STRESS INDUCED ASTROGLIAL PLASTICITY

Cortical Astroglial Plasticity in Response to Stress and Antidepressant Treatment

by

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Abstract

Major depressive disorder is among the leading causes of disability worldwide, with an estimated 350 million people affected. An emerging body of work has suggested a potential role for astroglial cells in both the etiology and treatment of depression. Much of this work has focused on changes within the hippocampus and amygdala. However, recent evidence has shown decreases in the expression of proteins associated with glial plasticity, such as glial fibrillary acidic protein (GFAP), in cortical astroglial cells of depressed-suicide human patients that were examined post-mortem (Torres-Platas et al., 2011). This is supported by evidence from rodent models that have suggested that cortical astroglial cells may be involved in the pathophysiology of depression (Banasr et al., 2007, 2008). Using transgenic mice (AldH-L1) that express green fluorescent protein (GFP) in astroglial cells, we employed a chronic unpredictable stress (CUS) paradigm in conjunction with chronic administration of the selective serotonin reuptake inhibitor (SSRI), fluoxetine, as an antidepressant treatment (ADT). Changes in behaviour and in cortical astroglia were examined using a variety of techniques across all groups. As expected, CUS significantly increased anxiety and depressive-like behaviours, however ADT did not reverse the effects, and, in some cases exacerbated the effect of stress. Using immunohistochemistry to examine the brains we found that total number of astroglial cells (as marked by constitutive astroglial-specific GFP) in the cortex did not change in response to stress or treatment, but their expression of intermediate filament proteins did. Moreover, stress induced markers associated with astroglial stem cell potential, including Sox2 and Vimentin. Finally, gene expression changes were examined using translating ribosome affinity purification (TRAP) and major pathway clusters were identified around changes in extracellular matrices, glutamatergic signalling, growth factors and drug addiction. Together, these data suggest tremendous plastic responsiveness of astroglial cells in response to chronic stress. Future studies will examine the causal involvement of differentially expressed pathways in response to stress.

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Cortical Astroglial Plasticity in Response to Stress and Antidepressant Treatment

Depression is among the leading causes of disability worldwide, with an estimated 350 million people affected (Collins et al., 2011; World Health Organization, 2015). While the clinical presentation of major depressive disorder can vary depending on the individual and the severity of the illness, it is commonly characterized by: low mood, feelings of hopelessness, anhedonia, changes in appetite, irritability, changes in sleep, fatigue, and loss of energy (American Psychiatric Association, 2013). Not surprisingly, depression is commonly co-morbid with many neurological diseases, along with numerous metabolic, immune and vascular conditions (Sacher et al., 2012). Adding to the complexity, depression can manifest in many different forms such that there are large individual differences in the expression of symptoms and likely, the etiology of the disease (Singh & Gotlib, 2014); for example, some individuals show increased sleep and appetite, while others show decreased sleep and anorexia (Krishnan & Nestler, 2008). Such heterogeneity presents a challenge not only for the diagnosis and treatment, but also in the ability to predict the long-term outcomes of depression.

Currently, treatments for depression include psychotherapy, anti-depressant medications and in some extreme cases, electroconvulsive therapy. While the various drug options are effective in a significant number of depressed individuals, an estimated 10-30% are treatment resistant (Al-Harbi, 2012). Furthermore, many patients need to try several treatments before finding an effective one. Today, treatment options largely stem from the monoamine deficiency theory of depression, which essentially argues that depression is a result of monoamine neurotransmitter (mostly serotonin, but also dopamine and noradrenaline) imbalances (Heinger, Delgado, & Charney, 1996). As a result, there are at

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least twenty antidepressant drugs presently available, all of which target the monoaminergic systems (e.g., selective serotonin re-uptake inhibitors). However, the monoamine theory of depression has fallen out of favour because serotonin levels have been shown to increase immediately following SSRI administration; yet, symptom relief is not observed until 3-5 weeks of continuous treatment (Lacasse & Leo, 2005; Heinger, Delgado, & Charney, 1996; Abdallah, Sanacora, Duman, & Krystal, 2015). Moreover, depleting serotonin levels through the alteration of tryptophan, a precursor to the neurotransmitter serotonin, have yielded no consistent results on depressive symptomatology. For example, Yatham et al. (2012) found that only about half of patients in remission following the use of antidepressant medication relapse after tryptophan depletion. These inconsistencies, together with the accumulating evidence pointing towards tremendous neuroplastic changes that occur in depression, have led to new theories speculating that disturbances of plasticity, including dendritic arborization, neurogenesis and synaptic organization underlie the etiology and treatment of depression (Rajkowska, & Miguel-Hidalgo, 2007; Pittenger, & Duman, 2008).

Support for the neuroplasticity hypothesis of depression comes from studies demonstrating structural changes in the brain and that these are reversible with successful treatment (Abdallah et al., 2015; Banasr & Duman, 2008). Brain-imaging studies have provided some insight into the neurobiology of depression, showing evidence of gray matter volumetric reductions in the rostral anterior cingulate cortex, hippocampus, striatum, basal ganglia, insula, subregions of the prefrontal cortex, and amygdala of depressed patients (Singh & Gotlib, 2014; Sacher et al., 2012; Hamilton, Siemer, & Gotlib, 2008; Videbech & Ravnkilde, 2004). Furthermore, the hippocampus, an area critical for learning and memory,

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is smaller in some depressed individuals and this reduction is correlated with both the duration and number of depressive episodes (Sheline, Sanghavi, Mintun, & Gado, 1999).

Stress, Depression and the Brain

In terms of etiological origins, major depressive disorder likely results from a complex interaction between genes and environmental risk factors, in particular psychosocial stressors and pre-and perinatal factors (Wong & Licinio, 2001). Indeed, numerous studies have demonstrated a strong link between exposure to stress and the development and exacerbation of depression (Kiyohara & Yoshimasu, 2009; Banasr et al., 2007; Kreisel et al., 2014). Research has demonstrated that various psychogenic and neurogenic stressors provoke monoaminergic and hormonal changes that have been aligned with depressive symptomology (Pittenger & Duman, 2008). Most notably, psychogenic stressors caused anhedonic changes that were correlated with hypothalamic and extra-hypothalamic alterations of neurotransmission (Banasr et al., 2007). More recently, studies have begun to look beyond monoamines and hormones and focus on glutamatergic systems and the neuroplastic processes they regulate with regards to depression (Abdallah et al., 2014; Li et al., 2011). One recent study conducted by Yuen and colleagues (2012) showed that repeated exposure to chronic unpredictable stress altered prefrontal cortex glutamatergic neurotransmission, leading to deficits in cognitive processes, including impaired performance on a temporal order recognition memory task. Importantly, excessive glutamate has a well-established role in perpetuating excitotoxicity, which of course, leads to changes in synaptic strength and connections (e.g., decreased dendritic branching) and

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eventually possible neuronal degeneration, thereby contributing not only to depression but a host of other pathology (Abdallah et al., 2014; Pettinger & Duman, 2008).

Increasing evidence now indicates that stress induces divergent changes in different brain regions (de Kloet, Joëls, & Holsboer, 2005; McEwen, 2007). One recent study using chronic unpredictable stress showed significant decreases in cell proliferation (by approximately 35%) in the neocortex, coupled with anhedonia (Banasr, Valentine, Li, Gourley, Taylor, & Duman, 2007). Furthermore, analysis of cell phenotype demonstrated that there were decreases in the number of oligodendrocytes and endothelial cells. However, chronic antidepressant administration reversed both of these effects (Banasr et al., 2007). Chronic stress has also been shown to cause atrophy of dendrites in the CA3 region, suppress neurogenesis of dentate gyrus granule neurons, and impair hippocampal-dependent cognitive functions (McEwen, 1999; Joëls, Karst, Krugers, & Lucassen, 2007). High levels of corticosterone or chronic stress can impair long-term potentiation (LTP) and facilitate long-term depression (LTD) induced by electrical stimulation in the hippocampus (Kim & Diamond, 2002). However, chronic stress has also been shown to enhance amygdala-dependent fear conditioning and anxiety-like behavior (Conrad, LeDoux, Magariños, & McEwen, 1999). This may in fact be correlated to the stress-induced dendritic growth and spinogenesis observed in this region (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005). Thus, stressor exposure can provoke actual structural brain changes that are critical for synaptic plasticity; however, these changes appear to be brain region specific. On the whole, it is believed that the stressor induced reductions in hippocampal-cortical plasticity, coupled with augmented amygdaloid plasticity can contribute to depressive pathology (Rial et al., 2016; Kreisel et al., 2014).

Ketamine and Depression

Ketamine is an N-Methyl-D-Aspartate (NMDA) receptor antagonist and is most commonly used as an analgesic or anesthetic. However, more recently researchers have been excited by the drug's apparent use as a rapid-acting antidepressant. Berman and colleagues (2000) were the first to report that a single subanesthetic (0.5 mg/kg) dose of ketamine rapidly reduced standardized depression scores in patients with major depressive disorder. Subsequently, there have been consistent findings that ketamine not only acts quickly as an antidepressant (within hours), but that the effects can be sustained from several days to weeks (Mathew et al., 2012; Niciu, Henter, Luckenbaugh, Zarate, & Charney, 2014). Importantly, ketamine has been shown to be effective in patients that had been previously treatment resistant; and due to the rapidity of its effects (within 1 hour in many cases) ketamine is of considerable potential benefit for suicidal patients (Reinstatler & Youssef, 2015; Al-Harbi, 2012).

Ketamine modulates the glutamate neurotransmitter system, which is intimately involved in regulating synaptic connectivity and hence, neuroplasticity (Abdallah et al., 2015; Berman et al., 2000). Ketamine administration rapidly triggers these events, which might mitigate the deleterious impact of stressors upon neuronal processes (Abdallah et al., 2015; Li et al., 2011). Specifically, ketamine restored stressor perturbed prefrontal synaptic connectivity through several overlapping processes. These included blocking NMDA receptors on GABAergic interneurons, activation of AMPA and NMDA receptors, increased production of brain-derived neurotrophic factor (BDNF) and mammalian target of rapamycin complex 1 (mTOR) (Abdallah et al., 2014).

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One major problem with ketamine as a potential therapeutic is that it is also a drug of abuse that causes an intense high, as well as hallucinations. Because of its illicit recreational properties, its potential for use as a clinical intervention is limited. Focus has now begun on developing more selective NMDA receptor antagonists, free of ketamine's adverse effects (Abdallah et al.; Niciu et al., 2014). However, a better understanding of the neurocircuits through which ketamine acts would greatly inform the surge towards novel “neuroplastic” acting antidepressants.

Until very recently, the role of glial cells in depression and the impact of antidepressants on these cells has received little attention despite the fact that astroglial cells are key players in most, if not all of the neuroplastic changes associated with depressive symptoms (Vaccarino et al., 2007). Astrocytes are key regulators of extracellular glutamate levels in the brain and are critically involved in synaptogenesis and dendritic remodeling (Sofroniew & Vinters, 2010). Hence, these often overlooked cells are logical targets for the development of novel antidepressants and will form the focus of the present thesis.

Astrocytes and Depression

Astroglial cells are found throughout the central nervous system (CNS) outnumbering neurons by up to 50 times, depending on the species, they are the most abundant cell type in the brain and exert many essential and complex functions. Astroglial cells encompass several subtypes, including both protoplasmic astrocytes, known for the typical star-shaped pattern as well as astroglial stem cells, exhibiting a more radial-like morphology. Throughout the lifespan, astroglia play pivotal roles in metabolizing neurotransmitters, modifying cell connectivity and providing trophic support to neurons; as

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neural stem cells, astroglia give rise to astrocytes, oligodendrocytes and neurons, both under basal conditions and in response to injury (Czeh & Di Benedetto, 2012; Sofroniew & Vinters, 2010).

Several studies have investigated the potential role of glial cells in the prefrontal cortex in the etiology and treatment of depression (Banasr & Duman, 2008; Cotter et al., 2002). Postmortem studies from patients with major depressive disorder have consistently shown a decreased density of glial cells in the prefrontal cortex (Rajkowska & Miguel-Hidalgo, 2007; Cotter, Mackay, Chana, Beasley, Landau, & Everall, 2002). Similarly, rats that are subjected to chronic unpredictable stress (CUS), an animal model for depression, show impairments in cortical glial function and gliogenesis (Banasr et al., 2010). Exposure to various stressors induced a 20% decrease in glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed in astrocytes (Banasr et al., 2010). Furthermore, glial ablation in the prefrontal cortex is sufficient to induce depressive-like behaviours similar to those seen after CUS (Banasr & Duman, 2008). Researchers injected an astroglial specific toxin (L-Alpha-Aminoadipic Acid) or a neurotoxin into the prefrontal cortex of rats. Interestingly, the glial ablation resulted in depressive-like behaviours, including anhedonia, similar to those seen after CUS, whereas the neurotoxin had no such effects (Banasr & Duman, 2008). Other authors have found that mice exposed to CUS exhibit a decreased number of microglia in the hippocampal dentate gyrus and to a lesser extent, the medial prefrontal cortex (Kreisel et al., 2014), raising the possibility that multiple glial subtypes might be involved in depression.

A recent review postulates that antidepressants activate astrocytes, priming them to carry out specific functions that result in the reactivation of cortical plasticity and

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readjustment of abnormal neuronal networks (Czeh & Di Benedetto, 2012). For example, antidepressants affect numerous astrocytic functions, such as the availability of various neurotransmitters (e.g., serotonin, glutamate or GABA), the regulation of energy homeostasis and control over the blood-brain barrier integrity (Czeh & Di Benedetto, 2012). In fact, one study conducted by Sun and colleagues (2012) illustrated that gap junction dysfunction in the prefrontal cortex induced depressive-like behaviours in rats exposed to stress. Specifically, the animals with behavioural deficits in both the sucrose preference test (SPT) and novelty suppressed feeding test (NSFT), exhibited significant decreases in diffusion of gap junction channel-permeable dye and expression of connexin 43 (Sun et al., 2012). Another study examined how antidepressants affect neurotrophic/ growth factor expression by preparing astrocyte and neuron enriched cultures from the neonatal rat cortex (Kajitani et al., 2012). The researchers found that the treatment of cortical astrocyte cultures, not neuronal ones, with amitriptyline (a tricyclic antidepressant) increased the expression of multiple neurotrophic/growth factors such as FGF-2, BDNF, GDNF, and VEGF (Kajitani et al., 2012). Interestingly, these increases were mostly found in astrocyte cultures from the hippocampus, an area implicated in the efficacy of antidepressants. Collectively, these studies indicate significant changes in glia in the depressed brain, particularly in the hippocampus and the prefrontal cortex. However, the nature and therapeutic relevance of these changes remains largely unknown and most of the work to date has focused on hippocampal and amygdalar changes, despite an obvious role for the cortex in cognitive processes underlying depressive symptomatology. The goal of the proposed research thesis is therefore to profile cortical astroglial responses to CUS and antidepressant treatment. Specifically, to assess overall number and protein/gene expression

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patterns of cortical astroglia after CUS and then examine reversal with antidepressant treatment. The following research goals have been made: 1) Previous studies have shown decreases in astroglial GFAP expression, however we hypothesize that there are no differences in astroglial number, but rather this is specific to GFAP. Thus, we have employed transgenic mice that express green fluorescent protein (GFP) in all astroglial cells and expect no differences in the number of GFP⁺ cells. 2) Because cortical glutamate transmission has been shown to change with depressive symptoms, we hypothesize that astroglial expression of the enzyme glutamine synthetase will also change in response to CUS and fluoxetine treatment. 3) Because increased cortical gliogenesis and neurogenesis have been shown in response to fluoxetine treatment, and because astroglial cells can act as neural stem cells, we hypothesize CUS and fluoxetine will change cortical astroglial expression of Sox2 (a transcription factor associated with astroglial stem cell potential) and vimentin (an intermediate filament protein associated with a radial morphology and stem cell state). These are some of the only markers associated with astroglial cell plasticity. 4) Because astroglial cells are key players in neuroplastic changes, we hypothesize that astroglial gene expression pathways associated with neuroplasticity will be significantly changed in response to CUS. 5) Finally, we hypothesize that CUS will increase depressive and anxiety behaviours and that fluoxetine will reverse these changes. Furthermore, some or all of aforementioned astroglial changes will be correlated with anxiety and depressive-like behavioral outcomes.

Method

Experimental Animals and Procedure

Seventy-two male C57/BL6- AldHL1-L10GFP transgenic mice (Breeding Colony, Carleton University, Ottawa) ages 7-30 weeks, were single housed in standard (27cm X 21cm X 14cm) fully transparent polypropylene cages. Thirty-six animals were placed in a stress room, while the remaining thirty-six were placed in a shared non-stress room (*Refer to Figure A for animal groups*). The animals were placed in each room 48 hours prior to the first day of the experiment in order to acclimate. Basic cage enrichment only was provided (i.e., nesting material and shelter) in order to not block the effects of the stressors. The mice were maintained on a 12-hour light/dark cycle in a temperature controlled (21 degrees) environment with food and water available as desired (with the exception of an active stressor). All animal use procedures were approved by the Carleton University Committee for Animal Care, according to the guidelines set by the Canadian Council for the Use and Care of Animals in Research.

	VEHICLE	FLUOXETINE
STRESS	7 GFP+ mice 11 GFP- mice	8 GFP+ mice 10 GFP- mice
CONTROL	7 GFP+ mice 11 GFP- mice	7 GFP+ mice 11 GFP- mice

Figure A. Group Assignment. Using transgenic male mice ($n = 18$ per group) that express green fluorescent protein (GFP) on a ribosomal protein, L10, under the control of the

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astrocytic promotor, AldH-L1, animals were randomly assigned to one of two groups (stress or control). They were then given one of two treatment conditions (fluoxetine or vehicle).

Chronic Uncontrollable Stress (CUS)

This experiment used a CUS paradigm adapted from Dr. Ronald Duman's laboratory (Yale University) to induce an anxiety and depressive-like behavioural phenotype. The animals in the stress group were exposed to various mild stressors (2-3 per 24-hour period) that are uncontrollable in both time and duration to prevent habituation. The exposure to these stressors commenced on day 1 and continued through to day 35 of the experiment. The continued exposure throughout the entire 5-week period allowed for the investigation of neuroadaptations that result from stress of a more chronic nature. Stressors consisted of the following conditions: altered light/dark cycle, food and water deprivation, isolation, cage tilt (30 degrees), exposure to predator odor, soiled bedding (200 ml water added to bedding material), no bedding, swim for 10 minutes and lastly, restraint (for 15 minutes, in commercially available restraint cones that have an opening in the front for ventilation). Stressors were applied in a random and variable order, for various durations (up to certain time points in accordance with the protocol). Animals were monitored immediately after each procedure, and again one hour later. They were observed for general condition, decreased locomotor activity or lethargy, decreased grooming and other unusual behaviors judged consistent with distress. Mice were monitored continuously during both the restraint and swimming procedures. Animals that were not in the stress group were simply not exposed to the experimental stressors.

Injection Protocol

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All mice received intraperitoneal injections of either vehicle or Fluoxetine (i.e., Prozac, Sigma-Aldrich) daily for the last 21 days of the experiment (*Refer to Figure B for experimental timeline*). At a dose of 10mg/kg, the injections occurred at approximately the same time each day (noon). The animals were monitored closely after each treatment for any signs of distress or discomfort.

Figure B. *Experimental timeline for the 5-week study.* Stress animals received stressors from day 1 through 35. Injections began for all animals on day 15 and continued until the end of experiment. Behavioural testing was performed towards the end of the experiment. All animals were sacrificed on day 36 (i.e., perfusions and rapid decapitations).

Sucrose Consumption Test

In order to assess whether or not exposure to chronic unpredictable stress is associated with depressive-like behaviours, a sucrose consumption test was conducted as a measure of anhedonia in the mice. First, animals were given a water consumption test to control for overall changes in consumption volume not specific to sucrose: following overnight water deprivation, normal water consumption was measured during a 1-hour test period. Bottles were weighed to measure this volume before and after the test (i.e., bottle weight before –

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bottle weight after = total consumed). Mice were then habituated to a 1% sucrose solution (replaced water bottle) for a 48-hour period. Habituation is important in order to avoid neophobia. This period was again followed by overnight water deprivation. For the test, the sucrose bottle was given back for 1-hour, again, weighing the bottles before and after the test. Normal ad libitum access to food was maintained throughout both habituation and testing periods. Access to water bottles resumed immediately after the test. All solutions were made fresh each day. Mice remained single housed throughout the process. Consumption for the both water and sucrose were calculated according to the following formula: intake / body weight. The test was conducted in the final week (5) of the experiment.

Forced Swim Test

In order to assess depressive-like effects that may be induced by CUS (e.g., learned helplessness), the forced swim test was used according to standard published procedures with minor modifications. Mice were individually placed in a glass cylinder (15.5cm diameter) that contains a depth of approximately 10cm of temperature-controlled water (23-25 °C) for a period of 5 minutes. Time immobile (i.e., floating while making only necessary movements that require the animal to keep their head above the water), latency to become immobile (time at which animal first shows signs of immobility) and time spent swimming (i.e., active, horizontal movements) were recorded on a video camera and scored by an independent observer blind to conditions of the experiment. Immediately following the task, animals were dried off using a paper towel and placed back in their home cage. This test was performed last, on the final day of the experiment

Open Field Test

In order to measure anxiety and exploratory behaviours the open field test was used. It is a standard and commonly used measure of rodent exploratory locomotion in a novel open space. Mice were placed in the corner of a brightly lit (650 lux) white Plexiglas box (50 x 50cm with 35cm high walls) and videotaped with AnyMaze Video Tracking System for 5 minutes. Time spent in the pre-defined zones (periphery and center) was recorded. Anxiety and exploratory behaviors were measured and included the amount of time an animal spends in a given zone, as well as motor activity. Decreased time spent in the center zone is indicative of anxiety behavior. The open field test was conducted during the second last day of the experiment. The testing area was cleaned with 70% ethanol between trials.

Elevated Plus Maze

Another measure of anxiety behaviour that was used included the elevated plus maze, which has been extensively characterized and validated for rodents. In the test, mice were allowed to freely explore a novel, elevated, plus-formed maze in which two opposing arms have walls (closed arms) and the other two arms are devoid of walls (open arms). Generally, mice will spend less time exploring the open arms compared to the closed arms. Open arm avoidance is considered to correlate with anxiety in this model. During testing, each mouse was placed in the center of the maze, facing an open arm. The dimensions of these arms are 30 x 5cm, with two enclosed with 25cm walls, and elevated 30cm from the floor. Illumination in all parts of the maze was 40 lux. The mouse was then recorded exploring the maze for a

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total of 5 minutes, using AnyMaze Video Tracking System. The amount of time spent in the open and closed arms was recorded. This test was performed on the last day of the experiment. The testing area was cleaned with 70% ethanol between trials.

Animal Sacrifice and Tissue Analyses with Immunohistochemistry

Upon completion of the experiment, on day 36 all animals were sacrificed. Half of the animals were intraperitoneally injected with 0.15mL of sodium pentobarbital and given a toe pinch to determine level of anesthesia. Blood was then flushed using 5mL of saline through the left ventricle to the right atrium followed by 40mL of 4% paraformaldehyde to fix the tissue. Brains were then extracted and placed into vials containing 4% paraformaldehyde and put on ice. Following a twenty-four hour period, the brains were transferred to a 30% sucrose solution at 4°C. Brains were then flash frozen and sectioned on a ThermoFisher cryostat at a 30µm thickness and adhered to electrostatic slides in alternation, totaling 25 sister section slide representations of the entire brain. Sections were processed for immunohistochemistry in order to analyze astroglial cells and astrocytic-mediated protein expression with emphasis on the cortex. Sections were blocked in PBS containing 0.3% Triton (PBS-T) with 10% horse serum (10% HS/PBS-T), and subsequently incubated in primary antibody overnight at the appropriate concentrations (*see antibody Table 1*). Sections were then washed thoroughly and incubated in a fluorescein conjugated secondary antibody of the appropriate species. See antibody table for more details.

The remaining half of the homozygous negative animals were decapitated with brains and trunk blood taken for hormonal and neurochemical determinations (i.e., corticosterone levels as measured by ELISA, in order to verify stress hormone level responsivity).

Antibody	Manufacturer	Species	Dilution
GFAP	Sigma	Mouse	1:500
GFP	Abcam	Chicken	1:1000
Sox2	Chemicon/Millipore	Goat	1:500
GS	Sigma	Mouse	1:500
Vimentin	Sigma	Mouse	1:500
Donkey Anti-Mouse	Invitrogen Alexa Fluor 546, 594	N/A	1:500
Donkey Anti-Chicken	Invitrogen Alexa Fluor 488	N/A	1:1000
Donkey Anti-Goat	Invitrogen Alexa Fluor 546, 594	N/A	1:500

Table 1. List of antibodies used for immunohistochemistry.

Corticosterone ELISA

Corticosterone assays were performed using trunk blood collected at time of sacrifice in experiment 1. Blood samples were collected in EDTA coated eppendorf tubes, spun for 10minutes at 4°C and the serum collected and stored at -80°C until processing. Corticosterone levels were determined by ELISA using the Assay Design kit (Corticosterone #900-097, Lot# D1260724), and quantified on a microplate reader set to 405 nm and to 570-590 nm for correction. Quantifications were normalized to standards and outliers removed using the MyAssays.com automated software.

Cell Counting and Microscope Analyses

Unbiased estimates of cell number were obtained *via* a Zeiss AxioImager M2 motorized fluorescent microscope with ApoTome (Carl Zeiss, Thornwood, NY, USA) attached to a motorized stage and connected to a computer running the Stereoinvestigator Software (MicroBrightfield, Colchester, VT, USA). Serial coronal sections (one every 25 µm)

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were used for all counts. Contours of the entire cortex were drawn. Cells were counted for expression of individual and/or co-expressed markers, as appropriate, using the optical fractionator probe with a 40X objective. Sampling grid size was optimized in order to obtain a relatively constant number of cells sampled (minimum 200 cells/cortex counted). This systematic yet unbiased method provides an estimate of cell density and number that is independent from cell size, shape, orientation, tissue shrinkage and spatial distribution of the cells (Schmitz & Hof, 2005). Tri-dimensional sampling boxes ($150\ \mu\text{m} \times 150\ \mu\text{m} \times 30\ \mu\text{m}$) with 3 out of 6 exclusion borders (Gundersen et al., 1988; West, 1993) were automatically placed by StereoInvestigator at each grid intersection point. The total number of cells counted per stain was reported via the program output data.

Translating Ribosome Affinity Purification

Translating Ribosome Affinity Purification (TRAP) was conducted in homogenate samples of cortex obtained from AldH- EGFP-L10 control and stress mice, which express green fluorescent protein in ribosomes of AldH⁺ cells, thus allowing for the immunoprecipitation of polysomes directly from astrocytes (Heiman et al, 2008; Doyle et al 2008; Dougherty, 2010). TRAP methods were conducted as previously published. Briefly, two adult male mice were decapitated and the cortex was quickly dissected on ice in a dissection buffer containing cyclohexamide (100 $\mu\text{g}/\text{ml}$, Sigma, dissolved in methanol, American Bioanalytical) in order to immediately freeze ribosome/mRNA (polysome) complexes. Samples were then homogenized in extraction buffer (Heiman et al, 2008) using a Teflon-glass homogenizer (Fisher #K8855100020) and then centrifuged for 10 minutes at 2000xg at 4°C. NP-40 (AG Scientific # P1505) and DHPC (Avanti # 850306P) were added to

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the collected supernatant and incubated for 5 minutes on ice before centrifugation for 15 minutes at 20000xg until pelleted. The lysate supernatant was extracted, 30mM DHPC was added, and then incubated with magnetic beads overnight. Magnetic beads (Streptavidin MyOne T1 Dynabeads; Invitrogen # 65601) were previously coated with Protein L (Fisher # PI-29997) for 35 minutes at room temperature (1 $\mu\text{g}/\mu\text{l}$ in 1x PBS), collected on a magnetic rack (DynaMag-2; Invitrogen #123-21D) and washed 5 times in 1xPBS containing 3% protease-free Bovine Serum Albumin (Jackson Immuno #001-000-162) and then subsequently incubated with 50 μg each of anti-GFP antibodies (HtzGFP_04 (clone19F7) and HtzGFP_02 (clone 19C8): Memorial Sloan-Kettering Monoclonal Antibody Facility) overnight at 4°C and then washed again, prior to being incubated with the lysate. Following antibody incubation, beads were again collected on a magnetic rack, and unbound fragments were also collected and saved for RNA extraction step as total homogenate controls. Bound fragments were washed in a polysome buffer (Heiman et al., 2008) and then resuspended in lysis buffer (Stratagene #400753) followed by RNA cleanup and extraction. All RNA samples were extracted using Stratagene's Absolutely RNA Nanoprep kit (Stratagene #400753) as per kit directions, including the DNASE treatment steps. Following RNA extraction, unbound and bound samples were snap frozen and stored at -80 until use.

Statistical Analyses

All data was analyzed using a 2 (control vs. chronic uncontrollable stress) x 2 (saline vs. fluoxetine) between-subjects analyses of variance (ANOVA) design. If a significant interaction was found then ANOVAs were followed by post-hoc comparisons with Bonferonni correction for non-orthogonal tests ($p < 0.05$). All data was evaluated using the

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Stat-View (version 6.0) statistical software package available from the SAS Institute, Inc., and differences were considered statistically significant when $p < 0.05$. Differentially expressed genes from RNAseq results were identified following reference genome alignment conducted by Dr. Coppola at Yale University using published pipelines (Mariani et al., 2015). Differentially expressed genes were further interrogated for differentially expressed pathways using REACTOME, KEGG and WikiPathways.

Results

Behavioural Analyses

Depressive Behaviours

Sucrose Consumption Test and Weight. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on sucrose consumption, a measure of anhedonia. Sucrose consumption was calculated using the following formula: Sucrose consumption/ body weight (1-hour period only following 24-hour deprivation). The ANOVA revealed a significant main effect of stress $F(1, 68) = 32.5, p < 0.05$ (*Figure 1*).

In order to assess specificity of these effects to an appetitive substance, we also measured water consumption relative to body weight and found no significant differences in water consumption across groups (*Figure 2*). This amount of sucrose was inversely related to weight (*Figure 3*).

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Figure 1. *Sucrose Consumption.* There was a significant main effect of stress, such that the stress animals consumed more sucrose water (relative to body weight) than the control animals. Data expressed as mean $\pm$ SEM. * $p < 0.05$ versus all other groups.

Figure 2. *Water Consumption.* Mice did not statistically differ in their total consumption of water relative to body weight regardless of stress or treatment condition. Data expressed as mean $\pm$ SEM.

Figure 3. Animal Weight. There was a significant main effect of stress found, such that the stress animals weighed significantly less than the control animals. Data expressed as mean $\pm$ SEM. $*p < 0.05$ main effect of stress.

Forced Swim Test. A two-way between-subjects analyses of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on the forced swim test, a measure of depressive-like behaviour. Time spent immobile and latency to immobility were assessed (in seconds). There was no significant interaction between stress and treatment on time spent immobile $F(1,65) = 0.535$, $p = 0.467$. However, a main effect of stress was found, such that animals in the stress group spent more time immobile (i.e., not swimming) than animals in the control group $F(1,68) = 38.737$, $p = .001$. A main effect of treatment was also found, such that animals injected with fluoxetine spent more time immobile than animals given a vehicle injection $F(1, 68) = 7.06$, $p = 0.01$ (Figure 4).

There was no significant interaction between stress and treatment on latency to

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become immobile $F(1,65) = .013, p = .908$. However, the stress animals had a significantly lower latency to immobility, meaning they stopped swimming faster, as compared to the control animals $F(1,68) = 66.178, p = .001$. There was no significant effect of treatment on latency to become immobile $F(1,68) = .047, p = .829$ (Figure 5).

Figure 4. *Time Spent Immobile on FST.* There was a main effect of both stress and treatment. Mice that were in the stress group spent more time immobile compared to mice in the control group. Additionally, mice treated with fluoxetine spent more time immobile than those given a vehicle injection. Data expressed as mean $\pm$ SEM.

Figure 5. *Latency to Immobility on FST.* There was a significant main effect of stress found, such that the stress animals stopped swimming faster than the control animals. Data expressed as mean $\pm$ SEM. * $p < .05$ main effect of stress.

Anxiety Behaviours

Open Field Test. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on the open field test, a measure of anxiety-like behaviour. Time spent in the centre (in seconds), as well as other motor and mobility measures were assessed. Results showed a statistically significant interaction between stress and treatment on total time spent in the centre zone $F(1,65) = 5.38$, $p = 0.024$. Post-hoc tests showed that Control/Vehicle animals spent more time in the centre than all other groups (*Figure 6*).

To evaluate locomotor activity total distance, average speed, and total distance/centre distance were examined. There was no statistically significant interaction between the effects of stress and treatment on total distance travelled $F(1,65) = 0.073$, $p > 0.05$. However, a

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statistically significant main effect of stress was found on total distance travelled $F(1,68) = 19.759, p < 0.001$ (*Figure 7*). There was no main effect of treatment on total distance travelled $F(1,68) = .227, p = .636$. There was also no statistically significant interaction between the effects of stress and treatment on the average speed during the test $F(1,65) = 0.066, p = 0.798$. However, a statistically significant main effect of stress was found on the average speed $F(1,68) = 19.6, p < 0.001$ (*Figure 8*). There was no main effect of treatment on the average speed $F(1,68) = 0.214, p = .645$.

Because mice in the stress condition were hyperactive as compared to controls and because this could obscure the anxiety behavior results, we also examined the distance travelled in the centre relative to total distance. Results showed a statistically significant interaction between the effects of stress and treatment on centre distance/total distance $F(1,65) = 5.38, p = 0.024$, suggesting that the effects of stress and treatment on anxiety behavior occur despite of hyperactivity. Subsequent simple effects tests showed that animals given a vehicle injection travelled more in the centre than any other group (*Figure 9*).

Figure 6. *Time Spent in the Centre Zone of the OF.* There was a significant interaction found between stress and treatment on the total time spent in the centre. Control animals given a vehicle injection spent more time in the centre than any other group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ stress by treatment interaction.

Figure 7. *Total Distance Travelled in the OF.* There was a significant main effect of stress found, such that the stress animals travelled or moved more than the control animals. Data expressed as mean $\pm$ SEM. * $p < 0.001$ main effect of stress.

Figure 8. *Average Speed in the OF.* There was a significant main effect of stress found, such that the stress animals moved faster on average compared to the control animals. Data expressed as mean $\pm$ SEM. * $p < 0.001$ main effect of stress.

Figure 9. *Centre Distance/Total Distance.* There was a significant interaction found between stress and treatment on centre distance/total distance. Control animals given a vehicle injection spent more time in the centre than any other group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ stress by treatment interaction.

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Elevated Plus Maze. A two-way between-subjects analyses of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on the elevated plus maze, a measure of anxiety-like behaviour. Time spent in the open arms was measured (in seconds). There was no statistically significant interaction between the effects of stress and treatment on total time spent in the open zone $F(1,65) = 0.414, p = 0.522$. However, a statistically significant main effect of stress was found on time spent in the open zone $F(1,68) = 6.509, p = 0.013$ (Figure 10). There was no main effect of treatment on time spent in the open zone $F(1,68) = 0.766, p = 0.382$.

Figure 10. *Time Spent in the Open Zone of the EPM.* There was a significant main effect of stress, such that animals in the stress group spent less time in the open zone compared to those in the control group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ main effect of stress.

Hormone Assay

Corticosterone Concentration. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment

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(fluoxetine vs. vehicle) on corticosterone concentration. Because circulating glucocorticoid concentrations should rise above baseline levels in response to stress, this analysis was used in order to validate the effects of our CUS paradigm. As expected, there was a statistically significant main effect of stress on corticosterone concentrations $F(1,14) = 8.495$, $p = 0.013$ (Figure 11).

Figure 11. *CORT Serum Concentration.* There was a significant main effect of stress, such that animals in the stress group had higher basal serum concentrations compared to those in the control group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ main effect of stress.

Immunohistochemical Analyses

Cortical Volume. A two-way between-subjects analyses of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total cortical volume (measured via Stereo Investigator). There were no significant differences observed (Figure 12).

Figure 12. *Cortical Volume.* There were no significant changes in total measured cortical volume across the groups, $p > 0.05$.

Astroglial GFP Quantification. A two-way between-subjects analyses of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total number of astroglial cells in the cortex as measured by green fluorescent protein (GFP). There were no significant differences observed GFP $F(1,7) = 2.59$, $p = 0.1515$ (Figure 13).

Figure 13. *GFP Total Number.* There were no significant changes in total number of astroglial cells as measured by GFP across the groups, $p > 0.05$.

GFAP Quantification. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total number of cells expressing the intermediate filament protein glial fibrillary acidic protein (GFAP). There was no statistically significant interaction between the effects of stress and treatment on total number of GFAP positive cells $F(1,10) = 0.002$, $p = 0.968$. However, a statistically significant main effect of stress was found on the number of cortical astroglial cells expressing GFAP $F(1,10) = 5.617$, $p = 0.0393$ (Figure 14). There was no main effect of treatment $F(1,10) = 0.798$, $p = 0.392$.

Figure 14. GFAP Total Number. Representative photo of DAPI-GFP-GFAP co-expression (63X+Oil, cortex). There was a significant main effect of stress, such that animals in the stress group had less GFAP+ cells in the cortex compared to those in the control group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ main effect of stress.

Glutamine Synthetase Quantification. A two-way between-subjects analyses of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total number of astroglial cells in the cortex expressing

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glutamine synthetase (GS), the enzyme that converts glutamate to glutamine. There were no significant differences observed $F(1,9) = 1.2, p = 0.3018$ (Figure 15).

Figure 15. *GS Total Number.* There were no significant changes in total number of astroglial cells expressing GS across the groups, $p > 0.05$.

SOX2 Quantification. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total number of astroglial cells in the cortex expressing the transcription factor Sox2 that is a marker of quiescent stem cells. Results showed a statistically significant interaction between the effects of stress and treatment on Sox2 expression $F(1,7) = 7.379, p = 0.0299$. Subsequent simple effects tests showed that Stress/Vehicle mice had higher levels of expression compared to any other group (Figure 16).

Figure 16. *Sox2 Cortical Expression.* Representative photo of DAPI-GFP-SOX2 co-expression (63X+Oil, cortex). There was a significant interaction found between stress and treatment on expression of Sox2 in cortical astroglial cells. Stress animals given a vehicle injection had higher levels of expression compared to any other group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ stress by treatment interaction.

Vimentin Quantification. A two-way between-subjects analysis of variance (ANOVA) was conducted to assess the effect of stress (stress vs. control) and treatment (fluoxetine vs. vehicle) on total number of astroglial cells in the cortex expressing the intermediate filament protein Vimentin that is a marker of radial morphology. Results showed a statistically significant interaction between the effects of stress and treatment on Vimentin expression $F(1,12) = 24.56, p = 0.001$. Subsequent simple effects tests showed that Stress/Vehicle mice had higher levels of expression compared to any other group (*Figure 17*).

Figure 17. *Vimentin Cortical Expression.* Representative photo of GFP-VIMENTIN co-expression with co-localization plot (63X+Oil, cortex). There was a significant interaction found between stress and treatment on expression of Vimentin in cortical astroglial cells. Stress animals given a vehicle injection had higher levels of expression compared to any other group. Data expressed as mean $\pm$ SEM. * $p < 0.05$ stress by treatment interaction.

Translating Ribosome Affinity Purification

In order to profile astroglial gene expression in response to stress, we performed TRAP to isolate cortical astroglial-specific transcripts. Whole genome RNAseq on an Illumina platform revealed 1061 differentially expressed genes in response to CUS (see Table

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2). Further analysis of all differentially expressed genes using KEGG, REACTOME and WikiPathways revealed four major clusters of pathways significantly implicated in the astroglial response to stress. These clusters included pathways involved in glutamatergic signaling, drug addiction, growth factor signaling and pathways involved in the formation of extra-cellular matrices (*see Table 3*).

Table 2. List of differentially expressed genes in response to stress (cortex).

Gene Symbols	EnsemblID	Significance
Pde10a	ENSMUSG000000023868	4.90E-08
Egr3	ENSMUSG000000033730	5.30E-08
Synpo2	ENSMUSG000000050315	5.30E-08
Prkcd	ENSMUSG000000021948	1.19E-07
Homer1	ENSMUSG000000007617	2.38E-07
Dach1	ENSMUSG000000055639	1.38E-06
Grm2	ENSMUSG000000023192	1.51E-06
9330132A10Rik	ENSMUSG000000043498	5.98E-06
Osbpl8	ENSMUSG000000020189	6.08E-06
Rtn4r	ENSMUSG000000043811	1.21E-05
Fam171a2	ENSMUSG000000034685	1.36E-05
C1qtnf4	ENSMUSG000000040794	1.36E-05
Barhl2	ENSMUSG000000034384	1.36E-05
Ano8	ENSMUSG000000034863	1.73E-05
Dgkb	ENSMUSG000000036095	1.98E-05
Ttr	ENSMUSG000000061808	1.99E-05
Qk	ENSMUSG000000062078	2.26E-05
Ephb6	ENSMUSG000000029869	2.26E-05
Fibcd1	ENSMUSG000000026841	4.70E-05
C1ql2	ENSMUSG000000036907	4.72E-05
Fam126b	ENSMUSG000000038174	5.03E-05
Tmem259	ENSMUSG000000013858	5.03E-05
Sh3rf2	ENSMUSG000000057719	5.03E-05
Pde7b	ENSMUSG000000019990	6.51E-05
Zdbf2	ENSMUSG000000027520	6.93E-05
Lmtk3	ENSMUSG000000062044	8.97E-05
Nebi	ENSMUSG000000053702	9.34E-05
Mfge8	ENSMUSG000000030605	1.18E-04
Plxdc1	ENSMUSG000000017417	2.11E-04
Abhd16a	ENSMUSG000000007036	2.25E-04
Trh	ENSMUSG000000005892	2.25E-04
Cdkn1a	ENSMUSG000000023067	2.39E-04
Mbnl1	ENSMUSG000000027763	2.53E-04
Kdm7a	ENSMUSG000000042599	2.90E-04
Wdfy1	ENSMUSG000000073643	3.15E-04
Syndig1l	ENSMUSG000000071234	3.64E-04
Cpeb3	ENSMUSG000000039652	3.64E-04

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Akap5	ENSMUSG000000021057	4.02E-04
Mcoln1	ENSMUSG000000004567	4.02E-04
Lrrn2	ENSMUSG000000026443	4.13E-04
Cggbp1	ENSMUSG000000054604	4.13E-04
Vstm2l	ENSMUSG000000037843	4.13E-04
Slc8a2	ENSMUSG000000030376	4.13E-04
Syt3	ENSMUSG000000030731	4.13E-04
Map2	ENSMUSG000000015222	4.31E-04
Igsf8	ENSMUSG000000038034	4.53E-04
Hsd11b1	ENSMUSG000000016194	4.63E-04
Grp	ENSMUSG000000024517	4.63E-04
Baiap3	ENSMUSG000000047507	4.78E-04
Cyp4f15	ENSMUSG000000073424	4.78E-04
Adam15	ENSMUSG000000028041	4.78E-04
Fosl2	ENSMUSG000000029135	4.78E-04
Plppr3	ENSMUSG000000035835	5.13E-04
Sema6b	ENSMUSG000000001227	5.13E-04
Sod3	ENSMUSG000000072941	5.13E-04
Fosb	ENSMUSG000000003545	5.13E-04
Reps2	ENSMUSG000000040855	5.21E-04
Spns1	ENSMUSG000000030741	5.61E-04
Peg10	ENSMUSG000000092035	5.70E-04
Crkl	ENSMUSG000000006134	5.77E-04
Ctsz	ENSMUSG000000016256	5.77E-04
Gas6	ENSMUSG000000031451	5.81E-04
Alpl	ENSMUSG000000028766	6.46E-04
Fjx1	ENSMUSG000000075012	6.80E-04
Grin1	ENSMUSG000000026959	7.34E-04
P4htm	ENSMUSG000000006675	7.57E-04
Nell2	ENSMUSG000000022454	7.59E-04
Rc3h2	ENSMUSG000000075376	7.59E-04
Man2b2	ENSMUSG000000029119	7.59E-04
Galns	ENSMUSG000000015027	7.90E-04
8030462N17Rik	ENSMUSG000000047466	8.01E-04
Vasn	ENSMUSG000000039646	8.12E-04
Pigt	ENSMUSG000000017721	8.12E-04
Prss12	ENSMUSG000000027978	8.20E-04
Nyap2	ENSMUSG000000054976	8.76E-04
Cyp46a1	ENSMUSG000000021259	8.76E-04
Slc45a1	ENSMUSG000000039838	8.76E-04

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Eea1	ENSMUSG00000036499	9.42E-04
Olfr2	ENSMUSG00000032172	1.00E-03
Gaa	ENSMUSG00000025579	1.00E-03
Txndc15	ENSMUSG00000021497	1.00E-03
B4galnt4	ENSMUSG00000055629	1.00E-03
Clock	ENSMUSG00000029238	1.04E-03
Adgrb1	ENSMUSG00000034730	1.05E-03
Os9	ENSMUSG00000040462	1.06E-03
Egfl7	ENSMUSG00000026921	1.09E-03
Sulf2	ENSMUSG00000006800	1.09E-03
Tmem238	ENSMUSG00000030431	1.09E-03
Unc5a	ENSMUSG00000025876	1.09E-03
Esr1	ENSMUSG00000019768	1.13E-03
Ncoa7	ENSMUSG00000039697	1.15E-03
Tmem145	ENSMUSG00000043843	1.15E-03
Chordc1	ENSMUSG00000001774	1.26E-03
Gpaa1	ENSMUSG00000022561	1.29E-03
Marf1	ENSMUSG00000060657	1.29E-03
Strn	ENSMUSG00000024077	1.29E-03
Tcf7l2	ENSMUSG00000024985	1.30E-03
Podxl2	ENSMUSG00000033152	1.30E-03
Abcc8	ENSMUSG00000040136	1.30E-03
Grn	ENSMUSG00000034708	1.30E-03
Grin2c	ENSMUSG00000020734	1.30E-03
Ubxn7	ENSMUSG00000053774	1.30E-03
Fyttd1	ENSMUSG00000022800	1.30E-03
Bcan	ENSMUSG00000004892	1.30E-03
Igfbp7	ENSMUSG00000036256	1.30E-03
Pcnx2	ENSMUSG00000060212	1.30E-03
Cln6	ENSMUSG00000032245	1.35E-03
Ephb3	ENSMUSG00000005958	1.36E-03
Celsr2	ENSMUSG00000068740	1.36E-03
Mgp	ENSMUSG00000030218	1.47E-03
Trove2	ENSMUSG00000018199	1.48E-03
Utp14b	ENSMUSG00000079470	1.52E-03
Gm15487	ENSMUSG00000080242	1.52E-03
Zdhhc8	ENSMUSG00000060166	1.53E-03
Fam13b	ENSMUSG00000036501	1.53E-03
Gad2	ENSMUSG00000026787	1.54E-03
Taok1	ENSMUSG00000017291	1.61E-03

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Neu1	ENSMUSG00000007038	1.61E-03
Ypel2	ENSMUSG00000018427	1.61E-03
H2-D1	ENSMUSG00000073411	1.63E-03
Tenm2	ENSMUSG00000049336	1.63E-03
Icam5	ENSMUSG00000032174	1.63E-03
Ache	ENSMUSG00000023328	1.64E-03
NA	ENSMUSG00000072829	1.66E-03
Fsd1l	ENSMUSG00000054752	1.75E-03
Pcnx3	ENSMUSG00000054874	1.78E-03
Skil	ENSMUSG00000027660	1.84E-03
Wasl	ENSMUSG00000029684	1.92E-03
Fgf1	ENSMUSG00000036585	1.92E-03
Spint2	ENSMUSG00000074227	1.94E-03
Tm2d3	ENSMUSG00000078681	1.94E-03
Vat1l	ENSMUSG00000046844	1.94E-03
Mbnl2	ENSMUSG00000022139	1.96E-03
Lrfr4	ENSMUSG00000045045	1.96E-03
Srsf10	ENSMUSG00000028676	1.96E-03
Adgrb2	ENSMUSG00000028782	1.99E-03
Tmem63b	ENSMUSG00000036026	2.01E-03
Lrrc4b	ENSMUSG00000047085	2.01E-03
Chid1	ENSMUSG00000025512	2.01E-03
Hook3	ENSMUSG00000037234	2.03E-03
Pomgnt2	ENSMUSG00000066235	2.10E-03
Rabac1	ENSMUSG00000003380	2.10E-03
Gspt1	ENSMUSG00000062203	2.18E-03
Usp45	ENSMUSG00000040455	2.19E-03
Irs2	ENSMUSG00000038894	2.20E-03
Sil1	ENSMUSG00000024357	2.47E-03
Fam69b	ENSMUSG00000036186	2.47E-03
Ctsa	ENSMUSG00000017760	2.50E-03
Srebf1	ENSMUSG00000020538	2.52E-03
Cpeb2	ENSMUSG00000039782	2.58E-03
Gng7	ENSMUSG00000048240	2.61E-03
Xylt2	ENSMUSG00000020868	2.62E-03
Pphln1	ENSMUSG00000036167	2.62E-03
Gm1673	ENSMUSG00000070858	2.67E-03
Tnnt1	ENSMUSG00000064179	2.67E-03
Tmem205	ENSMUSG00000040883	2.67E-03
Unc13c	ENSMUSG00000062151	2.67E-03

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Rbms3	ENSMUSG000000039607	2.67E-03
Pcsk2	ENSMUSG000000027419	2.75E-03
Chst12	ENSMUSG000000036599	2.75E-03
Aebp1	ENSMUSG000000020473	2.76E-03
Crtac1	ENSMUSG000000042401	2.83E-03
Fam132a	ENSMUSG000000023571	2.85E-03
Isl1	ENSMUSG000000042258	2.87E-03
Faah	ENSMUSG000000034171	2.87E-03
Fgfr3	ENSMUSG000000054252	2.87E-03
Dlg2	ENSMUSG000000052572	2.87E-03
Grik4	ENSMUSG000000032017	3.12E-03
Wnt4	ENSMUSG000000036856	3.14E-03
Igsf21	ENSMUSG000000040972	3.14E-03
B4gat1	ENSMUSG000000047379	3.29E-03
Daglb	ENSMUSG000000039206	3.35E-03
Ccdc186	ENSMUSG000000035173	3.38E-03
Zbtb33	ENSMUSG000000048047	3.38E-03
Serpina3n	ENSMUSG000000021091	3.40E-03
Abca3	ENSMUSG000000024130	3.40E-03
Slitrk1	ENSMUSG000000075478	3.45E-03
Pamr1	ENSMUSG000000027188	3.66E-03
Peg3	ENSMUSG000000002265	3.68E-03
Slc2a8	ENSMUSG000000026791	3.69E-03
Ssr2	ENSMUSG000000041355	3.69E-03
Fndc3a	ENSMUSG000000033487	3.75E-03
Slc2a6	ENSMUSG000000036067	3.78E-03
Plbd2	ENSMUSG000000029598	3.78E-03
Sema4f	ENSMUSG000000000627	3.93E-03
Prr7	ENSMUSG000000034686	3.96E-03
Bbx	ENSMUSG000000022641	3.96E-03
Tmem240	ENSMUSG000000084845	3.96E-03
Nupl1	ENSMUSG000000063895	3.99E-03
Foxp2	ENSMUSG000000029563	3.99E-03
Ctsl	ENSMUSG000000021477	4.08E-03
Ptprn	ENSMUSG000000026204	4.18E-03
Agt	ENSMUSG000000031980	4.18E-03
D8Ert82e	ENSMUSG000000050271	4.26E-03
Megf8	ENSMUSG000000045039	4.27E-03
Sdf2	ENSMUSG00000002064	4.33E-03
Atp13a2	ENSMUSG000000036622	4.33E-03

STRESS INDUCED ASTROGLIAL PLASTICITY

Cadm4	ENSMUSG000000054793	4.33E-03
Rlim	ENSMUSG000000056537	4.33E-03
Pptc7	ENSMUSG000000038582	4.39E-03
Filip1	ENSMUSG000000034898	4.42E-03
Tnks1bp1	ENSMUSG000000033955	4.48E-03
Cstf2	ENSMUSG000000031256	4.48E-03
Slc17a7	ENSMUSG000000070570	4.50E-03
Hgsnat	ENSMUSG000000037260	4.51E-03
Cacna1a	ENSMUSG000000034656	4.51E-03
Rsph9	ENSMUSG000000023966	4.52E-03
02-Mar	ENSMUSG000000073481	4.55E-03
Bscl2	ENSMUSG000000071657	4.56E-03
Evc2	ENSMUSG000000050248	4.58E-03
Ndnf2	ENSMUSG000000070520	4.58E-03
Clu	ENSMUSG000000022037	4.83E-03
Lgi3	ENSMUSG000000033595	4.83E-03
Cnpy3	ENSMUSG000000023973	4.83E-03
Stoml1	ENSMUSG000000032333	4.83E-03
Cck	ENSMUSG000000032532	4.83E-03
Gpr101	ENSMUSG000000036357	4.83E-03
Tmed9	ENSMUSG000000058569	4.92E-03
Rbm27	ENSMUSG000000024491	4.92E-03
Ermn	ENSMUSG000000026830	4.92E-03
Adam11	ENSMUSG000000020926	4.92E-03
Rcn2	ENSMUSG000000032320	4.92E-03
Tmem59l	ENSMUSG000000035964	5.09E-03
Pigk	ENSMUSG000000039047	5.16E-03
C1ra	ENSMUSG000000055172	5.16E-03
Rpn1	ENSMUSG000000030062	5.22E-03
Chpf	ENSMUSG000000032997	5.31E-03
Med13	ENSMUSG000000034297	5.31E-03
Npdc1	ENSMUSG000000015094	5.31E-03
Nusap1	ENSMUSG000000027306	5.31E-03
Txlng	ENSMUSG000000038344	5.31E-03
Ddr1	ENSMUSG00000003534	5.33E-03
Sgtb	ENSMUSG000000042743	5.38E-03
Slc6a7	ENSMUSG000000052026	5.38E-03
Cdh10	ENSMUSG000000022321	5.38E-03
2610002M06Rik	ENSMUSG000000031242	5.38E-03
Adcy2	ENSMUSG000000021536	5.43E-03

STRESS INDUCED ASTROGLIAL PLASTICITY

Setd7	ENSMUSG000000037111	5.44E-03
Clstn1	ENSMUSG000000039953	5.44E-03
Cdc73	ENSMUSG000000026361	5.64E-03
Slc1a6	ENSMUSG000000005357	5.64E-03
Kcng2	ENSMUSG000000059852	5.64E-03
Cspg5	ENSMUSG000000032482	5.64E-03
Slc5a7	ENSMUSG000000023945	5.75E-03
Necab1	ENSMUSG000000040536	5.96E-03
Ncln	ENSMUSG000000020238	6.06E-03
Gmfb	ENSMUSG000000062014	6.06E-03
Gabpa	ENSMUSG000000008976	6.06E-03
Prkar2b	ENSMUSG000000002997	6.08E-03
Rarb	ENSMUSG000000017491	6.08E-03
Zc2hc1a	ENSMUSG000000043542	6.11E-03
Slc7a10	ENSMUSG000000030495	6.11E-03
Svop	ENSMUSG000000042078	6.13E-03
Slc27a1	ENSMUSG000000031808	6.13E-03
Arhgap6	ENSMUSG000000031355	6.13E-03
Tnxb	ENSMUSG000000033327	6.28E-03
Cep131	ENSMUSG000000039781	6.29E-03
Kcnh2	ENSMUSG000000038319	6.38E-03
Cpsf6	ENSMUSG000000055531	6.43E-03
Lrfr3	ENSMUSG000000036957	6.43E-03
Plxna1	ENSMUSG000000030084	6.55E-03
Grid1	ENSMUSG000000041078	6.58E-03
Clcc1	ENSMUSG000000027884	6.58E-03
Plekkg1	ENSMUSG000000040624	6.62E-03
Dbpht2	ENSMUSG000000029878	6.62E-03
Fam102b	ENSMUSG000000040339	6.62E-03
Atp6v0a1	ENSMUSG000000019302	6.63E-03
Slit1	ENSMUSG000000025020	6.68E-03
Cacnb4	ENSMUSG000000017412	6.68E-03
Itih3	ENSMUSG000000006522	6.74E-03
Zfp148	ENSMUSG000000022811	6.79E-03
Lrrc58	ENSMUSG000000034158	6.79E-03
Pcdhb9	ENSMUSG000000051242	6.79E-03
Sema6c	ENSMUSG000000038777	6.79E-03
Lrch2	ENSMUSG000000031290	6.79E-03
Cdh24	ENSMUSG000000059674	6.81E-03
Ncoa2	ENSMUSG000000005886	6.85E-03

STRESS INDUCED ASTROGLIAL PLASTICITY

Ythdf3	ENSMUSG000000047213	6.88E-03
Rxrg	ENSMUSG000000015843	6.90E-03
Adora2a	ENSMUSG000000020178	6.90E-03
Tmem263	ENSMUSG000000060935	6.90E-03
Nfkbia	ENSMUSG000000021025	6.90E-03
Kcnj4	ENSMUSG000000044216	6.90E-03
Dgcr2	ENSMUSG000000003166	6.90E-03
Rfx3	ENSMUSG000000040929	6.90E-03
Gpr107	ENSMUSG000000000194	6.90E-03
Zfp770	ENSMUSG000000040321	6.90E-03
Spred1	ENSMUSG000000027351	6.90E-03
Smyd1	ENSMUSG000000055027	6.90E-03
Cd4	ENSMUSG000000023274	6.90E-03
Tecr	ENSMUSG000000031708	6.90E-03
Porcn	ENSMUSG000000031169	6.90E-03
Sh3bgrl	ENSMUSG000000031246	6.90E-03
Diaph2	ENSMUSG000000034480	6.90E-03
Tap2	ENSMUSG000000024339	6.99E-03
Dagla	ENSMUSG000000035735	7.01E-03
Mmp17	ENSMUSG000000029436	7.13E-03
Aplp1	ENSMUSG000000006651	7.13E-03
Gabra2	ENSMUSG000000000560	7.18E-03
Lrfr1	ENSMUSG000000030600	7.20E-03
Zfp24	ENSMUSG000000051469	7.20E-03
Cd248	ENSMUSG000000056481	7.22E-03
Prex2	ENSMUSG000000048960	7.23E-03
Sec11a	ENSMUSG000000025724	7.23E-03
Chga	ENSMUSG000000021194	7.23E-03
Maml3	ENSMUSG000000061143	7.23E-03
Amn	ENSMUSG000000021278	7.25E-03
Lpcat4	ENSMUSG000000027134	7.63E-03
Slc16a11	ENSMUSG000000040938	7.70E-03
Gde1	ENSMUSG000000033917	7.70E-03
Adra1d	ENSMUSG000000027335	7.73E-03
Gm13339	ENSMUSG000000082884	7.76E-03
Ggt7	ENSMUSG000000027603	7.76E-03
Ergic3	ENSMUSG000000005881	7.76E-03
Spink8	ENSMUSG000000050074	7.76E-03
Slmap	ENSMUSG000000021870	7.79E-03
Ccdc42	ENSMUSG000000045915	7.83E-03

STRESS INDUCED ASTROGLIAL PLASTICITY

Adra1b	ENSMUSG000000050541	7.89E-03
Glmp	ENSMUSG000000001418	7.91E-03
Tmem201	ENSMUSG000000044700	7.96E-03
Chrna4	ENSMUSG000000027577	7.96E-03
Arsa	ENSMUSG000000022620	7.99E-03
Nr3c1	ENSMUSG000000024431	8.17E-03
Gpr162	ENSMUSG000000038390	8.17E-03
Naa30	ENSMUSG000000036282	8.41E-03
Lama5	ENSMUSG000000015647	8.50E-03
Bcam	ENSMUSG000000002980	8.62E-03
Foxo1	ENSMUSG000000044167	8.65E-03
Agfg1	ENSMUSG000000026159	8.78E-03
Kcnk12	ENSMUSG000000050138	8.78E-03
Lin7c	ENSMUSG000000027162	8.78E-03
Grik5	ENSMUSG000000003378	8.78E-03
Alg1	ENSMUSG000000039427	8.83E-03
Btbd17	ENSMUSG000000000202	8.85E-03
Inadl	ENSMUSG000000061859	8.85E-03
Bicd1	ENSMUSG000000003452	8.85E-03
Rcn3	ENSMUSG000000019539	8.85E-03
Ankrd63	ENSMUSG000000078137	8.90E-03
Atg9a	ENSMUSG000000033124	8.93E-03
Scap	ENSMUSG000000032485	8.93E-03
Gtf2a1	ENSMUSG000000020962	8.95E-03
Mib1	ENSMUSG000000024294	9.04E-03
Usp29	ENSMUSG000000051527	9.04E-03
Kirrel3	ENSMUSG000000032036	9.04E-03
Cyp2d22	ENSMUSG000000061740	9.08E-03
Cacna1g	ENSMUSG000000020866	9.09E-03
Hdac9	ENSMUSG000000004698	9.15E-03
Nexn	ENSMUSG000000039103	9.36E-03
Avl9	ENSMUSG000000029787	9.36E-03
Slc27a4	ENSMUSG000000059316	9.43E-03
Fam117b	ENSMUSG000000041040	9.46E-03
Galnt16	ENSMUSG000000021130	9.46E-03
Nrp1	ENSMUSG000000025810	9.46E-03
Npy	ENSMUSG000000029819	9.47E-03
Fam71e1	ENSMUSG000000051113	9.47E-03
Phf6	ENSMUSG000000025626	9.47E-03
Abca7	ENSMUSG000000035722	9.53E-03

STRESS INDUCED ASTROGLIAL PLASTICITY

Aff4	ENSMUSG000000049470	9.53E-03
Samd4	ENSMUSG000000021838	9.58E-03
Sash1	ENSMUSG000000015305	9.58E-03
Lgmn	ENSMUSG000000021190	9.69E-03
Atf2	ENSMUSG000000027104	9.69E-03
NA	ENSMUSG000000086084	9.69E-03
Htr1d	ENSMUSG000000070687	9.69E-03
Dnm3	ENSMUSG000000040265	9.70E-03
Celsr1	ENSMUSG000000016028	9.90E-03
Ephb2	ENSMUSG000000028664	9.90E-03
Lamb2	ENSMUSG000000052911	9.90E-03
Plcx2	ENSMUSG000000087141	9.98E-03
Fcho2	ENSMUSG000000041685	1.00E-02
Eif5a2	ENSMUSG000000050192	1.00E-02
Amotl1	ENSMUSG000000013076	1.00E-02
Por	ENSMUSG000000005514	1.03E-02
Chrm4	ENSMUSG000000040495	1.04E-02
Ccdc88a	ENSMUSG000000032740	1.04E-02
Rpn2	ENSMUSG000000027642	1.05E-02
Abcb9	ENSMUSG000000029408	1.05E-02
Plxnb1	ENSMUSG000000053646	1.05E-02
Gabrd	ENSMUSG000000029054	1.06E-02
Cfap97	ENSMUSG000000031631	1.06E-02
Entpd2	ENSMUSG000000015085	1.06E-02
Naglu	ENSMUSG000000001751	1.06E-02
Prkcsh	ENSMUSG000000003402	1.06E-02
Zfp317	ENSMUSG000000057551	1.06E-02
Abca2	ENSMUSG000000026944	1.07E-02
Lrp10	ENSMUSG000000022175	1.07E-02
Spty2d1	ENSMUSG000000049516	1.07E-02
Ipcef1	ENSMUSG000000064065	1.08E-02
St3gal5	ENSMUSG000000056091	1.08E-02
Sel1l3	ENSMUSG000000029189	1.08E-02
Syncrip	ENSMUSG000000032423	1.08E-02
Pcsk1n	ENSMUSG000000039278	1.08E-02
Arl14ep	ENSMUSG000000027122	1.09E-02
Rabgap1l	ENSMUSG000000026721	1.10E-02
Nenf	ENSMUSG000000037499	1.10E-02
Atf6b	ENSMUSG000000015461	1.10E-02
Islr2	ENSMUSG000000051243	1.11E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Prrt3	ENSMUSG000000045009	1.11E-02
Ubxn2b	ENSMUSG000000028243	1.12E-02
Ogfod3	ENSMUSG000000025169	1.13E-02
Igfbp2	ENSMUSG000000039323	1.13E-02
Atp9a	ENSMUSG000000027546	1.13E-02
Dlx6os1	ENSMUSG000000090063	1.14E-02
1500015O10Rik	ENSMUSG000000026051	1.14E-02
Chat	ENSMUSG000000021919	1.14E-02
Tmem176b	ENSMUSG000000029810	1.17E-02
Clptm1	ENSMUSG000000002981	1.17E-02
Gcc2	ENSMUSG000000038039	1.20E-02
Erp29	ENSMUSG000000029616	1.20E-02
Lcat	ENSMUSG000000035237	1.20E-02
Ireb2	ENSMUSG000000032293	1.20E-02
Glb1	ENSMUSG000000045594	1.20E-02
Slc13a5	ENSMUSG000000020805	1.21E-02
Ext2	ENSMUSG000000027198	1.21E-02
Pde1c	ENSMUSG000000004347	1.22E-02
Smpd1	ENSMUSG000000037049	1.23E-02
Pld3	ENSMUSG000000003363	1.23E-02
Smad5	ENSMUSG000000021540	1.25E-02
Tspyl5	ENSMUSG000000038984	1.25E-02
Kcnb1	ENSMUSG000000050556	1.25E-02
NA	ENSMUSG000000090378	1.26E-02
Slc7a4	ENSMUSG000000022756	1.26E-02
Scg5	ENSMUSG000000023236	1.26E-02
Sv2a	ENSMUSG000000038486	1.26E-02
Slc2a1	ENSMUSG000000028645	1.26E-02
AW551984	ENSMUSG000000038112	1.26E-02
Prkch	ENSMUSG000000021108	1.26E-02
Spry4	ENSMUSG000000024427	1.26E-02
Wfs1	ENSMUSG000000039474	1.26E-02
Cpt1c	ENSMUSG000000007783	1.26E-02
Klhl13	ENSMUSG000000036782	1.27E-02
Lin7a	ENSMUSG000000019906	1.28E-02
Ddost	ENSMUSG000000028757	1.30E-02
Syt5	ENSMUSG000000004961	1.30E-02
Larp4b	ENSMUSG000000033499	1.30E-02
Fam107a	ENSMUSG000000021750	1.30E-02
Apoe	ENSMUSG000000002985	1.30E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Tcap	ENSMUSG00000007877	1.30E-02
Plxnb2	ENSMUSG000000036606	1.31E-02
Nek7	ENSMUSG000000026393	1.31E-02
Clcn2	ENSMUSG000000022843	1.31E-02
Emc1	ENSMUSG000000078517	1.31E-02
Rsf1	ENSMUSG000000035623	1.31E-02
Rnf4	ENSMUSG000000029110	1.32E-02
Crk	ENSMUSG000000017776	1.32E-02
Sytl1	ENSMUSG000000028860	1.32E-02
Galnt18	ENSMUSG000000038296	1.32E-02
Psd3	ENSMUSG000000030465	1.32E-02
Slc35c2	ENSMUSG000000017664	1.32E-02
Kcnc3	ENSMUSG000000062785	1.32E-02
Nucb1	ENSMUSG000000030824	1.32E-02
Gpr37l1	ENSMUSG000000026424	1.33E-02
Ppp1r1b	ENSMUSG000000061718	1.33E-02
Chrn3	ENSMUSG000000031492	1.34E-02
Abcb7	ENSMUSG000000031333	1.34E-02
Nppc	ENSMUSG000000026241	1.35E-02
Sp4	ENSMUSG000000025323	1.35E-02
Klf12	ENSMUSG000000072294	1.35E-02
H2-K1	ENSMUSG000000061232	1.35E-02
Sema4a	ENSMUSG000000028064	1.35E-02
Atp1a1	ENSMUSG000000033161	1.35E-02
Zic1	ENSMUSG000000032368	1.35E-02
Fam199x	ENSMUSG000000042595	1.35E-02
F3	ENSMUSG000000028128	1.37E-02
Clcn7	ENSMUSG000000036636	1.37E-02
Rprd1a	ENSMUSG000000040446	1.37E-02
Sez6l2	ENSMUSG000000030683	1.37E-02
Mtpn	ENSMUSG000000029840	1.38E-02
Fgfr1	ENSMUSG000000031565	1.38E-02
Txndc5	ENSMUSG000000038991	1.38E-02
Clspn	ENSMUSG000000042489	1.39E-02
Srsf7	ENSMUSG000000024097	1.40E-02
G6pc3	ENSMUSG000000034793	1.41E-02
Nov	ENSMUSG000000037362	1.43E-02
Slc35f6	ENSMUSG000000029175	1.43E-02
C530008M17Rik	ENSMUSG000000036377	1.43E-02
Ly6h	ENSMUSG000000022577	1.44E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

C1ql3	ENSMUSG000000049630	1.44E-02
Fam173a	ENSMUSG000000057411	1.45E-02
Arhgap5	ENSMUSG000000035133	1.46E-02
Ptpn4	ENSMUSG000000026384	1.48E-02
Chrm1	ENSMUSG000000032773	1.48E-02
Rims1	ENSMUSG000000041670	1.50E-02
Fbln1	ENSMUSG000000006369	1.50E-02
Tiparp	ENSMUSG000000034640	1.50E-02
Ntn5	ENSMUSG000000070564	1.50E-02
Tomm70a	ENSMUSG000000022752	1.50E-02
Yif1b	ENSMUSG000000030588	1.51E-02
Kdelr1	ENSMUSG000000002778	1.51E-02
Epha7	ENSMUSG000000028289	1.52E-02
Wnk1	ENSMUSG000000045962	1.52E-02
Ankrd52	ENSMUSG000000014498	1.53E-02
Cldn5	ENSMUSG000000041378	1.53E-02
Map3k13	ENSMUSG000000033618	1.53E-02
Fkbp2	ENSMUSG000000056629	1.53E-02
Tox4	ENSMUSG000000016831	1.56E-02
Slc39a12	ENSMUSG000000036949	1.56E-02
Elavl2	ENSMUSG000000008489	1.56E-02
Elavl1	ENSMUSG000000040028	1.56E-02
Syp	ENSMUSG000000031144	1.56E-02
Dgat2	ENSMUSG000000030747	1.56E-02
Adgrg1	ENSMUSG000000031785	1.56E-02
Bcl2	ENSMUSG000000057329	1.58E-02
Pfkfb3	ENSMUSG000000026773	1.58E-02
Adck2	ENSMUSG000000046947	1.59E-02
Tmem94	ENSMUSG000000020747	1.59E-02
Aup1	ENSMUSG000000068328	1.59E-02
Dr1	ENSMUSG000000029265	1.59E-02
Smg1	ENSMUSG000000030655	1.60E-02
Prdx4	ENSMUSG000000025289	1.60E-02
Hbb-bt	ENSMUSG000000073940	1.60E-02
Mob1b	ENSMUSG000000006262	1.60E-02
Cckbr	ENSMUSG000000030898	1.60E-02
AW549877	ENSMUSG000000041935	1.61E-02
Cyb5d1	ENSMUSG000000044795	1.61E-02
Clvs1	ENSMUSG000000041216	1.61E-02
Omg	ENSMUSG000000049612	1.61E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Pnoc	ENSMUSG000000045731	1.61E-02
Enpp2	ENSMUSG000000022425	1.64E-02
Sez6	ENSMUSG000000000632	1.65E-02
Cyp7b1	ENSMUSG000000039519	1.65E-02
Tpst2	ENSMUSG000000029344	1.65E-02
Fkrp	ENSMUSG000000048920	1.66E-02
Sik2	ENSMUSG000000037112	1.66E-02
Tmem132a	ENSMUSG000000024736	1.66E-02
L1cam	ENSMUSG000000031391	1.66E-02
Matn4	ENSMUSG000000016995	1.67E-02
Asphd1	ENSMUSG000000046378	1.67E-02
Pcolce	ENSMUSG000000029718	1.67E-02
Creld1	ENSMUSG000000030284	1.69E-02
Astn2	ENSMUSG000000028373	1.70E-02
Slc37a4	ENSMUSG000000032114	1.70E-02
Fam208a	ENSMUSG000000040651	1.70E-02
Trps1	ENSMUSG000000038679	1.70E-02
Lama4	ENSMUSG000000019846	1.71E-02
Ptger3	ENSMUSG000000040016	1.71E-02
Cntfr	ENSMUSG000000028444	1.71E-02
Clstn3	ENSMUSG000000008153	1.71E-02
S100pbb	ENSMUSG000000040928	1.71E-02
Creb1	ENSMUSG000000025958	1.72E-02
Psen2	ENSMUSG000000010609	1.72E-02
Atp6v0c	ENSMUSG000000024121	1.72E-02
Rasd2	ENSMUSG000000034472	1.73E-02
Lrp1	ENSMUSG000000040249	1.73E-02
Mpp5	ENSMUSG000000021112	1.73E-02
Scn8a	ENSMUSG000000023033	1.73E-02
Ccdc50	ENSMUSG000000038127	1.73E-02
Scai	ENSMUSG000000035236	1.73E-02
Lrp3	ENSMUSG000000001802	1.73E-02
Rbm26	ENSMUSG000000022119	1.74E-02
Ago2	ENSMUSG000000036698	1.74E-02
Slc10a4	ENSMUSG000000029219	1.74E-02
Vgf	ENSMUSG000000037428	1.74E-02
Sp3	ENSMUSG000000027109	1.75E-02
Shisa9	ENSMUSG000000022494	1.75E-02
Sppl2b	ENSMUSG000000035206	1.76E-02
Nfat5	ENSMUSG000000003847	1.76E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Ttll7	ENSMUSG000000036745	1.76E-02
Wbscr17	ENSMUSG000000034040	1.77E-02
Lzts1	ENSMUSG000000036306	1.77E-02
Tmem101	ENSMUSG000000020921	1.77E-02
Wdr26	ENSMUSG000000038733	1.80E-02
Fam169a	ENSMUSG000000041817	1.80E-02
Oaf	ENSMUSG000000032014	1.80E-02
Sorbs1	ENSMUSG000000025006	1.81E-02
Slc22a17	ENSMUSG000000022199	1.81E-02
Sstr3	ENSMUSG000000044933	1.83E-02
Adrb1	ENSMUSG000000035283	1.83E-02
Gmps	ENSMUSG000000027823	1.83E-02
Enah	ENSMUSG000000022995	1.84E-02
Ptprk	ENSMUSG000000019889	1.86E-02
Atraid	ENSMUSG000000013622	1.86E-02
Man2b1	ENSMUSG000000005142	1.86E-02
Cdipt	ENSMUSG000000030682	1.86E-02
Ptprs	ENSMUSG000000013236	1.87E-02
Gnal	ENSMUSG000000024524	1.87E-02
Apmmap	ENSMUSG000000033096	1.87E-02
Ctsf	ENSMUSG000000083282	1.87E-02
Sp1	ENSMUSG000000001280	1.88E-02
Hrh3	ENSMUSG000000039059	1.88E-02
Dkk3	ENSMUSG000000030772	1.88E-02
Nlgn2	ENSMUSG000000051790	1.88E-02
Gm5578	ENSMUSG000000029633	1.88E-02
Sst	ENSMUSG000000004366	1.88E-02
Ago3	ENSMUSG000000028842	1.91E-02
Lats1	ENSMUSG000000040021	1.91E-02
Spg7	ENSMUSG000000000738	1.92E-02
Ranbp2	ENSMUSG000000003226	1.92E-02
Tnks	ENSMUSG000000031529	1.92E-02
Ntsr2	ENSMUSG000000020591	1.94E-02
Cnga4	ENSMUSG000000030897	1.94E-02
Kcnh3	ENSMUSG000000037579	1.94E-02
Rab37	ENSMUSG000000020732	1.96E-02
Armcl	ENSMUSG000000027599	1.96E-02
Rfx4	ENSMUSG000000020037	1.98E-02
Rabep1	ENSMUSG000000020817	1.98E-02
Ptk7	ENSMUSG000000023972	1.98E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Wipf1	ENSMUSG000000075284	1.98E-02
Lingo1	ENSMUSG000000049556	1.98E-02
Fcgrt	ENSMUSG000000003420	1.98E-02
Adgrb3	ENSMUSG000000033569	2.01E-02
Grina	ENSMUSG000000022564	2.01E-02
Snx16	ENSMUSG000000027534	2.01E-02
Klhl34	ENSMUSG000000047485	2.03E-02
Tcea1	ENSMUSG000000033813	2.03E-02
Ptpa	ENSMUSG000000027303	2.03E-02
Nr2f2	ENSMUSG000000030551	2.03E-02
Dnajc16	ENSMUSG000000040697	2.04E-02
Lin54	ENSMUSG000000035310	2.04E-02
Aplp2	ENSMUSG000000031996	2.05E-02
Bdp1	ENSMUSG000000049658	2.07E-02
Pdlim5	ENSMUSG000000028273	2.07E-02
Wnt6	ENSMUSG000000033227	2.07E-02
Dcbld1	ENSMUSG000000019891	2.07E-02
Ddx6	ENSMUSG000000032097	2.09E-02
Cnnm2	ENSMUSG000000064105	2.11E-02
Dbp	ENSMUSG000000059824	2.11E-02
Chic1	ENSMUSG000000031327	2.11E-02
Slc9a1	ENSMUSG000000028854	2.12E-02
Cacna1b	ENSMUSG000000004113	2.12E-02
Klk8	ENSMUSG000000064023	2.12E-02
Arid4b	ENSMUSG000000039219	2.13E-02
Fbxl6	ENSMUSG000000022559	2.13E-02
Fkbp10	ENSMUSG000000001555	2.14E-02
Spaca6	ENSMUSG000000080316	2.14E-02
Pcdh1	ENSMUSG000000051375	2.14E-02
Cpne3	ENSMUSG000000028228	2.14E-02
Slc30a3	ENSMUSG000000029151	2.14E-02
Slc12a4	ENSMUSG000000017765	2.14E-02
Zc3h12c	ENSMUSG000000035164	2.14E-02
C1galt1c1	ENSMUSG000000048970	2.14E-02
Epha5	ENSMUSG000000029245	2.14E-02
Trpc4	ENSMUSG000000027748	2.15E-02
Plppr2	ENSMUSG000000040563	2.16E-02
Efna3	ENSMUSG000000028039	2.18E-02
Pitpnc1	ENSMUSG000000040430	2.19E-02
Tmem151a	ENSMUSG000000061451	2.19E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Fads3	ENSMUSG000000024664	2.19E-02
Tmem9	ENSMUSG000000026411	2.19E-02
Pth1r	ENSMUSG000000032492	2.19E-02
Rab9b	ENSMUSG000000043463	2.19E-02
Vcpip1	ENSMUSG000000045210	2.20E-02
NA	ENSMUSG000000074910	2.20E-02
Ptbp3	ENSMUSG000000028382	2.20E-02
Mfsd12	ENSMUSG000000034854	2.20E-02
Zhx1	ENSMUSG000000022361	2.20E-02
Ubac2	ENSMUSG000000041765	2.21E-02
Ankrd13c	ENSMUSG000000039988	2.22E-02
Ppfibp1	ENSMUSG000000016487	2.22E-02
Slc4a3	ENSMUSG000000006576	2.22E-02
Gm13394	ENSMUSG000000083773	2.22E-02
Cpe	ENSMUSG000000037852	2.23E-02
NA	ENSMUSG000000071286	2.24E-02
Camk2d	ENSMUSG000000053819	2.24E-02
Tmed1	ENSMUSG000000032180	2.24E-02
Atp6ap1	ENSMUSG000000019087	2.24E-02
Pdpk1	ENSMUSG000000024122	2.26E-02
Ttbk2	ENSMUSG000000090100	2.27E-02
Nr2c2	ENSMUSG000000005893	2.27E-02
Dhrs7b	ENSMUSG000000042569	2.27E-02
Pon2	ENSMUSG000000032667	2.28E-02
Pcdhb17	ENSMUSG000000046387	2.28E-02
Gabbr2	ENSMUSG000000039809	2.28E-02
Sgce	ENSMUSG000000004631	2.28E-02
Tmem246	ENSMUSG000000039611	2.29E-02
Xiap	ENSMUSG000000025860	2.29E-02
mt-Co3	ENSMUSG000000064358	2.30E-02
Top1	ENSMUSG000000070544	2.33E-02
Hipk3	ENSMUSG000000027177	2.37E-02
Fam63b	ENSMUSG000000042444	2.37E-02
Caprin1	ENSMUSG000000027184	2.37E-02
Ankrd45	ENSMUSG000000044835	2.41E-02
Zfp449	ENSMUSG000000073176	2.42E-02
Slc3a2	ENSMUSG000000010095	2.42E-02
Fzd9	ENSMUSG000000049551	2.43E-02
Pigo	ENSMUSG000000028454	2.44E-02
Pou3f4	ENSMUSG000000056854	2.44E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Pla2g7	ENSMUSG000000023913	2.46E-02
Brinp1	ENSMUSG000000028351	2.46E-02
Plod3	ENSMUSG000000004846	2.46E-02
Wnk3	ENSMUSG0000000041245	2.46E-02
Ncstn	ENSMUSG000000003458	2.47E-02
Rgma	ENSMUSG000000070509	2.49E-02
Sppl3	ENSMUSG000000029550	2.49E-02
Map3k19	ENSMUSG000000051590	2.49E-02
9330159F19Rik	ENSMUSG000000004360	2.49E-02
Gm15440	ENSMUSG000000051107	2.49E-02
Trib1	ENSMUSG000000032501	2.49E-02
Fndc3b	ENSMUSG000000039286	2.49E-02
Tsc22d2	ENSMUSG000000027806	2.49E-02
Ccdc107	ENSMUSG000000028461	2.49E-02
Dusp4	ENSMUSG000000031530	2.49E-02
Scpep1	ENSMUSG000000000278	2.53E-02
Gpalpp1	ENSMUSG000000022008	2.53E-02
Plagl1	ENSMUSG000000019817	2.54E-02
Rtn4rl1	ENSMUSG000000045287	2.54E-02
Magi3	ENSMUSG000000052539	2.54E-02
Msi2	ENSMUSG000000069769	2.54E-02
Olfml2b	ENSMUSG000000038463	2.55E-02
B230219D22Rik	ENSMUSG000000045767	2.55E-02
Mapk1ip1l	ENSMUSG000000021840	2.55E-02
Xpnpep3	ENSMUSG000000022401	2.55E-02
Larp4	ENSMUSG000000023025	2.55E-02
NA	ENSMUSG000000008686	2.55E-02
Mydgf	ENSMUSG000000019579	2.55E-02
Plpp7	ENSMUSG000000051373	2.55E-02
Tyro3	ENSMUSG000000027298	2.55E-02
Slc35a2	ENSMUSG000000031156	2.56E-02
Gabrb1	ENSMUSG000000029212	2.57E-02
Atrx	ENSMUSG000000031229	2.57E-02
Pcyox1l	ENSMUSG000000024579	2.57E-02
Ddrgk1	ENSMUSG000000068290	2.57E-02
Ackr2	ENSMUSG000000044534	2.58E-02
Tmod2	ENSMUSG000000032186	2.58E-02
Rybp	ENSMUSG000000072872	2.59E-02
Slc39a6	ENSMUSG000000024270	2.61E-02
Glg1	ENSMUSG000000003316	2.61E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

A830010M20Rik	ENSMUSG00000044060	2.63E-02
Tram1l1	ENSMUSG00000044528	2.64E-02
Zfp521	ENSMUSG00000024420	2.64E-02
Pcp4l1	ENSMUSG00000038370	2.65E-02
Fnbp1l	ENSMUSG00000039735	2.66E-02
Agpat4	ENSMUSG00000023827	2.66E-02
Fn1	ENSMUSG00000026193	2.68E-02
Kitl	ENSMUSG00000019966	2.68E-02
Col11a2	ENSMUSG00000024330	2.68E-02
Mecp2	ENSMUSG00000031393	2.68E-02
Vstm2b	ENSMUSG00000039257	2.69E-02
Mbtps1	ENSMUSG00000031835	2.69E-02
Nhs	ENSMUSG00000059493	2.69E-02
Zfp37	ENSMUSG00000028389	2.69E-02
Mex3c	ENSMUSG00000037253	2.71E-02
Rnf145	ENSMUSG00000019189	2.72E-02
Il12rb2	ENSMUSG00000018341	2.72E-02
Heca	ENSMUSG00000039879	2.72E-02
Zmiz1	ENSMUSG00000007817	2.73E-02
AU040320	ENSMUSG00000028830	2.74E-02
Tmem97	ENSMUSG00000037278	2.75E-02
Dolk	ENSMUSG00000075419	2.75E-02
Gm12696	ENSMUSG00000084159	2.75E-02
Arih1	ENSMUSG00000025234	2.76E-02
Adgrl3	ENSMUSG00000037605	2.77E-02
Gpkow	ENSMUSG00000031148	2.77E-02
Pdgfrb	ENSMUSG00000024620	2.77E-02
Trem2	ENSMUSG00000023992	2.77E-02
BC017158	ENSMUSG00000030780	2.78E-02
Plekhh3	ENSMUSG00000035172	2.78E-02
Rictor	ENSMUSG00000050310	2.78E-02
Adgra3	ENSMUSG00000029090	2.81E-02
Cep170	ENSMUSG00000057335	2.81E-02
Slit3	ENSMUSG00000056427	2.81E-02
B2m	ENSMUSG00000060802	2.81E-02
Abcb8	ENSMUSG00000028973	2.83E-02
Clcn6	ENSMUSG00000029016	2.84E-02
Mum1l1	ENSMUSG00000042515	2.84E-02
Chgb	ENSMUSG00000027350	2.87E-02
Hbb-bs	ENSMUSG00000052305	2.87E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Cxzc4	ENSMUSG00000044365	2.88E-02
Col8a2	ENSMUSG00000056174	2.88E-02
Aftph	ENSMUSG00000049659	2.88E-02
Lfng	ENSMUSG00000029570	2.88E-02
Serpini1	ENSMUSG00000027834	2.89E-02
Gpnmb	ENSMUSG00000029816	2.89E-02
Them6	ENSMUSG00000056665	2.89E-02
Ramp2	ENSMUSG00000001240	2.89E-02
Ddx3y	ENSMUSG00000069045	2.90E-02
Dnajb11	ENSMUSG00000004460	2.91E-02
2510009E07Rik	ENSMUSG00000043391	2.93E-02
Ciart	ENSMUSG00000038550	2.94E-02
Dennd5b	ENSMUSG00000030313	2.94E-02
Zranb2	ENSMUSG00000028180	2.94E-02
Sox5	ENSMUSG00000041540	2.95E-02
Zfp654	ENSMUSG00000047141	2.96E-02
Ganab	ENSMUSG00000071650	2.96E-02
Srgap1	ENSMUSG00000020121	2.96E-02
Fam196b	ENSMUSG00000069911	2.97E-02
Itga3	ENSMUSG00000001507	3.00E-02
Synj2bp	ENSMUSG00000090935	3.00E-02
Slc26a8	ENSMUSG00000036196	3.00E-02
Whsc1	ENSMUSG00000057406	3.00E-02
Zeb2	ENSMUSG00000026872	3.00E-02
mt-Nd6	ENSMUSG00000064368	3.00E-02
Rbm20	ENSMUSG00000043639	3.04E-02
H2-T23	ENSMUSG00000067212	3.05E-02
Trim44	ENSMUSG00000027189	3.06E-02
Gabra5	ENSMUSG00000055078	3.07E-02
Lrrn3	ENSMUSG00000036295	3.09E-02
Tmem50a	ENSMUSG00000028822	3.12E-02
Reep2	ENSMUSG00000038555	3.12E-02
Ptp4a1	ENSMUSG00000026064	3.13E-02
Esrrg	ENSMUSG00000026610	3.13E-02
Lims1	ENSMUSG00000019920	3.13E-02
Vmp1	ENSMUSG00000018171	3.13E-02
Rspo2	ENSMUSG00000051920	3.13E-02
Pbx3	ENSMUSG00000038718	3.13E-02
Apbb2	ENSMUSG00000029207	3.14E-02
Pdk4	ENSMUSG00000019577	3.14E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Ctsb	ENSMUSG000000021939	3.14E-02
Atp2c2	ENSMUSG000000034112	3.14E-02
Hcn2	ENSMUSG000000020331	3.16E-02
Tmem184b	ENSMUSG000000009035	3.16E-02
Mid2	ENSMUSG000000000266	3.16E-02
Notch3	ENSMUSG000000038146	3.20E-02
Emc9	ENSMUSG000000022217	3.22E-02
St13	ENSMUSG000000022403	3.22E-02
Cacul1	ENSMUSG000000033417	3.22E-02
mt-Atp6	ENSMUSG000000064357	3.24E-02
Gulp1	ENSMUSG000000056870	3.24E-02
Ubfd1	ENSMUSG000000030870	3.29E-02
Ddah1	ENSMUSG000000028194	3.30E-02
Wls	ENSMUSG000000028173	3.30E-02
Cdh20	ENSMUSG000000050840	3.32E-02
Sid1t1	ENSMUSG000000022696	3.32E-02
Haghl	ENSMUSG000000061046	3.32E-02
Dzank1	ENSMUSG000000037259	3.33E-02
Wscd1	ENSMUSG000000020811	3.34E-02
Cpq	ENSMUSG000000039007	3.34E-02
Igf2r	ENSMUSG000000023830	3.34E-02
Sec11c	ENSMUSG000000024516	3.35E-02
Gdap1	ENSMUSG000000025777	3.39E-02
Nxph3	ENSMUSG000000046719	3.42E-02
Mier3	ENSMUSG000000032727	3.42E-02
App	ENSMUSG000000022892	3.42E-02
NA	ENSMUSG000000067833	3.42E-02
Htra1	ENSMUSG000000006205	3.42E-02
Ldlr	ENSMUSG000000032193	3.42E-02
Arhgap20	ENSMUSG000000053199	3.42E-02
Mamld1	ENSMUSG000000059401	3.42E-02
Prkar2a	ENSMUSG000000032601	3.43E-02
Ormdl3	ENSMUSG000000038150	3.43E-02
Cidea	ENSMUSG000000024526	3.43E-02
Tmem121	ENSMUSG000000049036	3.44E-02
Luzp1	ENSMUSG000000001089	3.46E-02
Itm2c	ENSMUSG000000026223	3.46E-02
Erlec1	ENSMUSG000000020311	3.46E-02
Mtss1	ENSMUSG000000022353	3.46E-02
Arhgap31	ENSMUSG000000022799	3.46E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Cadm3	ENSMUSG00000005338	3.47E-02
Frmpd4	ENSMUSG000000049176	3.47E-02
Myh11	ENSMUSG000000018830	3.48E-02
NA	ENSMUSG000000078191	3.48E-02
Adgrl1	ENSMUSG000000013033	3.49E-02
Tab3	ENSMUSG000000035476	3.50E-02
Vtn	ENSMUSG000000017344	3.50E-02
Cyp51	ENSMUSG000000001467	3.52E-02
Il17ra	ENSMUSG000000002897	3.53E-02
Gm2606	ENSMUSG000000078300	3.55E-02
Gm13340	ENSMUSG000000083563	3.56E-02
Fxyd6	ENSMUSG000000066705	3.56E-02
Stk26	ENSMUSG000000031112	3.56E-02
Zfp322a	ENSMUSG000000046351	3.57E-02
Mfsd13a	ENSMUSG000000025227	3.57E-02
Gca	ENSMUSG000000026893	3.58E-02
Nrg2	ENSMUSG000000060275	3.58E-02
Cst3	ENSMUSG000000027447	3.58E-02
Ttc39b	ENSMUSG000000038172	3.58E-02
Scn1b	ENSMUSG000000019194	3.60E-02
Nrxn2	ENSMUSG000000033768	3.60E-02
Pten	ENSMUSG000000013663	3.61E-02
Ankrd6	ENSMUSG000000040183	3.62E-02
Zmat4	ENSMUSG000000037492	3.62E-02
mt-Nd2	ENSMUSG000000064345	3.63E-02
Scrg1	ENSMUSG000000031610	3.65E-02
Rapgef5	ENSMUSG000000041992	3.66E-02
mt-Cytb	ENSMUSG000000064370	3.66E-02
Secisbp2l	ENSMUSG000000035093	3.67E-02
Tmem59	ENSMUSG000000028618	3.69E-02
Meis1	ENSMUSG000000020160	3.70E-02
Sorcs3	ENSMUSG000000063434	3.70E-02
Vav3	ENSMUSG000000033721	3.70E-02
Nr4a3	ENSMUSG000000028341	3.70E-02
Sun1	ENSMUSG000000036817	3.70E-02
Tusc3	ENSMUSG000000039530	3.70E-02
Tmem160	ENSMUSG000000019158	3.71E-02
Ubash3b	ENSMUSG000000032020	3.74E-02
Fam120c	ENSMUSG000000025262	3.74E-02
Flrt1	ENSMUSG000000047787	3.74E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Zscan26	ENSMUSG000000022228	3.75E-02
Pcdhb12	ENSMUSG000000043458	3.76E-02
Lrpap1	ENSMUSG000000029103	3.77E-02
Fuca1	ENSMUSG000000028673	3.77E-02
Slc24a3	ENSMUSG000000063873	3.81E-02
Hapln4	ENSMUSG000000007594	3.81E-02
Lrfrn2	ENSMUSG000000040490	3.81E-02
Prkaa2	ENSMUSG000000028518	3.82E-02
NA	ENSMUSG000000073868	3.82E-02
Dscaml1	ENSMUSG000000032087	3.83E-02
Dzip3	ENSMUSG000000064061	3.85E-02
Ptgds	ENSMUSG000000015090	3.87E-02
Tmem131	ENSMUSG000000026116	3.87E-02
Dlk2	ENSMUSG000000047428	3.87E-02
Kcnj9	ENSMUSG000000038026	3.88E-02
St8sia5	ENSMUSG000000025425	3.88E-02
Paqr7	ENSMUSG000000037348	3.88E-02
Orai1	ENSMUSG000000049686	3.88E-02
Grin2b	ENSMUSG000000030209	3.88E-02
Kctd12b	ENSMUSG000000041633	3.88E-02
Hist1h1c	ENSMUSG000000036181	3.92E-02
Crif1	ENSMUSG000000007888	3.92E-02
Saraf	ENSMUSG000000031532	3.93E-02
mt-Co2	ENSMUSG000000064354	3.93E-02
Cdc27	ENSMUSG000000020687	3.94E-02
Zswim6	ENSMUSG000000032846	3.94E-02
H13	ENSMUSG000000019188	3.94E-02
Clptm1l	ENSMUSG000000021610	3.95E-02
Far1	ENSMUSG000000030759	3.95E-02
Tex264	ENSMUSG000000040813	3.96E-02
Mesdc2	ENSMUSG000000038503	3.98E-02
Efna2	ENSMUSG00000003070	3.98E-02
Lgals3bp	ENSMUSG000000033880	3.98E-02
mt-Nd4	ENSMUSG000000064363	3.98E-02
Mlc1	ENSMUSG000000035805	3.98E-02
Rpgrip1l	ENSMUSG000000033282	3.98E-02
Rnf215	ENSMUSG000000003581	4.01E-02
Vimp	ENSMUSG000000075701	4.01E-02
Zfx	ENSMUSG000000079509	4.01E-02
Psap	ENSMUSG000000004207	4.01E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

09-Mar	ENSMUSG00000040502	4.02E-02
Slc52a2	ENSMUSG00000022560	4.02E-02
Zkscan1	ENSMUSG00000029729	4.02E-02
Braf	ENSMUSG00000002413	4.02E-02
Grm7	ENSMUSG00000056755	4.02E-02
C330007P06Rik	ENSMUSG00000006423	4.02E-02
Shisa5	ENSMUSG00000025647	4.03E-02
Slc45a4	ENSMUSG00000079020	4.05E-02
Pofut2	ENSMUSG00000020260	4.06E-02
Ubxn2a	ENSMUSG00000020634	4.06E-02
Tprn	ENSMUSG00000048707	4.06E-02
Tmem55a	ENSMUSG00000028221	4.06E-02
Shroom2	ENSMUSG00000045180	4.06E-02
Rorb	ENSMUSG00000036192	4.08E-02
Tenm4	ENSMUSG00000048078	4.10E-02
Bsg	ENSMUSG00000023175	4.11E-02
Tmem40	ENSMUSG00000059900	4.11E-02
Pdp1	ENSMUSG00000049225	4.11E-02
Slc7a8	ENSMUSG00000022180	4.15E-02
Mier1	ENSMUSG00000028522	4.15E-02
Cnot6	ENSMUSG00000020362	4.16E-02
Tsku	ENSMUSG00000049580	4.16E-02
Boc	ENSMUSG00000022687	4.18E-02
BC004004	ENSMUSG00000052712	4.18E-02
Rnf26	ENSMUSG00000053128	4.20E-02
Ralgps2	ENSMUSG00000026594	4.21E-02
NA	ENSMUSG00000051554	4.21E-02
Gpc4	ENSMUSG00000031119	4.21E-02
Mansc1	ENSMUSG00000032718	4.22E-02
Atp1a2	ENSMUSG00000007097	4.23E-02
Zfp397	ENSMUSG00000024276	4.23E-02
Angel1	ENSMUSG00000021257	4.23E-02
C4b	ENSMUSG00000073418	4.23E-02
Rab8b	ENSMUSG00000036943	4.24E-02
Ddx17	ENSMUSG00000055065	4.24E-02
Pank3	ENSMUSG00000018846	4.27E-02
Rgs9	ENSMUSG00000020599	4.27E-02
Agpat1	ENSMUSG00000034254	4.27E-02
Serpinh1	ENSMUSG00000070436	4.27E-02
Srek1	ENSMUSG00000032621	4.28E-02

STRESS INDUCED ASTROGLIAL PLASTICITY

Mfsd5	ENSMUSG000000045665	4.28E-02
Marcks	ENSMUSG000000069662	4.30E-02
Nufip2	ENSMUSG000000037857	4.30E-02
Hdgfrp3	ENSMUSG000000025104	4.33E-02
Arhgap36	ENSMUSG000000036198	4.35E-02
Otud6b	ENSMUSG000000040550	4.36E-02
Srsf1	ENSMUSG000000018379	4.37E-02
Tsga10	ENSMUSG000000060771	4.41E-02
Serpine2	ENSMUSG000000026249	4.41E-02
Camk4	ENSMUSG000000038128	4.41E-02
Tm2d2	ENSMUSG000000031556	4.41E-02
Tmem254a	ENSMUSG000000072676	4.43E-02
B930095G15Rik	ENSMUSG000000085133	4.43E-02
Snap23	ENSMUSG000000027287	4.43E-02
Dcun1d1	ENSMUSG000000027708	4.43E-02
Gm16485	ENSMUSG000000078117	4.43E-02
Ano10	ENSMUSG000000037949	4.43E-02
Tspan3	ENSMUSG000000032324	4.46E-02
Pum2	ENSMUSG000000020594	4.49E-02
Dapk3	ENSMUSG000000034974	4.51E-02
Tbc1d4	ENSMUSG000000033083	4.51E-02
Snap29	ENSMUSG000000022765	4.51E-02
Txndc12	ENSMUSG000000028567	4.51E-02
Etv6	ENSMUSG000000030199	4.51E-02
Plekhh2	ENSMUSG000000026123	4.54E-02
Dhcr24	ENSMUSG000000034926	4.54E-02
Erbin	ENSMUSG000000021709	4.54E-02
Acta2	ENSMUSG000000035783	4.56E-02
Gm4631	ENSMUSG000000078899	4.56E-02
Zfp91	ENSMUSG000000024695	4.57E-02
Gm21988	ENSMUSG000000040904	4.58E-02
Ptprn2	ENSMUSG000000056553	4.58E-02
Dscam	ENSMUSG000000050272	4.58E-02
Fzd2	ENSMUSG000000050288	4.58E-02
Cebpb	ENSMUSG000000056501	4.58E-02
Sorcs2	ENSMUSG000000029093	4.58E-02
Lrrtm1	ENSMUSG000000060780	4.58E-02
Atp6ap2	ENSMUSG000000031007	4.58E-02
Tbl1x	ENSMUSG000000025246	4.58E-02
Pcyox1	ENSMUSG000000029998	4.61E-02

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Gm13341	ENSMUSG000000083863	4.63E-02
Itch	ENSMUSG000000027598	4.63E-02
Sqle	ENSMUSG000000022351	4.63E-02
Gdf10	ENSMUSG000000021943	4.64E-02
Trim33	ENSMUSG000000033014	4.66E-02
Creg1	ENSMUSG000000040713	4.67E-02
Pds5b	ENSMUSG000000034021	4.67E-02
Scarf2	ENSMUSG000000012017	4.68E-02
Dad1	ENSMUSG000000022174	4.68E-02
Klhl24	ENSMUSG000000062901	4.68E-02
Rsrc1	ENSMUSG000000034544	4.70E-02
Zfp760	ENSMUSG000000067928	4.71E-02
Ptchd2	ENSMUSG000000041544	4.73E-02
Tmem43	ENSMUSG000000030095	4.73E-02
Islr	ENSMUSG000000037206	4.74E-02
Nrip1	ENSMUSG000000048490	4.76E-02
Nell1	ENSMUSG000000055409	4.80E-02
Slco4a1	ENSMUSG000000038963	4.80E-02
Lrrtm4	ENSMUSG000000052581	4.80E-02
Cldn22	ENSMUSG000000038064	4.80E-02
Alg2	ENSMUSG000000039740	4.80E-02
Tnpo1	ENSMUSG000000009470	4.83E-02
Hhatl	ENSMUSG000000032523	4.83E-02
E330009J07Rik	ENSMUSG000000037172	4.84E-02
Tmem176a	ENSMUSG000000023367	4.84E-02
Ctsd	ENSMUSG000000007891	4.84E-02
Cul4b	ENSMUSG000000031095	4.84E-02
Ackr1	ENSMUSG000000037872	4.85E-02
Naa50	ENSMUSG000000022698	4.85E-02
Il34	ENSMUSG000000031750	4.85E-02
Aph1a	ENSMUSG000000015750	4.86E-02
NA	ENSMUSG000000049494	4.86E-02
A830018L16Rik	ENSMUSG000000057715	4.86E-02
Cyb5r1	ENSMUSG000000026456	4.86E-02
Ntm	ENSMUSG000000059974	4.86E-02
Tmem165	ENSMUSG000000029234	4.86E-02
Lamp5	ENSMUSG000000027270	4.88E-02
Bbox1	ENSMUSG000000041660	4.91E-02
Tmem218	ENSMUSG000000032121	4.91E-02
Dhrs7	ENSMUSG000000021094	4.91E-02

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Slc35b2	ENSMUSG000000037089	4.91E-02
Abhd3	ENSMUSG000000002475	4.91E-02
Cyp26b1	ENSMUSG000000063415	4.91E-02
Gm9769	ENSMUSG000000040078	4.92E-02
Yod1	ENSMUSG000000046404	4.95E-02
Hsd17b11	ENSMUSG000000029311	4.95E-02
Tpr	ENSMUSG000000006005	4.96E-02
Kdelr2	ENSMUSG000000079111	4.96E-02
Lrg1	ENSMUSG000000037095	4.97E-02
Nbl1	ENSMUSG000000041120	4.97E-02
Lct	ENSMUSG000000026354	4.98E-02
Pik3c2a	ENSMUSG000000030660	4.99E-02
Dpp8	ENSMUSG000000032393	4.99E-02
Zc3h12b	ENSMUSG000000035045	4.99E-02
Cacna1h	ENSMUSG000000024112	5.00E-02
Cd34	ENSMUSG000000016494	5.00E-02
Rspo3	ENSMUSG000000019880	5.00E-02
Fstl4	ENSMUSG000000036264	5.00E-02
Trip11	ENSMUSG000000021188	5.00E-02
Nol4	ENSMUSG000000041923	5.00E-02
Zmat3	ENSMUSG000000027663	5.00E-02
Clstn2	ENSMUSG000000032452	5.00E-02
Fam3a	ENSMUSG000000031399	5.00E-02

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Table 3. Four major clusters of pathways significantly implicated in cortical astroglial response to stress in adult male mice.

TOP CATEGORIES OF PATHWAY CHANGES RELATED TO STRESS IN THE CORTEX OF ADULT MALE MICE				
	<i>p</i> -value	<i>q</i> -value	Pathway	Source
Growth Factor Signaling	4.23E-06	7.75E-04	Signaling by NGF	Reactome
	1.59E-05	1.29E-03	NGF signaling via TRKA from plasma membrane	Reactome
	6.05E-05	3.23E-03	Downstream signaling of activated FGFR	Reactome
	8.13E-05	3.83E-03	Signaling by FGFR	Reactome
	2.26E-04	9.06E-03	Signaling by FGFR in disease	Reactome
	9.87E-04	1.94E-02	Signaling by EGFR	Reactome
Drug Addiction Pathways	5.32E-06	7.75E-04	Nicotine addiction- mus musculus (mouse)	KEGG
	5.27E-05	3.01E-03	Cocaine addiction- mus musculus (mouse)	KEGG
	9.01E-04	1.90E-02	Amphetamine addiction- mus musculus (mouse)	KEGG
	1.32E-03	2.19E-02	Morphine addiction- mus musculus (mouse)	KEGG
	8.27E-03	7.25E-02	Opioid signaling	Reactome
Glutamatergic Pathways	1.28E-03	2.17E-02	Post NMDA receptor activation events	Reactome
	2.63E-03	3.34E-02	Activation of NMDA receptor upon glutamate binding & postsynaptic events	Reactome
	8.49E-03	7.25E-02	Glutamatergic synapse- mus musculus (mouse)	KEGG
Extracellular Matrix Pathways	2.08E-03	2.87E-02	Other glycan degradation - Mus musculus (mouse)	KEGG
	5.16E-03	5.50E-02	Extracellular matrix organization	Reactome
	5.40E-03	5.61E-02	Glycosphingolipid metabolism	Reactome
	6.44E-03	6.44E-02	ECM proteoglycans	Reactome
	1.20E-03	2.17E-02	N-Glycan biosynthesis - Mus musculus (mouse)	KEGG

Discussion

In the current study we examined the effects of a rodent model of depression on cortical astroglial cell expression of proteins associated with plastic states and related gene expression profiles. Although stress is a normal and albeit important physiological reaction, chronic and uncontrollable stressors (such as job loss, death of a loved one, etc.) have been linked to both the onset of depression and exacerbation of symptoms. The use of the chronic uncontrollable stress (CUS) paradigm employed in this study allowed us to examine neuroadaptations or compensations (i.e., plasticity) that may result from stress of a more chronic nature. Furthermore, chronic administration of the selective serotonin reuptake inhibitor (SSRI), fluoxetine, in both control and stressed animals may elucidate potential mechanisms for the reversal of stress effects.

The chronic uncontrollable stress paradigm (adapted from the Duman laboratory) was used to induce an anxiety and depressive-like behavioural phenotype. Indeed, the present study found that exposure to chronic uncontrollable stress had significant effects on both depressive and anxiety-like behaviours. However, contrary to our initial hypothesis, fluoxetine administration did not reverse the behavioural effects of stress. In fact, in many instances fluoxetine actually augmented the effects of stress such that it increased depressive and anxiety-like behaviours in control mice. While seemingly paradoxical, this finding may be explained by strain differences in responsivity to fluoxetine. Certainly, anti-depressants show individual heterogeneity in their effectiveness in human patients. In mice, several studies have shown general strain differences in responses to antidepressant treatments, and in particular, studies have shown that fluoxetine treatment does not show behavioural effects in the C57/B6, DBA/2, 129SvEv strains, however it is effective in Balb/c mice (Dulawa, Holick,

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Gundersen, & Hen, 2004). Further studies with different anti-depressant drugs shown to be effective in C57/B6 (such as imipramine) will allow us to examine the astroglial response to reversing the effects of stress (Strekalova et al., 2013).

While an emerging body of work has suggested a potential role for astroglial cells in both the etiology and treatment of depression, much of this work concentrates on changes within the hippocampus and amygdala (Czeh, Simon, Schmelting, Hiemke, & Fuchs, 2006; Bowley, Drevets, Öngür, & Price, 2002; Fuchs, Czeh, Koe, Michaelis, & Lucassen, 2004). However, since the very nature of depression intimately involves alterations in thought processing and cognition, it seemed rational that the cortex would be primarily involved along with the hippocampus and amygdala. This is further justified by evidence from post-mortem tissue of depressed-suicide patients, which has shown decreased expression of glial fibrillary acidic protein (GFAP) in cortical astroglial cells, a protein associated with glial plasticity (Torres-Platas et al., 2011). Interestingly, decreases in cortical GFAP levels have also been reported in animal models of depression, leading authors to conclude that there is a decrease in the total number of cortical astroglial cells in depression/models of depression. However, in the current study we employed a mouse that constitutively expresses GFP in all astroglial cells and although we saw a decrease in GFAP⁺ cells, we found no differences in total cell number when we assessed GFP. All GFAP⁺ cells co-expressed GFP, however only 40-50% of GFP⁺ cells were GFAP⁺, indicating that GFAP only represents a fragment of the astroglial population. This finding highlights the importance in interpreting protein expression as a marker of total cell population number rather than specific protein expression levels.

Several studies in recent years have focused on the role of glutamate in depressive behaviors, in part due to the identification of ketamine as a rapid-acting anti-depressant

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treatment. Because astroglial cells recycle upwards of 90% of glutamate in the brain, we examined whether astroglial GS, the enzyme responsible for glutamine production from glutamate, expression levels changed in response to stress. We found no significant differences in the total number of astroglial cells expressing GS, suggesting that there were no changes in glutamate breakdown. However, GS expression is only an indirect measure of glutamate/glutamine turnover, with the assumption that enzyme levels represent use. Therefore, it is possible that GS enzyme levels do not represent activity per se; further studies would need to examine glutamate and glutamine levels directly in order to ascertain efficiency of astroglial recycling of glutamate into glutamine.

Previous studies have shown increases in gliogenesis and neurogenesis in response to stress and anti-depressant treatment (Rajkowska, & Miguel-Hidalgo, 2007; Banasr, & Duman, 2007). Because a subpopulation of astroglial cells show neural stem cell capability, we examined whether proteins associated with stem cell state were changed in response to stress and fluoxetine. When we examined the transcription factor Sox2, we found an increase in response to stress that was reversed with fluoxetine administration. This reversal by fluoxetine suggests that Sox2 expression was not related to any of our behavioural results, as we did not see any reversal of stress-induced behavioural differences by fluoxetine. When we examined vimentin expression, an intermediate filament protein associated with radial extensions in astroglia, we found significant increases in the Stress/Vehicle animals, particularly in the superficial layers of the cortex and the PFC. These findings are potentially exciting because vimentin is typically down regulated in the cortex of the adult brain, where astroglial cells are protoplasmic and generally star-shaped. Up regulation of vimentin suggests increased astroglial cell stem cell capability and a general increase in plastic state. Further analysis

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using a marker of active cell division for example, Ki67, in conjunction with GFP expression, would provide insight as to astroglial proliferation levels. This is particularly important given the same pattern was observed in both Sox2 and Vimentin cortical expression levels.

In addition to protein expression profiles, we also used TRAP technology to examine astroglial-specific transcriptome changes in response to stress. We found a large number of differentially expressed genes that were significantly related to 98 known signaling/expression pathways. Of these 98, four major ontology clusters were observed: growth factors, drug addiction, glutamatergic signaling, and extracellular matrices (ECM). These findings are particularly relevant, as all of these phenomena have been related to depression in humans. Growth factors such as BDNF and FGF2 have been causally implicated in depression and anxiety as has glutamate and glutamate signaling (as aforementioned). Drug addiction has long been related to mood and anxiety disorders, with high-levels of co-morbidity and well defined phenomena such as stress-induced relapse (Volkow, 2004). Finally ECM's have recently been implicated in psychiatric disorders in humans, where changes in cortical perineuronal nets (PNN), the major ECM in the CNS, have been identified in post-mortem tissue of depressed and schizophrenic patients, among other psychiatric illnesses (Mauney et al., 2013; Hercher, Turecki, Mechawar, 2009). Astroglial cells are key players in the formation and modulation of these PNNs (Kwok, Dick, Wang, & Fawcett, 2011). Whether PNNs are changed in animal models of psychiatric disease remains to be determined, however the current study would suggest that they are changed in response to CUS, at least at the level of gene expression. Future studies in our laboratory will examine PNN protein expression levels and assess the causal involvement of PNNs in stress and rehabilitation by degrading PNNs using Chondroitinase ABC.

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The primary objective of this research thesis was therefore to assess how cortical astroglial cells change in response to stress and antidepressant treatment in order to examine novel astrocytic factors involved in depressive-like behaviours. We have demonstrated significant changes in astroglial cells in several functional domains including stem cell capability, intermediate filament protein expression and have identified transcriptomic changes associated with neuroplasticity and addiction. Future studies will continue to elucidate the causal role of astroglial changes in response to stress on behavioural phenotypes.

Research using animal models of psychiatric illness is imperative to find novel treatments for human disease. However, animal models are only a first step towards translation. Findings such as our described changes in cortical astroglial cells need to be validated at least indirectly in post-mortem human tissue (for example, changes in stem cell potential) before we can even begin to conceive of testing new therapies. Interestingly, the current study shows that the strain of mice used (C57/B6) do not respond to fluoxetine, and yet other strains of mice have been shown to respond therapeutically to fluoxetine. This is inline with approximately half of individuals not responding to fluoxetine as well. Therefore, future animal studies might compare strains that respond to strains that do not respond and search for related biomarkers that could then be used in humans to predict which antidepressant would be most effective for that individual. This would allow psychiatrists to use a blood test to determine the best course of treatment and therefore reduce the amount of time before reduction of symptoms.

References

- Abdallah, C., Sanacora, G., Duman, R. S., & Krystal, J. H. (2015). Ketamine and Rapid-Acting Antidepressants: A Window into a New Neurobiology for Mood Disorder Therapeutics. *Annual Review of Medicine*, 66, 509–523.
- Al-Harbi, K. S. (2012). Treatment-resistant depression: therapeutic trends, challenges, and future directions. *Patient Preference and Adherence*, 6, 369–388. doi: 10.2147/PPA.S29716
- American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.
- Banasr, M., Chowdhury, G., Terwilliger, R., Newton, S., Duman, R., Behar, K., & Sanacora, G. (2010). Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioural deficits by the glutamate-modulating drug riluzole. *Molecular Psychiatry*, 15, 501-511. doi:10.1038/mp. 2008.106
- Banasr, M., & Duman, R. S. (2007). Regulation of neurogenesis and gliogenesis by stress and antidepressant treatment. *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, 6, 311-320.
- Banasr, M., & Duman, R. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviours. *Biological Psychiatry*, 64, 863-870. doi:10.1016/j.biopsych.2008.06.008
- Banasr, M., Valentine, G., Li, X., Gourley, S., Taylor, J., & Duman, R. (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biological Psychiatry*, 62, 496-504.

STRESS INDUCED ASTROGLIAL PLASTICITY

- Berman, R., Cappiello, A., Anand, A., Oren, D., Heninger, G., Charney, D., & Krystal, J. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, 47, 351–354.
- Bowley, M. P., Drevets, W. C., Öngür, D., & Price, J. L. (2002). Low glial numbers in the amygdala in major depressive disorder. *Biological psychiatry*, 52, 404-412.
- Collins, P. Y., Patel, V., Joestl, S. S., March, D., Insel, T. R., & Daar, A. S. (2011). Grand challenges in global mental health: A consortium of researchers, advocates and clinicians announces here research priorities for improving the lives of people with mental illness around the world, and calls for urgent action and investment. *Nature*, 475, 27–30. doi:10.1038/475027a
- Conrad, C., LeDoux, J., Magariños, A., McEwen, B. (1999). Repeated restraint stress facilitates fear conditioning independently of causing hippocampal CA3 dendritic atrophy. *Behavioural Neuroscience*, 113, 902–913.
- Cotter, D., Mackay, D., Chana, G., Beasley, C., Landau, S., & Everall, I. (2002). Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cerebral Cortex*, 12, 386-394.
- Czéh, B., & Di Benedetto, B. (2012). Antidepressants act directly on astrocytes: evidence and functional consequences. *European Neuropsychopharmacology*, 23, 171-185. doi:10.1016/j.euroneuro.2012.04.017
- Czéh, B., Simon, M., Schmelting, B., Hiemke, C., & Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology*, 31, 1616-1626.
- de Kloet, E., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease.

STRESS INDUCED ASTROGLIAL PLASTICITY

Nature Review Neuroscience, 6, 463–475.

- Doyle, J. P., Dougherty, J. D., Heiman, M., Schmidt, E. F., Stevens, T. R., Ma, G., ... Heintz, N. (2008). Application of BACarray for comparative analysis of CNS cell types. *Cell*, 135, 749–762. doi:10.1016/j.cell.2008.10.029
- Dougherty, J. D., Schmidt, E. F., Nakajima, M., & Heintz, N. (2010). Analytical approaches to RNA profiling data for the identification of genes enriched in specific cells. *Nucleic acids research*, 38, 4218-4230.
- Dulawa, S. C., Holick, K. A., Gundersen, B., & Hen, R. (2004). Effects of chronic fluoxetine in animal models in anxiety and depression. *Neuropsychopharmacology*, 29.
- Fuchs, E., Czéh, B., Kole, M. H., Michaelis, T., & Lucassen, P. J. (2004). Alterations of neuroplasticity in depression: the hippocampus and beyond. *European Neuropsychopharmacology*, 14, S481-S490.
- Gundersen, H., Bendtsen, T., Korbo, L., Marcussen, N., Moller, A., Nielsen, K., Nyengaard, B., Pakkenberg, B., Sorensen, B., Vesterby, A., & West, M. (1988). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *APMIS*, 96, 379-394. doi:10.1111/j.1699-0463.1988.tb05320.x
- Hamilton, J. P., Siemer, M., & Gotlib, I. H. (2008). Amygdala volume in Major Depressive Disorder: A meta-analysis of magnetic resonance imaging studies. *Molecular Psychiatry*, 13, 993–1000. doi:10.1038/mp.2008.57
- Hawton, K., & van Heeringen, K. (2009). Suicide. *Lancet*, 373, 1372–81. doi:10.1016/S0140-6736(09)60372-X
- Heiman, M., Schaefer, A., Gong, S., Peterson, J., Day, M., Ramsey, K. E., ... Heintz, N. (2008). Development of a BACarray translational profiling approach for the molecular

STRESS INDUCED ASTROGLIAL PLASTICITY

characterization of CNS cell types. *Cell*, 135, 738–748. doi:10.1016/j.cell.2008.10.028

Heinger, G., Delgado, P., & Charney, D. (1996). The revised monoamine theory of depression: A modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry*, 29, 2-11. doi:10.1055/s-2007-979535

Hercher, C., Turecki, G., Mechawar, N. (2009). Through the looking glass: examining neuroanatomical evidence for cellular alterations in major depression. *Journal of Psychiatry*, 43, 947–961.

Joëls, M., Karst, H., Krugers, H., & Lucassen, P. (2007). Chronic stress: implications for neuronal morphology, function and neurogenesis. *Frontiers in Neuroendocrinology*, 28, 72–96.

Kajitani, N., Hisaoka-Nakashima, K., Morioka, N., Okada-Tsuchioka, M., Kaneko, M., Kasai, M., Chiyo, S., Nakata, Y., & Takebayashi, M. (2012). Antidepressant acts on astrocytes leading to an increase in the expression of neurotrophic/growth factors: differential regulation of FGF-2 by noradrenaline. *PLoS ONE*, 7, e51197. doi:10.1371/journal.pone.0051197

Krishnan, V., & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature*, 455, 894–902. <http://doi.org.proxy.library.carleton.ca/10.1038/nature07455>

Kim, J., & Diamond, D. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nature Review Neuroscience*, 3, 453–462.

Kiyohara, C., & Yoshimasu, K. (2009). Molecular epidemiology of major depressive disorder. *Environmental Health and Preventive Medicine*, 14, 71–87. doi:10.1007 /s12199-008-0073-6

STRESS INDUCED ASTROGLIAL PLASTICITY

- Kreisel, T., Frank, M., Licht, T., Reshef, R., Zidon, O., Baratta, M., Maier, S., & Yirmiya, R. (2014). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Molecular Psychiatry*, *19*, 699-709. doi:10.1038/mp.2013.155
- Kwok, J. C., Dick, G., Wang, D., & Fawcett, J. W. (2011). Extracellular matrix and perineuronal nets in CNS repair. *Developmental neurobiology*, *71*, 1073-1089.
- Lacasse, J. R., & Leo, J. (2005). Serotonin and Depression: A disconnect between the advertisements and the scientific literature. *PLoS Medicine*, *2*, e392. doi:10.1371/journal.pmed.0020392
- Li, N., Liu, R.-J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Aghajanian, G., & Duman, R. S. (2011). Glutamate NMDA receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry*, *69*, 754–761.
- Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., ... Vaccarino, F. M. (2015). FOXP1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. *Cell*, *162*, 375–390. doi: 10.1016/j.cell.2015.06.034
- Mathew, S. J., Shah, A., Lapidus, K., Clark, C., Jarun, N., Ostermeyer, B., & Murrough, J. W. (2012). Ketamine for treatment-resistant unipolar depression: current evidence. *CNS Drugs*, *26*, 189–204. doi:10.2165/11599770
- Mauney, S. A., Athanas, K. M., Pantazopoulos, H., Shaskan, N., Passeri, E., Berretta, S., & Woo, T. U. W. (2013). Developmental pattern of perineuronal nets in the human prefrontal cortex and their deficit in schizophrenia. *Biological psychiatry*, *74*, 427-435.
- McEwen, B. (1999). Stress and hippocampal plasticity. *Annual Review Neuroscience*, *22*, 105-

122.

- McEwen, B. (2007). Physiology and neurobiology of stress adaptation: Central role of the brain. *Physiological Reviews*, 87, 873-904.
- Mitra, R., Jadhav, S., McEwen, B., Vyas, A., & Chattarji, S. (2005). Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 9371–9376.
- Niciu, M. J., Henter, I. D., Luckenbaugh, D. A., Zarate, C. A., & Charney, D. S. (2014). Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annual Review of Pharmacology and Toxicology*, 54, 119–139.
- Pittenger, C., & Duman, R. (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology Reviews*, 33, 88-109.
- Rajkowska, G., & Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS & Neurological Disorders Drug Targets*, 6, 219–233.
- Reinstatler, L., & Youssef, N. (2015). Ketamine as a potential treatment for suicidal ideation: A systematic review of the literature. *Drugs in Research and Development*, 15, 37-43.
doi:10.1007/s40268-015-0081-0
- Rial, D., Lemos, C., Pinheiro, H., Duarte, J., Gonçalves, F., Real, J., Prediger, R., Gonçalves, N., Gomes, C., Canas, P., Agostinho, P., & Cunha, R. (2016). Depression as a glial-based synaptic dysfunction. *Frontiers in Cellular Neuroscience*, 9, doi:10.3389/fncel.2015.00521
- Sacher J, Neumann J, Fünfstück T, Soliman A, Villringer A, Schroeter ML. (2012). Mapping

STRESS INDUCED ASTROGLIAL PLASTICITY

the depressed brain: a meta-analysis of structural and functional alterations in major depressive disorder. *Journal of Affective Disorders*, 140, 142–148.

Schmitz, C., & Hof, P. (2005). Design-based stereology in neuroscience. *Neuroscience*, 130, 813-831. doi:10.1016/j.neuroscience.2004.08.050

Sheline, Y., Sanghavi, M., Mintun, M., & Gado, M. (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *The Journal of Neuroscience*, 19, 5034-5043.

Singh, M. K., & Gotlib, I. H. (2014). The Neuroscience of Depression: Implications for Assessment and Intervention. *Behaviour Research and Therapy*, 62, 60–73. doi:10.1016/j.brat.2014.08.008

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathologica*, 119, 7–35.

Strekalova, T., Anthony, D. C., Dolgov, O., Anokhin, K., Kubatiev, A., Steinbusch, H. M., & Schroeter, C. (2013). The differential effects of chronic imipramine or citalopram administration on physiological and behavioral outcomes in naïve mice. *Behavioural brain research*, 245, 101-106.

Sun, J.-D., Liu, Y., Yuan, Y.-H., Li, J., & Chen, N.-H. (2012). Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology*, 37, 1305–1320.

Torres-Platas, S. G., Hercher, C., Davoli, M. A., Maussion, G., Labonté, B., Turecki, G., & Mechawar, N. (2011). Astrocytic Hypertrophy in Anterior Cingulate White Matter of Depressed Suicides. *Neuropsychopharmacology*, 36, 2650–2658. <http://doi.org.proxy.library.carleton.ca/10.1038/npp.2011.154>

STRESS INDUCED ASTROGLIAL PLASTICITY

- Videbech, P., & Ravnkilde, B. (2004). Hippocampal volume and depression: a meta-analysis of MRI studies. *The American Journal of Psychiatry*, 161, 1957–1966.
- Volkow, N. D. (2004). The reality of comorbidity: depression and drug abuse. *Biological psychiatry*, 56, 714-717.
- West, M. (1993). New stereological methods for counting neurons. *Neurobiology of Aging*, 14, 275-285. doi:10.1016/0197-4580(93)90112-O
- Wong, M., & Licinio, J. (2001). Research and treatment approaches to depression. *Nature Review Neuroscience*, 2, 343–51. doi:10.1038/35072566
- World Health Organization. The Global Burden of Disease: Update 2015. Retrieved from: <http://www.who.int/mediacentre/factsheets/fs369/en/>
- Yatham, L., Liddle, F., Sossi, V., et al. (2012). Positron emission tomography study of the effects of tryptophan depletion on brain serotonin₂ receptors in subjects recently remitted from major depression. *Arch Gen Psychiatry*, 69, 601-609. doi:10.1001/archgenpsychiatry.2011.1493
- Yuen, E. Y., Wei, J., Liu, W., Zhong, P., Li, X., & Yan, Z. (2012). Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*, 73, 962–977. doi:10.1016/j.neuron.2011.12.033