptyline is associated with a decrease in depression symptoms[94,95]. More specifically, in Linsey *et al*., patients treated with Nortriptyline for 6-weeks were found to have a significant decrease in depression compared to controls as assessed by the Hamilton Depression Scale, the Zung Depression Scale, a present state examination, and the Overall Depression Scale[95]. Nortriptyline was found to improve patients between 60-79% compared to placebo treated patients who improved on average by 30-40%, depending on the study[94,95]. Nortriptyline has also been found to reduce mortality[96] and increase independence on activities of daily living[97]. Unfortunately, despite its seemingly beneficial effect, it has many adverse side effects

including fast heart rate, blurred vision, urinary retention, constipation, insomnia, nausea, weight changes, decrease sex drive, and impotence.

2.2 Citalopram is an SSRI that is known to have good efficacy and tolerability in depressed patients, and has been thoroughly assessed in the literature. In one 6-week double-blind placebo controlled study by Andersen *et al.,* patients diagnosed with PSD as determined by a HDRS score of 13+, in accordance with the DSM-III-Revised diagnostic standards, were given 20 mg/day at bedtime (10mg/day if over 66 years of age). The study found citalopram to be both well-tolerate and effective in patients, especially those who became depressed within 7 weeks of stroke. Citalopram administration was also found to reduce emotionalism and to be effective regardless of lesion hemisphere[98]. In an earlier study by the same group, citalopram was found to significantly reduce crying episodes in those with PSD[99]. Additionally, citalopram has been found to act favorably in the elderly, a population that is at an especially high risk of PSD due to the fact that stroke is more prevalent in older populations[100]. Lastly, it is important to note that to date, the functional gain(s) associated with citalopram have yet to be assessed[93].

2.3 Fluoxetine, also known by the trade names Prozac and Sarafem, is an SSRI that has been shown to be effective in the treatment of PSD. This was demonstrated in a study by Wiart *et al.,* in which hemiplegic patients within 3-months of stroke onset, were treated with 20mg/day of Fluoxetine or a placebo for 6-weeks. At the conclusion of the trial, patients were assessed using MADRS, and the fluoxetine treated group was found to have a greater mean decreased in MADRS compared to placebo (16.6 vs 8.4, respectively)[101]. In this study it is important to note that no differences in motor, cognitive, and/or functional improvement were observed[101]. The conclusions of Wiart *et al.* were mimicked in a 2011 study by Chollet *et al*. in which patients 5-10 days post-stroke were treated with

a placebo or 20mg/day of fluoxetine[102]. Placebo-treated patients were found to have increased rates of depression as indicated by scores on MARDS and the FugiMeyer Scale. Fruehwald *et al.* conducted a double-blind randomized place-controlled study that produced complimentary results and indicated that fluoxetine may need to be administered for longer periods of time for its full benefits to be observed. During the study, fluoxetine (20 mg/day) or a placebo was administered for 3-months to patients beginning within two weeks of stroke onset. Treatment efficacy was assessed using the Scandinavian Stroke Scale, Mini-Mental State Exam, and the Barthel Index. The study results indicate that following 4-weeks of treatment, despite the fact that both the treatment and placebo groups improved, no significant difference was observed between the two groups, indicating a high degree of spontaneous recovery. Following 12-weeks of treatment the fluoxetine group showed further improvement, while the placebo group had symptom relapse. This difference was even more visible at the final 18-month assessment in which the placebo group showed a significant depression relapse. Despite the promising anti-depressant effects observed in PSD patients when administered fluoxetine, there have been studies that refute its efficacy. One such study was conducted by Robinson *et al.,* in which 12-weeks of active Fluoxetine treatment (10mg/day gradually increased to 40mg/day during the study) followed by 12-weeks of placebo treatment led to no significant difference between the fluoxetine and placebo treatment groups[94]. Although the majority of studies in the literature support the efficacy and use of fluoxetine to treat PSD, it is important to note that fluoxetine comes with a variety of side effects including, trouble sleeping, appetite loss, mania, seizure, and increased risk of suicidal behavior, which may make its use less appealing.

2.4 Sertraline, an SSRI also known as Zoloft, was first shown to be effective in treating PSD in a 2003 study by Spalletta *et al*[103]. Post-stroke patients with a diagnosis of MDD

according to the DSM-IV were given 50-100 mg/day of sertraline or a placebo and their improvement assessed by the Hamilton Depression Scale, the Mini Mental State Exam, and the Barthel Index. Of those treated with sertraline, 45% were found to not be depressed at the conclusion of the trial, while 20% presented with minor depression, and 35% still suffered from MDD. All participants treated with sertraline were found to have both cognitive and functional performance improvement at the conclusion of the study. Despite these promising findings, it is important to note that the significance of these findings are inhibited by the fact that the study was not double-blind, and was conducted using a small sample size (n=20)[103]. Similar efficacy was reported by Zifko *et al.*, in which 88% of patients treated with sertraline showed improvement on the Clinical Global Impression test. Additionally, the duration of time between treatment and improvement in this study was found to be 13.3 +/- 6 days, which is a much shorter duration that associated with most SSRIs[104]. The efficacy of sertraline in the prevention of PSD has also been examine and shown to significantly decrease the development of PSD such that only 10% of individuals treated with sertraline developed PSD while 30% of placebo treated patients developed PSD[105].

2.5 Venlafaxine is a serotonergic and noradrenergic reuptake inhibitor whose use in treating PSD was first explored in 1999 by Dahment *et al*. The findings of this investigation revealed that treating patients for 5-weeks with venlafaxine (75 mg/day dose for days 1-2, 150 mg/day for remainder of trial) resulted in a decrease in depression as indicated by MADRS (26.7 +/-5 baseline, 7.6 +/_ 2.2 post-treatment). Additionally, treatment with venlafaxine led to decreased neurological deficits as measured by the Modified Barthel Rehabilitation Score, the European Stroke Scale, and the Rankin Scale[106]. Similar results have also been reported in more recent studies[107,108]. One especially important finding was published by Cravello *et al*., which showed that

treatment with ventlafaxine was able to attenuate alexithymia, a condition which commonly accompanies PSD and is defined as the inability to recognize emotions and their subtleties and textures[108].

2.6 Mirtazapine, a noradrenergic and specific serotinergic antidepressant, is another drug that has been shown to be effective in the treatment of PSD. In 2004, Niedermaier *et al*., showed that administration of 30mg/day of mirtazapine beginning 1-day post-stroke was associated with a significant decreased in PSD, such that patients treated with mirtazapine had a 5.7% rate of PSD, while 40% of those untreated were diagnosed with PSD[109]. It is important to note that this study was not blinded and had no placebo group. One year later, another study was published showing that mirtazapine was associated with a rapid decrease in depression symptoms, in particular pathological laughing and crying, in patients who did not previously respond to or could not tolerate SSRI treatment[110]. Unfortunately, this study was a case study of 2-patients, thus making its results less applicable, and still leaving the efficacy of mirtazapine as a treatment for PSD unknown.

H. Influence on Recovery: In recent decades, there has been a decrease in stroke mortalities, primarily due to increased accessibility to medical care and medical advancements, leading to more survivors with residual impairments and disabilities[82,111]. Thus, the influence of PSD on recovery has become a topic of great interest. Stroke prognosis has been shown to be dependent on a number of baseline characteristics including age, gender, stroke severity, and the presence of post-stroke complications such as PSD[112,113]. Many of the symptoms attributed to PSD, such as sleep disturbances, loss of energy, sense of worthlessness, and psychomotor retardation, are also associated with reduced quality of life and are known to interfere with stroke recovery[114,115]; thus, it

comes as no surprise that PSD is negatively associated with a number of recovery metrics. To date, it has been shown that PSD can have a negative effect on quality of life[116-120], functional recovery, cognition, and time to stroke reoccurrence as briefly described below.

Quality of life is an important healthcare outcome which is of particular interest following stroke due to its ability to effect multiple domains of life[120]. PSD has been shown to be one of the strongest predictors of quality of life in stroke survivors[116-120], yet the exact aspects of quality of life that are most effected remain unclear. Quality of life is commonly assessed using short form-36, a patient-reported self-assessment that evaluates eight domains: vitality, physical functioning, bodily pain, general health perceptions, physical functioning, emotional functioning, social relations, and mental health. In patients with PSD, Zikic *et al*. found the domains of emotional functioning and social relations to be most impaired[121], while another study by Unalan *et al.* showed that the general health perception and vitality domains had the lowest scores[122]. Undoubtedly a more thorough investigation of the decline of quality of life associated with PSD is necessary in order for effective therapies and treatments to be developed.

Functional recovery is another means of assessing rehabilitation, but the results to date regarding the influence of PSD on functional recovery are inconsistent. A number of studies by Robinson and colleagues have failed to show a significant difference in functional outcome[123-127], but more recently there have been publications showing that PSD is indicative of poorer functional outcomes[38,128,129] and stroke reoccurrence[130]. Further support was published by Pohjasvaara *et al.* in a 2001 study which showed that individuals with PSD have poorer functional outcomes as indicated by Rankin Scale and Barthel Index scores (RS>11, BI<17)[128]. Despite the fact that those with PSD have poorer

functional outcomes, they do not necessarily have decreased functional improvement as shown by Van de Weg *et al*. In their study individuals suffering from PSD were found to have significantly lower functional scores during all study time points, but no functional improvement differences were observed between depressed and non-depressed patients[39]. Similar results were seen in Sinyor *et al.,* in which patients with PSD were found to have greater functional impairment at both admission and discharge from a rehabilitation program, but there were no significant differences observed in functional gains when compared to stroke only patients. Nannetti *et al.,* contradicted these results in a 2005 study which showed that stroke patients with PSD had similar functional improvement during hospitalization as stroke patients without PSD, but the functional improvement seen in these patients decreased after discharge[129].

PSD is also thought to influence stroke reoccurrence, as indicated by the results of a publication by Yuan *et al.,* in 2012 that showed that patients diagnosed with PSD 2-weeks post-stroke had an increased chance of reoccurrence of stroke at 1 year post-stroke, and that administration of antidepressants did not reduce this risk[131]. In addition, time to first ischemic stroke recurrence was found to be significantly shorter in patients with PSD, compared to stroke survivors who did not suffer from PSD[130]. In particular, stroke patients suffering from a subtype of depression called depression-executive dysfunction syndrome (DES), a condition in which patients have difficulty organizing, planning, and reduced interest in activities, had even shorter reoccurrence times than patients with PSD alone[130,132]. These findings reinforce the fact that individuals with PSD and DES are a high risk post-stroke patient group, and further confirms that doctors should actively treat PSD rather than allow self-recovery in order to lessen the chance of ischemic stroke reoccurrence.

It is well established in the literature that PSD is associated with increased incidence of mortality[133-135]. The first study to show this was published by Morris *et al.,* in 1993. The study showed that at 10 years post-stroke, those who had been diagnosed with PSD at 2 weeks post-stroke had a 3.4 times greater chance of mortality compared to stroke only controls[134]. These results were later confirmed by House *et al.* in which patients diagnosed with PSD at one month post-stroke were found to have a 2.4 greater chance of mortality at 12 months post-stroke[133]. Additionally, Williams *et al.,* the largest study examining the association between PSD and mortality to date, showed similar results. At a 3 years post-stroke assessment, veterans with PSD were found to have higher rates of mortality. However it is important to note that the authors of this study only diagnosed approximately 5% of post-stroke veterans with PSD, which suggests that PSD was likely underdiagnosed[135].

I. Hypothesized Mechanisms: There are currently multiple hypotheses as to the cause of PSD, but unfortunately they do not all point to a definite conclusion. The majority of those that do exist were generated in response to MDD, but are believed to be applicable to PSD as well. Current hypotheses for PSD suggest either a biological or psychosocial origin of depression, and are described below:

1. Biological Hypothesis: According to biological hypotheses, PSD is believed to occur as a result of brain lesion location, changes in neurotransmitter availability, the inflammatory cytokine response, gene polymorphisms, or deregulated neurogenesis.

1.1 Lesion Location Hypothesis: Many publications have reported variance in PSD prevalence and/or severity as a direct result of lesion location[136,137]. The first to report such findings was Robinson *et al.* who observed increased depression severity in

association with lesions in the left frontal lobe, and furthermore that left posterior lesions were associated with significantly lower depression scores than left anterior lesions[124,138,139]. However, there have also been multiple individual studies[34,140-144] and meta-analyses[142,145] in which left hemisphere strokes were not found to be associated with an increased risk of PSD. In fact, many studies have shown the exact opposite, suggesting instead that there is an association between right hemisphere lesions and PSD[146-149]. Still other studies have shown associations between different brain regions including the frontal cortex[150], temporal lobe[150], internal capsule[150,151], and the basal ganglia[151], but still no hemisphere association. More studies are necessary to elucidate whether or not lesion location and PSD incidence are connected, as the current findings are inconclusive.

1.2 Biogenic Amine Hypothesis: This hypothesis was first proposed in 1977 by two researchers at Johns Hopkins University, Robinson and Bloom, who hypothesized that depletion of serotonin, dopamine, and norepinephrine in the central nervous system is the pathophysiologic basis of depression[152]. Serotonin, dopamine, and norephinephrine neuronal bodies are located in the brainstem, while their axons reach the frontal cortex through the thalamus and basal ganglia. Thus, the authors proposed that decreased monoamine availability and synthesis was due to interruption of ascending biogenic amine containing axons, which further explains why production of monoamines is reduced even in uninjured areas of the brain. Support for this hypothesis has been found in a number of studies which have shown that serum and cerebral spinal fluid (CSF) levels of dopamine, norepinephrine, and serotonin are decreased in PSD patients[153]. This hypothesis is further supported by the fact that many antidepressants have been shown to be effective in alleviating depression in patients with MDD, however it remains unclear whether that is the case in PSD[152]. Lastly, if this hypothesis were confirmed true, it would provide further support for the influence of lesion location on the development of

PSD, such that if a lesion falls in a location that interrupts biogenic amine transport, bioamine production may also be reduced leading to depression.

1.3 Cytokine hypothesis: The cytokine hypothesis, which was first proposed by Spalletta *et al.* in 2006, suggests that depression occurs as a result of increased pro-inflammatory cytokine production. Increased pro-inflammatory cytokine availability amplifies the inflammatory process and ultimately causes widespread activation of the indoleamine 2,3-dioxygenate (IDO) enzyme[154-157]. Increased activation of the IDO enzyme leads to increased tryptophan, a serotonin precursor, metabolism and consequently decreased availability for serotonin synthesis. Decreased production of serotonin is believed to ultimately lead to depression[157]. Providing support for this hypothesis is the fact that patients suffering from MDD have been found to have elevated levels of the pro-inflammatory cytokines, IL-1, IL-6, IL-8, tumor necrosis factor-alpha, and interferon-y[158,159] and elevated pro-inflammatory cytokines have also been found in patients with PSD[158]. Additionally, Yang *et al.* showed that at 7 days and 6 months post-stroke levels of IL-18 are associated with the occurrence of PSD[160]. In animal studies, administration of IL-6 or other cytokine inducers, such as lipopolysaccharide, have also been shown to induce a depressive-like phenotype[161]. Although there are multiple publications that support the cytokine hypothesis there are a number of concerns that make validation difficult. To begin, most of the published studies measure cytokine levels in the plasma, which may not reflect cytokine activity within the central nervous system[157]. Furthermore, in addition to increased activation of pro-inflammatory cytokines, there are other molecular cascades activated following stroke that lead to increased anti-inflammatory cytokine production. In theory these anti-inflammatory cytokines should counterbalance the effects of inflammatory cytokines. Thus, it is clear that further studies are necessary before the validity of the cytokine hypothesis can be determined.

1.4 Gene Polymorphism Hypothesis: It has long been suspected that MDD may be a result of gene polymorphisms, as a result of interactions between predisposing genes and environment, and it appears that the same may be true for PSD. The first genetic study showing that polymorphisms to the serotonin transporter (SERT) gene may predispose stroke survivors to PSD was published in 2006 by Ramasubba *et al.* Their study examined the Serotonin gene-linked promoter region (5-HTTLPR), an allele variant that is subdivided into a short (s) and long (l) allele based on the presence or absence of a 43-base pair insertion/deletion polymorphism[162-164]. The study results showed that the s-allele was associated with increased odds of PSD[165]. These results were replicated in a larger study by Kohen *et al.* two years later, that also showed that stroke patients with the 5-HTTLPR s/s genotype have a three times greater chance of being diagnosed with PSD than l/l and l/xl genotype carriers. Kohen *et al.* also examined another SERT polymorphism, STin2 VNTR, and showed that those with the Stin2 9/12 and 12/12 alleles had four times greater odds of PSD than Stin2 10/10 genotype carriers[164]. Similar results were shown by Kim *et al.* such that the 5-HTTLPR s/s genotype was found to be associated with greater risk of PSD[166].

1.5 Neurogenesis Hypothesis: One of the newest hypotheses in the field of depression is the neurogenesis hypothesis, which proposes that neurogenesis in the subgranular zone of the dentate gyrus is negatively influenced by stressful experiences, such as stroke, and positively regulated by antidepressant treatment. Thus, dysregulation of neurogenesis is believed to play a key role in the pathology and treatment of PSD. There exists a wealth of knowledge supporting this hypothesis, specifically that stressful events can decrease hippocampal neuronal generation[167,168] and that the hippocampal volume of MDD patients is decreased compared to controls[169,170]. Additionally, increased proliferation of

hippocampal neuronal progenitor cells have been found in MDD patients treated with antidepressants[171-175] and/or electroconvulsive shock therapy[176]. Even more promising is the fact that newly generated hippocampal neurons have been found to integrate into the preexisting circuitry[177-180]. Another reason why researchers have been quick to support the neurogenesis hypothesis is that it helps to explain the delayed clinical results observed following antidepressant administration in claiming that maturation of hippocampal progenitors into mature dentate gyrus granule neurons and circuitry integration takes approximately 3-4 weeks.[181,182], and thus the delayed clinical efficacy following antidepressant administration is reasonable and expected. Despite the fact that a wealth of knowledge has been gathered to validate this hypothesis in regards to MDD, little work has been done in the field of PSD to date. One study that has provided proof for the legitimacy of the neurogenesis hypothesis in PSD was published by Wang *et al*. in 2008. This study showed that in a rat model of PSD, hippocampal proliferation and neurogenic rates were reduced compared to controls, and that this reduction was rescued by administration of citalopram[183].

2. Psychological Hypothesis: The psychological hypothesis of PSD is based on the fact that stroke associated social and psychological stressors cause depression[25]. It was not always clear whether PSD was a physiological reaction to the neurological deficits associated with stroke or a biologically mediated change, especially since many results that supported a biological hypothesis could not be replicated. The idea of a psychologically based hypotheses for PSD first arose in the 1970's, after stroke patients were found to have a higher occurrence of depression compared to orthopedic patients with similar functional deficits[184]. Another study that has provided support for a psychosocial hypothesis was published by Biran and Chatterjee. In their study a male patient experienced a left subcortical stroke and anosognosia, a condition in which a

patient lacks awareness of his/her disability. Anosognosia is incredibly rare in patients with PSD, and provides a perfect model in which to examine whether PSD is psychological mediated. The patient with anosognosia for hemiplegia was found to only recognize his deficits when engaging in activities that highlight their disability. Due to this particular patient's lack of constitutional symptoms, he was not aware of his disability yet he still experienced symptoms of depression[185], thereby suggesting that the mechanism of PSD cannot be purely biological.

J. Animal Models: Despite the fact that PSD is one of the most prevalent neuropsychiatric manifestations of stroke, development of relevant animal models has been difficult primarily due to the multifaceted nature of PSD (psychological, behavioral and vegetative)[186]. To date few animal models of PSD have been established in the literature, and those that do exist may not properly replicate PSD. Most of the current animal models focus on the post-acute stage of recovery, which only examines a small snap-shot of time. Additionally, the majority of PSD studies utilize young animals. Epidemiological studies have shown that ischemic strokes are most common during late middle age (50-70 years old)[187,188]; thus, it is advised that older animals (12-18 month rodents) be used in studies to properly model PSD and make findings translatable to a clinical population[189-192]. Of the models that do exist, the current models most frequently utilized are described below:

1. Middle Cerebral Artery Occlusion (MCAO): The MCAO animal model of stroke is one of the most widely used animal models of ischemic stroke. Results regarding whether MCAO alone can induce depression have been inconclusive, with some studies showing the induction of a depression-like phenotype[193,194], while other studies show no behaviors which suggest the presence of PSD[195-197].

2. MCAO + Chronic Mild Stress: The majority of current PSD models rely on MCAO in combination with a stressor[194,198]. Most commonly stress is induced via a chronic mild stress (CMS) paradigm which may include exposure to a variety of stressors including: food and water deprivation, a 45° cage tilt, overnight illumination, soiled cage, swimming in 4 °C water, and paired caging[199]. Following stroke + CMS, rodents have been found to have consistent and persistent depression-like behavior as observed during open field, sucrose preference, forced swim, and Morris Water Maze testing[194,198]. Additionally, stroke + CMS treated animals show atrophy, neurodegeneration, and decreased proliferation and differentiation of neuronal cells in the striatum and hippocampus similar to that observed in patients with PSD[194,200,201]. Despite its widespread use, unfortunately the mechanism(s) through which PSD develops in the MCAO + CMS model are still unknown.

3. MCAO+ Social Isolation: Social isolation has been shown to be a predictor of increased rates of depression, as well as morbidity and mortality[202-204]; thus, combining MCAO and social isolation is used as a means of modeling PSD. Following stroke, survivors often experience real and/or perceived social isolation due to physical, cognitive, and social functional deficits that often accompany stroke[205-207]. Interestingly, pre-stroke isolation has also been linked to poorer functional recover and increased incidence of post-stroke anxiety and PSD[6,22,203]. In 2014, O'Keefe *et al.* demonstrated this in a mouse model. Prior to induction of 60-minute MCAO, mice were pair-housed or individually housed, and following surgery all animals were individually housed. Study results indicated that a depression-like phenotype was present in all animals as shown by behavioral results from the forced swim and open field tests. Additionally, the depression-like phenotype was found to be much more prevalent in individually housed

animals compared to pair-housed mice. Further proving the detriment of social isolation following stoke, in that same year Verma *et al.* released a study showing that animals pair-housed before and after stroke had increased sociability, reduced immobility during the tail suspension test, and decreased atrophy compared to isolated littermates[208].

4. MCAO + Spatial Restrain Stress: Following stroke, survivors suffering from hemiplegia often become reliant on caregivers due to mobility deficits, thereby inducing psychological stress and physical restraint. Thus in order to generate a more clinically relevant model of PSD, Zhang *et al.* developed a model of PSD which involves a 60-minute MCAO coupled with spatial restraint stress for 2 hours/day for 2-4 weeks beginning 4 days post-stroke. This paradigm is believed to be reflective of the psychological strain and perceived/real mobility deficits experienced following stroke, both of which may play a role in the development of PSD. This model has shown great pathophysiological and etiological similarities with clinical PSD, as indicated by increased immobility on the forced swim test and tail suspension test, decreased levels of dopamine and serotonin in the brain, and responsiveness to the antidepressant imipramine[209].

K. Behavioral Tasks Use to Assess PSD in Animal Models: The majority of current behavioral tasks used to assess the presence of a depression-like phenotype in rodents were originally developed to determine the efficacy of pharmaceutical treatments to treat depression. Because there is no consensus on exactly what a depression-like state in mice entails, it is common for researchers to conduct a number of behavioral tests, called a test battery, in order to assess depression. Test batteries are often completed in order to assess different aspects of depression, which is especially useful due to PSD's multifaceted nature[195]. Unfortunately, behavioral tasks are known to be stressful, and may introduce

unintentional confounds into experimentation. Thus, it is recommended that test batteries begin with the least and end with the most stressful test to limit the influence of one behavioral test on subsequent tests[210]. Additionally, when possible, the completion of each behavioral task in the test battery should be separated by one day[210].

Today there exists a number of behavioral tasks commonly used to assess PSD, but previously the forced swim test (FST) and tail suspension test (TST) were thought to be the gold standards for detecting a depression-like phenotype. However, since both of these tests assess response to an acute inescapable stressor and thus provoke despair–based behavior rather than depression-like behavior, a number of other behavioral tests such as the sucrose preference test, splash test, the novelty-suppressed feeding test, and social interaction test, have become commonplace[211]. It should be noted that other behavioral tasks such as the open field and the elevated plus maze are used in some studies to measure depression, but the results from these tests are more indicative of anxiety levels than depressive-like behavior. Below the most commonly used behavioral tests to assess a depression-like phenotype are described.

1. Forced Swim Test (Porsolt swim test): A rodent behavioral task initially developed as a rodent screening test for potential antidepressant drugs, originally designed for rats and modified for use in mice[212,213]. During testing, rodents are placed in an inescapable transparent tank that is filled with room-temperature/warm water (24-30^{o}C) to a depth sufficient to prohibit the use of the rodent's tail for balance. During testing latency to, duration, and frequency of immobility (indicated by the absence of swimming/movement with the exception of minimal movement required for the animal to keep its head above water) are measured. Immobility during testing is interpreted as a failure to produce persistent escape-directed behavior and/or the development of a passive response to

stress[213]. It is important to note that not all strains of mice have the same baseline immobility on the FST. One strain of mice particularly non-responsive to the FST is the FVB strain that typically shows no immobility during test[214].

2. Tail Suspension Test: A rodent behavioral task developed by Steru *et al*., used to assess a depressive-like phenotype, based on the assumption that animals will actively try to escape aversive stimuli[215]. During testing animals are suspended above a solid surface using adhesive tape applied to the tail in such a way that escape or contact with nearby surfaces is not possible[216]. Throughout the typically 6-minute trial, duration of immobility is measured, as defined as a deficit of swimming, jumping, rearing, sniffing, and/or diving. Longer periods of immobility are thought to indicate a lack of "will to live" and thus are indicative of a depressive-like phenotype. Additionally, it is important to know that not all mouse strains are suitable for tail suspension testing, particularly those which are known to have vestibular deficits or tail climbing behavior (ex: C57BL/6J)[217]. Both the forced swim and tail suspension tests measure immobility, thus it is important to note that use of the tail suspension test has a number of advantages in comparison to the forced swim test including that: 1) testing poses no risk of hypothermia; 2) upon completion of testing, animals resume spontaneous activity immediately; 3) no post-testing care is required; and 4) results are not confounded by motor deficits that may be present post-stroke[218].

3. Sucrose Preference Test: This task assesses anhedonia, a lack of interest in rewarding stimuli, which is commonly observed in depressed individuals. The task specifically accesses the rodent's interest in seeking out a sweet, rewarding drink compared to water. On the first day of testing, mice are presented with 2 liquid diet feeding tubes, both containing water to allow for habituation to the presence of two water bottles and non-

lixit water consumption. After habituation, one of the animal's liquid feeding tubes is replaced with another liquid feeding tube filled with 2% sucrose dissolved in water (2g/100ml). Rodents are given free choice to drink from either feeding tube for anywhere from one to four days. In the case of multiple day trials, the location of feeding tubes are switched daily to eliminate location bias. During testing, consumption of water and 2% sucrose is assessed daily by weight, and the results averaged over the trial duration[195]. Typically, mice are biased towards the sweetened water, and failure to do so is indicative of anhedonia/depression. One potential confound to consider when completing the sucrose preference test is that preference may be influenced by metabolic, sensory, and appetitive influences, which may be present in genetically modified rodents.

4. Splash Test: The splash test is a behavioral test that assesses grooming as an index of self-care and motivational behavior. During testing the mouse's dorsal coat is sprayed with a 10% sucrose solution. Due to the solution's viscosity, the sucrose dirties the mouse's fur resulting in the initiation of grooming behavior. Latency to first grooming and duration of grooming is recorded manually for five minutes. A lack of grooming or delayed grooming is indicative of a depressive-like phenotype[219-221].

5. Novelty-Suppressed Feeding: Another means of assessing anhedonia beyond using the sucrose preference test is by conducting the novelty-suppressed feeding test. This test is based on rodents' innate fear of new spaces; thus, it assesses anhedonia by examining the conflict a mouse has when approaching and consuming food in a novel environment[195,222]. Twenty-four hours prior to testing, rodents are deprived of food, yet still have free access to water. During testing rodents are removed from their home cage and placed in a novel environment with a single food pellet in one corner. Latency to

approach and consume the food pellet is recorded. Anxious mice are known to be slower to approach and consume food than non-anxious mice[195,222].

6. Social Interaction Test: Social deficits are common in depressed individuals, thus assessing social interaction may be an appropriate means of detecting a depressive-like phenotype in rodents. During the social interaction test a rodent is allowed to explore an unfamiliar mouse in its home cage. Social interaction is defined by the amount of time the rodent spends around the congener in addition to the amount/duration of other social interaction behaviors (sniffing, following, grooming, biting, mounting). The only potential drawbacks to administering this test is that automated scoring is difficult, thereby making its results highly objective[223].

L. Conclusion: PSD is a condition effecting over 2 million people in the United States alone, and thus it is critical that research funds are dedicated to better understand its etiology and to developing prevention/treatment methods. Despite the fact that a wealth of research has been conducted in regards to stroke and depression, exploration of post-stroke depression as its own condition is relatively minimal. Fortunately, in recent years much more time and funding has been dedicated to PSD research, but sadly conclusions in the field are slowed by the sheer number of potential hypotheses, the fact that few animal models exist thereby limiting the generation of clinically translatable research, and that the efficacy of common depression treatments have not been assessed for PSD. Hopefully better awareness of the symptoms and prevalence of PSD may lead to more individuals being properly diagnosed and treated, and as a consequence, the impact PSD may be minimized.

CHAPTER II: Hypoxically preconditioned-bone marrow stromal cells attenuate post-stroke depression

A. Introduction: Each year over 800,000 strokes occur in the United States alone, resulting in a number of neuropsychiatric manifestations[3], the most common of which is depression, commonly referred to as post-stroke depression (PSD), which is found to occur in over one-third of stroke survivors[4-6]. Currently, over one-third of stroke survivors experience PSD[5,6,126], thereby leaving them at a greater chance of increased disability, delayed functional recovery, decreased cognitive function, morbidity, and mortality[134,135,224,225]. The detrimental effects associated with PSD are countless, and thus it is necessary that new and innovative treatments for PSD are investigated in order to improve long-term patient outcomes post-stroke.

Following stroke, survivors often experience significant distress and/or impairment of social, occupational, and/or other important functional areas. Social impairment is especially of concern, as social factors have a profound influence on stroke outcome[226], and have been shown to be associated with decreased quality of life and psychopathological states. Furthermore, social interaction has been shown to decrease neuronal damage associated with cerebral ischemia[227-229]; consequently, reduced social interaction is often detrimental. Thus, it comes as no surprise that a strong correlation exists between social deficits/isolation and PSD incidence. One hormone believed to be a psychosocial mediator of stroke outcome is oxytocin[230], a hormone secreted by the posterior lobe of the pituitary gland which is known to induce and facilitate social behaviors[227-229]. Oxytocin works by helping animals to overcome avoidance of proximity to others and by inhibiting defensive behavior, thereby allowing approach behavior[231-235]; therefore, changes in oxytocin level play a role in sociability. Although there have been few investigations into the role of oxytocin in stroke recovery and outcome, a recent

study by Karelina *et al.* provided evidence to suggest that social interaction and oxytocin are neuroprotective following stroke. Specifically, this study showed that socially housed stroke mice had reduced infarct volume, neuroinflammation, and oxidative stress. Furthermore, administration of an oxytocin receptor agonist eliminated the neuroprotective effect associated with social housing, and administration of endogenous oxytocin to socially isolated stroke mice reproduced the neuroprotective effect conferred by social housing[230]. Together, these data provide strong evidence for oxytocin as a mediator of stroke outcome.

To date very few resources have been dedicated to exploring PSD therapies. Rather, treatment options have been extrapolated from those utilized to treat major-depressive disorder. In order to fully explore the potential benefits of oxytocin, we decided to utilize stem cell transplantation, specifically of bone marrow stromal cells (BMSC). BMSCs are multipotent progenitor cells which are capable of differentiating into many cell types including neurons[236]. Treatment of neurological conditions with BMSCs has previously been shown to confer a number of positive benefits including enhanced cell migration to the site of injury[237], improved functional outcomes[238,239] and enhanced neurotrophic support[240,241]. Additionally, we utilized BMSCs hypoxically-precondition with sublethal hypoxia prior to transplantation[242], a strategy which we have previously shown to be associated not only increased cell survival, but also with increased oxytocin and oxytocin receptor expression when utilized on induced pluripotent/embryonic stem cell-derived neural progenitor cells[243]. Hypoxic pretreatment is also associated with a number of other therapeutic benefits that are believed to occur as a result of the activation of endogenous pro-survival trophic signals, such as BCL-2, hypoxic-inducible factor, erythropoietin receptor, neurofilament, synaptophysin, and VEGF[244,245]. Furthermore, transplantation of hypoxically-preconditioned BMSCs (HP-BMSCs) has been shown to down-regulate pro-

inflammatory cytokines/chemokines (ie. CC3, CC5, CC17, CCL4, CXCR3, CXCL10) and suppress microglia activity in the brain[246].

Due to the multitude of benefits associated with BMSC transplantation and hypoxic preconditioning, we are confident that their therapeutic use will be beneficial to both stroke recovery and outcome. Consequently, we hypothesize that treatment of stroke animals with HP-BMSCs will attenuate the depression-like phenotype commonly observed following stroke and attenuate the social deficits associated with stroke.

B. Methods

Transient focal ischemia animal model: C57BL/6 (20–26 g, 8-10 weeks old) mice were housed at room temperature with a 12 hr light/dark cycle in the pathogen-free Laboratory Animal Center for Research at Emory University. Occlusion of the distal branches of the middle cerebral artery (MCA) were performed according to previous procedures with minor modifications[246,247]. In brief, 8–10 week old C57BL6 mice were anesthetized using isoflurane (4% induction; 1.5% maintenance). The right MCA branches were permanently ligated using a 10-0 suture (Surgical Specialties Co., Reading, PA) accompanied by a bilateral 7-14 minute ligation of the common carotid arteries (CCA). During CCA occlusion, barrel cortex blood flow was reduced to less than 20% as measured by laser doppler scanning. Body temperature was monitored during surgery and maintained at 37.0°C using a temperature control unit and heating pads. The mortality rate due to ischemic surgery and/or anesthesia failure was approximately 7%. Animals were euthanized and decapitated 21 days after ischemic stroke. Brains were immediately removed, sectioned, and stored at −80°C for further processing. All experimental and surgical procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Emory University.

Isolation and Culture of BMSCs From Rats

BMSCs were isolated and harvested as previously described[248]. In brief, BMSCs were flushed from the fibias of postnatal day 21 Wistar rats (Charles River, Wilmington, MA, USA) using a 25-gauge needle. Mononuclear cells were suspended in Dulbecco's modified Eagle's medium (Cellgro, Manassas, VA, USA) supplemented with 15% fetal bovine serum (Sigma, St. Louis, MO, USA) and plated into dishes. Cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide. After 24 hours, non-adherent cells were discarded, and adherent cells were washed four times with phosphate-buffered saline solution (PBS; Sigma). Fresh complete medium was added and replaced every 2 days. Each primary culture was subcultured 1:3 when BMSCs were approximately 80% confluent. Fluorescence-activated cell sorting was performed to characterize the BMSC population using CD105, CD73, CD34, and CD45 markers (eBioscience, San Diego, CA, USA). All cells used in this study were freshly isolated within five passages and harvested for analysis when they were approximately 80–90% confluent.

Hypoxic preconditioning and administration of iPS-BMSCs

Hypoxic preconditioning of stem cells prior to transplantation was performed to enhance their survival and regenerative properties[241,248,249]. Cells were incubated under normoxic conditions or in a finely controlled ProOx C-chamber system (Biospherix, Redfield, NY, USA). For hypoxic preconditioning, the oxygen concentration in the chamber was maintained at 0.1–0.3% with a residual gas mixture composed of 5% carbon dioxide balanced with nitrogen for 24 hours followed by 1 hour of reoxygenation time prior to analysis and/or transplantation. BMSCs were rinsed with phosphate buffered saline (PBS; Sigma-Aldrich) and harvested by trypsinization using Trypsin-EDTA (Life Technologies,

Carlsbad, CA, USA). To increase the barrier permeability of nasopharyngeal mucosa and facilitate cell entry into the brain, all animals received 100 µL hyaluronidase (Sigma-Aldrich) dissolved in sterile PBS, 30 minutes prior to administration of cells. Five-microliter drops per nostril were given with 1-minute intervals until a total volume of 50 µl of cell suspension (containing 1×10^6 cells) was administered. BMSC were transplanted 1, 3, 7, and 10 days post-stroke as previously described[250,251].

Depression-like Behavior Tasks

Tail Suspension: A rodent behavioral task developed by Steru et al., used to assess depression, based on the assumption that animals will actively try to escape aversive stimuli[215]. One hour prior to testing, animals were brought into the testing room to allow for acclimation. Animals were suspended within a wire cage. A 17 cm length of general-purpose laboratory labeling tape (VWR, Radnor, PA) was used to suspend each mouse, approximately 2 cm was used to securely attach the animal about 3-4 mm from the end of the tail, while the remaining 15 cm were used for suspending each mouse. Each mouse was suspended such that their head was approximately 20-25 cm above the wire cage's base, and they were incapable of escaping or holding onto nearby surfaces. The use of climbstoppers was unnecessary as no tail climbing occurred during testing. Mice were observed and their behavior manually scored for 6 minutes. The duration of mobility was recorded. Mobility was deemed as any escape-oriented behavior (e.g. trying to reach apparatus walls, body shaking, movement of limbs similar to running), but did not include smaller movements that were confined to the front legs only. At the end of testing, the tape was gently removed from the mouse's tail and they were returned to their home-cage. Between sessions, the wire cage was thoroughly cleaned using a sterilizing solution. Tail suspension testing was completed on days 7 and 14 post-stroke.

Forced Swim Test: A rodent behavioral task that measures escape-related mobility behavior as a means of assessing depression. For this task, the rodent is placed in a transparent glass cylinder filled with room temperature-warm water (23-25 °C) with a minimum depth of 10 cm, so as not to allow the animal's tail or legs to be used for balance. Water levels must also be a minimum of 15 cm below the upper surface of the cylinder to prevent the animal from climbing out. Animals were brought into the testing room a minimum of 15 minutes prior to experimentation to allow for acclimation. When testing began, animals were held by their tails and slowly placed into the water, and their tails were only released once their body had completely entered the water, to minimize the chance of the animal's head becoming submerged. The rodent's behavior was recorded for a total of 6 minutes, but only the last 4 minutes were analyzed. Immobility duration, defined as an absence of activity and escape-oriented behaviors (swimming, jumping, rearing, sniffing, diving) was recorded. Once testing was completed, rodents were removed from the water and completely dried with paper towels to prevent hypothermia prior to being returned to their home cage. The forced swim test was completed on days 7 and 14 post-stroke.

Splash Test: The splash test is a behavioral test that assesses grooming as an index of self-care and motivational behavior. A lack of grooming or delayed grooming is indicative of a depressive-like phenotype. The splash test was adapted from Yalcin *et al*., and consists of spraying the mouse's dorsal coat with a 10% sucrose solution[252]. Due to the solution's viscosity, the sucrose dirties the mouse's fur resulting in the initiation of grooming behavior. Following the application of the sucrose solution, grooming duration was recorded over 5 minute. The splash test was completed on days 7 and 14 post-stroke.

Sucrose Preference Test: This task assesses anhedonia (lack of interest in rewarding stimuli), which is commonly observed in depressed individuals. The task specifically accesses the rodent's interest in seeking out a sweet, rewarding drink compared to unsweetened water. Bias towards the sweetened water is typical, and failure to do so is indicative of anhedonia/depression. For this task animals were individually housed. On the first day of testing, mice were presented with 2 liquid diet feeding tubes (Bio-Serv), both containing water to allow for habituation to the presence of two water bottles and non-lixit water consumption. On the second day of testing, one liquid feeding tube was filled with water, and the other with 2% (w/v) sucrose. Mice were given free choice to drink from either liquid feeding tube. Consumption of water and 2% sucrose water was assessed 24 hours later by weight.

Social Behavior Tasks

Social interaction test: Animals were placed into a test cage and social interaction, as defined as social sniffing, social grooming, and following/chasing behaviors, was recorded during a 10-minute testing period on days 21 and 28 post-stroke. Social sniffing was defined as the experimental animal sniffing any body part of its test partner. Social grooming was defined as the experimental animal licking and/or chewing the fur of its test partner while placing its forepaws on its partner's back or neck. Following/chasing behaviors were also recorded, and defined as any time when the experimental rat walked or ran in the direction of its test partner, who either stayed in place or moved away in response. Total social interaction was the total time spent completing social sniffing, grooming, and following/chasing.

*Social novelty test***:** This task assesses general sociability and response to social novelty. Rodents are more innately inclined to be social and investigate a novel intruder rather

than a known individual; thus a lack of these symptoms indicates deficits in sociability and/or social novelty. During testing, a mouse was placed into a three-chamber box and allowed to explore all of the chambers for 10 minutes, after which the mouse completed two 10-minute tests. During the first testing period, a non-littermate mouse (former stranger) locked in a wire cage was placed into the left chamber, and the experimental mouse was allowed to explore all three chambers. Exploration during this period was used to quantify sociability. During the second testing period, a different locked non-littermate mouse (new stranger) was placed into the right chamber. The time spent with the former stranger mouse and the new stranger mouse was recorded, and results were used to access the animal's response to social novelty.

Western Blot Analysis: Western blotting was used to detect the expression of depression-related genes after stroke in mice. After sacrifice, mice were subjected to transcardial perfusion using PBS. P enumbra and prefrontal cortex tissues were lysed in a buffer containing 0.02 M Na4P2O7, 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM EDTA (pH 8.0), 1 % Triton, 1 mM EGTA, 2 mM Na3VO4, and a protease inhibitor cocktail (Sigma, St. Louis, MO). The supernatant was collected after centrifugation at 15000g for 10 min at 4 °C. Protein concentration was determined with a bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL, USA). Equivalent amounts of total protein were separated by molecular weight on an SDS-polyacrylamide gradient gel and then transferred to a PVDF membrane. The blot was incubated in 10% nonfat dry milk for 1 h and then reacted with primary antibodies at 4 °C for overnight. The primary antibodies and their dilutions are as follows: rabbit anti-synapsin1 antibody (Cell Signaling, Danvers, MA, USA) 1:2000, rabbit anti-SNAP25 antibody (Cell Signaling) 1:1000, rabbit anti-PSD-95 antibody (Cell Signaling) 1:2000, mouse anti-actin (Sigma) 1:5000, rabbit anti-serotonin receptor (Millipore, Billerica, MA) 1:2500, goat anti-oxytocin

(Abcam, Cambridge, MA) 1:1000, and rabbit anti-oxytocin receptor (Santa Cruz Biotechnology, Dallas, TX) 1:500. After washing with Tris-buffered saline with Tween-20 (TBST), membranes were incubated with AP-conjugated or HRP-conjugated secondary antibodies (GE Healthcare, Piscataway, NJ, USA) for 2 h at room temperature. After final washing with TBST, the signals were detected with bromochloroidolylphosphate/nitroblue tetrazolium (BCIP/NBP) solution (Sigma) or film. Signal intensity was measured by ImageJ (NIH) and normalized to the actin signal intensity.

Statistical analysis: For comparison between 2 groups, a student 2-tailed t-test was used. Graph Prism version 5.0 was used to make graphs and to perform statistical analysis. One-way ANOVA analysis of variance was used for data analysis and multiple comparisons (4 groups) were corrected for with Bonferroni's test for pair-wise comparisons. Significance was assumed at a p-value of 0.05 in all statistical analyses. Randomization was performed, and the sample size was further determined using power analysis (Power and Precision 4; Biostat, Inc, Englewood, NJ, USA).

C. Results

Oxytocin/Oxytocin receptor in vitro upreglation following hypoxic-preconditioning

Following hypoxic-preconditioning, oxytocin and its receptor's *in vitro* expression in HP-BMSCs were assessed. HP-BMSCs were found to have significantly increased oxytocin and oxytocin receptor protein expression *in vitro* in comparison to normoxically treated BMSCs (N-BMSCs) (Fig. 2.2) . To ensure that increased expression was maintained, oxytocin and oxytocin receptor expression were examined at 0, 1, 3, and 24 hours after hypoxic preconditioning. Our results indicate that upregulation of expression of oxytocin

and the oxytocin receptor in HP-BMSCs remains present for at least 24 hours post-hypoxic treatment (Fig. 2.1a).

Depression-like behavior is present post-stroke, and attenuated by HP-BMSCs.
A series of behavioral tests capable of detecting a depression-like phenotype were utilized to detect PSD in our right barrel cortex transient ischemia mouse model.

Tail Suspension Test: The percent of total time during the test trial spent immobile was recorded at 7-days ($n_{control}$=16; n_{stroke}=34; $n_{HP\text{-}BMSC}$=21) and 14-days ($n_{control}$=16; n_{stroke}=34; $n_{HP\text{-}BMSC}$=21) post-stroke. The results revealed no difference between control and stroke mice at 7-days post-stroke, but a significant difference between both control and stroke+HP-BMSC treated mice and stroke and stroke+HP-BMSC treated mice. At 14-days post-stroke, significant differences were observed between control and stroke animals, and stroke+HP-BMSC treated animals (Fig. 2.2a-b).

Forced Swim Test: The percent of total time during the testing trial spent immobile was recorded at 7-days ($n_{control}$=23; n_{stroke}=30; $n_{HP\text{-}BMSC}$=16) and 14-days ($n_{control}$=23; n_{stroke}=23; $n_{HP\text{-}BMSC}$=23) post-stroke. Our results revealed a significant difference between control and stroke animals at 7-days post-stroke, but no difference between stroke+HP-BMSC animals despite the presence of a positive trend. By 14-days post-stroke, a significant difference in immobility duration was detected between controls and stroke mice and stroke+HP-BMSC treated mice (Fig. 2.2c-d).

Splash Test: After wetting the animal's dorsal coat with a 10% sucrose solution, grooming duration was measured at 7 ($n_{control}$=9; n_{stroke}=12; $n_{HP\text{-}BMSC}$=8) and 14 ($n_{control}$=9; n_{stroke}=13; $n_{HP\text{-}BMSC}$=10) days post-stroke. At 7-days post-stroke, a significant difference

in grooming duration was only found between control and stroke animals. At 14-days post-stroke a significant difference between the grooming durations of control and stroke mice remained, and a significant difference between control and stroke+HP-BMSC grooming duration was also found. Although no significant difference was found between stroke and stroke+HP-BMSC mice at any of the time-points measured, a trend of increased grooming duration was associated with HP-BMSC treatment (Fig. 2.2e).

Sucrose Preference: Due to the time intensive nature of testing, sucrose preference testing was only conducted at 21-days post-stroke($n_{control}$=10; n_{stroke}=7; $n_{HP\text{-}BMSC}$=6). A significant decrease in preference for consumption of the sucrose water was observed between controls and stroke animals, indicating anhedonia and a depressive-like phenotype. Additionally, stroke=HP-BMSC animals had a significantly increased preference for sucrose water, similar to controls, indicating attenuation of the depressive-like phenotype (Fig. 2.2f).

Social behavior is reduced post-stroke, but attenuated by HP-BMSC administration

Social behavior was assessed using the social interaction test and social novelty test (Fig. 2.3). The social interaction test, examines social sniffing, following, grooming, and total social interaction, as a means of assessing sociability. Social sniffing was significantly decreased in stroke mice compared to controls, and levels of social sniffing increased significantly in stroke animals treated with HP-BMSCs compared to stroke only animals (Fig. 2.3b). Similarly, social following and social grooming were found to be reduced in stroke animals compared to controls, but time spend socially following or grooming did not increase in stroke+HP-BMSC animals (Fig. 2.3 a,c). Lastly, total social interaction time was significantly decreased in stroke animals compared to both controls and stroke+HP-BMSC treated animals (Fig. 2.3d). The social novelty test revealed that

stroke animals have significantly less sociability and social novelty in comparison to controls, and levels of both increase significantly following administration of HP-BMSCs (Fig. 2.3d-e).

Assessment of depression-related proteins

Analysis of depression related proteins in the penumbra, the prefrontal cortex, and the thalamus were assessed via western blot. In accordance with the biogenic monoamine hypothesis, protein levels of dopamine, D2 (dopamine receptor), and S2 (serotonin receptor) were assessed. To examine in vivo upregulation of oxytocin and its receptor, these proteins were assessed as well. Lastly, protein implicated in connectivity including Synapsin-1, Synaptophysin, SNAP-25, and PSD-95 were examined. The results of these analyses are summarized below:

Prefrontal Cortex: No differences were detected in any of the protein examined (Figure 2.4).

Thalamus: No differences in any of the proteins examined except for SNAP-25 and PSD-95. For SNAP-25, a significant difference was detected between control and stroke mice, in addition to stroke and stroke+HP-BMSC mice. In the case of PSD-95, a significant difference was detected between control and stroke animals (Fig. 2.5).

Penumbra: A significant decrease in oxytocin levels was detected following stroke. Increased oxytocin levels were also observed following HP-BMSC administration, but these results were not significant. No differences were detected for the other proteins assessed (Fig. 2.6).

D. Discussion:

Stroke induces a depression-like phenotype that is attenuated by treatment with HP-BMSCs

Our study results indicate that our model of right barrel cortex transient focal ischemia induces a PSD-like state as indicated by behavioral analysis (Fig. 2.2). We utilized a series of tests to assess for a PSD-like phenotype, because PSD is a multi-faceted condition, the likes of which may not be easily detected by all behavioral analyses depending on the test sensitivity and the sub-set of symptoms present in mice. Specifically we conducted the forced swim, tail suspension, splash, and sucrose preference behavioral tasks. Results from both the forced swim test and the splash test indicated the presence of a depressive-like phenotype by 7-days post-stroke (Fig. 2.2 c, e), such that in the forced swim test stroke animals were immobile for a significantly greater period of time and stroke animals spent significantly less time grooming than controls. By 14-days post-stroke, these tests in addition to the tail suspension test, continued to indicate the presence of a depressive-like phenotype (Fig. 2.2 a, d, e). Additionally, sucrose preference was assessed at 21-days post-stroke, at which point our results indicated the continual presence of a depressive-like phenotype (Fig. 2.2f). Together, these results suggest that PSD onset is not immediate post-stroke, but rather appears to occur gradually with overt symptoms being easily detected 2 weeks post-stroke.

Our behavioral analyses also provided evidence that administration of HP-BMSCs at 1, 3, 7, and 10 days post-stroke is able to attenuate the depressive-like phenotype observed post-stroke (Fig. 2.2). Seven days post-stroke, HP-BMSC treated stroke mice had decrease immobility duration in the forced swim and tail suspension tests and increased grooming times in the splash test compared to stroke only animals; although, it is

important to note that these changes were only significant in the tail suspension test. Similar results were observed 14-days post-stroke, except that behavioral differences between stroke and stroke+HP-BMSC animals during the forced swim and tail suspension tests reached significance(Fig. 2.2 a, c, e);. Lastly, sucrose preference testing at 21 days post-stroke, showed that stroke animals administered HP-BMSCs had increased preference for the sweetened water, similar to levels observed in controls; thus indicating attenuation of anhedonia (Fig. 2.2f).

Reduced sociability post-stroke is rescued by administration of HP-BMSCs

In addition to conducting behavioral testing to assess for the presence of a depression-like phenotype post-stroke, we also utilized behavioral analysis to assess sociability, specifically via the social interaction test and social novelty test (Fig. 2.3). Sociability is an important parameter to assess because stroke survivors frequently experience social deficits post-stroke, and social deficits and social isolation strongly influence stroke outcome. In the social interaction test, social sniffing, grooming, and following, in addition to total interaction time between the test animal and a test partner were assessed (Fig. 2.3 a-d). Our analyses indicated that mice treated with HP-BMSCs were found to spend significantly more time sniffing their congener (Fig. 2.3b). Additionally stroke+HP-BMSC animals spent more time socially grooming and following their test partner, although the time spent completing these tasks was not significantly different (Fig. 2.3 a,c). Furthermore, we assessed the total time spent completing social activities, and found a significant difference between control and stroke animals, and a positive uptrend associated with HP-BMSC treatment (Fig. 2.3d).

The social novelty test, which assesses cognition in regards to general sociability and interest in social novelty, had complimentary results. The results from our testing

indicated that stroke animals are less sociable than controls, as indicated by less time exploring the former stranger mouse, and that treatment of stroke animals with HP-BMSCs restores sociability levels to those similar to controls (Fig. 2.3e). Similar results were shown for social novelty. Social novelty was defined as the amount of time spend exploring a 'new stranger' compared to a 'former stranger.' Consequently, stroke animals had reduced preference for social novelty compared to controls, such that they explored the 'new stranger' for a shorter period of time than controls, while treatment of stroke animals with HP-BMSCs had an increased preference for social novelty (Fig. 2.3f).

Together the results from the social interaction and social novelty tests suggest that sociability decreases following stroke, similarly to that observed in humans post-stroke. Furthermore, treatment of stroke animals with HP-BMSCs leads to increased sociability, which may contribute to stroke outcome. Additionally, it is important to note that our findings are particularly profound because all tested animals were group housed, which facilitates social behavior and is known to upregulate oxytocin. Thus, if animals were housed alone, as is often the case in humans following stroke, it is likely that the behavioral differences that occurred post-stroke would have been even more prominent. Additionally, it is important to note that we did not utilize cutoffs to exclude animals from our stroke group that did not show a depressive-like symptoms, thus potentially decreasing the significance of our results. It is advised that in future studies cut-off points be utilized, so that the effectiveness of treatment can be examined only in depressive-like phenotypes.

Biogenic amine availability is not altered in our model of PSD

In order to understand why the observed behavioral changes occurred post-stroke and following HP-BMSC administration, investigation of potential underlying mechanisms was necessary. We began by testing one of the most widespread depression hypothesis, the biogenic amine hypothesis, which claims that depletion of serotonin, dopamine, and norepinephrine in the central nervous system is the pathophysiologic basis of depression[152]. Normally this hypothesis is associated with changes in monoamine availability in the prefrontal cortex. Despite the fact that our stroke model injures the barrel cortex, not the prefrontal cortex, the biogenic amine hypothesis can still be used to explain the presence of PSD. This is because decreased monoamine availability and synthesis may be due to interruption of ascending biogenic amine containing axons. In order to explore whether monoamine availability was altered in our model of PSD, we examined monoamine associated protein expression (dopamine, dopamine receptor, and/or serotonin receptor) in the prefrontal cortex, the penumbra, and the thalamus (Fig. 2.4, 2.5, 2.6). We failed to detect any significant differences regardless of parameter examined or brain region. This suggests that alterations to monoamine availability, at least in regards to dopamine, the dopamine receptor, and/or the serotonin receptor, do not underlie the PSD-like phenoptype found in our animal model of stroke.

Connectivity is altered in the thalamus of our model of PSD

Next, we examined changes in connectivity within the prefrontal cortex, thalamus, and penumbra. Previously, researchers have shown that patients with MDD experience neural hyperconnectivity[253], which led us to examine expression levels of Synapsin-1, Synaptophysin, SNAP-25, and PSD-95, all of which are implicated in neuronal connectivity. No differences were detected in any of the proteins examined in the prefrontal cortex or the penumbra (Fig. 2.4, 2.6), but differences were detected in the thalamus (Fig. 2.5). Specifically, there were significant changes in SNAP-25 expression

between control and stroke mice, in addition to stroke and stroke+HP-BMSC mice. Furthermore, a significant difference in PSD-95 protein expression was found between control and stroke animals. How to interpret these results is unclear. One recent study has reported that patients with thalamic hyperactivity are more likely to have treatment resistant depression[254], but this does not appear to be the case in our animals, as their depression was attenuated by HP-BMSC administration. Another study showed increased resting-state neural networks in the prefrontal cortex and thalamus of patients with MDD[255], which partially aligns with our findings. To date, changes in thalamus connectivity have yet to be reported in patients with PSD, so further exploration and analysis is necessary.

Oxytocin is downregulated by stroke, and upregulated by HP-BMSC administation

We have previously shown that hypoxic pretreatment of induced pluripotent/embryonic stem cell-derived neural progenitor cells leads to oxytocin and oxytocin receptor upregulation[243], and we have presented in this paper that hypoxic pretreatment of BMSCs leads to oxytocin and oxytocin receptor upregulation *in vitro.* To confirm that this upregulation is present *in vivo,* we conducted western blot analysis. Although no differences in oxytocin and/or oxytocin receptor were detected in the prefrontal cortex or the thalamus (Fig. 2.4, 2.5), our analysis revealed that oxytocin protein expression significantly decreased in the penumbra following stroke, and did increase following HP-BMSC administration to levels close to control, but these results were not significant. (Fig. 2.6). We also examined the oxytocin receptor expression, and although trends similar to those of oxytocin were detected, these changes were not significant. This evidence coupled with our behavioral data suggesting that changes in oxytocin play a key role in the development of PSD, and that HP-BMSC treatment appears to positively influence oxytocin levels and attenuate post-stroke depression.

E. Summary and Future Directions: Our results provide strong support for intranasal delivery of HP-BMSCs as a viable treatment for PSD. According to our investigation, it appears that oxytocin serves as a PSD mediator, and HP-BMSCs confer favorable benefits due to their ability to upregulate oxytocin and its receptor. To better explore and confirm our conclusions, future studies will examine the effect of exogenous application of oxytocin to rescue the depressive-like phenotype found in mice post-stroke and to confirm oxytocin's role as a PSD mediator. Additionally, although we have not explored it in this investigation, recently published data indicates that oxytocin may be anti-inflammatory, as administration of exogenous oxytocin has been shown to alleviate tissue damage associated with ischemia[256-258]. Thus in future studies, we may want to examine inflammation in association with administration of HP-BMSCs and elevated oxytocin levels.

Through experimentation we somewhat ruled out monoamine availability as a potential mechanisms at work in our model, however a more thorough investigation is necessary to completely rule out monoamine availability changes. In future investigations, serotonin, dopamine, and norepinephrine transporters as well as their metabolites should be examined. Additional studies may include a thorough proteomic analysis to assess gene polymorphisms and/or gene up- or down-regulation following stroke. It is advisable that proteomic analysis be completed at multiple time-points to further explore the onset of PSD. Lastly, in the future our lab will complete a more in-depth analysis of inflammation post-stroke, as inflammation is believed to be a primary factor in depression. Hopefully with awareness of PSD etiology, and its developmental time-course, we will be able to the number of individuals struggling with PSD and reduce the negative outcomes associated with untreated PSD.

F. Figures

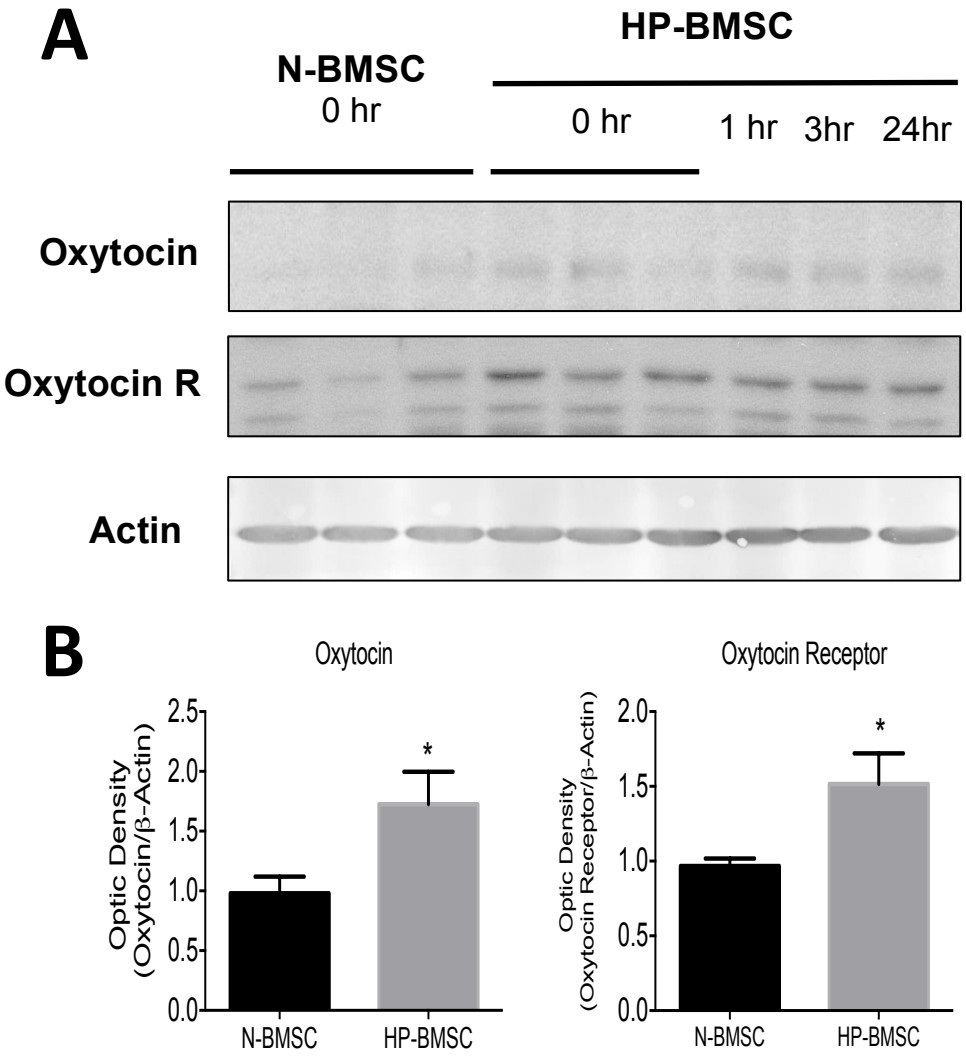

Figure 2.1: *In vitro* hypoxic treatment of BMSCs leads to increased expression of oxytocin and the oxytocin receptor. (a) BMSC cells were either normoxically (N-BMSC) or hypoxically (HP-BMSC) treated *in vitro*, after which the expression of oxytocin and the oxytocin receptor were measured, as shown at the 0 hour time-point. HP-BMSC treatment was found to increase protein expression of both oxytocin and its receptor. Maintenance of protein upregulation was monitored at 1, 3, and 24 hours post-treatment, and found to remain upregulated during all measured time-points. **(b)** Quantified results from the 0 hour time-point, confirm significant protein expression upregulation. *$P < 0.05$ vs. N-BMSC; n = 6-8 per group.

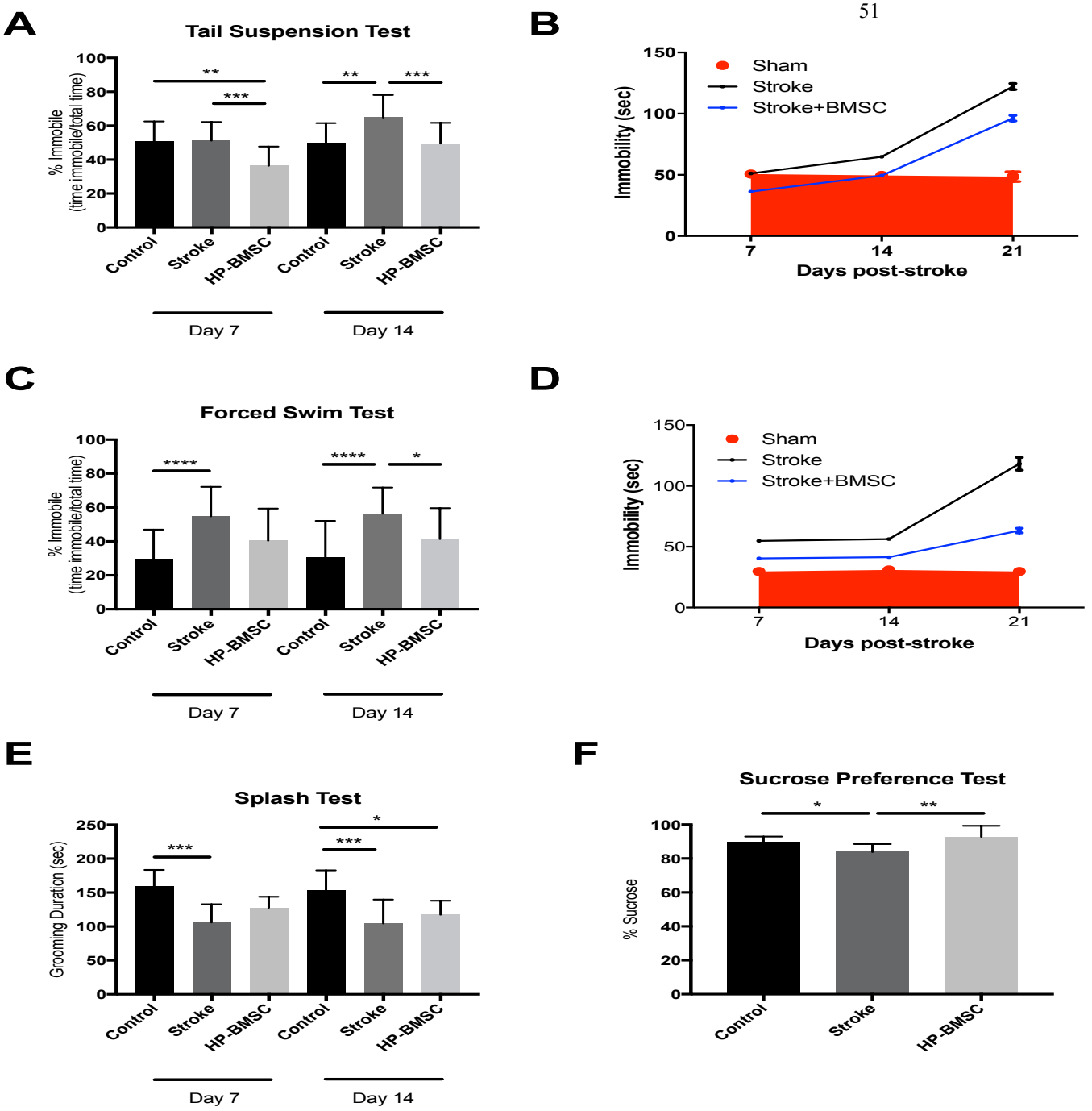

Figure 2.2: Our model of right barrel cortex transient ischemic stroke induces a depression-like phenotype that is attenuated by intranasal delivery of HP-BMSCs. (a) Tail suspension results indicate a depression-like phenotype at 14-days post-stroke, that is attenuated by treatment with HP-BMSCs. **(b)** Graph comparing tail suspension test results of the stroke and stroke + HP-BMSC animals compared to controls. Data indicates development of PSD-like symptoms is gradual. **(c)** Behavioral results from forced swim testing show the presence of a depression-like phenotype as early as 7-days post-stroke, which is attenuated by administration of HP-BMSCs. **(d)** Graph comparing forced swim test results of the stroke and stroke + HP-BMSC animals compared to controls. Data indicates development of PSD-like symptoms is gradual. **(e)** Splash test results indicate the presence of a depression-like phenotype, as indicated by decreased grooming, at both 7- and 14-days post-stroke. Again HP-BMSC intranasal delivery was found to attenuate the depression-like phenotype. **(f)** Results from sucrose preference task show that at 21-days post-stroke mice maintain a depressive-like phenotype, as indicated by a reduced consumption of sucrose water compared to controls, and this phenotype was rescued by treatment with HP-BMSCs. *P < 0.05 vs. control, **P < 0.01 vs. control, ***P < 0.001 vs. control; n = 15-25 per group (tail suspension, forced swim, splash test), n = 7-10 (sucrose preference).

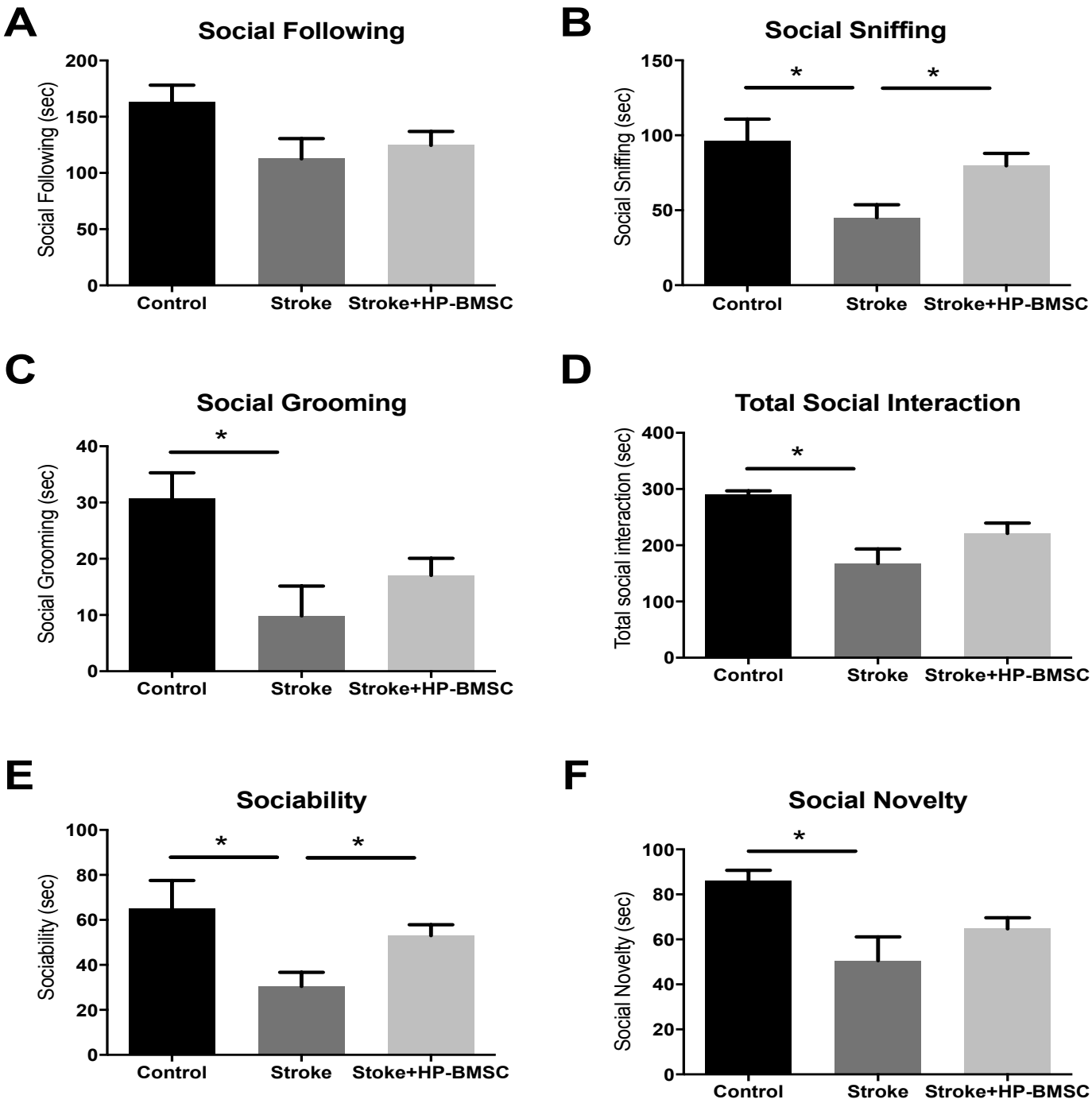

Figure 2.3: Sociability, as measured by the social novelty and social interaction test, is reduced following stroke, and is attenuated by HP-BMSC treatment. The social novelty test **(a-d)** assesses social following, sniffing, and grooming, in addition to total social interaction. Test results show significant decreases in social sniffing and grooming. These decreases were minimized by HP-BMSC treatment. Additionally total social interaction significantly decreased following stroke, but increased following HP-BMSC administration. **(e)** The social novelty test showed complimentary results such that sociability significantly decreased following stroke, and this change was attenuated by HP-BMSC administration. **(f)** Social novelty also significantly decreased post-stroke. *$P < 0.05$; n = 3-12 per group.

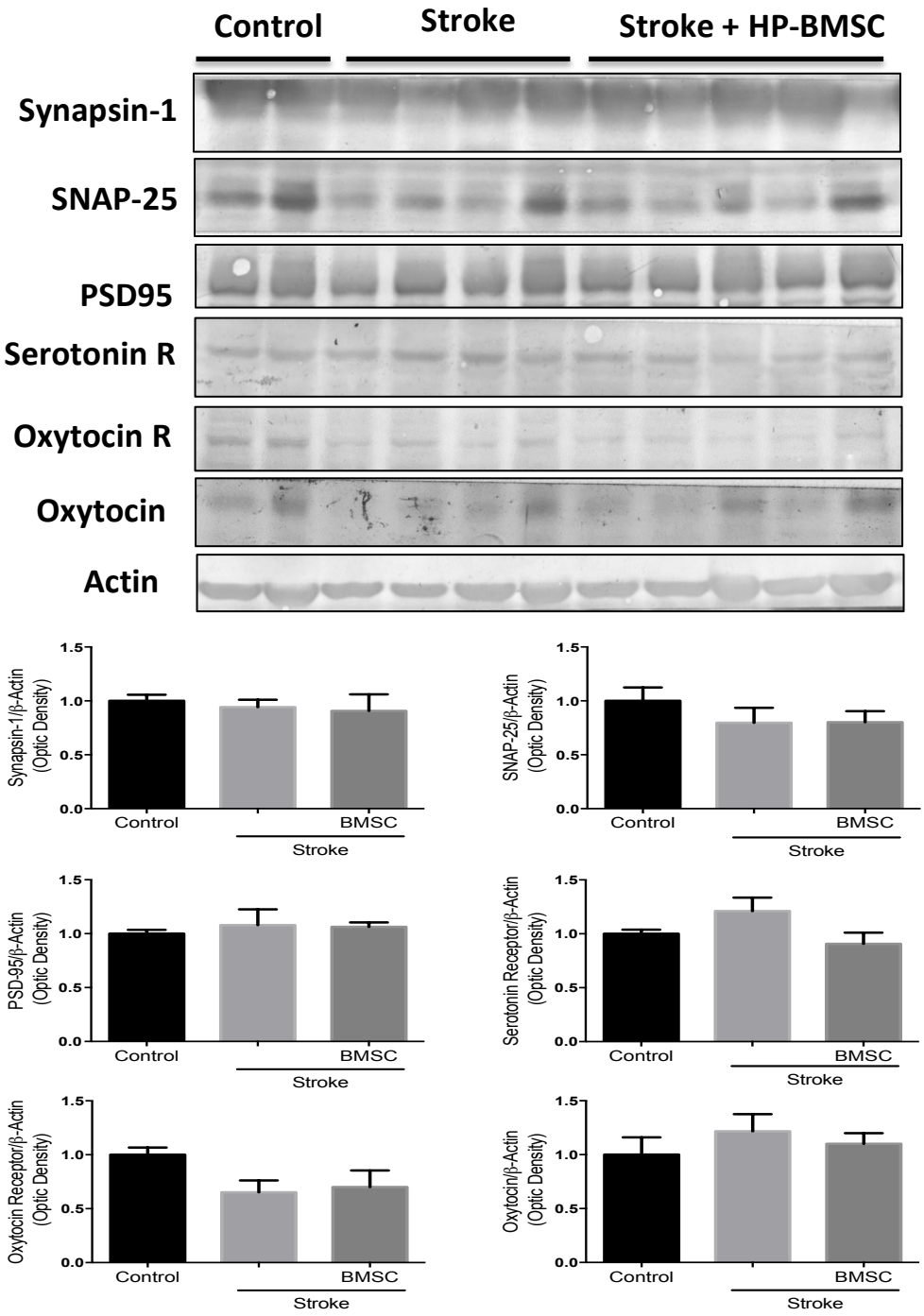

Figure 2.4: Intranasal delivery of HP-BMSCs does not alter the expression of depression-related genes in the prefrontal cortex. (a) Following stroke, mice were administered HP-BMSCs intranasally 1, 3, 7, and 10 days post-stroke. 21 days post-stroke mice were sacrificed and synapsin-1, SNAP-25, PSD95, serotonin receptor, oxytocin, and the oxytocin receptor protein levels were measured via western blot. **(b)** Quantified results from a show that no significance difference was detect for any of the parameters assessed. n = 2-6 per group.

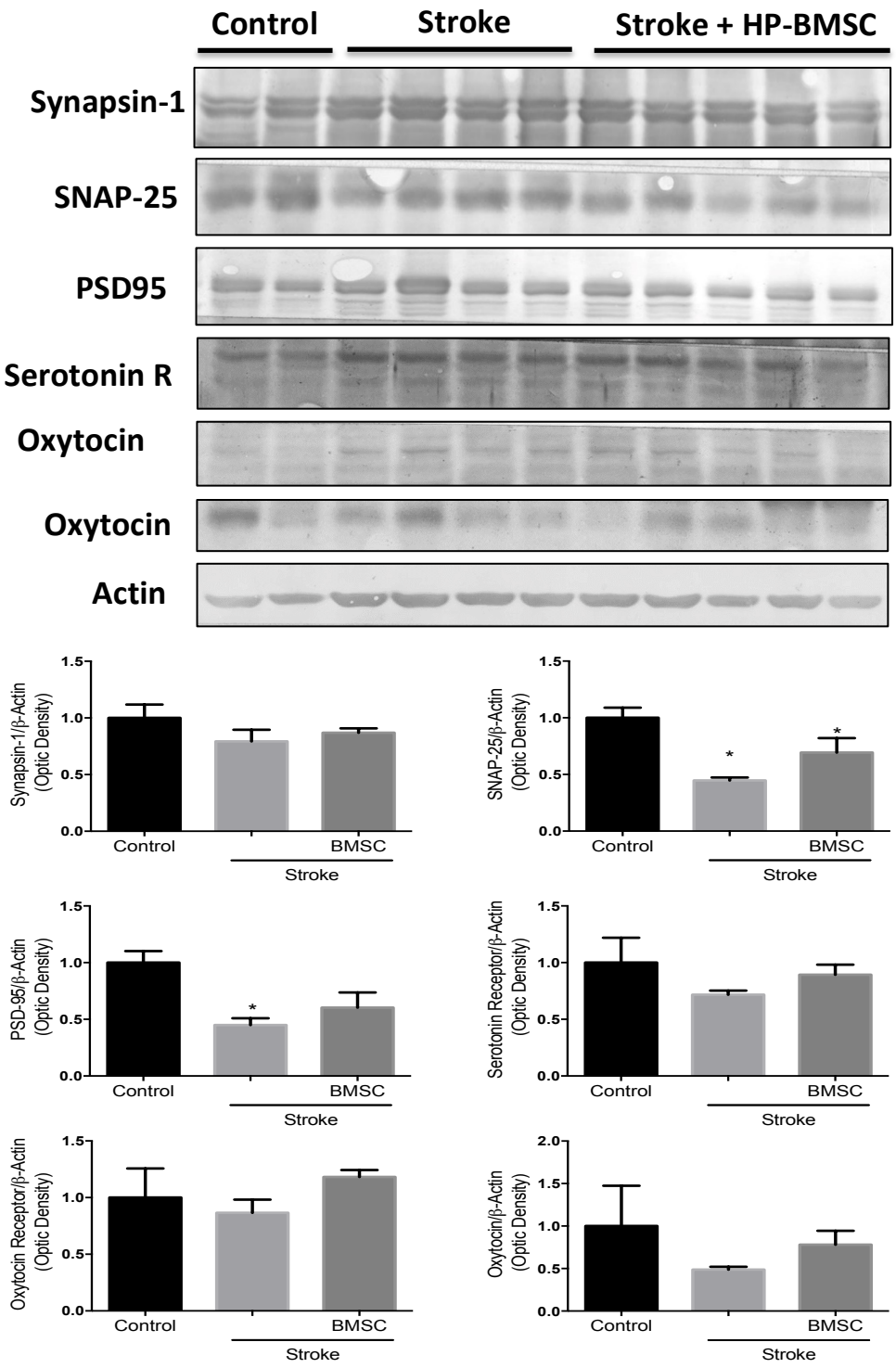

Figure 2.5: Stroke alters the expression of depression-related genes in the thalamus. (a) Following stroke, mice were administered HP-BMSCs intranasally 1, 3, 7, and 10 days post-stroke. 21 days post-stroke mice were sacrificed and synapsin-1, SNAP-25, PSD95, serotonin receptor, oxytocin, and the oxytocin receptor protein levels were measured via western blot. **(b)** Quantified results from the western blot analysis. The expression of SNAP-25 and PSD95 protein levels significantly decreased post-stroke. No significance difference in expression levels of synapsin-1, the serotonin receptor, the oxytocin receptor, and/or oxytocin were detected. . *$P < 0.05$; n = 2-6 per group.

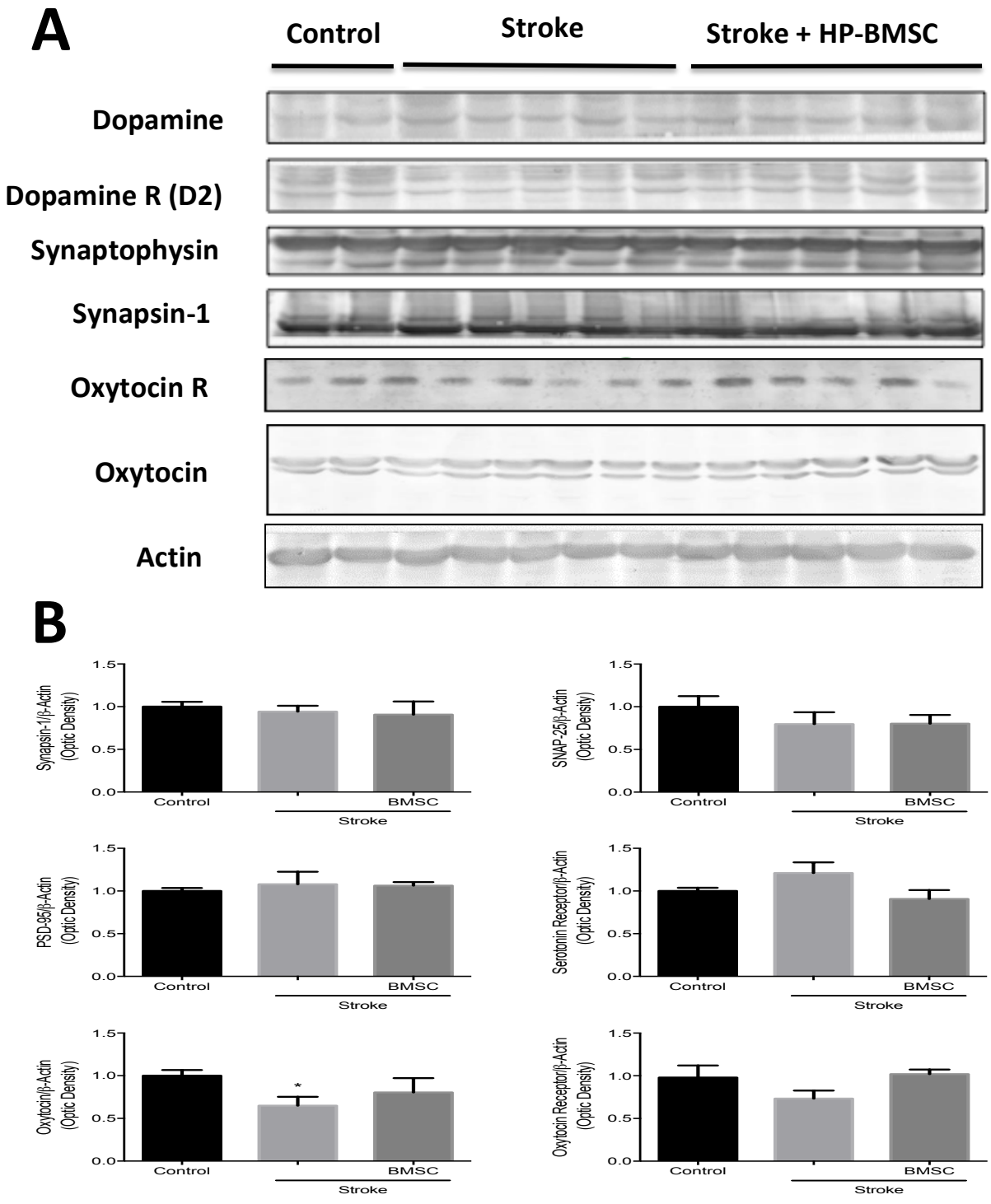

Figure 2.6: Intranasal delivery of HP-BMSCs alters oxytocin expression in the penumbra. (a) Following stroke, mice were administered HP-BMSCs intranasally 1, 3, 7, and 10 days post-stroke. 21 days post-stroke mice were sacrificed and synapsin-1, SNAP-25, PSD95, serotonin receptor, oxytocin, and the oxytocin receptor protein levels were measured via western blot. A significant increase was observed in stroke animals administered HP-BMSCs. **(b)** Quantified results from the western analysis. $^{*}P < 0.05$; n = 2-6 per group.

Chapter III. References

1 Mozaffarian, D. *et al.* Executive Summary: Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* **133**, 447-454, doi:10.1161/CIR.0000000000000366 (2016).

2 Mozaffarian, D. *et al.* Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* **131**, e29-322, doi:10.1161/CIR.0000000000000152 (2015).

3 Dafer, R. M., Rao, M., Shareef, A. & Sharma, A. Poststroke depression. *Topics in stroke rehabilitation* **15**, 13-21, doi:10.1310/tsr1501-13 (2008).

4 Robinson, R. G., Bolduc, P. L. & Price, T. R. Two-year longitudinal study of poststroke mood disorders: diagnosis and outcome at one and two years. *Stroke; a journal of cerebral circulation* **18**, 837-843 (1987).

5 Robinson, R. G. & Spalletta, G. Poststroke depression: a review. *Canadian journal of psychiatry. Revue canadienne de psychiatrie* **55**, 341-349 (2010).

6 Whyte, E. M., Mulsant, B. H., Vanderbilt, J., Dodge, H. H. & Ganguli, M. Depression after stroke: a prospective epidemiological study. *Journal of the American Geriatrics Society* **52**, 774-778, doi:10.1111/j.1532-5415.2004.52217.x (2004).

7 American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force. *Diagnostic and statistical manual of mental disorders : DSM-5*. Fifth edition. edn.

8 Stroke--1989. Recommendations on stroke prevention, diagnosis, and therapy. Report of the WHO Task Force on Stroke and other Cerebrovascular Disorders. *Stroke; a journal of cerebral circulation* **20**, 1407-1431 (1989).

9 American Psychiatric Association. & American Psychiatric Association. DSM-5 Task Force. *Diagnostic and statistical manual of mental disorders : DSM-5*. 5th edition. edn.

10 Klinedinst, N. J., Dunbar, S. B. & Clark, P. C. Stroke survivor and informal caregiver perceptions of poststroke depressive symptoms. *The Journal of neuroscience nursing : journal of the American Association of Neuroscience Nurses* **44**, 72-81, doi:10.1097/JNN.0b013e3182477944 (2012).

11 Brodaty, H., Withall, A., Altendorf, A. & Sachdev, P. S. Rates of depression at 3 and 15 months poststroke and their relationship with cognitive decline: the Sydney Stroke Study. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry* **15**, 477-486, doi:10.1097/JGP.0b013e3180590bca (2007).

12 Schubert, D. S., Taylor, C., Lee, S., Mentari, A. & Tamaklo, W. Detection of depression in the stroke patient. *Psychosomatics* **33**, 290-294, doi:10.1016/S0033-3182(92)71967-7 (1992).

13 Hackett, M. L., Yapa, C., Parag, V. & Anderson, C. S. Frequency of depression after stroke: a systematic review of observational studies. *Stroke; a journal of cerebral circulation* **36**, 1330-1340, doi:10.1161/01.STR.0000165928.19135.35 (2005).

14 Hackett, M. L. & Pickles, K. Part I: frequency of depression after stroke: an updated systematic review and meta-analysis of observational studies. *International journal of stroke : official journal of the International Stroke Society* **9**, 1017-1025, doi:10.1111/ijs.12357 (2014).

15 Whyte, E. M. & Mulsant, B. H. Post stroke depression: epidemiology, pathophysiology, and biological treatment. *Biological psychiatry* **52**, 253-264 (2002).

16 Santos, M. *et al.* The neuroanatomical model of post-stroke depression: towards a change of focus? *Journal of the neurological sciences* **283**, 158-162, doi:10.1016/j.jns.2009.02.334 (2009).
17 Gaete, J. M. & Bogousslavsky, J. Post-stroke depression. *Expert review of neurotherapeutics* **8**, 75-92, doi:10.1586/14737175.8.1.75 (2008).
18 De Ryck, A. *et al.* Risk factors for poststroke depression: identification of inconsistencies based on a systematic review. *Journal of geriatric psychiatry and neurology* **27**, 147-158, doi:10.1177/0891988714527514 (2014).
19 Andersen, G., Vestergaard, K., Ingemann-Nielsen, M. & Lauritzen, L. Risk factors for post-stroke depression. *Acta psychiatrica Scandinavica* **92**, 193-198 (1995).
20 Oladiji, J. O., Akinbo, S. R., Aina, O. F. & Aiyejusunle, C. B. Risk factors of post-stroke depression among stroke survivors in Lagos, Nigeria. *African journal of psychiatry* **12**, 47-51 (2009).
21 Carota, A. *et al.* A prospective study of predictors of poststroke depression. *Neurology* **64**, 428-433, doi:10.1212/01.WNL.0000150935.05940.2D (2005).
22 Ouimet, M. A., Primeau, F. & Cole, M. G. Psychosocial risk factors in poststroke depression: a systematic review. *Canadian journal of psychiatry. Revue canadienne de psychiatrie* **46**, 819-828 (2001).
23 Bour, A. *et al.* A one-year follow-up study into the course of depression after stroke. *The journal of nutrition, health & aging* **14**, 488-493 (2010).
24 Hackett, M. L. & Anderson, C. S. Predictors of depression after stroke: a systematic review of observational studies. *Stroke; a journal of cerebral circulation* **36**, 2296-2301, doi:10.1161/01.STR.0000183622.75135.a4 (2005).
25 Fang, J. & Cheng, Q. Etiological mechanisms of post-stroke depression: a review. *Neurological research* **31**, 904-909, doi:10.1179/174313209X385752 (2009).
26 Robinson, R. G. & Jorge, R. E. Post-Stroke Depression: A Review. *The American journal of psychiatry* **173**, 221-231, doi:10.1176/appi.ajp.2015.15030363 (2016).
27 American Psychiatric Association. & American Psychiatric Association. Task Force on DSM-IV. *Diagnostic and statistical manual of mental disorders : DSM-IV-TR*. 4th edition, text revision. edn.
28 American Psychiatric Association. & American Psychiatric Association. Task Force on Nomenclature and Statistics. *Quick reference to the diagnostic criteria from DSM-III*.
29 Gelenberg, A. J. & Hopkins, H. S. Assessing and treating depression in primary care medicine. *The American journal of medicine* **120**, 105-108, doi:10.1016/j.amjmed.2006.05.059 (2007).
30 American Psychiatric Association. *Diagnostic criteria from DSM-IV-TR*.
31 Gainotti, G., Azzoni, A. & Marra, C. Frequency, phenomenology and anatomical-clinical correlates of major post-stroke depression. *The British journal of psychiatry : the journal of mental science* **175**, 163-167 (1999).
32 Paradiso, S., Ohkubo, T. & Robinson, R. G. Vegetative and psychological symptoms associated with depressed mood over the first two years after stroke. *International journal of psychiatry in medicine* **27**, 137-157 (1997).
33 Teasell, R. *et al.* Evidence-Based Review of Stroke Rehabilitation: executive summary, 12th edition. *Topics in stroke rehabilitation* **16**, 463-488, doi:10.1310/tsr1606-463 (2009).
34 Berg, A., Palomaki, H., Lehtihalmes, M., Lonnqvist, J. & Kaste, M. Poststroke depression in acute phase after stroke. *Cerebrovascular diseases* **12**, 14-20, doi:47675 (2001).

35 Kellermann, M. *et al.* Screening for depressive symptoms in the acute phase of stroke. *General hospital psychiatry* **21**, 116-121 (1999).

36 Kotila, M., Numminen, H., Waltimo, O. & Kaste, M. Post-stroke depression and functional recovery in a population-based stroke register. The Finnstroke study. *European journal of neurology* **6**, 309-312 (1999).

37 Paolucci, S. *et al.* The Italian multicenter observational study on post-stroke depression (DESTRO). *Journal of neurology* **253**, 556-562, doi:10.1007/s00415-006-0058-6 (2006).

38 Sinyor, D. *et al.* Post-stroke depression: relationships to functional impairment, coping strategies, and rehabilitation outcome. *Stroke; a journal of cerebral circulation* **17**, 1102-1107 (1986).

39 van de Weg, F. B., Kuik, D. J. & Lankhorst, G. J. Post-stroke depression and functional outcome: a cohort study investigating the influence of depression on functional recovery from stroke. *Clinical rehabilitation* **13**, 268-272 (1999).

40 Morris, P. L., Robinson, R. G. & Raphael, B. Prevalence and course of depressive disorders in hospitalized stroke patients. *International journal of psychiatry in medicine* **20**, 349-364 (1990).

41 Gainotti, G. *et al.* The Post-Stroke Depression Rating Scale: a test specifically devised to investigate affective disorders of stroke patients. *Journal of clinical and experimental neuropsychology* **19**, 340-356, doi:10.1080/01688639708403863 (1997).

42 Gillen, R., Tennen, H., McKee, T. E., Gernert-Dott, P. & Affleck, G. Depressive symptoms and history of depression predict rehabilitation efficiency in stroke patients. *Archives of physical medicine and rehabilitation* **82**, 1645-1649, doi:10.1053/apmr.2001.26249 (2001).

43 Diamond, P. T., Holroyd, S., Macciocchi, S. N. & Felsenthal, G. Prevalence of depression and outcome on the geriatric rehabilitation unit. *American journal of physical medicine & rehabilitation / Association of Academic Physiatrists* **74**, 214-217 (1995).

44 Eastwood, M. R., Rifat, S. L., Nobbs, H. & Ruderman, J. Mood disorder following cerebrovascular accident. *The British journal of psychiatry : the journal of mental science* **154**, 195-200 (1989).

45 Townend, B. S. *et al.* Longitudinal prevalence and determinants of early mood disorder post-stroke. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* **14**, 429-434, doi:10.1016/j.jocn.2006.01.025 (2007).

46 Wilkinson, P. R. *et al.* A long-term follow-up of stroke patients. *Stroke; a journal of cerebral circulation* **28**, 507-512 (1997).

47 Fedoroff, J. P., Starkstein, S. E., Parikh, R. M., Price, T. R. & Robinson, R. G. Are depressive symptoms nonspecific in patients with acute stroke? *The American journal of psychiatry* **148**, 1172-1176, doi:10.1176/ajp.148.9.1172 (1991).

48 Beck, A. T., Weissman, A., Lester, D. & Trexler, L. The measurement of pessimism: the hopelessness scale. *Journal of consulting and clinical psychology* **42**, 861-865 (1974).

49 Beck, A. T. S., R.A. *Manual for the Beck Hopelessness Scale*. (Psychological Corp., 1989).

50 Beck, A. T., Steer, R. A., Kovacs, M. & Garrison, B. Hopelessness and eventual suicide: a 10-year prospective study of patients hospitalized with suicidal ideation. *The American journal of psychiatry* **142**, 559-563, doi:10.1176/ajp.142.5.559 (1985).

51 Beck, A. T., Ward, C. H., Mendelson, M., Mock, J. & Erbaugh, J. An inventory for measuring depression. *Archives of general psychiatry* **4**, 561-571 (1961).

52 Mahoney, F. I. & Barthel, D. W. Functional Evaluation: The Barthel Index. *Maryland state medical journal* **14**, 61-65 (1965).

53 Shah, S., Vanclay, F. & Cooper, B. Improving the sensitivity of the Barthel Index for stroke rehabilitation. *Journal of clinical epidemiology* **42**, 703-709 (1989).

54 Radloff, S. A. The CES-D scale: a self-report depression scale for research in the general population. *Applied Psychological Measurement* **1**, 385-401 (1977).

55 Irwin, M., Artin, K. H. & Oxman, M. N. Screening for depression in the older adult: criterion validity of the 10-item Center for Epidemiological Studies Depression Scale (CES-D). *Archives of internal medicine* **159**, 1701-1704 (1999).

56 Shrout, P. E. & Yager, T. J. Reliability and validity of screening scales: effect of reducing scale length. *Journal of clinical epidemiology* **42**, 69-78 (1989).

57 Van Dam, N. T. & Earleywine, M. Validation of the Center for Epidemiologic Studies Depression Scale--Revised (CESD-R): pragmatic depression assessment in the general population. *Psychiatry research* **186**, 128-132, doi:10.1016/j.psychres.2010.08.018 (2011).

58 Lewinsohn, P. M. S., J.R.; Roberts R.E.; Allen N.B. Center for Epidemiological Studies-Depression Scale (CES-D) as a screening instrument for depression among community-residing older adults. *Psychology and Aging* **12**, 277-287 (1997).

59 Yesavage, J. A. *et al.* Development and validation of a geriatric depression screening scale: a preliminary report. *Journal of psychiatric research* **17**, 37-49 (1982).

60 Sheikh, J. I., YeSavage, J.A. . Geriatric Depression Scale (GDS): recent evidence and development of a shorter version. *Clinical Gerontology: A Guide to Assessment and Intervention* **5**, 165-173 (1986).

61 Hoyl, M. T. *et al.* Development and testing of a five-item version of the Geriatric Depression Scale. *Journal of the American Geriatrics Society* **47**, 873-878 (1999).

62 Yesavage, J. A. Geriatric Depression Scale. *Psychopharmacology bulletin* **24**, 709-711 (1988).

63 Rinaldi, P. *et al.* Validation of the five-item geriatric depression scale in elderly subjects in three different settings. *Journal of the American Geriatrics Society* **51**, 694-698 (2003).

64 Hamilton, M. A rating scale for depression. *Journal of neurology, neurosurgery, and psychiatry* **23**, 56-62 (1960).

65 Hamilton, M. Assessment of change in psychiatric state by means of rating scales. *Proceedings of the Royal Society of Medicine* **59 Suppl**, 10-13 (1966).

66 Hamilton, M. Development of a rating scale for primary depressive illness. *The British journal of social and clinical psychology* **6**, 278-296 (1967).

67 Hamilton, M. Standardised assessment and recording of depressive symptoms. *Psychiatria, neurologia, neurochirurgia* **72**, 201-205 (1969).

68 Hamilton, M. Rating depressive patients. *The Journal of clinical psychiatry* **41**, 21-24 (1980).

69 Zigmond, A. S. & Snaith, R. P. The hospital anxiety and depression scale. *Acta psychiatrica Scandinavica* **67**, 361-370 (1983).

70 Whelan-Goodinson, R., Ponsford, J. & Schonberger, M. Validity of the Hospital Anxiety and Depression Scale to assess depression and anxiety following traumatic brain injury as compared with the Structured Clinical Interview for

DSM-IV. *Journal of affective disorders* **114**, 94-102, doi:10.1016/j.jad.2008.06.007 (2009).

71 Folstein, M. F., Folstein, S. E. & McHugh, P. R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* **12**, 189-198 (1975).

72 Folstein, M. F., Robins, L. N. & Helzer, J. E. The Mini-Mental State Examination. *Archives of general psychiatry* **40**, 812 (1983).

73 Tombaugh, T. N. & McIntyre, N. J. The mini-mental state examination: a comprehensive review. *Journal of the American Geriatrics Society* **40**, 922-935 (1992).

74 Montgomery, S. & Asberg, M. A new depression scale designed to be sensitive to change. *British Journal of Psychiatry* **134**, 382-389 (1979).

75 Kroenke, K., Spitzer, R. L. & Williams, J. B. The Patient Health Questionnaire-2: validity of a two-item depression screener. *Medical care* **41**, 1284-1292, doi:10.1097/01.MLR.0000093487.78664.3C (2003).

76 Kroenke, K., Spitzer, R. L. & Williams, J. B. The PHQ-9: validity of a brief depression severity measure. *Journal of general internal medicine* **16**, 606-613 (2001).

77 Williams, L. S. *et al.* Performance of the PHQ-9 as a screening tool for depression after stroke. *Stroke; a journal of cerebral circulation* **36**, 635-638, doi:10.1161/01.STR.0000155688.18207.33 (2005).

78 Wing, J., Cooper, J. E. & Sartorius, N. *The Description Classification of Psychiatric Symptoms. An Instruction Manual for the PSE and CATEGO System*. (Cambridge University Press, 1974).

79 Zung, W. W. A Self-Rating Depression Scale. *Archives of general psychiatry* **12**, 63-70 (1965).

80 Zung, W. W., Richards, C. B. & Short, M. J. Self-rating depression scale in an outpatient clinic. Further validation of the SDS. *Archives of general psychiatry* **13**, 508-515 (1965).

81 Shafer, A. B. Meta-analysis of the factor structures of four depression questionnaires: Beck, CES-D, Hamilton, and Zung. *Journal of clinical psychology* **62**, 123-146, doi:10.1002/jclp.20213 (2006).

82 Paolucci, S. Epidemiology and treatment of post-stroke depression. *Neuropsychiatric disease and treatment* **4**, 145-154 (2008).

83 Kwan, J. & Sandercock, P. In-hospital care pathways for stroke. *The Cochrane database of systematic reviews*, CD002924, doi:10.1002/14651858.CD002924.pub2 (2004).

84 de Man-van Ginkel, J. M. *et al.* An efficient way to detect poststroke depression by subsequent administration of a 9-item and a 2-item Patient Health Questionnaire. *Stroke; a journal of cerebral circulation* **43**, 854-856, doi:10.1161/STROKEAHA.111.640276 (2012).

85 Steinwachs, D. M., Collins-Nakai, R. L., Cohn, L. H., Garson, A., Jr. & Wolk, M. J. The future of cardiology: utilization and costs of care. *Journal of the American College of Cardiology* **35**, 91B-98B (2000).

86 El Husseini, N. *et al.* Depression and antidepressant use after stroke and transient ischemic attack. *Stroke; a journal of cerebral circulation* **43**, 1609-1616, doi:10.1161/STROKEAHA.111.643130 (2012).

87 Gustafson, Y., Nilsson, I., Mattsson, M., Astrom, M. & Bucht, G. Epidemiology and treatment of post-stroke depression. *Drugs & aging* **7**, 298-309 (1995).

88 Gordon, W. A. & Hibbard, M. R. Poststroke depression: an examination of the literature. *Archives of physical medicine and rehabilitation* **78**, 658-663 (1997).

89 Kneebone, II & Dunmore, E. Psychological management of post-stroke depression. *The British journal of clinical psychology / the British Psychological Society* **39 (Pt 1)**, 53-65 (2000).

90 Lincoln, N. B. & Flannaghan, T. Cognitive behavioral psychotherapy for depression following stroke: a randomized controlled trial. *Stroke; a journal of cerebral circulation* **34**, 111-115 (2003).

91 Lipsey, J. R., Robinson, R. G., Pearlson, G. D., Rao, K. & Price, T. R. Nortriptyline treatment of post-stroke depression: a double-blind study. *Lancet* **1**, 297-300 (1984).

92 Khawam, E. A., Laurencic, G. & Malone, D. A., Jr. Side effects of antidepressants: an overview. *Cleveland Clinic journal of medicine* **73**, 351-353, 356-361 (2006).

93 Turner-Stokes, L. & Hassan, N. Depression after stroke: a review of the evidence base to inform the development of an integrated care pathway. Part 2: Treatment alternatives. *Clinical rehabilitation* **16**, 248-260 (2002).

94 Robinson, R. G. *et al.* Nortriptyline versus fluoxetine in the treatment of depression and in short-term recovery after stroke: a placebo-controlled, double-blind study. *The American journal of psychiatry* **157**, 351-359, doi:10.1176/appi.ajp.157.3.351 (2000).

95 Lipsey, J. R. & Robinson, R. G. Nortriptyline for post-stroke depression. *Lancet* **1**, 803 (1984).

96 Jorge, R. E., Robinson, R. G., Arndt, S. & Starkstein, S. Mortality and poststroke depression: a placebo-controlled trial of antidepressants. *The American journal of psychiatry* **160**, 1823-1829, doi:10.1176/appi.ajp.160.10.1823 (2003).

97 Narushima, K. & Robinson, R. G. The effect of early versus late antidepressant treatment on physical impairment associated with poststroke depression: is there a time-related therapeutic window? *The Journal of nervous and mental disease* **191**, 645-652, doi:10.1097/01.nmd.0000092197.97693.d2 (2003).

98 Andersen, G., Vestergaard, K. & Lauritzen, L. Effective treatment of poststroke depression with the selective serotonin reuptake inhibitor citalopram. *Stroke; a journal of cerebral circulation* **25**, 1099-1104 (1994).

99 Andersen, G., Vestergaard, K. & Riis, J. O. Citalopram for post-stroke pathological crying. *Lancet* **342**, 837-839 (1993).

100 Tan, J. Y. & Levin, G. M. Citalopram in the treatment of depression and other potential uses in psychiatry. *Pharmacotherapy* **19**, 675-689 (1999).

101 Wiart, L., Petit, H., Joseph, P. A., Mazaux, J. M. & Barat, M. Fluoxetine in early poststroke depression: a double-blind placebo-controlled study. *Stroke; a journal of cerebral circulation* **31**, 1829-1832 (2000).

102 Chollet, F. *et al.* Fluoxetine for motor recovery after acute ischaemic stroke (FLAME): a randomised placebo-controlled trial. *The Lancet. Neurology* **10**, 123-130, doi:10.1016/S1474-4422(10)70314-8 (2011).

103 Spalletta, G. & Caltagirone, C. Sertraline treatment of post-stroke major depression: an open study in patients with moderate to severe symptoms. *Functional neurology* **18**, 227-232 (2003).

104 Zifko, U. A., Rupp, M. & Schwarz, S. [Sertraline in the treatment of post-stroke depression--results of an open multicenter study]. *Wiener medizinische Wochenschrift* **152**, 343-348 (2002).

105 Rasmussen, A. *et al.* A double-blind, placebo-controlled study of sertraline in the prevention of depression in stroke patients. *Psychosomatics* **44**, 216-221, doi:10.1176/appi.psy.44.3.216 (2003).

106 Dahmen, N., Marx, J., Hopf, H. C., Tettenborn, B. & Roder, R. Therapy of early

poststroke depression with venlafaxine: safety, tolerability, and efficacy as determined in an open, uncontrolled clinical trial. *Stroke; a journal of cerebral circulation* **30**, 691-692 (1999).

107 Kucukalic, A., Bravo-Mehmedbasic, A., Kulenovic, A. D. & Suljic-Mehmedika, E. Venlafaxine efficacy and tolerability in the treatment of post-stroke depression. *Psychiatria Danubina* **19**, 56-60 (2007).

108 Cravello, L., Caltagirone, C. & Spalletta, G. The SNRI venlafaxine improves emotional unawareness in patients with post-stroke depression. *Human psychopharmacology* **24**, 331-336, doi:10.1002/hup.1021 (2009).

109 Niedermaier, N., Bohrer, E., Schulte, K., Schlattmann, P. & Heuser, I. Prevention and treatment of poststroke depression with mirtazapine in patients with acute stroke. *The Journal of clinical psychiatry* **65**, 1619-1623 (2004).

110 Kim, S. W. *et al.* Mirtazapine treatment for pathological laughing and crying after stroke. *Clinical neuropharmacology* **28**, 249-251 (2005).

111 Sarti, C., Rastenyte, D., Cepaitis, Z. & Tuomilehto, J. International trends in mortality from stroke, 1968 to 1994. *Stroke; a journal of cerebral circulation* **31**, 1588-1601 (2000).

112 Appelros, P., Nydevik, I. & Viitanen, M. Poor outcome after first-ever stroke: predictors for death, dependency, and recurrent stroke within the first year. *Stroke; a journal of cerebral circulation* **34**, 122-126 (2003).

113 Saposnik, G. *et al.* Variables associated with 7-day, 30-day, and 1-year fatality after ischemic stroke. *Stroke; a journal of cerebral circulation* **39**, 2318-2324, doi:10.1161/STROKEAHA.107.510362 (2008).

114 Graven, C. *et al.* From rehabilitation to recovery: protocol for a randomised controlled trial evaluating a goal-based intervention to reduce depression and facilitate participation post-stroke. *BMC neurology* **11**, 73, doi:10.1186/1471-2377-11-73 (2011).

115 Dwyer Hollender, K. Screening, diagnosis, and treatment of post-stroke depression. *The Journal of neuroscience nursing : journal of the American Association of Neuroscience Nurses* **46**, 135-141, doi:10.1097/JNN.0000000000000047 (2014).

116 Niemi, M. L., Laaksonen, R., Kotila, M. & Waltimo, O. Quality of life 4 years after stroke. *Stroke; a journal of cerebral circulation* **19**, 1101-1107 (1988).

117 Ahlsio, B., Britton, M., Murray, V. & Theorell, T. Disablement and quality of life after stroke. *Stroke; a journal of cerebral circulation* **15**, 886-890 (1984).

118 Astrom, M., Asplund, K. & Astrom, T. Psychosocial function and life satisfaction after stroke. *Stroke; a journal of cerebral circulation* **23**, 527-531 (1992).

119 Kim, P., Warren, S., Madill, H. & Hadley, M. Quality of life of stroke survivors. *Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation* **8**, 293-301 (1999).

120 King, R. B. Quality of life after stroke. *Stroke; a journal of cerebral circulation* **27**, 1467-1472 (1996).

121 Zikic, T. R. *et al.* The effect of post stroke depression on functional outcome and quality of life. *Acta clinica Croatica* **53**, 294-301 (2014).

122 Unalan, D., Ozsoy, S., Soyuer, F. & Ozturk, A. Poststroke depressive symptoms and their relationship with quality of life, functional status, and severity of stroke. *Neurosciences* **13**, 395-401 (2008).

123 Robinson, R. G. & Szetela, B. Mood change following left hemispheric brain injury. *Annals of neurology* **9**, 447-453, doi:10.1002/ana.410090506 (1981).

124 Robinson, R. G., Starr, L. B., Lipsey, J. R., Rao, K. & Price, T. R. A two-year longitudinal study of post-stroke mood disorders: dynamic changes in associated

variables over the first six months of follow-up. *Stroke; a journal of cerebral circulation* **15**, 510-517 (1984).

125 Robinson, R. G. & Price, T. R. Post-stroke depressive disorders: a follow-up study of 103 patients. *Stroke; a journal of cerebral circulation* **13**, 635-641 (1982).

126 Robinson, R. G., Starr, L. B., Kubos, K. L. & Price, T. R. A two-year longitudinal study of post-stroke mood disorders: findings during the initial evaluation. *Stroke; a journal of cerebral circulation* **14**, 736-741 (1983).

127 Herrmann, N., Black, S. E., Lawrence, J., Szekely, C. & Szalai, J. P. The Sunnybrook Stroke Study: a prospective study of depressive symptoms and functional outcome. *Stroke; a journal of cerebral circulation* **29**, 618-624 (1998).

128 Pohjasvaara, T., Vataja, R., Leppavuori, A., Kaste, M. & Erkinjuntti, T. Depression is an independent predictor of poor long-term functional outcome post-stroke. *European journal of neurology* **8**, 315-319 (2001).

129 Nannetti, L., Paci, M., Pasquini, J., Lombardi, B. & Taiti, P. G. Motor and functional recovery in patients with post-stroke depression. *Disability and rehabilitation* **27**, 170-175, doi:10.1080/09638280400009378 (2005).

130 Sibolt, G. *et al.* Post-stroke depression and depression-executive dysfunction syndrome are associated with recurrence of ischaemic stroke. *Cerebrovascular diseases* **36**, 336-343, doi:10.1159/000355145 (2013).

131 Yuan, H. W. *et al.* Poststroke depression and risk of recurrent stroke at 1 year in a Chinese cohort study. *PloS one* **7**, e46906, doi:10.1371/journal.pone.0046906 (2012).

132 Vataja, R. *et al.* Depression-executive dysfunction syndrome in stroke patients. *The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry* **13**, 99-107, doi:10.1176/appi.ajgp.13.2.99 (2005).

133 House, A., Knapp, P., Bamford, J. & Vail, A. Mortality at 12 and 24 months after stroke may be associated with depressive symptoms at 1 month. *Stroke; a journal of cerebral circulation* **32**, 696-701 (2001).

134 Morris, P. L., Robinson, R. G., Andrzejewski, P., Samuels, J. & Price, T. R. Association of depression with 10-year poststroke mortality. *The American journal of psychiatry* **150**, 124-129, doi:10.1176/ajp.150.1.124 (1993).

135 Williams, L. S., Ghose, S. S. & Swindle, R. W. Depression and other mental health diagnoses increase mortality risk after ischemic stroke. *The American journal of psychiatry* **161**, 1090-1095 (2004).

136 Astrom, M., Adolfsson, R. & Asplund, K. Major depression in stroke patients. A 3-year longitudinal study. *Stroke; a journal of cerebral circulation* **24**, 976-982 (1993).

137 Robinson, R. G., Starr, L. B., Lipsey, J. R., Rao, K. & Price, T. R. A two-year longitudinal study of poststroke mood disorders. In-hospital prognostic factors associated with six-month outcome. *The Journal of nervous and mental disease* **173**, 221-226 (1985).

138 Robinson, R. G., Kubos, K. L., Starr, L. B., Rao, K. & Price, T. R. Mood Disorders in Stroke Patients - Importance of Location of Lesion. *Brain : a journal of neurology* **107**, 81-93, doi:Doi 10.1093/Brain/107.1.81 (1984).

139 Robinson, R. G., Lipsey, J. R., Rao, K. & Price, T. R. Two-year longitudinal study of post-stroke mood disorders: comparison of acute-onset with delayed-onset depression. *The American journal of psychiatry* **143**, 1238-1244, doi:10.1176/ajp.143.10.1238 (1986).

140 Snaphaan, L., van der Werf, S., Kanselaar, K. & de Leeuw, F. E. Post-stroke

depressive symptoms are associated with post-stroke characteristics. *Cerebrovascular diseases* **28**, 551-557, doi:10.1159/000247598 (2009).

141 Berg, A., Palomaki, H., Lehtihalmes, M., Lonnqvist, J. & Kaste, M. Poststroke depression: an 18-month follow-up. *Stroke; a journal of cerebral circulation* **34**, 138-143 (2003).

142 Carson, A. J. *et al.* Depression after stroke and lesion location: a systematic review. *Lancet* **356**, 122-126, doi:10.1016/S0140-6736(00)02448-X (2000).

143 Nys, G. M. *et al.* Early depressive symptoms after stroke: neuropsychological correlates and lesion characteristics. *Journal of the neurological sciences* **228**, 27-33, doi:10.1016/j.jns.2004.09.031 (2005).

144 Aben, I. *et al.* Focal or generalized vascular brain damage and vulnerability to depression after stroke: a 1-year prospective follow-up study. *International psychogeriatrics / IPA* **18**, 19-35, doi:10.1017/S104161020500270X (2006).

145 Yu, L. *et al.* Relationship between post-stroke depression and lesion location: a meta-analysis. *The Kaohsiung journal of medical sciences* **20**, 372-380, doi:10.1016/S1607-551X(09)70173-1 (2004).

146 Dam, H., Pedersen, H. E. & Ahlgren, P. Depression among patients with stroke. *Acta psychiatrica Scandinavica* **80**, 118-124 (1989).

147 MacHale, S. M., O'Rourke, S. J., Wardlaw, J. M. & Dennis, M. S. Depression and its relation to lesion location after stroke. *Journal of neurology, neurosurgery, and psychiatry* **64**, 371-374 (1998).

148 Schwartz, J. A. *et al.* Depression in stroke rehabilitation. *Biological psychiatry* **33**, 694-699 (1993).

149 Finset, A., Goffeng, L., Landro, N. I. & Haakonsen, M. Depressed mood and intra-hemispheric location of lesion in right hemisphere stroke patients. *Scandinavian journal of rehabilitation medicine* **21**, 1-6 (1989).

150 Zhang, T. *et al.* A prospective cohort study of lesion location and its relation to post-stroke depression among Chinese patients. *Journal of affective disorders* **136**, e83-87, doi:10.1016/j.jad.2011.06.014 (2012).

151 Morris, P. L., Robinson, R. G., Raphael, B. & Hopwood, M. J. Lesion location and poststroke depression. *The Journal of neuropsychiatry and clinical neurosciences* **8**, 399-403, doi:10.1176/jnp.8.4.399 (1996).

152 Krishnan, V. & Nestler, E. J. The molecular neurobiology of depression. *Nature* **455**, 894-902, doi:10.1038/nature07455 (2008).

153 Bryer, J. B. *et al.* Reduction of CSF monoamine metabolites in poststroke depression: a preliminary report. *The Journal of neuropsychiatry and clinical neurosciences* **4**, 440-442, doi:10.1176/jnp.4.4.440 (1992).

154 Hu, B., Hissong, B. D. & Carlin, J. M. Interleukin-1 enhances indoleamine 2,3-dioxygenase activity by increasing specific mRNA expression in human mononuclear phagocytes. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* **15**, 617-624, doi:10.1089/jir.1995.15.617 (1995).

155 Pemberton, L. A., Kerr, S. J., Smythe, G. & Brew, B. J. Quinolinic acid production by macrophages stimulated with IFN-gamma, TNF-alpha, and IFN-alpha. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* **17**, 589-595, doi:10.1089/jir.1997.17.589 (1997).

156 Babcock, T. A. & Carlin, J. M. Transcriptional activation of indoleamine dioxygenase by interleukin 1 and tumor necrosis factor alpha in interferon-treated epithelial cells. *Cytokine* **12**, 588-594, doi:10.1006/cyto.1999.0661 (2000).

157 Spalletta, G. *et al.* The etiology of poststroke depression: a review of the

literature and a new hypothesis involving inflammatory cytokines. *Molecular psychiatry* **11**, 984-991, doi:10.1038/sj.mp.4001879 (2006).

158 Mossner, R. *et al.* Consensus paper of the WFSBP Task Force on Biological Markers: biological markers in depression. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* **8**, 141-174, doi:10.1080/15622970701263303 (2007).

159 Levine, J. *et al.* Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology* **40**, 171-176, doi:26615 (1999).

160 Yang, L. *et al.* The serum interleukin-18 is a potential marker for development of post-stroke depression. *Neurological research* **32**, 340-346, doi:10.1179/016164110X12656393665080 (2010).

161 O'Connor, J. C. *et al.* Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Molecular psychiatry* **14**, 511-522, doi:10.1038/sj.mp.4002148 (2009).

162 Heils, A. *et al.* Allelic variation of human serotonin transporter gene expression. *Journal of neurochemistry* **66**, 2621-2624 (1996).

163 Wendland, J. R., Martin, B. J., Kruse, M. R., Lesch, K. P. & Murphy, D. L. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular psychiatry* **11**, 224-226, doi:10.1038/sj.mp.4001789 (2006).

164 Kohen, R. *et al.* Association of serotonin transporter gene polymorphisms with poststroke depression. *Archives of general psychiatry* **65**, 1296-1302, doi:10.1001/archpsyc.65.11.1296 (2008).

165 Ramasubbu, R., Tobias, R., Buchan, A. M. & Bech-Hansen, N. T. Serotonin transporter gene promoter region polymorphism associated with poststroke major depression. *The Journal of neuropsychiatry and clinical neurosciences* **18**, 96-99, doi:10.1176/jnp.18.1.96 (2006).

166 Kim, J. M. *et al.* Serotonergic and BDNF genes and risk of depression after stroke. *Journal of affective disorders* **136**, 833-840, doi:10.1016/j.jad.2011.09.029 (2012).

167 Gould, E., Cameron, H. A., Daniels, D. C., Woolley, C. S. & McEwen, B. S. Adrenal hormones suppress cell division in the adult rat dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **12**, 3642-3650 (1992).

168 Gould, E., Woolley, C. S. & McEwen, B. S. Adrenal steroids regulate postnatal development of the rat dentate gyrus: I. Effects of glucocorticoids on cell death. *The Journal of comparative neurology* **313**, 479-485, doi:10.1002/cne.903130308 (1991).

169 Cobb, J. A. *et al.* Hippocampal volume and total cell numbers in major depressive disorder. *Journal of psychiatric research* **47**, 299-306, doi:10.1016/j.jpsychires.2012.10.020 (2013).

170 Boldrini, M. *et al.* Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **38**, 1068-1077, doi:10.1038/npp.2013.5 (2013).

171 Boldrini, M. *et al.* Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **34**, 2376-2389, doi:10.1038/npp.2009.75 (2009).

172 Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of*

neuroscience : the official journal of the Society for Neuroscience **20**, 9104-9110 (2000).

173 Manev, H., Uz, T., Smalheiser, N. R. & Manev, R. Antidepressants alter cell proliferation in the adult brain in vivo and in neural cultures in vitro. *European journal of pharmacology* **411**, 67-70 (2001).

174 Santarelli, L. *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805-809, doi:10.1126/science.1083328 (2003).

175 van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* **2**, 266-270, doi:10.1038/6368 (1999).

176 Madsen, T. M. *et al.* Increased neurogenesis in a model of electroconvulsive therapy. *Biological psychiatry* **47**, 1043-1049 (2000).

177 Eckenhoff, M. F. & Rakic, P. Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **8**, 2729-2747 (1988).

178 Markakis, E. A. & Gage, F. H. Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. *The Journal of comparative neurology* **406**, 449-460 (1999).

179 Rozental, R. *et al.* Differentiation of hippocampal progenitor cells in vitro: temporal expression of intercellular coupling and voltage- and ligand-gated responses. *Developmental biology* **167**, 350-362, doi:10.1006/dbio.1995.1029 (1995).

180 van Praag, H. *et al.* Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030-1034, doi:10.1038/4151030a (2002).

181 Encinas, J. M., Vaahtokari, A. & Enikolopov, G. Fluoxetine targets early progenitor cells in the adult brain. *Proc Natl Acad Sci U S A* **103**, 8233-8238, doi:10.1073/pnas.0601992103 (2006).

182 Kempermann, G., Jessberger, S., Steiner, B. & Kronenberg, G. Milestones of neuronal development in the adult hippocampus. *Trends in neurosciences* **27**, 447-452, doi:10.1016/j.tins.2004.05.013 (2004).

183 Wang, S. H., Zhang, Z. J., Guo, Y. J., Teng, G. J. & Chen, B. A. Hippocampal neurogenesis and behavioural studies on adult ischemic rat response to chronic mild stress. *Behavioural brain research* **189**, 9-16, doi:10.1016/j.bbr.2007.11.028 (2008).

184 Folstein, M. F., Maiberger, R. & McHugh, P. R. Mood disorder as a specific complication of stroke. *Journal of neurology, neurosurgery, and psychiatry* **40**, 1018-1020 (1977).

185 Biran, I. & Chatterjee, A. Depression with anosognosia following a left subcortical stroke. *Clinical neurology and neurosurgery* **105**, 99-101 (2003).

186 Loubinoux, I. *et al.* Post-stroke depression: mechanisms, translation and therapy. *Journal of cellular and molecular medicine* **16**, 1961-1969, doi:10.1111/j.1582-4934.2012.01555.x (2012).

187 Mishra, N. K. *et al.* Influence of age on outcome from thrombolysis in acute stroke: a controlled comparison in patients from the Virtual International Stroke Trials Archive (VISTA). *Stroke; a journal of cerebral circulation* **41**, 2840-2848, doi:10.1161/STROKEAHA.110.586206 (2010).

188 Feigin, V. L., Lawes, C. M., Bennett, D. A. & Anderson, C. S. Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. *The Lancet. Neurology* **2**, 43-53 (2003).

189 Badan, I. *et al.* Accelerated glial reactivity to stroke in aged rats correlates with reduced functional recovery. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* **23**, 845-854, doi:10.1097/01.WCB.0000071883.63724.A7 (2003).

190 Wang, R. Y., Wang, P. S. & Yang, Y. R. Effect of age in rats following middle cerebral artery occlusion. *Gerontology* **49**, 27-32, doi:66505 (2003).

191 Zhang, L. *et al.* Functional recovery in aged and young rats after embolic stroke: treatment with a phosphodiesterase type 5 inhibitor. *Stroke; a journal of cerebral circulation* **36**, 847-852, doi:10.1161/01.STR.0000158923.19956.73 (2005).

192 Won, S. J. *et al.* Influence of age on the response to fibroblast growth factor-2 treatment in a rat model of stroke. *Brain research* **1123**, 237-244, doi:10.1016/j.brainres.2006.09.055 (2006).

193 Kato, M., Iwata, H., Okamoto, M., Ishii, T. & Narita, H. Focal cerebral ischemia-induced escape deficit in rats is ameliorated by a reversible inhibitor of monoamine oxidase-a: implications for a novel animal model of post-stroke depression. *Biological & pharmaceutical bulletin* **23**, 406-410 (2000).

194 Kim, Y. R. *et al.* Studies on the animal model of post-stroke depression and application of antipsychotic aripiprazole. *Behavioural brain research* **287**, 294-303, doi:10.1016/j.bbr.2015.03.062 (2015).

195 Powell, T. R., Fernandes, C. & Schalkwyk, L. C. Depression-Related Behavioral Tests. *Current protocols in mouse biology* **2**, 119-127, doi:10.1002/9780470942390.mo110176 (2012).

196 Deplanque, D., Venna, V. R. & Bordet, R. Brain ischemia changes the long term response to antidepressant drugs in mice. *Behavioural brain research* **219**, 367-372, doi:10.1016/j.bbr.2011.01.003 (2011).

197 Winter, B. *et al.* Anxious and hyperactive phenotype following brief ischemic episodes in mice. *Biological psychiatry* **57**, 1166-1175, doi:10.1016/j.biopsych.2005.02.010 (2005).

198 Wang, S. H., de Oliveira Alvares, L. & Nader, K. Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nat Neurosci* **12**, 905-912, doi:10.1038/nn.2350 (2009).

199 Willner, P., Towell, A., Sampson, D., Sophokleous, S. & Muscat, R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology* **93**, 358-364 (1987).

200 Hill, M. N., Hellemans, K. G., Verma, P., Gorzalka, B. B. & Weinberg, J. Neurobiology of chronic mild stress: parallels to major depression. *Neuroscience and biobehavioral reviews* **36**, 2085-2117, doi:10.1016/j.neubiorev.2012.07.001 (2012).

201 Sahay, A. & Hen, R. Adult hippocampal neurogenesis in depression. *Nat Neurosci* **10**, 1110-1115, doi:10.1038/nn1969 (2007).

202 Barry, L. C., Kasl, S. V., Lichtman, J., Vaccarino, V. & Krumholz, H. M. Social support and change in health-related quality of life 6 months after coronary artery bypass grafting. *Journal of psychosomatic research* **60**, 185-193, doi:10.1016/j.jpsychores.2005.06.080 (2006).

203 Boden-Albala, B., Litwak, E., Elkind, M. S., Rundek, T. & Sacco, R. L. Social isolation and outcomes post stroke. *Neurology* **64**, 1888-1892, doi:10.1212/01.WNL.0000163510.79351.AF (2005).

204 Ikeda, A. *et al.* Social support and stroke and coronary heart disease: the JPHC study cohorts II. *Stroke; a journal of cerebral circulation* **39**, 768-775, doi:10.1161/STROKEAHA.107.496695 (2008).

205 House, J. S., Landis, K. R. & Umberson, D. Social relationships and health.

Science **241**, 540-545 (1988).

206 Glass, T. A., Matchar, D. B., Belyea, M. & Feussner, J. R. Impact of social support on outcome in first stroke. *Stroke; a journal of cerebral circulation* **24**, 64-70 (1993).

207 Venna, V. R. & McCullough, L. D. "Won't you be my neighbor?": deciphering the mechanisms of neuroprotection induced by social interaction. *Stroke; a journal of cerebral circulation* **42**, 3329-3330, doi:10.1161/STROKEAHA.111.632570 (2011).

208 Verma, R., Friedler, B. D., Harris, N. M. & McCullough, L. D. Pair housing reverses post-stroke depressive behavior in mice. *Behavioural brain research* **269**, 155-163, doi:10.1016/j.bbr.2014.04.044 (2014).

209 Zhang, G. *et al.* Combined use of spatial restraint stress and middle cerebral artery occlusion is a novel model of post-stroke depression in mice. *Scientific reports* **5**, 16751, doi:10.1038/srep16751 (2015).

210 Lad, H. V. *et al.* Behavioural battery testing: evaluation and behavioural outcomes in 8 inbred mouse strains. *Physiology & behavior* **99**, 301-316, doi:10.1016/j.physbeh.2009.11.007 (2010).

211 Cryan, J. F., Markou, A. & Lucki, I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends in pharmacological sciences* **23**, 238-245 (2002).

212 Jiao, J., Nitzke, A. M., Doukas, D. G., Seiglie, M. P. & Dulawa, S. C. Antidepressant response to chronic citalopram treatment in eight inbred mouse strains. *Psychopharmacology* **213**, 509-520, doi:10.1007/s00213-010-2140-0 (2011).

213 Porsolt, R. D., Le Pichon, M. & Jalfre, M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**, 730-732 (1977).

214 Jacobson, L. H. & Cryan, J. F. Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. *Behavior genetics* **37**, 171-213, doi:10.1007/s10519-006-9106-3 (2007).

215 Steru, L., Chermat, R., Thierry, B. & Simon, P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* **85**, 367-370 (1985).

216 Castagne, V., Moser, P. & Porsolt, R. D. in *Methods of Behavior Analysis in Neuroscience Frontiers in Neuroscience* (ed J. J. Buccafusco) (2009).

217 Bergner, C. L. *et al.* Mouse models for studying depression-like states and antidepressant drugs. *Methods Mol Biol* **602**, 267-282, doi:10.1007/978-1-60761-058-8_16 (2010).

218 Castagne, V., Moser, P., Roux, S. & Porsolt, R. D. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. *Current protocols in pharmacology / editorial board, S.J. Enna* **Chapter 5**, Unit 5 8, doi:10.1002/0471141755.ph0508s49 (2010).

219 Piato, A. L. *et al.* Effects of Marapuama in the chronic mild stress model: further indication of antidepressant properties. *Journal of ethnopharmacology* **118**, 300-304, doi:10.1016/j.jep.2008.04.018 (2008).

220 Yalcin, I., Aksu, F. & Belzung, C. Effects of desipramine and tramadol in a chronic mild stress model in mice are altered by yohimbine but not by pindolol. *European journal of pharmacology* **514**, 165-174, doi:10.1016/j.ejphar.2005.03.029 (2005).

221 Yalcin, I., Aksu, F., Bodard, S., Chalon, S. & Belzung, C. Antidepressant-like effect of tramadol in the unpredictable chronic mild stress procedure: possible involvement of the noradrenergic system. *Behavioural pharmacology* **18**, 623-

631, doi:10.1097/FBP.0b013e3282eff109 (2007).
222 Samuels, B. A. & Hen, R. in *Mood and Anxiety Related Phenotypes in Mice* Vol. 42 (ed T. D. Gould) Ch. Chapter 7: Novelty-Suppressed Feeding in the Mouse, 107-121 (Humana Press, 2009).
223 Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J. & Woodgett, J. R. Assessment of social interaction behaviors. *Journal of visualized experiments : JoVE*, doi:10.3791/2473 (2011).
224 Downhill, J. E., Jr. & Robinson, R. G. Longitudinal assessment of depression and cognitive impairment following stroke. *The Journal of nervous and mental disease* **182**, 425-431 (1994).
225 Paolucci, S. *et al.* Poststroke depression and its role in rehabilitation of inpatients. *Archives of physical medicine and rehabilitation* **80**, 985-990 (1999).
226 McDade, T. W., Hawkley, L. C. & Cacioppo, J. T. Psychosocial and behavioral predictors of inflammation in middle-aged and older adults: the Chicago health, aging, and social relations study. *Psychosomatic medicine* **68**, 376-381, doi:10.1097/01.psy.0000221371.43607.64 (2006).
227 Craft, T. K. *et al.* Social interaction improves experimental stroke outcome. *Stroke; a journal of cerebral circulation* **36**, 2006-2011, doi:10.1161/01.STR.0000177538.17687.54 (2005).
228 Weil, Z. M. *et al.* Social isolation potentiates cell death and inflammatory responses after global ischemia. *Molecular psychiatry* **13**, 913-915, doi:10.1038/mp.2008.70 (2008).
229 Karelina, K. *et al.* Social isolation alters neuroinflammatory response to stroke. *Proc Natl Acad Sci U S A* **106**, 5895-5900, doi:10.1073/pnas.0810737106 (2009).
230 Karelina, K. *et al.* Oxytocin mediates social neuroprotection after cerebral ischemia. *Stroke; a journal of cerebral circulation* **42**, 3606-3611, doi:10.1161/STROKEAHA.111.628008 (2011).
231 Carter, C. S. & Altemus, M. Integrative functions of lactational hormones in social behavior and stress management. *Annals of the New York Academy of Sciences* **807**, 164-174 (1997).
232 Insel, T. R., Gingrich, B. S. & Young, L. J. Oxytocin: who needs it? *Progress in brain research* **133**, 59-66 (2001).
233 Pedersen, C. A. Oxytocin control of maternal behavior. Regulation by sex steroids and offspring stimuli. *Annals of the New York Academy of Sciences* **807**, 126-145 (1997).
234 Young, L. T., Bakish, D. & Beaulieu, S. The neurobiology of treatment response to antidepressants and mood stabilizing medications. *Journal of psychiatry & neuroscience : JPN* **27**, 260-265 (2002).
235 Uvnas-Moberg, K. Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology* **23**, 819-835 (1998).
236 Pittenger, M. F. *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* **284**, 143-147 (1999).
237 Hu, X. *et al.* Hypoxic preconditioning enhances bone marrow mesenchymal stem cell migration via Kv2.1 channel and FAK activation. *American journal of physiology. Cell physiology* **301**, C362-372, doi:10.1152/ajpcell.00013.2010 (2011).
238 Orlic, D. *et al.* Bone marrow cells regenerate infarcted myocardium. *Nature* **410**, 701-705, doi:10.1038/35070587 (2001).
239 Zhang, Z. G., Zhang, L., Jiang, Q. & Chopp, M. Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. *Circulation research* **90**, 284-288 (2002).

240 Lv, W., Li, W. Y., Xu, X. Y., Jiang, H. & Bang, O. Y. Bone marrow mesenchymal stem cells transplantation promotes the release of endogenous erythropoietin after ischemic stroke. *Neural regeneration research* **10**, 1265-1270, doi:10.4103/1673-5374.162759 (2015).

241 Theus, M. H. *et al.* In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. *Experimental neurology* **210**, 656-670, doi:10.1016/j.expneurol.2007.12.020 (2008).

242 Francis, K. R. & Wei, L. Human embryonic stem cell neural differentiation and enhanced cell survival promoted by hypoxic preconditioning. *Cell death & disease* **1**, e22, doi:10.1038/cddis.2009.22 (2010).

243 Wei, J. W. *et al.* A Systematic Review and Meta-Analysis of Perforator-Pedicled Propeller Flaps in Lower Extremity Defects: Identification of Risk Factors for Complications. *Plastic and reconstructive surgery*, doi:10.1097/PRS.0000000000002392 (2016).

244 Samoilov, M. O. *et al.* The adaptive effects of hypoxic preconditioning of brain neurons. *Neuroscience and behavioral physiology* **33**, 1-11 (2003).

245 Sharp, F. R. & Bernaudin, M. HIF1 and oxygen sensing in the brain. *Nature reviews. Neuroscience* **5**, 437-448, doi:10.1038/nrn1408 (2004).

246 Wei, L., Fraser, J. L., Lu, Z. Y., Hu, X. & Yu, S. P. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiology of disease* **46**, 635-645, doi:10.1016/j.nbd.2012.03.002 (2012).

247 Wei, L., Rovainen, C. M. & Woolsey, T. A. Ministrokes in rat barrel cortex. *Stroke; a journal of cerebral circulation* **26**, 1459-1462 (1995).

248 Hu, X. *et al.* Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *J Thorac Cardiovasc Surg* **135**, 799-808 (2008).

249 Yu, S. P., Wei, Z. & Wei, L. Preconditioning strategy in stem cell transplantation therapy. *Translational stroke research* **4**, 76-88 (2013).

250 Wei, N. *et al.* Delayed intranasal delivery of hypoxic-preconditioned bone marrow mesenchymal stem cells enhanced cell homing and therapeutic benefits after ischemic stroke in mice. *Cell transplantation* **22**, 977-991, doi:10.3727/096368912X657251 (2013).

251 Danielyan, L. *et al.* Intranasal delivery of cells to the brain. *European journal of cell biology* **88**, 315-324, doi:10.1016/j.ejcb.2009.02.001 (2009).

252 Yalcin, I., Belzung, C. & Surget, A. Mouse strain differences in the unpredictable chronic mild stress: a four-antidepressant survey. *Behavioural brain research* **193**, 140-143, doi:10.1016/j.bbr.2008.04.021 (2008).

253 Leuchter, A. F., Cook, I. A., Hunter, A. M., Cai, C. & Horvath, S. Resting-state quantitative electroencephalography reveals increased neurophysiologic connectivity in depression. *PloS one* **7**, e32508, doi:10.1371/journal.pone.0032508 (2012).

254 Yamamura, T. *et al.* Association of thalamic hyperactivity with treatment-resistant depression and poor response in early treatment for major depression: a resting-state fMRI study using fractional amplitude of low-frequency fluctuations. *Translational psychiatry* **6**, e754, doi:10.1038/tp.2016.18 (2016).

255 Greicius, M. D. *et al.* Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological psychiatry* **62**, 429-437, doi:10.1016/j.biopsych.2006.09.020 (2007).

256 Dusunceli, F. *et al.* Oxytocin alleviates hepatic ischemia-reperfusion injury in rats. *Peptides* **29**, 1216-1222, doi:10.1016/j.peptides.2008.02.010 (2008).

257 Houshmand, F., Faghihi, M. & Zahediasl, S. Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. *Peptides* **30**, 2301-2308, doi:10.1016/j.peptides.2009.09.010 (2009).

258 Ondrejcakova, M., Ravingerova, T., Bakos, J., Pancza, D. & Jezova, D. Oxytocin exerts protective effects on in vitro myocardial injury induced by ischemia and reperfusion. *Canadian journal of physiology and pharmacology* **87**, 137-142, doi:10.1139/Y08-108 (2009).

Appendix: Diffuse Axonal Injury in Traumatic Brain Injury and the Potential for Remyelination Through Stem Cell Therapy

A. Introduction: Traumatic Brain Injury (TBI) is a devastating "silent epidemic," affecting approximately 1.7 million people annually in the United States[1,2], approximately 43% of which result in mortality[3]. For those that survive, the effects of TBI last long beyond the initial insult. Consequently, there are 5.3 million Americans with a TBI-attributed disability[4], and less than 50% of TBI survivors are capable of leading independent lives[3].

TBI is a heterogeneous condition due to the multitude of mechanisms that produce injury and the array of associated symptoms and pathologies[5]. At the time of initial injury, damage to neurons, glia, and axons occurs (primary axonomy)[6,7], but the most detrimental damage transpires as a result of a complex secondary cascade (secondary axonomy), one result of which is white matter degeneration[8,9]. This degeneration, clinically deemed diffuse axonal injury (DAI), is the most common TBI pathology, regardless of injury severity[10], and is the leading cause of post-traumatic neurological disability[11]. DAI interferes with signal transduction, axon integrity, and network processing[8,12], leading to neurological impairments, including long-term memory loss, emotional disturbances, unconsciousness, and mortality [13-15].

Due to the diffuse nature of DAI, development and implementation of treatments has been difficult. To date, the exploration of clinically effective neuroprotective treatments has been largely unsuccessful; thus, the development of neurorestorative therapies is necessary in order to decrease TBI associated mortalities and improve post-injury quality of life. It was long thought that repair within the mature adult brain was not possible, but

recent findings indicate that multipotent neural stem/progenitor cells (NSPCs) persist in the adult brain[4,16]. Stem cell mediated oligodendrogenesis holds potential as a neurorestorative therapy mandating further investigation.

B. Injury Biomechanics and Pathophysiology of DAI: DAI is the result of inertial forces, caused by rapid angular, rotational, and/or translational acceleration/deceleration and the continued propagation of force throughout the brain[15,17]. Tissue-to-tissue friction causes an irreversible shearing of axons and widespread brain injury[18,19], resulting in damage to both the cell's membrane and cytoskeleton[10,20]. This damage manifests as a variety of identifiable cellular and structural changes. The extent of shearing injury is highly correlated with the size of the human brain, such that the greater the mass of the brain, the higher the shearing strain present between tissues[10,21]. Shearing injury causes lesions to form at common loci throughout the brain's white matter, most frequently in the corpus callosum, cerebral hemispheres, and brainstem [8,15,22,23].

Beyond direct tissue damage, TBI is also associated with subcellular changes including increased membrane permeability and altered cellular metabolism[24,25]. Due to axonal stretching and the disruption of neuronal cell membranes as a direct result of TBI, an indiscriminate influx of ions occurs[26], leading to the release of excitatory amino acids (EAAs), one of which is glutamate [27,28]. Once released, glutamate binds to and activates kainate, N-methyl-D-aspartate (NMDA), and d-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors[28], while membrane depolarization activates voltage dependent Ca^{2+} and Na^{+} channels[29]. Coupled together, these chemical and physical insults act synergistically, resulting in further depolarization and Ca^{2+}-dependent injury [24]. Excess Ca^{2+} accumulates in the mitochondria, resulting in mitochondrial injury, adenosine triphosphate (ATP) depletion[30,31], and impaired membrane

pumps/transporters/exchanges, leading to further disruption of ionic homeostases involving Na^+, K^+, and Ca^{2+} [8]. Increased intracellular Ca^{2+} leads to aberrant activation of proteases, phosphatases[32,33], and lipid peroxidases[29]. Following the activation of these enzymes, excitotoxicity ensues, resulting in damage to the cell's cytoskeleton and the production of reactive free fatty acids and free radicals, both of which have further deleterious effects within the cell [29].

Beyond the known intracellular changes, there are other associated structural changes that occur selectively within the white matter of the brain. Due to its structural organization and highly anisotropic arrangement[15], white matter is naturally prone to damage[34,35]. Under normal circumstances, the viscoelastic nature of the brain is beneficial, but during injury this property makes the brain more susceptible to impairment[10,36]. Normally axons are complaint when stretched, but in the case of rapid acceleration/deceleration, axons become brittle[10,37]. Rapid axonal stretching causes axonal misalignment and axons ripple due to cytoskeletal damage, loss of resilience, and axonal microtubule damage[20,38]. Misalignment prohibits the restoration and relaxation of microtubules to their original state. To rectify this problem, microtubules depolymerize in a process known as axonal relaxation. Unfortunately, this interrupts axonal transport and induces swelling via accumulation of organelles and vesicles[10]. This prolonged axonal swelling can result in white matter degeneration[20].

C. Pathology of DAI: DAI typically presents in deep and subcortical white matter structures, and is characterized by a number of notable neuroanatomical changes. Two of the primary indicators of DAI pathology are axonal varicosities and axonal bulbs. Axonal varicosities are defined as swellings along otherwise intact axons[20]. Varicosities are thought to result from the accumulation of transported materials[38,39], and thus indicate

a partial disruption of axonal transport[11,20]. Axonal bulbs, also known as reactive axonal swellings or retraction bulbs, are apparent at the end of disconnected axons, and are indicative of complete axonal disconnection[40]. In addition to these indicators of DAI, during the posttraumatic period of injury (1-2 days) other pathological features including microscopic tears and microvascular ruptures appear[41]. Long-term pathological features include the presence of microglial stars and Wallerian degeneration, both of which are indicative of axonal disintegration and a loss of membrane integrity[8]. Additionally, demyelination normally manifests within two years of injury[8].

D. Diagnosis of Axonal Damage after DAI:

1. Clinical Diagnosis: Due to its microscopic nature, diagnosing axonal damage in patients can be challenging, leading to widespread under-diagnosis by the medical community[42]. Clinicians rely primarily on observable manifestations and radiological examinations to diagnose DAI.

A primary indication that an individual may have suffered a DAI is coma. DAI is one of the primary causes of post-traumatic unconsciousness, whether it persists for minutes or days, although it is not present in all cases[43]. The duration of coma has been shown to positively correlate with the severity of DAI[44].

Radiological examinations, including computed tomography (CT) and magnetic resonance imaging (MRI), are another means of diagnosis. Unfortunately, these techniques are only sensitive enough to detect white matter tears and parenchymal hemorrhages, which are more common in focal injuries, and may only be present in more severe cases of DAI[42]. It is not unusual for CT and MRI scans to underestimate white matter damage, or yield normal results despite widespread microscopic pathology[45,46].

More recently, some laboratories have begun to integrate MRI with diffusion tensor imaging (DTI) which, by examining functional anisotropy and diffusivity, allows for quantification of white matter damage[46]. Utilization of DTI is particularly well-tuned for DAI diagnosis and treatment. Studies indicate that irregularities detected via DTI directly correlate with the cognitive impairments observed post-TBI[47-49]. Another imaging technique currently being utilized is susceptibility-weighed imaging (SWI), which is able to identify micro-bleeds, a marker of DAI[50-52]. SWI examines the magnetic susceptibility differences of tissues, thereby creating a new type of MRI contrast and consequently improving the sensitivity of MRI imaging[53,54].

Another method of diagnosing DAI is through analysis of S-100B and neuron-specific enolase (NSE), both of which are elevated in the serum and cerebrospinal fluid of DAI patients[55-57]. S-100B is an acidic calcium binding protein which is localized to glial and Schwann cells, and NSE is a glycolytic enzyme found in the cytoplasm of neurons[58]. Although elevation of either is indicative of DAI, S-100B is more highly correlated with injury severity[59].

2. Post-mortem Diagnosis: Positive diagnosis of DAI is only possible post-mortem. The gold standard for identifying DAI is immunostaining for beta-amyloid precursor protein (β-APP), which identifies axons with impaired fast axonal transport[60]. β-APP is a particularly sensitive marker that can be used to identify DAI before other more noticeable pathologies, such as axonal swelling or Wallerian degeneration, become apparent[61-63]. Positive β-APP staining can be observed 1-3 hours after trauma, although a more recent study by Hortobagyi *et al.* suggests that axonal damage may be detected as early as 35 minutes following severe TBI[64].

In addition to β-APP, there are a number of other markers that can be used to identify DAI with lower fidelity including synaptic protein-25 (SNAP-25), chromagranin A, cathepsin D, and neurofilament-68 (NF-68). SNAP-25, a protein involved in synaptic exocytosis[65,66], labels swollen axons, but has also been shown to label uninjured neurons, perhaps due to normal axonal transport[67]. Thus, this marker is insufficient to confirm injury without the visible presence of swelling[67]. Chromagranin A is a glycoprotein that labels dense-core synaptic vesicles and is transported via anterograde axonal flow[62,68]. Cathespin D is an endopeptidase that plays a role in apoptosis. Both chromagranin A and cathepsin D are present in injured axons, but their staining is less intense than that associated with β-APP[62]. Certain neurofilaments, most commonly NF-68, are also used to detect DAI because they have minimally concomitant immunoreactivity with the cell nucleus, soma, and dendritic processes[69]. Decreased levels of NF-68 have been found in the lateral controlled cortical impact[70] and lateral rotational animal models of TBI[71,72], but the decreased presence of NF-68 has not been consistently observed in human studies [67].

E. Myelin Degeneration after DAI: Due to oligodendrocyte damage, widespread demyelination occurs following DAI[15,73,74]. Myelin, a fatty substance which sheaths axons throughout the brain[75], acts to both electrically insulate and increase the speed of signaling transmission. Myelin debris is a known trigger of inflammation, and the fact that there is currently no effective and/or efficient way to clear debris from the central nervous system (CNS), may explain why chronic white matter inflammation can persist for years following TBI[73,76]. Microglia and macrophages do remove myelin debris, but this process is slow and may take weeks or longer[77]. Recently, Wen *et al.* showed that in a closed-head weight drop model of TBI, peak quantities of myelin debris are present on day 7 and day 14 following injury in the brainstem and the cerebral cortex/hippocampus, respectively, and debris remains present in the brainstem, cerebral cortex, and

hippocampus on day 28. This data supports the conclusion that myelin debris persists in the CNS following DAI[78].

There is also evidence to support the idea that myelin debris plays an inhibitive role in remyelination. The adult CNS naturally has a population of precursor cells that are capable of proliferation, migration, and differentiation into oligodendrocytes following injury[79,80]. However, for unknown reasons, differentiation and maturation of oligodendrocyte progenitor cells (OPCs) becomes arrested in the presence of myelin debris[81,82], despite the fact that recruitment of OPCs and macrophages is not affected [83]. One possible explanation for the observed arrest is that new myelin sheets cannot be formed in the presence of myelin debris, and therefore debris must be removed before remyelination can occur[81,84].

F. Oligodendrocytes in DAI: Due to their naturally high metabolic rate and consequently high oxygen and ATP demands, oligodendrocytes, the myelin forming cells of the CNS, are especially susceptible to molecular homeostasis disruptions caused by injury[85]. Following DAI, oligodendrocytes frequently die due to hypoxia, excitotoxicity, oxidative stress, exposure to inflammatory cytokines, and/or a lack of metabolic activities[86,87]. Loss of oligodendrocytes results in demyelination, impaired axonal function, and ultimately, cognitive impairment[83,88,89]. The negative consequences of oligodendrocyte loss and the associated demyelination are further compounded by the fact that unmyelinated axons are more susceptible to damage[34,90]. Following injury, many axons are left unmyelinated, leaving axons near the site of injury at a greater risk of future impairment. Independent of myelination, oligodendrocytes also play a supportive role in axonal function and survival, which further compounds the detriment associated with their loss[91-93].

G. Endogenous Oligodendrogenesis after Brain Injury: A straightforward potential treatment for DAI and demyelination is remyelination. Unfortunately injured and mature oligodendrocytes are incapable of forming new myelin sheets[94,95]. However, as part of the brain's inherent repair mechanisms, levels of activated NSPCs and NG2-positive oligodendrocyte precursor cells have been shown to increase following a variety of insults including traumatic, seizure-inducing, ischemic, and demyelinating brain injuries[74,96-98]. Furthermore, increased cell proliferation in the adult subventricular zone (SVZ) has been demonstrated in a number of rodent models of TBI including the lateral fluid percussion injury[99-101], closed-head weight drop injury[102], acceleration-impact injury[103], and controlled cortical impact model[99,104]. A proportion of these activated NSPCs are OPCs, which under normal conditions, serve to myelinate previously unmyelinated axons in both the white and grey matter of the brain[105-107], but in the case of neuronal injury, they attempt to remyelinate injured axons. Consequently, following injury a limited number of OPCs proliferate, are recruited to the site of injury, and differentiate into myelinating oligodendrocytes[89]. Unfortunately, endogenous myelin repair yields minimal results due to the relatively small number of OPCs produced and the fact that increased rates of proliferation rapidly decline following injury[108].

H. Regulation of outgrowth and neuritogenesis: Neurite outgrowth or neuritogenesis and its inhibition are under balanced control during neurodevelopment[109]. Disruption of this control occurs in pathological states of the adult CNS. After injury, axonal regeneration is inhibited in the adult mammalian CNS. CNS myelin inhibits outgrowth because it contains several growth-inhibitory factors, including myelin-associated

glycoprotein (MAG), oligodendrocyte myelin glycoprotein, Nogo, and chondroitin sulfate proteoglycans[110-112].

Among inhibitory genes, the RhoA-ROCK pathway is critical to the control of neurite outgrowth. RhoA activation leads to growth cone collapse and neurite inhibition in many cells and primary neurons[113]. Inhibiting RhoA, using C3 transferase, miR-133b overexpression, or a dominant-negative approach, promotes neurite outgrowth, and this has been observed even in the presence of inhibitory factors[114-117]. Previous reports have shown that ROCK inhibitors C3 transferase and Y-27632 can promote axonal regeneration in spinal cord injury and cortico-spinal tract lesion[111,115]. Inhibiting ROCK with Y-27632 promotes neurite outgrowth and protects neurons from excitotoxicity-induced death[118]. Inhibition of RhoA/ROCK pathway has also shown potential to induce neural differentiation[119,120]. Additionally, a recent transcriptomic profiling study suggests that RhoA inhibition might serve as a neuroprotective treatment via the inhibition of glial scar formation. Their investigation revealed that application of a Rho kinase inhibitor, fasudil, to astrocytes results in extensive changes to gene expression in biological processes regulating cellular shape and motility, and increased excitatory amino acid transporter 2 expression[121].

In recent years, stem cells and stem cell-derived neural progenitor cells have emerged as a regenerative medicine for TBI, stroke, Parkinson's disease and other neurological disorders. Consequently, strategies that promote axonal outgrowth and neuronal differentiation appear to have promising benefits in cell-based therapy. Based on the above information, we recently explored whether RhoA inhibition can affect neuritogenesis and differentiation of neural stem cells[122]. In neural stem cells (NSCs)

isolated from the subventricular zone (SVZ) of the mouse, the Rho inhibitor C3 transferase was tested. Western blot analysis showed that C3 treatment had no effect on mitogen-activated protein kinase (MAPK) or protein kinase B (Akt) expression levels, but significantly increased phospho-Akt and phospho-MAPK in NSCs. MAP-2 and NF-L, mature neuron markers, significantly increased in C3-treated NSC cultures compared to untreated controls that underwent the same neuronal differentiation protocol. Markers of astrocytes (GFAP) and oligodendrocytes (myelin basic protein and proteolipid protein) were not significantly changed following C3 treatment. Interestingly, pretreatment of NSCs with C3 transferase dramatically increased neurite outgrowth during the consequent period of neuronal differentiation. Neurite outgrowth of NSCs is normally reduced when NSCs are cultured on myelin substrate, however we demonstrate that myelin-induced inhibition was diminished by C3 transferase. Six days post plating the neurite length of C3-pretreated cells was 3 times longer than those of control cells on myelin substrate. Consistently, the ROCK inhibitor Y-27632 increased neurite outgrowth of NSCs[122]. Similarly, Lim *et al.* has also explored the role of Rho/ROCK inhibition on neuronal induction. Their results indicate that inhibition via Y-27632 can block the inhibitory effect of chrondroiten sulfate proteoglycan on neuronal induction of mesenchymal stromal/stem cells, more specifically on the necessary morphological changes that occur during neuronal induction. Additionally, pretreatment with Y-27632 resulted in increased, although not significant, levels of neurite-like structures[120]. Together, these results support that the Rho signaling pathway plays an important role in neurite development, neuronal differentiation, and migration.

I. Cellular Therapy and Preconditioning Strategy of Transplanted Cells: Widespread cell loss in the CNS represents a challenging clinical problem, primarily due to damage to irreplaceable circuitry and the permanent loss of neuronal substrates. Recent

advancements in cellular therapies have proven stem cell therapy to be an effective means of treating CNS related injuries[123]. Cell replacement using exogenous cells has been shown to bolster trophic support to host tissues and promote functional recovery following CNS injury[124]. Furthermore, transplantation of stem cells aids in fortification of endogenous neurogenesis and angiogenesis, both of which are critical for sub-acute and chronic recovery from stroke which similarly results in massive cell loss in the CNS[125,126]. More recently, challenged by many failures of neuroprotective treatments in clinical trials, research attention has been directed toward tailoring cellular therapies for neurorestoration. Compelling evidence now demonstrates that cell transplantation can provide much needed trophic support to the post-injury brain and specific cell types needed for brain tissue repair.

A major and serious problem in cell transplantation therapy is the low survival rate of transplanted cells in the hazardous post-injury environment[127-129]. About 70-90% of implanted neurons in the striatum die after transplantation into the brain[127,130], and the majority of mouse embryonic stem (ES) cells die after transplantation into the injured spinal cord[131]. Endogenous and environmental factors, such as hypoxia/ischemia and reactive oxygen species induction, may be contributing factors. Apoptosis has been identified as an important mechanism resulting in loss of these cells[128]. The poor survival of implanted cells has severely hampered the efficiency and application of stem cell therapy. To resolve this issue and promote the therapeutic potential of cell transplantation, we and others have developed a number of approaches to enhance the tolerance and regenerative properties of transplanted cells. It was found that exposure to sub-lethal hypoxia or ischemia alters gene expression and activates intracellular signaling pathways favorable for cell survival and regenerative processes[132]. These protective effects have been demonstrated *in vitro* and *in vivo*[133]. For example, following hypoxic

preconditioning (HP), expression of hypoxia-inducible factor-1α (HIF-1α) is upregulated, conferring cytoprotective and angiogenic effects. Hypoxia has also been found to prolong the half-life of several mRNAs, such as HIF-1α, vascular endothelial growth factor (VEGF) and erythropoietin (EPO)[134,135]. Chronic hypoxia has also been shown to stimulate and maintain angiogenesis in the postnatal developing brain[136]. We and others have shown that hypoxic preconditioning of mouse or human bone marrow mesenchymal stem cells (BMSCs), ES cell, and iPS cell-derived NPCs prior to transplantation allows for greater survival and engraftment of these cells into the ischemic heart and brain of adult rats[137-141]. HP-primed mouse and/or human ES cells show up-regulated HIF-1α and EPO signaling and increased expression/secretion of growth and survival factors[137,139,142]. A multitude of preconditioning strategies including anoxia, carbon monoxide, and hydrogen sulfide along with non-hypoxic preconditioning using stromal-derived factor-1 (SDF-1) and insulin-like growth factor-1 (IGF-1) have also been explored with promising results[143-145]. Thus, the preconditioning strategy for graft cells is likely an effective and feasible means of promoting cell survival and regeneration including axonal outgrowth after transplantation into the ischemic and TBI brain.

J. Stem Cell Derived Oligodendrogenesis: The brain's natural response is insufficient to repair the demyelination caused by DAI despite endogenous upregulation of OPCs following injury. No drugs in clinical trials have demonstrated effective neuroprotection against DAI[15,40]. The difficulty in developing effective treatments is widely attributed to the heterogeneity of TBI pathology and variability in patient ancillary treatments[146-149]. Exploration of alternative therapeutic options remains imperative[15,40]. One such

promising approach to ameliorate the loss of white matter following injury is through enhancing regeneration via stem cell therapy.

Using self-renewable pluripotent stem cells is one promising method of generating new oligodendrocytes. A breadth of research has already illustrated that stem cells are able to survive and differentiate into neurons following implantation into rodent models of TBI[150-152] and that stem cell implantation is associated with improved motor function[151] and cognition[152]. More recently, successful intravenous and intracerebral administration of marrow stromal cells, multipotent cells from bone marrow and adipose tissue, have been shown to improve functional outcomes[150,153,154] and to promote endogenous cellular proliferation following TBI[155]. Thus, it is believed that NSPCs may serve as a therapeutic means of repairing DAI associated demyelination.

Few investigators have examined the potential for oligodendrocyte generation in the CNS following TBI via stem cell therapy. In contrast, cell based approaches have been thoroughly explored in the field of spinal cord injury (SCI). Research has demonstrated that implanted human embryonic stem cell (hESC) derived oligodendrocytes can survive, remyelinate injured axons[156-159], and improve functional recovery following SCI[160,161]. Liu *et al*. demonstrated this in an *in vitro* study in which retinoic acid-induced embryonic stem cells generated oligodendrocytes capable of myelinating an adult rat chemically demyelinated spinal cord and a myelin-deficient shirerer (shi/shi) mutant mouse[156]. Similarly, Yasuda *et al.* provided evidence that oligodendrocytes derived from grafted NSPCs are able to myelinate, and that this remyelination is associated with locomotor and electrophysiological recovery[159]. Other studies have focused on the most appropriate time-point to implant OPCs. Karimi-Abdolrezaee *et al.* transplanted OPCs at either two (subacute) or eight (chronic) weeks post-injury with a number of growth factors including

minocycline, an anti-inflammatory, and cyclosporine A, an immunosuppressant, following an aneurysm clip compression model of SCI. Investigators were able to differentiate oligodendrocytes with more success at 2-weeks than at 8-weeks post-SCI. Differential success was attributed to the presence of inhibitory obstacles in the spinal cord environment which may have influenced OPC survival and migration[162]. This data, and that gathered by other investigators, may help determine the most beneficial time point for stem cell implantation.

Despite its relative novelty, there have been a few studies in which stem cell therapy was utilized to repair DAI associated white matter damage. Xu *et al*. has provided promising support for the future use of stem cell generated oligodendrocytes in the treatment of DAI. The authors demonstrated that transplanted human OPCs are capable of replacing and/or remodeling myelin, and contribute to axonal regeneration following DAI[163]. Thus, this study provides support that stem cell derived oligodendrocytes may also be utilized in the treatment of DAI. Recently, Yang *et al*., showed that OPCs can be induced from mouse/rat fibroblasts via direct lineage conversion as a result of reprogramming following exposure to transcription factors including Sox2, Oligo2, and Zfp536[164]. Additionally, Braun *et al.* showed that hippocampal NSCs can be differentiated via Ascl1-mediated conversion into mature oligodendrocytes, which were shown to enhanced remyelination in a diphtheria-toxin induced genetic model of demyelination[165].

K. Neurotrophic Factors Provide Trophic Support to Endogenous Neural Stem Cells: When using stem cell derived oligodendrocytes as a neurorestorative treatment for TBI, it is crucial to consider the influence of neurotrophic factors on oligodendrocyte biogenesis, survival, and proliferation. There is evidence to support the idea that the improved functional outcomes observed following stem cell therapy in TBI models may

be partially attributed to endogenous release of neurotrophic factors[166,167]. One such study by Mahmood *et al.* showed that following marrow stromal cell (MSC) administration, only 4-7% of grafted stem cells expressed a neuronal phenotype, yet functional outcomes were significantly improved[155]. It was also observed that following MSC application, significant increases in both brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) were detected. This led investigators to draw the conclusion that MSC are unlikely to be solely responsible for the neurological improvements associated with MSC administration, and that instead, the functional improvements observed might be partially due to endogenous release of BDNF and NGF[166].

Since the recognition of endogenous release of BDNF and NGF in conjunction with stem cell transplantation[100,102,104], the release of other endogenous neurotrophic factors by OPCs and oligodendrocytes including NGF glial-cell-line-derived neurotrophic factor, neurotrophin-3, midkine, hepatocyte growth factor, activin A, and transforming growth factor-β2 have been noted[152,168]. Release of these trophic factors differentially promotes survival, differentiation, maturation, and proliferation of hESC derived neurons and/or oligodendrocytes, and are associated with improved functional outcomes following TBI[169-171]. Therefore, application of neurotrophic factors may prove to be a critical component for success in the generation of oligodendrocytes and myelin via stem cell therapy.

L. Conclusion: TBI is an epidemic that affects millions worldwide. One of the most detrimental aspects of TBI is its associated DAI, which leads to extensive axonal abnormalities and demyelination, and accordingly signal transmission deficits and network processing disturbances. Despite our increasing knowledge of TBI's

pathobiology, there are currently no clinically relevant treatments available. It is likely that advancements in the use of stem cell derived oligodendrocytes in the treatment of spinal cord injury, and the known beneficial effects of neurotrophic factors on oligodendrocyte biogenesis, may provide the basis for the development of future neurorestorative TBI treatments.

References

1 Consensus conference. Rehabilitation of persons with traumatic brain injury. NIH Consensus Development Panel on Rehabilitation of Persons With Traumatic Brain Injury. *Jama* **282**, 974-983 (1999).

2 Goldstein, M. Traumatic brain injury: a silent epidemic. *Annals of neurology* **27**, 327, doi:10.1002/ana.410270315 (1990).

3 Chelly, H. *et al.* Diffuse axonal injury in patients with head injuries: an epidemiologic and prognosis study of 124 cases. *The Journal of trauma* **71**, 838-846, doi:10.1097/TA.0b013e3182127baa (2011).

4 Langlois, J. A. & Sattin, R. W. Traumatic brain injury in the United States: research and programs of the Centers for Disease Control and Prevention (CDC). *The Journal of head trauma rehabilitation* **20**, 187-188 (2005).

5 Saatman, K. E. *et al.* Classification of traumatic brain injury for targeted therapies. *Journal of neurotrauma* **25**, 719-738, doi:10.1089/neu.2008.0586 (2008).

6 Hicks, R., Soares, H., Smith, D. & McIntosh, T. Temporal and spatial characterization of neuronal injury following lateral fluid-percussion brain injury in the rat. *Acta neuropathologica* **91**, 236-246 (1996).

7 Golding, E. M. Sequelae following traumatic brain injury - The cerebrovascular perspective. *Brain Res Rev* **38**, 377-388, doi:Pii S0165-0173(02)00141-8 Doi 10.1016/S0165-0173(02)00141-8 (2002).

8 Meythaler, J. M., Peduzzi, J. D., Eleftheriou, E. & Novack, T. A. Current concepts: diffuse axonal injury-associated traumatic brain injury. *Archives of physical medicine and rehabilitation* **82**, 1461-1471 (2001).

9 Thompson, H. J. *et al.* Lateral fluid percussion brain injury: a 15-year review and evaluation. *Journal of neurotrauma* **22**, 42-75, doi:10.1089/neu.2005.22.42 (2005).

10 Smith, D. H. & Meaney, D. F. Axonal damage in traumatic brain injury. *Neuroscientist* **6**, 483-495 (2000).

11 Adams, J. H. *et al.* Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology* **15**, 49-59 (1989).

12 Bonnelle, V. *et al.* Salience network integrity predicts default mode network function after traumatic brain injury. *P Natl Acad Sci USA* **109**, 4690-4695, doi:Doi 10.1073/Pnas.1113455109 (2012).

13 Inglese, M. *et al.* Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. *Journal of neurosurgery* **103**, 298-303, doi:10.3171/jns.2005.103.2.0298 (2005).

14 Povlishock, J. T. & Katz, D. I. Update of neuropathology and neurological recovery after traumatic brain injury. *The Journal of head trauma rehabilitation* **20**, 76-94 (2005).

15 Johnson, V. E., Stewart, W. & Smith, D. H. Axonal pathology in traumatic brain injury. *Experimental neurology* **246**, 35-43, doi:10.1016/j.expneurol.2012.01.013 (2013).

16 Gage, F. H., Kempermann, G., Palmer, T. D., Peterson, D. A. & Ray, J. Multipotent progenitor cells in the adult dentate gyrus. *Journal of neurobiology* **36**, 249-266 (1998).

17 Browne, K. D., Chen, X. H., Meaney, D. F. & Smith, D. H. Mild traumatic brain injury and diffuse axonal injury in swine. *Journal of neurotrauma* **28**, 1747-1755, doi:10.1089/neu.2011.1913 (2011).

18 Adams, J. H., Graham, D. I., Murray, L. S. & Scott, G. Diffuse axonal injury due

to nonmissile head injury in humans: an analysis of 45 cases. *Annals of neurology* **12**, 557-563, doi:10.1002/ana.410120610 (1982).

19 Gennarelli, T. A. *et al.* Diffuse axonal injury and traumatic coma in the primate. *Annals of neurology* **12**, 564-574, doi:10.1002/ana.410120611 (1982).

20 Tang-Schomer, M. D., Patel, A. R., Baas, P. W. & Smith, D. H. Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **24**, 1401-1410, doi:10.1096/fj.09-142844 (2010).

21 Smith, R. Experimental models suggest therapies for human diabetes. *Lancet* **347**, 1175 (1996).

22 Maas, A. I., Stocchetti, N. & Bullock, R. Moderate and severe traumatic brain injury in adults. *The Lancet. Neurology* **7**, 728-741, doi:10.1016/S1474-4422(08)70164-9 (2008).

23 Kawai, N. *et al.* Focal neuronal damage in patients with neuropsychological impairment after diffuse traumatic brain injury: evaluation using (1)(1)C-flumazenil positron emission tomography with statistical image analysis. *Journal of neurotrauma* **27**, 2131-2138, doi:10.1089/neu.2010.1464 (2010).

24 Barkhoudarian, G., Hovda, D. A. & Giza, C. C. The molecular pathophysiology of concussive brain injury. *Clinics in sports medicine* **30**, 33-48, vii-iii, doi:10.1016/j.csm.2010.09.001 (2011).

25 Zhang, Y. P. *et al.* Traumatic brain injury using mouse models. *Transl Stroke Res* **5**, 454-471, doi:10.1007/s12975-014-0327-0 (2014).

26 Farkas, O., Lifshitz, J. & Povlishock, J. T. Mechanoporation induced by diffuse traumatic brain injury: an irreversible or reversible response to injury? *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 3130-3140, doi:10.1523/JNEUROSCI.5119-05.2006 (2006).

27 Faden, A. I., Demediuk, P., Panter, S. S. & Vink, R. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science* **244**, 798-800 (1989).

28 Katayama, Y., Becker, D. P., Tamura, T. & Hovda, D. A. Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. *Journal of neurosurgery* **73**, 889-900, doi:10.3171/jns.1990.73.6.0889 (1990).

29 Werner, C. & Engelhard, K. Pathophysiology of traumatic brain injury. *British journal of anaesthesia* **99**, 4-9, doi:10.1093/bja/aem131 (2007).

30 Xiong, Y., Gu, Q., Peterson, P. L., Muizelaar, J. P. & Lee, C. P. Mitochondrial dysfunction and calcium perturbation induced by traumatic brain injury. *Journal of neurotrauma* **14**, 23-34 (1997).

31 Juurlink, B. H. & Paterson, P. G. Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. *The journal of spinal cord medicine* **21**, 309-334 (1998).

32 Farkas, O. & Povlishock, J. T. Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage. *Progress in brain research* **161**, 43-59, doi:10.1016/S0079-6123(06)61004-2 (2007).

33 Kermer, P., Klocker, N. & Bahr, M. Neuronal death after brain injury. Models, mechanisms, and therapeutic strategies in vivo. *Cell and tissue research* **298**, 383-395 (1999).

34 Reeves, T. M., Phillips, L. L. & Povlishock, J. T. Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery

following traumatic brain injury. *Experimental neurology* **196**, 126-137, doi:10.1016/j.expneurol.2005.07.014 (2005).

35 Staal, J. A. & Vickers, J. C. Selective vulnerability of non-myelinated axons to stretch injury in an in vitro co-culture system. *Journal of neurotrauma* **28**, 841-847, doi:10.1089/neu.2010.1658 (2011).

36 McKee, A. C., Daneshvar, D. H., Alvarez, V. E. & Stein, T. D. The neuropathology of sport. *Acta neuropathologica* **127**, 29-51, doi:10.1007/s00401-013-1230-6 (2014).

37 Smith, D. H., Wolf, J. A., Lusardi, T. A., Lee, V. M. & Meaney, D. F. High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **19**, 4263-4269 (1999).

38 Smith, D. H. *et al.* Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. *Journal of neuropathology and experimental neurology* **58**, 982-992 (1999).

39 Christman, C. W., Grady, M. S., Walker, S. A., Holloway, K. L. & Povlishock, J. T. Ultrastructural studies of diffuse axonal injury in humans. *Journal of neurotrauma* **11**, 173-186 (1994).

40 Smith, D. H., Hicks, R. & Povlishock, J. T. Therapy development for diffuse axonal injury. *Journal of neurotrauma* **30**, 307-323, doi:10.1089/neu.2012.2825 (2013).

41 Imajo, T. & Roessman, U. Diffuse axonal injury. *The American journal of forensic medicine and pathology* **5**, 217-222 (1984).

42 Smith, D. H., Meaney, D. F. & Shull, W. H. Diffuse axonal injury in head trauma. *The Journal of head trauma rehabilitation* **18**, 307-316 (2003).

43 Corbo, J. & Tripathi, P. Delayed presentation of diffuse axonal injury: a case report. *Annals of emergency medicine* **44**, 57-60, doi:10.1016/S0196064403012095 (2004).

44 Takaoka, M. *et al.* Semiquantitative analysis of corpus callosum injury using magnetic resonance imaging indicates clinical severity in patients with diffuse axonal injury. *Journal of neurology, neurosurgery, and psychiatry* **73**, 289-293 (2002).

45 Cecil, K. M. *et al.* Proton magnetic resonance spectroscopy for detection of axonal injury in the splenium of the corpus callosum of brain-injured patients. *Journal of neurosurgery* **88**, 795-801, doi:10.3171/jns.1998.88.5.0795 (1998).

46 Arfanakis, K. *et al.* Diffusion tensor MR imaging in diffuse axonal injury. *Am J Neuroradiol* **23**, 794-802 (2002).

47 Levin, H. S. *et al.* Diffusion tensor imaging of mild to moderate blast-related traumatic brain injury and its sequelae. *Journal of neurotrauma* **27**, 683-694, doi:10.1089/neu.2009.1073 (2010).

48 Sugiyama, K. *et al.* Clinical utility of diffusion tensor imaging for evaluating patients with diffuse axonal injury and cognitive disorders in the chronic stage. *Journal of neurotrauma* **26**, 1879-1890, doi:10.1089/neu.2008-0839 10.1089/neu.2008.0839. (2009).

49 Smits, M. *et al.* Microstructural brain injury in post-concussion syndrome after minor head injury. *Neuroradiology* **53**, 553-563, doi:10.1007/s00234-010-0774-6 (2011).

50 Scheid, R., Preul, C., Gruber, O., Wiggins, C. & von Cramon, D. Y. Diffuse axonal injury associated with chronic traumatic brain injury: evidence from T2*-weighted gradient-echo imaging at 3 T. *AJNR. American journal of neuroradiology* **24**, 1049-1056 (2003).

51 Beauchamp, M. H. *et al.* Detecting traumatic brain lesions in children: CT versus MRI versus susceptibility weighted imaging (SWI). *Journal of neurotrauma* **28**, 915-927, doi:10.1089/neu.2010.1712 (2011).

52 Chastain, C. A. *et al.* Predicting outcomes of traumatic brain injury by imaging modality and injury distribution. *Journal of neurotrauma* **26**, 1183-1196, doi:10.1089/neu.2008.0650 (2009).

53 Haacke, E. M., Xu, Y., Cheng, Y. C. & Reichenbach, J. R. Susceptibility weighted imaging (SWI). *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine* **52**, 612-618, doi:10.1002/mrm.20198 (2004).

54 Mittal, S., Wu, Z., Neelavalli, J. & Haacke, E. M. Susceptibility-weighted imaging: technical aspects and clinical applications, part 2. *AJNR. American journal of neuroradiology* **30**, 232-252, doi:10.3174/ajnr.A1461 (2009).

55 Herrmann, M. *et al.* Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as demonstrated in cranial computerized tomography. *Journal of neurotrauma* **17**, 113-122 (2000).

56 Pleines, U. E. *et al.* S-100 beta reflects the extent of injury and outcome, whereas neuronal specific enolase is a better indicator of neuroinflammation in patients with severe traumatic brain injury. *Journal of neurotrauma* **18**, 491-498, doi:10.1089/089771501300227297 (2001).

57 Savola, O. *et al.* Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. *The Journal of trauma* **56**, 1229-1234; discussion 1234 (2004).

58 Li, X. Y. & Feng, D. F. Diffuse axonal injury: novel insights into detection and treatment. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia* **16**, 614-619, doi:10.1016/j.jocn.2008.08.005 (2009).

59 de Kruijk, J. R., Leffers, P., Menheere, P. P., Meerhoff, S. & Twijnstra, A. S-100B and neuron-specific enolase in serum of mild traumatic brain injury patients. A comparison with health controls. *Acta neurologica Scandinavica* **103**, 175-179 (2001).

60 Koo, E. H. *et al.* Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proc Natl Acad Sci U S A* **87**, 1561-1565 (1990).

61 Ng, H. K., Mahaliyana, R. D. & Poon, W. S. The pathological spectrum of diffuse axonal injury in blunt head trauma: assessment with axon and myelin strains. *Clinical neurology and neurosurgery* **96**, 24-31 (1994).

62 Sherriff, F. E., Bridges, L. R. & Sivaloganathan, S. Early detection of axonal injury after human head trauma using immunocytochemistry for beta-amyloid precursor protein. *Acta neuropathologica* **87**, 55-62 (1994).

63 Li, G. L., Farooque, M., Holtz, A. & Olsson, Y. Changes of beta-amyloid precursor protein after compression trauma to the spinal cord: an experimental study in the rat using immunohistochemistry. *Journal of neurotrauma* **12**, 269-277 (1995).

64 Hortobagyi, T. *et al.* Traumatic axonal damage in the brain can be detected using beta-APP immunohistochemistry within 35 min after head injury to human adults. *Neuropathology and applied neurobiology* **33**, 226-237, doi:10.1111/j.1365-2990.2006.00794.x (2007).

65 Oyler, G. A. *et al.* The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *The Journal of cell biology* **109**, 3039-3052 (1989).

66 Sollner, T. *et al.* SNAP receptors implicated in vesicle targeting and fusion. *Nature* **362**, 318-324, doi:10.1038/362318a0 (1993).
67 Sherriff, F. E., Bridges, L. R., Gentleman, S. M., Sivaloganathan, S. & Wilson, S. Markers of axonal injury in post mortem human brain. *Acta neuropathologica* **88**, 433-439 (1994).
68 Winkler, H. & Fischer-Colbrie, R. The chromogranins A and B: the first 25 years and future perspectives. *Neuroscience* **49**, 497-528 (1992).
69 Grady, M. S. *et al.* The use of antibodies targeted against the neurofilament subunits for the detection of diffuse axonal injury in humans. *Journal of neuropathology and experimental neurology* **52**, 143-152 (1993).
70 Posmantur, R., Hayes, R. L., Dixon, C. E. & Taft, W. C. Neurofilament 68 and neurofilament 200 protein levels decrease after traumatic brain injury. *Journal of neurotrauma* **11**, 533-545 (1994).
71 Xiaoshengi, H., Guitao, Y., Xiang, Z. & Zhou, F. A morphological study of diffuse axonal injury in a rat model by lateral head rotation trauma. *Acta neurologica Belgica* **110**, 49-56 (2010).
72 He, X. *et al.* Content change of neurofilament protein subunits in experimental brain diffuse axonal injury by lateral head rotation. *Chinese journal of traumatology = Zhonghua chuang shang za zhi / Chinese Medical Association* **3**, 45-49 (2000).
73 Smith, C. *et al.* The neuroinflammatory response in humans after traumatic brain injury. *Neuropathology and applied neurobiology* **39**, 654-666, doi:10.1111/nan.12008 (2013).
74 Zhang, R., Chopp, M. & Zhang, Z. G. Oligodendrogenesis after cerebral ischemia. *Frontiers in cellular neuroscience* **7**, 201, doi:10.3389/fncel.2013.00201 (2013).
75 Ludwin, S. K. The pathobiology of the oligodendrocyte. *Journal of neuropathology and experimental neurology* **56**, 111-124 (1997).
76 Johnson, V. E. *et al.* Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain : a journal of neurology* **136**, 28-42, doi:10.1093/brain/aws322 (2013).
77 Fawcett, J. W. & Asher, R. A. The glial scar and central nervous system repair. *Brain research bulletin* **49**, 377-391 (1999).
78 Wen, L. *et al.* The occurrence of diffuse axonal injury in the brain: associated with the accumulation and clearance of myelin debris. *Neural regeneration research* **9**, 1902-1906, doi:10.4103/1673-5374.145358 (2014).
79 Horner, P. J. *et al.* Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **20**, 2218-2228 (2000).
80 Franklin, R. J. Why does remyelination fail in multiple sclerosis? *Nature reviews. Neuroscience* **3**, 705-714, doi:10.1038/nrn917 (2002).
81 Robinson, S. & Miller, R. H. Contact with central nervous system myelin inhibits oligodendrocyte progenitor maturation. *Developmental biology* **216**, 359-368, doi:10.1006/dbio.1999.9466 (1999).
82 Wolswijk, G. Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **18**, 601-609 (1998).
83 Kotter, M. R., Li, W. W., Zhao, C. & Franklin, R. J. Myelin impairs CNS remyelination by inhibiting oligodendrocyte precursor cell differentiation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**,

328-332, doi:10.1523/JNEUROSCI.2615-05.2006 (2006).

84 Kotter, M. R., Zhao, C., van Rooijen, N. & Franklin, R. J. Macrophage-depletion induced impairment of experimental CNS remyelination is associated with a reduced oligodendrocyte progenitor cell response and altered growth factor expression. *Neurobiology of disease* **18**, 166-175, doi:10.1016/j.nbd.2004.09.019 (2005).

85 McTigue, D. M. & Tripathi, R. B. The life, death, and replacement of oligodendrocytes in the adult CNS. *Journal of neurochemistry* **107**, 1-19, doi:10.1111/j.1471-4159.2008.05570.x (2008).

86 McDonald, J. W., Althomsons, S. P., Hyrc, K. L., Choi, D. W. & Goldberg, M. P. Oligodendrocytes from forebrain are highly vulnerable to AMPA/kainate receptor-mediated excitotoxicity. *Nat Med* **4**, 291-297, doi:Doi 10.1038/Nm0398-291 (1998).

87 Salter, M. G. & Fern, R. NMDA receptors are expressed in developing oligodendrocyte processes and mediate injury. *Nature* **438**, 1167-1171, doi:Doi 10.1038/Nature04301 (2005).

88 Nave, K. A. & Trapp, B. D. Axon-glial signaling and the glial support of axon function. *Annual review of neuroscience* **31**, 535-561, doi:10.1146/annurev.neuro.30.051606.094309 (2008).

89 Kou, Z. & VandeVord, P. J. Traumatic white matter injury and glial activation: from basic science to clinics. *Glia* **62**, 1831-1855, doi:10.1002/glia.22690 (2014).

90 Langham, J., Goldfrad, C., Teasdale, G., Shaw, D. & Rowan, K. Calcium channel blockers for acute traumatic brain injury. *The Cochrane database of systematic reviews*, CD000565, doi:10.1002/14651858.CD000565 (2000).

91 Nave, K. A. OPINION Myelination and the trophic support of long axonss. *Nature Reviews Neuroscience* **11**, 275-283, doi:Doi 10.1038/Nrn2797 (2010).

92 Funfschilling, U. *et al.* Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature* **485**, 517-U130, doi:Doi 10.1038/Nature11007 (2012).

93 Morrison, B. M., Lee, Y. & Rothstein, J. D. Oligodendroglia: metabolic supporters of axons. *Trends Cell Biol* **23**, 644-651, doi:Doi 10.1016/J.Tcb.2013.07.007 (2013).

94 Gensert, J. M. & Goldman, J. E. Endogenous progenitors remyelinate demyelinated axons in the adult CNS. *Neuron* **19**, 197-203 (1997).

95 Franklin, R. J. M. & Ffrench-Constant, C. Remyelination in the CNS: from biology to therapy. *Nature Reviews Neuroscience* **9**, 839-855, doi:Doi 10.1038/Nrn2480 (2008).

96 Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z. & Lindvall, O. Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nat Med* **8**, 963-970, doi:Doi 10.1038/Nm747 (2002).

97 Parent, J. M., Valentin, V. V. & Lowenstein, D. H. Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. *Journal of Neuroscience* **22**, 3174-3188 (2002).

98 Picard-Riera, N. *et al.* Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *P Natl Acad Sci USA* **99**, 13211-13216, doi:Doi 10.1073/Pnas.192314199 (2002).

99 Chirumamilla, S., Sun, D., Bullock, M. R. & Colello, R. J. Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. *Journal of neurotrauma* **19**, 693-703, doi:Doi 10.1089/08977150260139084 (2002).

100 Urrea, C. *et al.* Widespread cellular proliferation and focal neurogenesis after traumatic brain injury in the rat. *Restor Neurol Neuros* **25**, 65-76 (2007).
101 Bye, N. *et al.* Neurogenesis and Glial Proliferation Are Stimulated Following Diffuse Traumatic Brain Injury in Adult Rats. *J Neurosci Res* **89**, 986-1000, doi:Doi 10.1002/Jnr.22635 (2011).
102 Villasana, L. E., Westbrook, G. L. & Schnell, E. Neurologic impairment following closed head injury predicts post-traumatic neurogenesis. *Experimental neurology* **261**, 156-162, doi:10.1016/j.expneurol.2014.05.016 (2014).
103 Bye, N. *et al.* Neurogenesis and glial proliferation are stimulated following diffuse traumatic brain injury in adult rats. *J Neurosci Res* **89**, 986-1000, doi:10.1002/jnr.22635 (2011).
104 Theus, M. H., Ricard, J., Bethea, J. R. & Liebl, D. J. EphB3 Limits the Expansion of Neural Progenitor Cells in the Subventricular Zone by Regulating p53 During Homeostasis and Following Traumatic Brain Injury. *Stem cells* **28**, 1231-1242, doi:Doi 10.1002/Stem.449 (2010).
105 Fancy, S. P., Chan, J. R., Baranzini, S. E., Franklin, R. J. & Rowitch, D. H. Myelin regeneration: a recapitulation of development? *Annual review of neuroscience* **34**, 21-43, doi:10.1146/annurev-neuro-061010-113629 (2011).
106 Young, K. M. *et al.* Oligodendrocyte dynamics in the healthy adult CNS: evidence for myelin remodeling. *Neuron* **77**, 873-885, doi:10.1016/j.neuron.2013.01.006 (2013).
107 Fields, R. D. White matter in learning, cognition and psychiatric disorders. *Trends in neurosciences* **31**, 361-370, doi:10.1016/j.tins.2008.04.001 (2008).
108 Fleiss, B. *et al.* Impact of Injured Tissue on Stem Cell Fate. *Stem Cells Biol Reg*, 43-56, doi:Doi 10.1007/978-1-4899-7399-3_3 (2014).
109 Skaper, S. D. Neuronal growth-promoting and inhibitory cues in neuroprotection and neuroregeneration. *Annals of the New York Academy of Sciences* **1053**, 376-385, doi:10.1196/annals.1344.032 (2005).
110 Mellough, C. B., Cho, S., Wood, A. & Przyborski, S. Neurite formation by neurons derived from adult rat hippocampal progenitor cells is susceptible to myelin inhibition. *Neurochemistry international* **59**, 333-340, doi:10.1016/j.neuint.2011.01.015 (2011).
111 Schmandke, A., Schmandke, A. & Strittmatter, S. M. ROCK and Rho: biochemistry and neuronal functions of Rho-associated protein kinases. *Neuroscientist* **13**, 454-469, doi:10.1177/1073858407303611 (2007).
112 Kubo, T. & Yamashita, T. Rho-ROCK inhibitors for the treatment of CNS injury. *Recent patents on CNS drug discovery* **2**, 173-179 (2007).
113 Gallo, G. Myosin II activity is required for severing-induced axon retraction in vitro. *Experimental neurology* **189**, 112-121, doi:10.1016/j.expneurol.2004.05.019 (2004).
114 Yuan, X. B. *et al.* Signalling and crosstalk of Rho GTPases in mediating axon guidance. *Nature cell biology* **5**, 38-45, doi:10.1038/ncb895 (2003).
115 Lu, X. C. *et al.* MiR-133b Promotes neurite outgrowth by targeting RhoA expression. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* **35**, 246-258, doi:10.1159/000369692 (2015).
116 Lingor, P. *et al.* Inhibition of Rho kinase (ROCK) increases neurite outgrowth on chondroitin sulphate proteoglycan in vitro and axonal regeneration in the adult optic nerve in vivo. *Journal of neurochemistry* **103**, 181-189, doi:10.1111/j.1471-4159.2007.04756.x (2007).
117 Gopalakrishnan, S. M. *et al.* Role of Rho kinase pathway in chondroitin sulfate

proteoglycan-mediated inhibition of neurite outgrowth in PC12 cells. *J Neurosci Res* **86**, 2214-2226, doi:10.1002/jnr.21671 (2008).

118 Jeon, B. T. *et al.* The Rho-kinase (ROCK) inhibitor Y-27632 protects against excitotoxicity-induced neuronal death in vivo and in vitro. *Neurotoxicity research* **23**, 238-248, doi:10.1007/s12640-012-9339-2 (2013).

119 Niederost, B., Oertle, T., Fritsche, J., McKinney, R. A. & Bandtlow, C. E. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **22**, 10368-10376 (2002).

120 Lim, H. S. & Joe, Y. A. A ROCK Inhibitor Blocks the Inhibitory Effect of Chondroitin Sulfate Proteoglycan on Morphological Changes of Mesenchymal Stromal/Stem Cells into Neuron-Like Cells. *Biomolecules & therapeutics* **21**, 447-453, doi:10.4062/biomolther.2013.041 (2013).

121 Lau, C. L. *et al.* Transcriptomic profiling of astrocytes treated with the Rho kinase inhibitor fasudil reveals cytoskeletal and pro-survival responses. *Journal of cellular physiology* **227**, 1199-1211, doi:10.1002/jcp.22838 (2012).

122 Gu, H., Yu, S. P., Gutekunst, C. A., Gross, R. E. & Wei, L. Inhibition of the Rho signaling pathway improves neurite outgrowth and neuronal differentiation of mouse neural stem cells. *International journal of physiology, pathophysiology and pharmacology* **5**, 11-20 (2013).

123 Ogle, M. E., Yu, S. P. & Wei, L. Primed for lethal battle: A step forward to enhance the efficacy and efficiency of stem cell transplantation therapy. *The Journal of thoracic and cardiovascular surgery* **138**, 527 (2009).

124 Yu, S. P., Wei, Z. & Wei, L. Preconditioning strategy in stem cell transplantation therapy. *Translational stroke research* **4**, 76-88 (2013).

125 Li, Y., Yu, S. P., Mohamad, O., Genetta, T. & Wei, L. Sublethal transient global ischemia stimulates migration of neuroblasts and neurogenesis in mice. *Translational stroke research* **1**, 184-196 (2010).

126 Wei, L., Fraser, J. L., Lu, Z.-Y., Hu, X. & Yu, S. P. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiology of disease* **46**, 635-645 (2012).

127 Brundin, P. *et al.* Survival of intracerebrally grafted rat dopamine neurons previously cultured in vitro. *Neurosci Lett* **61**, 79-84 (1985).

128 Zhang, M. *et al.* Cardiomyocyte grafting for cardiac repair: graft cell death and anti-death strategies. *J Mol Cell Cardiol* **33**, 907-921 (2001).

129 Reinecke, H. & Murry, C. E. Cell grafting for cardiac repair. *Methods Mol Biol* **219**, 97-112 (2003).

130 Zawada, W. M. *et al.* Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. *Brain research* **786**, 96-103 (1998).

131 McDonald, J. W. *et al.* Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med* **5**, 1410-1412 (1999).

132 Anrather, J. & Hallenbeck, J. M. Biological networks in ischemic tolerance - rethinking the approach to clinical conditioning. *Transl Stroke Res* **4**, 114-129, doi:10.1007/s12975-012-0244-z (2013).

133 Keep, R. F., Wang, M. M., Xiang, J., Hua, Y. & Xi, G. Full steam ahead with remote ischemic conditioning for stroke. *Transl Stroke Res* **5**, 535-537, doi:10.1007/s12975-014-0363-9 (2014).

134 Jones, N. M. & Bergeron, M. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. *Journal of cerebral blood flow and*

metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism **21**, 1105-1114 (2001).

135 Paulding, W. R. & Czyzyk-Krzeska, M. F. Hypoxia-induced regulation of mRNA stability. *Adv Exp Med Biol* **475**, 111-121 (2000).

136 Taguchi, A. *et al.* Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J Clin Invest* **114**, 330-338 (2004).

137 Theus, M. H. *et al.* In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. *Experimental neurology* **210**, 656-670 (2008).

138 Wei, L., Keogh, C. L., Whitaker, V. R., Theus, M. H. & Yu, S. P. Angiogenesis and stem cell transplantation as potential treatments of cerebral ischemic stroke. *Pathophysiology* **12**, 47-62 (2005).

139 Hu, X. *et al.* Transplantation of hypoxia-preconditioned mesenchymal stem cells improves infarcted heart function via enhanced survival of implanted cells and angiogenesis. *J Thorac Cardiovasc Surg* **135**, 799-808 (2008).

140 Rosova, I., Dao, M., Capoccia, B., Link, D. & Nolta, J. A. Hypoxic Preconditioning Results in Increased Motility and Improved Therapeutic Potential of Human Mesenchymal Stem Cells. *Stem cells* (2008).

141 Wang, J. A. *et al.* Hypoxic preconditioning attenuates hypoxia/reoxygenation-induced apoptosis in mesenchymal stem cells. *Acta pharmacologica Sinica* **29**, 74-82, doi:10.1111/j.1745-7254.2008.00716.x (2008).

142 Francis, K., Wallace, G., Li, Y., Wei, L. & Yu, S. P. Novel use of hypoxic preconditioning to enhance the survival of human embryonic stem cells after transplantation to the ischemic brain. *Society for Neurosci. Abstr.* **113.3.** (2007).

143 Lu, D. Y., Mahmood, A., Zhang, R. L., Li, Y. & Chopp, M. Upregulation of neurogenesis and reduction in functional deficits following administration of DETA/NONOate, a nitric oxide donor, after traumatic brain injury in rats. *Journal of neurosurgery* **99**, 351-361, doi:Doi 10.3171/Jns.2003.99.2.0351 (2003).

144 Pasha, Z. *et al.* Preconditioning enhances cell survival and differentiation of stem cells during transplantation in infarcted myocardium. *Cardiovascular research* (2007).

145 Xie, X. *et al.* Transplantation of mesenchymal stem cells preconditioned with hydrogen sulfide enhances repair of myocardial infarction in rats. *The Tohoku journal of experimental medicine* **226**, 29-36 (2012).

146 Xiong, Y., Mahmood, A. & Chopp, M. Emerging treatments for traumatic brain injury. *Expert opinion on emerging drugs* **14**, 67-84, doi:10.1517/14728210902769601 (2009).

147 Xiong, Y., Mahmood, A. & Chopp, M. Animal models of traumatic brain injury. *Nature reviews. Neuroscience* **14**, 128-142, doi:10.1038/nrn3407 (2013).

148 Narayan, R. K. *et al.* Clinical trials in head injury. *Journal of neurotrauma* **19**, 503-557, doi:10.1089/089771502753754037 (2002).

149 Beauchamp, K., Mutlak, H., Smith, W. R., Shohami, E. & Stahel, P. F. Pharmacology of traumatic brain injury: where is the "golden bullet"? *Molecular medicine* **14**, 731-740, doi:10.2119/2008-00050.Beauchamp (2008).

150 Mahmood, A., Lu, D., Wang, L. & Chopp, M. Intracerebral transplantation of marrow stromal cells cultured with neurotrophic factors promotes functional recovery in adult rats subjected to traumatic brain injury. *Journal of neurotrauma* **19**, 1609-1617, doi:10.1089/089771502762300265 (2002).

151 Riess, P. *et al.* Transplanted neural stem cells survive, differentiate, and improve

neurological motor function after experimental traumatic brain injury. *Neurosurgery* **51**, 1043-1052; discussion 1052-1044 (2002).

152 Gao, J. *et al.* Transplantation of primed human fetal neural stem cells improves cognitive function in rats after traumatic brain injury. *Experimental neurology* **201**, 281-292, doi:10.1016/j.expneurol.2006.04.039 (2006).

153 Lu, D. *et al.* Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* **12**, 559-563 (2001).

154 Mahmood, A. *et al.* Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* **49**, 1196-1203; discussion 1203-1194 (2001).

155 Mahmood, A., Lu, D. & Chopp, M. Marrow stromal cell transplantation after traumatic brain injury promotes cellular proliferation within the brain. *Neurosurgery* **55**, 1185-1193 (2004).

156 Liu, S. *et al.* Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. *Proc Natl Acad Sci U S A* **97**, 6126-6131 (2000).

157 Keirstead, H. S. *et al.* Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 4694-4705, doi:10.1523/JNEUROSCI.0311-05.2005 (2005).

158 Nistor, G. I., Totoiu, M. O., Haque, N., Carpenter, M. K. & Keirstead, H. S. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia* **49**, 385-396, doi:10.1002/glia.20127 (2005).

159 Yasuda, A. *et al.* Significance of remyelination by neural stem/progenitor cells transplanted into the injured spinal cord. *Stem cells* **29**, 1983-1994, doi:10.1002/stem.767 (2011).

160 Erceg, S. *et al.* Transplanted oligodendrocytes and motoneuron progenitors generated from human embryonic stem cells promote locomotor recovery after spinal cord transection. *Stem cells* **28**, 1541-1549, doi:10.1002/stem.489 (2010).

161 Sharp, J., Frame, J., Siegenthaler, M., Nistor, G. & Keirstead, H. S. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. *Stem cells* **28**, 152-163, doi:10.1002/stem.245 (2010).

162 Karimi-Abdolrezaee, S., Eftekharpour, E., Wang, J., Morshead, C. M. & Fehlings, M. G. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 3377-3389, doi:10.1523/JNEUROSCI.4184-05.2006 (2006).

163 Xu, L. Y. *et al.* Transplantation of human oligodendrocyte progenitor cells in an animal model of diffuse traumatic axonal injury: survival and differentiation. *Stem Cell Res Ther* **6**, doi:Artn 93
Doi 10.1186/S13287-015-0087-0 (2015).

164 Yang, N. *et al.* Generation of oligodendroglial cells by direct lineage conversion. *Nature biotechnology* **31**, 434-439, doi:10.1038/nbt.2564 (2013).

165 Braun, S. M. *et al.* Programming Hippocampal Neural Stem/Progenitor Cells into Oligodendrocytes Enhances Remyelination in the Adult Brain after Injury. *Cell reports*, doi:10.1016/j.celrep.2015.05.024 (2015).

166 Mahmood, A., Lu, D. & Chopp, M. Intravenous administration of marrow stromal cells (MSCs) increases the expression of growth factors in rat brain after

traumatic brain injury. *Journal of neurotrauma* **21**, 33-39, doi:10.1089/089771504772695922 (2004).

167 Uzun, G., Subhani, D. & Amor, S. Trophic factors and stem cells for promoting recovery in stroke. *Journal of vascular and interventional neurology* **3**, 3-12 (2010).

168 Zhang, Y. W., Denham, J. & Thies, R. S. Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. *Stem cells and development* **15**, 943-952, doi:10.1089/scd.2006.15.943 (2006).

169 Dai, X. *et al.* The trophic role of oligodendrocytes in the basal forebrain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 5846-5853 (2003).

170 Wilkins, A., Majed, H., Layfield, R., Compston, A. & Chandran, S. Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 4967-4974 (2003).

171 Bakshi, A. *et al.* Neural progenitor cells engineered to secrete GDNF show enhanced survival, neuronal differentiation and improve cognitive function following traumatic brain injury. *The European journal of neuroscience* **23**, 2119-2134, doi:10.1111/j.1460-9568.2006.04743.x (2006).