Disc1 Mutant Mice SubJECTED to Chronic Social Defeat Stress as a Model of Gene-Environment Interaction in Schizophrenia and Depression

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Pharmacology
University of Toronto

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Master of Science

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2009

Abstract

Human genetic data suggests DISC1 (Disrupted-in-schizophrenia 1) is a susceptibility gene for schizophrenia and depression. Disc1 Q31L-/- mutants show depression-like behaviour and Disc1 L100P-/- mutants schizophrenia-like behaviour. Heterozygous mutants show an intermediate phenotype. In a gene-environment interaction study, we exposed heterozygotes to chronic social defeat (CSD) stress and phenotyped behaviour. Disc1, Bdnf(III) and Pde4b mRNA levels were also measured. Moreover, as epigenetic mechanisms may mediate some effects of CSD, we also exposed wildtype mice to CSD concurrently with the histone deacetylase inhibitor valproate. We found that CSD increased anxiety in L100P-/+ mutants, and that levels of Disc1, Bdnf(III) and Pde4b mRNA were higher in this mutant. Valproate treatment did not correct CSD-induced behavioural changes. In conclusion, we have demonstrated an interaction between a strong susceptibility gene for psychiatric disease and an environmental manipulation similar to stressors known to affect mental illness.
Acknowledgements

First and foremost, I thank my supervisor, Dr. Albert H.C. Wong, for this tremendous learning experience. I appreciate his guidance, patience and advice in addressing the challenges presented to me over the past few years. Further helpful advice has come from my advisor, Dr. Paul J. Fletcher, to whom I also extend my sincerest gratitude.

Secondly, I am thoroughly grateful to Dr. Tatiana V. Lipina for her expertise in the chronic social defeat paradigm and animal behaviour testing, which guided me in this work. I also thank her for her help with some of the experimental work. I further extend my gratitude to Dr. John C. Roder for the use of his facilities and equipment. This work could not have been completed without the collaboration of the members of Dr. Roder’s lab.

Furthermore, I thank all members of the Wong lab for their contribution to my learning experience both in the lab and outside of it. Each member’s unique repertoire of skills, knowledge and humour has made them invaluable friends in addition to colleagues. I also thank my other colleagues at CAMH for their support. In particular, members from the labs of Dr. Kennedy and Dr. Fletcher are thanked for their sharing of equipment and morale.

Finally, I thank my family and friends. I thank my sister Dipa for constantly challenging my views, inspiring me to fight, making me laugh until my stomach hurts, and for her unwavering support in all aspects of my life. I sincerely thank my parents for their emotional and financial support, which has allowed me to pursue my passions. Their selfless sacrifices have made it possible for me to aim without limits. I thank all of my friends deeply for their understanding, patience and humour. In particular, I thank Mawahib, Alexandre, Frankie, Xiaoxi, Anjali, Alicia, Allison and Judy for keeping me sane these past few years through endless entertainment, coffee breaks and 3AM-phone/-MSN conversations.
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<tbody>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>Bdnf(III)/BDNF</td>
<td>brain-derived neurotrophic factor (transcript III)</td>
</tr>
<tr>
<td>CA</td>
<td>closed arm(s)</td>
</tr>
<tr>
<td>CAMKII</td>
<td>Ca2+/calmodulin-dependent protein kinase II</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>ChIP</td>
<td>chromatin immunoprecipitation</td>
</tr>
<tr>
<td>cLH</td>
<td>congenitally learned helpless</td>
</tr>
<tr>
<td>cNLH</td>
<td>congenitally not learned helpless</td>
</tr>
<tr>
<td>COMT</td>
<td>catechol-O-methyl transferase</td>
</tr>
<tr>
<td>CP</td>
<td>central platform</td>
</tr>
<tr>
<td>CpG</td>
<td>cytosine and guanine separated by a phosphate group; site of DNA methylation (at the cytosine)</td>
</tr>
<tr>
<td>CRL</td>
<td>Charles River Laboratories</td>
</tr>
<tr>
<td>CS</td>
<td>conditioned stimulus</td>
</tr>
<tr>
<td>CSD</td>
<td>chronic social defeat</td>
</tr>
<tr>
<td>CSM</td>
<td>chronic stress model</td>
</tr>
<tr>
<td>$C_T$</td>
<td>cycle threshold</td>
</tr>
<tr>
<td>DA</td>
<td>dopamine</td>
</tr>
<tr>
<td>dB</td>
<td>decibel</td>
</tr>
<tr>
<td>Disc1/DISC1</td>
<td>disrupted-in-schizophrenia 1</td>
</tr>
</tbody>
</table>
DNA  deoxyribonucleic acid
DNMT  DNA methyl transferase
DRD2  dopamine receptor D2
EE  environmental enrichment
eIF2B  guanine nucleotide exchange factor for eukaryotic initiation factor 2
eIF3  eukaryotic initiation factor 3
ENU  N-ethyl-N-nitrosourea
EPM  elevated plus maze
ES  effect size
FEZ1  fasciculation and elongation protein zeta-1
fMRI  functional magnetic resonance imaging
FR  Flinders Resistant
FS  Flinders Sensitive
FST  forced swim test
$G \times E$  gene-environment interaction
GABA  gamma-aminobutyric acid
Gapdh  glyceraldehyde 3-phosphate dehydrogenase
GEI  gene-environment interaction
GLM  general linear model
Grin  glutamate receptor, ionotropic, N-methyl-D-aspartate
H3  histone 3
HDAC  histone deacetylase
HPA  hypothalamus-pituitary-adrenal gland
HSD  honestly significant differences
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>IB</td>
<td>interaction behaviour test</td>
</tr>
<tr>
<td>LHM</td>
<td>learned helplessness model</td>
</tr>
<tr>
<td>LI</td>
<td>latent inhibition</td>
</tr>
<tr>
<td>LIS1</td>
<td>lissencephaly-1</td>
</tr>
<tr>
<td>MANOVA</td>
<td>multivariate ANOVA</td>
</tr>
<tr>
<td>MDD</td>
<td>major depressive disorder</td>
</tr>
<tr>
<td>Met</td>
<td>methionine</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>MZ</td>
<td>monozygotic</td>
</tr>
<tr>
<td>NAc</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NMDA</td>
<td>$N$-methyl-$D$-aspartic acid</td>
</tr>
<tr>
<td>NPE</td>
<td>non pre-exposed</td>
</tr>
<tr>
<td>NRG1</td>
<td>neuregulin 1</td>
</tr>
<tr>
<td>NUDEL</td>
<td>nuclear distribution element-like protein</td>
</tr>
<tr>
<td>OA</td>
<td>open arm(s)</td>
</tr>
<tr>
<td>OF</td>
<td>open field test</td>
</tr>
<tr>
<td>PCP</td>
<td>phencyclidine</td>
</tr>
<tr>
<td>Pde4b/PDE4B</td>
<td>phosphodiesterase 4b</td>
</tr>
<tr>
<td>PE</td>
<td>pre-exposed</td>
</tr>
<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
</tr>
<tr>
<td>PPI</td>
<td>prepulse inhibition</td>
</tr>
<tr>
<td>PT</td>
<td>partition test</td>
</tr>
<tr>
<td>QTL</td>
<td>quantitative trait loci</td>
</tr>
<tr>
<td>RE</td>
<td>relative expression</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>REM</td>
<td>rapid eye movement</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNE</td>
<td>relative normalized expression</td>
</tr>
<tr>
<td>rRNA</td>
<td>ribosomal RNA</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SC</td>
<td>sucrose consumption test</td>
</tr>
<tr>
<td>SCZ</td>
<td>schizophrenia</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSN</td>
<td>sociability and social novelty test</td>
</tr>
<tr>
<td>T0</td>
<td>no defeat sessions</td>
</tr>
<tr>
<td>T10</td>
<td>ten defeat sessions</td>
</tr>
<tr>
<td>T20</td>
<td>twenty defeat sessions</td>
</tr>
<tr>
<td>TCP</td>
<td>Toronto Centre for Phenogenomics</td>
</tr>
<tr>
<td>TIA-1</td>
<td>poly(A)-binding protein</td>
</tr>
<tr>
<td>TJL</td>
<td>The Jackson Laboratory</td>
</tr>
<tr>
<td>TM</td>
<td>transmembrane</td>
</tr>
<tr>
<td>TRAX</td>
<td>translin-associated factor X</td>
</tr>
<tr>
<td>UCR2</td>
<td>upstream conserved region 2</td>
</tr>
<tr>
<td>US</td>
<td>unconditioned stimulus</td>
</tr>
<tr>
<td>Val</td>
<td>valine</td>
</tr>
</tbody>
</table>
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Appendix A. Definitions for Specific Behaviours in Behavioural Tests.

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1 Introduction

1.1 Statement of Problem

Schizophrenia (SCZ) and major depressive disorder (MDD) are complex diseases that have significant impact on patients’ ability to function in their daily lives. SCZ affects approximately 1% of the population while MDD is much more common, with a lifetime prevalence of 7-12% in men and 20-25% in women (Ressler and Mayberg, 2007). There are likely many candidate genes that are important in the aetiology and pathophysiology of these diseases (Rapoport et al., 2005; Hovatta et al., 1999), but genetic factors alone cannot predict disease risk, nor fully explain disease in all individuals. Heritability figures reported for SCZ hover around 80% (Wong et al., 2005; Cardno and Gottesman, 2000) and only 21-45% for MDD (Kendler et al., 1992). Moreover, concordance among monozygotic (MZ) twin pairs (genetically identical) ranges between 41-65% for SCZ (Cardno and Gottesman, 2000) and is around 53% for MDD (McGuffin, Katz and Rutherford, 1991); thus DNA sequence alone cannot explain the presence of disease in the affected twin. Environmental factors affect the risk of developing both MDD (Fava and Kendler, 2000; Sullivan et al., 2000) and SCZ (Oh and Petronis, 2008; Krabbendam and van Os, 2005; Moghaddam and Jackson, 2004).

Animal models are essential for investigating disease aetiology, for understanding pathological mechanisms, and for identifying new treatment targets. For example, since human genetic data implicates DISC1 (Disrupted-in-schizophrenia 1) as a susceptibility gene in SCZ and MDD (Blackwood et al., 2001; Millar et al., 2000), mutant Disc1 mouse models can help to study neurobiological mechanisms by which Disc1 gene sequence variants can affect behaviour in
these illnesses. The effect of environmental factors can also be simulated in animal models such as the maternal inflammation model for SCZ (Nawa and Takei, 2006) or the learned helplessness model for MDD (Porsolt, 2000). To date, there have been few attempts to directly test gene-environment interactions in animal models of psychiatric disease, and such approaches are essential to better understand the aetiology of SCZ and MDD. These models also permit investigation of epigenetic mechanisms affecting disease risk and progression, since there is strong evidence that the environment can act via epigenetic mechanisms (Oh and Petronis, 2008).

Here we propose to model putative interactions between mutations in the mouse Disc1 gene and a psychological stressor, chronic social defeat (CSD), to simulate the social stress associated with precipitating psychotic and depressive episodes in humans (Björkqvist, 2001), using two lines of heterozygous Disc1 mutant mice. The homozygous counterparts of these mutants display behavioural phenotypes consistent with endophenotypes of SCZ and MDD: Q31L -/- mutants display depression-like behaviour and L100P -/- mice display schizophrenia-like behaviour (Clapcote et al., 2007). The heterozygous Q31L -/+ and L100P -/+ mutants show little or no change in behaviour (Clapcote et al., 2007), but as these animals carry one copy of the respective mutated Disc1 gene, we hypothesize an altered vulnerability to CSD stress exists. We expect that depression-like and psychosis-like symptoms in the heterozygous mutants will be exacerbated upon exposure to CSD.
1.2 Research Objectives

Our primary objective is to investigate whether the ENU-induced Disc1 Q31L and L100P mutations render heterozygous mutant mice more vulnerable to CSD stress. We will also investigate whether CSD and/or the Disc1 mutation alters expression of three strong candidate genes for SCZ and MDD – *Disc1, Bdnf* and *Pde4b* – which may be mediated by epigenetic mechanisms. By demonstrating that Disc1 genotype modulates susceptibility to environmental social stress in an animal model, we hope to provide the foundation for further work aimed at identifying specific molecular components of this interaction that may be suitable targets for improved treatments for these disorders. While identification of susceptibility genes is of course a necessary step in understanding disease aetiology, gene therapy is not yet feasible. It is more likely that other molecular targets mediating the effect of environment on susceptibility gene networks, will be amenable to pharmacological or psychological interventions.

1.3 Hypotheses

I. Since Disc1 Q31L-/- have more abnormalities in depression-related behavioural tests, and Disc1 L100P-/- have more abnormalities in psychosis-related behavioural tests, we hypothesize that CSD will exacerbate depression-like behaviours in Disc1 Q31L -/+ mutant mice and psychosis-like behaviours in Disc1 L100P -/+ mice.

II. Since social and psychological stress can increase the risk of developing MDD, and can trigger episodes of depression in MDD and psychosis in SCZ, we hypothesize that heterozygous ENU Disc1 mutant mice will be more vulnerable to the behavioural effects of CSD. We expect that Disc1 Q31L -/+ mutant mice will be more sensitive than L100P -/+
mutants to CSD, since the effect of social and psychological stressors is greater in MDD versus SCZ (Myin-Germeys et al., 2003; Paykel, 1978).

III. We hypothesize that Disc1, Bdnf and Pde4b mRNA levels will be altered by CSD, and that these effects will be dependent on Disc1 genotype and brain region, and be correlated with alterations in behaviour.

IV. We hypothesize that chronic treatment with valproate, a common mood stabilizer used to treat bipolar disorder and an anticonvulsant that is a histone deacetylase (HDAC) inhibitor, will improve behavioural deficits induced by CSD.

1.4 Experimental Outline

To test our hypotheses, we exposed heterozygous males from each of the two lines of mutants and wildtype littermates to the CSD paradigm, and then utilized traditional behavioural tests for phenotypic characterization. Control data was obtained for naive heterozygous mice in each line by Tatiana Lipina in the laboratory of Dr. John C. Roder. All data were obtained using the same test equipment and programs used in the current work.

Separate cohorts were used for molecular analyses to prevent detection of any changes in expression due to stress from behavioural testing (Figure 1.1). These “no-behaviour” cohorts of mice were sacrificed 24 hours after the last defeat exposure. Individually-housed controls were used for the genetic work: mice from each line were housed across from a stranger in a social defeat box, but the partition was never removed so there was never any direct social contact. The molecular-genetic work consisted of measuring Disc1, Bdnf (transcript III) and Pde4b mRNA
levels in the hypothalamus, hippocampus, striatum and prefrontal cortex using real-time RT-PCR, expression normalized to *Gapdh* and *β-actin* levels.

Because we had a limited number of social defeat boxes available to us at any one time, experiments were repeated to generate sufficient replicates in each group condition. The environment and testing parameters were controlled as much as possible to reproduce the test conditions over time. Moreover, we posit that any significant gene, environment, treatment or gene-environment effects observed in spite of possible variability between batches of animals separated in time makes our analysis more stringent, since temporal variability should increase noise in our data.

**Figure 1.1. Experimental outline.** Separate cohorts were used for behavioural testing and molecular-genetic work. Behavioural control data was provided by Tatiana Lipina (Roder lab).
1.5 The Aetiology of Schizophrenia and Major Depressive Disorder

SCZ and MDD are complex diseases, polygenic and multifactorial in origin (Gottesman and Gould, 2003). These psychopathologies – and mental illness in general – are highly complex in that the disease mechanisms are highly diverse. Consequently, one popular and traditional scientific approach has been reductionistic, in which researchers focus on genetic, environmental, and – more recently – epigenetic components in the aetiology and progression of SCZ and MDD.

1.5.1 Genetic Heritability

There is a large body of evidence for a genetic component in neuropsychiatric disease, be it heritability as observed in traditional family or twin studies or a predisposing gene variant in population association or linkage studies. Classical twin studies show that the heritability for SCZ is around 80% (Wong et al., 2005; Cardno and Gottesman, 2000) and that the heritability for MDD ranges somewhere between 21-45% for MDD (Kendler et al., 1992). The development of more efficient, high-throughput genetic techniques in recent times has led to an explosion in population association and linkage studies. Such studies have been key in identifying specific candidate genes in SCZ and MDD. A few of these are discussed below.

1.5.1.1 Disrupted -in-schizophrenia 1 (DISC1)

DISC1 is a candidate susceptibility gene for both SCZ and MDD. DISC1 encodes a full-length protein (~100 kDa) with various isoforms via alternative splicing that have not yet been
characterized (Figure 1.2). DISC1 protein is predicted to consist of a globular N-terminal domain and a helical C-terminal domain which has the potential to form a coiled-coil by interaction with another protein (Millar et al., 2000). The putative structure of DISC1 is compatible with a role in the nervous system and it is important to note that DISC1 is localized primarily within regions of the brain implicated in the pathogenesis of psychiatric illnesses (Porteous and Millar, 2006). Numerous functions of DISC1 have been proposed in various pathways. The consensus to date is that DISC1 acts as a molecular scaffold, interacting with many other proteins required for neuronal migration, neurite outgrowth, signal transduction, cAMP signalling, cytoskeletal modulation, and translational regulation (Millar et al., 2000). Some of the more well-established interactions are with NUDEL, LIS1, FEZ1, TRAX, PDE4B and BDNF (Chubb et al., 2008; Hennah et al., 2006).

The DISC1 region (not yet named DISC1) was first reported by St Clair et al. when they found a Scottish family with a high occurrence of major mental illness, where a t(1;11) translocation co-segregated with various psychopathologies such as SCZ, schizoaffective disorder and recurring MDD (1990). Millar et al. then fine-mapped the breakpoint of the balanced (1;11) (q42.1; q14.3) translocation and found DISC1 (2000). The group reported that the translocation affects the coding region and truncates the protein product of DISC1.

**Figure 1.2. Exon/intron schematic of DISC1 gene.** Adapted from Ishizuka et al., 2006. DISC1 contains 13 major exons and some minor exons that allow for alternative splicing. Several isoforms have been identified, although not yet fully characterized.
Since the discovery of DISC1, numerous independent linkage studies in diverse populations have found evidence implicating it as an important susceptibility factor both in psychosis and mood disorders. Linkage studies in Finnish populations have found evidence for linkage of SCZ and schizoaffective disorder to the 1q32.2-q41 region of chromosome 1, proximal to the DISC1 gene (Hovatta et al., 1999; Ekelund et al., 2000; Ekelund et al., 2004). A genome-wide linkage scan in a sample of patients with schizoaffective disorder from the UK and Ireland revealed that the highest peak occurred at 1q42, close to the DISC1 locus (Hamshere et al., 2005). Moreover, a study of 52 Taiwanese families with at least two affected schizophrenic siblings reported the segregation of a marker located near the breakpoint and DISC1 (D1S251) with SCZ, schizoaffective disorder, and other nonaffective psychotic disorders (Hwu et al., 2003).

Association studies in independent population samples also provide strong evidence implicating DISC1 in psychiatric illness. For example, the T-A allele combination of the single nucleotide polymorphisms (SNPs) of HEP3, a common haplotype containing two SNPs spanning from intron 1 to exon 2 of DISC1 at the 5’ end, has been reported to be significantly under-transmitted to affected individuals in one Finnish study (Hennah et al., 2003; 2005). It is also reported that the HEP3 haplotype displayed association with poorer performance on short-term visual memory and attention tests in affected and unaffected subjects, leading the authors to hypothesize that DISC1 affects short-term visual memory functions in addition to contributing to sensitivity to SCZ and associated disturbances (Hennah et al., 2005). In addition, a large Scottish case-control study found a strong association of the C-A allele combination of the SNPs of HEP3 with SCZ (Zhang et al., 2006). Another common haplotype, HEP1, consisting of three SNPs near the translocation breakpoint of DISC1, was found to be overrepresented among schizophrenic individuals in a Finnish population-based twin cohort study (Cannon et al., 2005). The study also
found an association with impairments in short- and long-term memory functioning and reduced grey matter density in the prefrontal cortex, as well as a trend toward association with reduced hippocampal volume (Cannon et al., 2005). Haplotype HEP1 has also been reported to be underrepresented in patients with schizoaffective disorder in a case-control study (Hodgkinson et al., 2004). This study found association of multiple haplotypes contained within four haplotype blocks extending between exon 1 and exon 9 of DISC1 with SCZ, schizoaffective disorder, and bipolar disorder (Hodgkinson et al., 2004).

1.5.1.2 Phosphodiesterase 4B (PDE4B)

PDE4B is a cyclic AMP (cAMP)-specific phosphodiesterase that regulates the cellular concentrations of cAMP through hydrolysis of the cyclic nucleotide. cAMP is a second messenger implicated in learning, memory, and mood (Numata et al., 2008a; Millar et al., 2005). DISC1 has been demonstrated to interact with PDE4B. Specifically, DISC1 interacts with the UCR2 domain of PDE4B and may regulate cAMP signalling through modulating PDE4B activity. Elevation of cellular cAMP leads to dissociation of PDE4B from DISC1 and an increase in PDE4B activity (Millar et al., 2005). It is therefore suggested that one functional role of DISC1 is to sequester PDE4B in resting cells and release it in an activated state in response to elevated intracellular levels of cAMP (Millar et al., 2005). The ENU-induced Disc1 mutant lines developed by Clapcote and colleagues (see sections 1.6.3.2 and 1.6.4.2) carry missense point mutations located within binding sites for PDE4B (Millar et al., 2007). Reduced binding between DISC1 and PDE4B in overexpression systems was found in both mutants, with the greatest deficit in the L100P -/- line (Clapcote et al., 2007).
Several linkage and association studies suggest that the *PDE4B* gene is also an independent candidate risk factor for SCZ. For example, disruption to *PDE4B* on chromosome 1 was found in a Scottish pair of cousins with a t(1;16) chromosomal translocation where one was diagnosed with SCZ and the other with another psychotic disorder (Millar *et al.*, 2005). A PDE4B haplotype was also shown to confer a protective effect against SCZ in females, implicating PDE4B further as a genetic risk factor (Pickard *et al.*, 2007). Several SNPs and haplotypes in *PDE4B* have also been found to be associated with SCZ in large populations of Caucasian, African American and Japanese patients (Fatemi *et al.*, 2008; Numata *et al.*, 2008b). Finally, lower levels of PDE4B isoforms have been observed in post-mortem brain tissue from patients diagnosed with SCZ and bipolar disorder (Fatemi *et al.*, 2008).

Several studies also offer evidence for a role of PDE4B in MDD. For example, a significantly higher level of expression of *PDE4B* mRNA has been observed in the peripheral blood leukocytes of drug-naïve MDD patients and *PDE4B* expression appears to significantly decrease after antidepressant treatment (Numata *et al.*, 2008a). The authors of this study also tried to validate their findings with two case-controlled association analyses in the Japanese population and found conflicting results: one analysis found significant allelic associations of four SNPs and a significant haplotypic association between *PDE4B* and MDD, while the other independent analysis could not reproduce these results (Numata *et al.*, 2008a). Furthermore, mice deficient in PDE4B and PDE4D have been developed, and they display a behavioural profile similar to wildtype mice that have been treated with antidepressants (O'Donnell & Zhang, 2004). In addition, studies have also reported that antidepressants up-regulate expression of PDE4 isoforms in rodent brain and the PDE4 inhibitor rolipram displays antidepressant-like effects in addition to antipsychotic-like (Millar *et al.*, 2007).
1.5.1.3 Brain-Derived Neurotrophic Factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that is important for the development and maintenance of adult neurons, playing a crucial role in the growth, differentiation and survival of neurons in the CNS (Xu et al., 2008; Naoe et al., 2007). The Bdnf gene contains five short 5’ noncoding exons in mice, each of which can undergo alternative splicing to form several mRNA transcripts (Tsankova et al., 2006). Bdnf exists in especially high abundance in the hippocampus, a brain region with a high degree of neural plasticity, suggesting it is an important regulator of synaptic plasticity (Naoe et al., 2007; Shoval and Weizman, 2005). There is in fact convincing evidence to implicate BDNF in hippocampal long-term potentiation, learning and memory (Egan et al., 2003). BDNF is also a key regulator of the mesolimbic dopamine pathway where it promotes dopamine release in the nucleus accumbens through TrkB receptor activation on dopaminergic nerve terminals (Berton et al., 2006). Many postmortem, pharmacogenetic and association studies have investigated the role of BDNF in the pathologies of SCZ and MDD.

Postmortem studies suggest there are indeed changes in BDNF levels in the brains of schizophrenic patients. In particular, there are reports of reduced levels of BDNF mRNA and protein in the prefrontal cortex (Hashimoto et al., 2005; Weickert et al., 2003), significantly decreased BDNF levels in the hippocampus and significantly increased BDNF levels in the cerebral cortex (Durany et al., 2001) of schizophrenic postmortem brains. Abnormal expression of BDNF and its receptor has also been reported in the corticolimbic system of schizophrenics: higher levels of BDNF were detected in the anterior cingulate cortex and hippocampus of patients compared to controls (Takahashi et al., 2000).
There are three important polymorphisms for BDNF that are most often investigated in association studies: (1) a dinucleotide microsatellite repeat polymorphism, (GT)n, in the promoter region (Proschel et al., 1992); (2) a C132T polymorphism located in the 5’ noncoding region of the gene that influences changes in expression (Xu et al., 2007); and (3) a SNP in the gene causing a valine to methionine substitution at codon 66 (Val66Met) that has been reported to affect memory and hippocampal function (Egan et al., 2003). Positive association between the (GT)n repeat polymorphism and SCZ has been reported for early-onset patients (Xu et al., 2008), and significantly higher frequencies of the T allele have been found in schizophrenic patients in Japanese, Hungarian and Chinese populations (Kunugi et al. 2003; Szekeres et al., 2003; He et al., 2005).

A genetic association between the BDNF Val66Met polymorphism and SCZ has been found in separate Japanese, Scottish and Spanish populations (Numata et al., 2006; Neves-Pereira et al., 2005; Rosa et al., 2006). The polymorphism has been implicated in the age of onset of SCZ (Numata et al., 2006) and reduced performance on neuropsychological tests for visuospatial skills, such as the Rey-Osterrieth Complex Figure Test copy subtest, the Wechsler Adult Intelligence Scale–Revised (WAIS-R) block design subtest, the WAIS-R object assembly subtest, and the Benton Judgment of Line Orientation, in schizophrenic patients (Ho et al., 2006). An MRI study also reported the polymorphism to be associated with hippocampal volume in schizophrenic patients (Szeszko et al., 2005). Furthermore, people with the Met allele have increased vulnerability to MDD when exposed to psychosocial stress (aan het Rot, Mathew and Charney, 2009). This is thought to be due to an excessive response by a hypersensitive hippocampus as people with the Met allele seem to have smaller hippocampi at birth and display hippocampal hypoactivity in a resting state, hippocampal hyperactivation during learning, and
poor hippocampus-dependent memory function (aan het Rot, Mathew and Charney, 2009). A transgenic mouse study using the Val66Met SNP variant found that BDNF\textsuperscript{Met/Met} neurons had defective BDNF secretion; these mice exhibited increased anxiety-related behaviours, implicating BDNF in conferring a predisposition to psychiatric conditions (Chen \textit{et al.}, 2006).

More evidence of a role for BDNF in MDD comes from a study that found abnormally low serum-BDNF levels in patients, and from post-mortem studies that report low levels of BDNF in the hippocampus and PFC of symptomatic depressed patients (aan het Rot, Mathew and Charney, 2009) and in suicide victims (Dwivedi \textit{et al.}, 2003). It appears that BDNF in the brain is important for antidepressant treatment response as studies have shown that pharmacologically-induced antidepressant-like responses are accompanied by an upregulation of BDNF levels in corticolimbic portions of mouse frontal cortex (Schroeder \textit{et al.}, 2007) and in the mouse hippocampus (Shieh \textit{et al.}, 2008). The regulatory mechanisms mediating BDNF expression changes are yet to be elucidated, but there is some evidence to suggest epigenetic factors such as chromatin remodelling are at play (Tsankova \textit{et al.}, 2006; see section 1.5.3.3).

1.5.2 The Role of the Environment in Schizophrenia and Depression

The environment has consistently been found to play a significant role in the susceptibility to and progression of SCZ and MDD. Traditionally, environmental contribution to disease in humans has been investigated using MZ twin pairs (Oh and Petronis, 2008). Population analyses have also been used in psychiatric illness, especially when investigating the influence of social factors (Frith and Frith, 2007). Furthermore, animal studies have been influential in gaining evidence for environmental influence as well as elucidating molecular mechanisms that may be involved.
1.5.2.1 Evidence for Environmental Influence

SCZ has been repeatedly found to be higher in incidence in urbanized areas compared to rural areas (Krabbendam and van Os, 2005). It has been argued that at least some of the urban-rural differences in SCZ are more likely to be of genetic, rather than environmental, origin (Oh and Petronis, 2008), however there is a strong case for the added environmental stress and social isolation in urban areas having causative effects on mental illness (Malmström et al., 1999). Childhood trauma, particularly molestation and physical abuse, has also been heavily implicated in SCZ. One study even found evidence suggesting a dose-response type relationship between the two where the experience of two or more trauma types during childhood was significantly more predictive of psychosis later in life (Shevlin et al., 2008). In addition to face validity, there is overwhelming evidence that environmental factors such as significant negative life events or physical harm may also bring about depression or related symptoms (aan het Rot, Mathew and Charney, 2009; Hyman, 2007; Frith and Frith, 2007) – see section 1.5.3.2.

Two well-established environmental animal models for abnormal development are the maternal care and the maternal inflammation models. Several studies have found that early post-natal maternal care deprivation in rats leads to behavioural and hypothalamus-pituitary-adrenal response abnormalities in the adult animal that are reminiscent of behavioural abnormalities observed in human patients of SCZ and MDD (Weaver et al., 2004). There is also good evidence that pre-natal exposure to stress such as maternal inflammation leading to immune activation, leads to abnormalities such as disrupted LI, dopaminergic hyperfunction and altered limbic morphology in the adult offspring (Zuckerman et al., 2003).
1.5.2.2 Social Stress and Mental Disease

Stress is widely believed to be a factor that contributes to the aetiology, pathophysiology, and treatment outcome of most psychiatric disorders (Moghaddam and Jackson, 2004). In particular, we know that stressful life events appear to play a major role in the development of MDD and the onset of psychosis/psychotic episodes in patients (van Winkel, Stefanis and Myin-Germeys, 2008; Charney and Manji, 2004; Kendler et al., 2001; Kessler, 1997). Such events can include threats to one’s life, bodily harm, death of a loved one, loss of a job and prolonged isolation among many other possibilities (Hyman, 2007; Frith and Frith, 2007). Urbanicity also involves social stress (section 1.5.3.1).

In the human brain, stressful experiences are processed by emotional circuits that include the amygdala, the mesolimbic and mesocortical dopamine projections, and the orbital and medial prefrontal cortex (Hyman, 2007; Lang and Davis, 2006; Berton et al., 2006; Cardinal et al., 2002; Barbas, 2000). These circuits output to the hypothalamus, brainstem, and other brain regions to produce physiological responses such as the release of stress hormones, behavioural responses such as aggressive, submissive, or flight behaviours, and the formation of memories of these negative experiences (Hyman, 2007; Barbas, 2000). The propensity for mental disease, and in particular mood and anxiety disorders, to develop is significantly greater when there is abnormal functioning of the emotion processing circuits in the human brain (Hyman, 2007; Ressler and Mayberg, 2007; Drevets, 2000). For example, it has been shown that cerebral blood flow to the amygdala is abnormally high, indicating overactivation of the region, in patients with MDD, (Drevets, 2000; Drevets et al., 1992). Furthermore, the PFC is often found to be dysregulated in emotional disorders (Ressler and Mayberg, 2007). Many of the symptoms of SCZ are also attributed to disturbances in patients’ ability to accurately process emotionally
salient sensory information (Laviolette, 2007). For example, several fMRI studies have shown that there is a failure to activate the amygdala and the medial prefrontal cortex in schizophrenic patients in response to affective visual stimuli (Takahashi et al., 2004; Schneider et al., 1998; Williams et al., 2004).

Animal studies support the hypothesis that social stress is a causative factor in mental disease. For example, social isolation after defeat leads to persistent behavioural and physiological deficits/changes that are not present in animals that are housed in groups after defeat (see section 1.7.1). The CSD paradigm is thought to emulate some aspects of social stress as SCZ- and, particularly, MDD-related endophenotypes are often observed in animal CSD studies (see section 1.7.1).

1.5.2.3 Negative Environmental and Social Stimuli May Lead to Stress Sensitization

One hypothesis that connects environmental (and social) stressors to changes at the molecular level that may mediate behavioural and biological changes in patients is the concept of “behavioural sensitization” or “stress sensitization” (van Winkel, Stefanis and Myin-Germeys, 2008; Yuii, Suzuki and Kurachi, 2007). The concept is based on the premise that exposure to psychosocial or environmental stress may progressively increase the behavioural and biological response to subsequent exposures due to a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis (Figure 1.3). This ultimately contributes to dopamine sensitization in mesolimbic areas and increased stress-induced striatal dopamine release (van Winkel, Stefanis and Myin-Germeys, 2008).
Stress is thought to activate the hypothalamus and thereby the HPA axis by stimulating local synthesis and release of corticotropin-releasing factor (aan het Rot, Mathew and Charney, 2009). The axis may also be activated through other regions in the brain, such as the amygdala which is heavily involved in processing emotional stimuli (see section 1.5.3.2). MDD patients often display faulty processing of environmental threats and exaggerated stress reactions, and also have elevated levels of corticotropin-releasing factor (aan het Rot, Mathew and Charney, 2009), suggesting a hypersensitive HPA axis and supporting the behavioural/stress sensitization hypothesis. In animal studies, maternal care deprivation has been shown to lead to permanent changes in the HPA axis (Meaney and Szyf, 2005).

Figure 1.3. The hypothalamic–pituitary–adrenal axis. Reproduced from aan het Rot, Mathew and Charney, 2009.
1.5.3 Epigenetics: Aetiological Factor and Vehicle for Environmental Influence

1.5.3.1 Epigenetic Modifications

Epigenetic modifications are modifications to genomic DNA and/or chromatin structure that may result in modified gene expression (Petronis et al., 2000; Henikoff and Matzke, 1997). Epigenetic mechanisms are heritable, but potentially reversible, and are influenced by developmental stage, tissue type, environmental factors and stochastic events (Petronis et al., 2003). Because they are dynamic and only partially stable, epigenetic modifications are crucial processes by which environmental influences can exert their effects on an organism’s phenotype. Chromatin remodelling is the most common form of epigenetic modification affecting the expression of genomic DNA (Figure 1.4). DNA methylation is generally associated with gene silencing: addition of methyl groups at CpG sites converts cytosine to 5-methylcytosine, and the gene is turned off. Both de novo methylation for altering gene expression and maintenance methylation occur in the cell and methylation patterns can be maintained during DNA replication and inherited across generations (Goto and Monk, 1998). Another heavily-investigated form of chromatin remodelling consists of acetylation, methylation, ubiquitylation, phosphorylation and/or sumoylation of histone tails to influence gene expression by altering the packing of genomic DNA (Shilatifard, 2006; Berger, 2002).
1.5.3.2 Epigenetics in Mental Disease

Traditionally, neuropathology has been assumed to be the result of gene-environment interplay, but epigenetics has recently emerged as a crucial factor in the aetiology of psychiatric diseases. Studies in MZ twin pairs discordant for SCZ show that DNA methylation may influence susceptibility to SCZ (Petronis 2001; Petronis, 2006). One recent study found loss of DNA methylation of schizophrenia-related retrovirus-2, a heavily-methylated and placentally-expressed endogenous retroviral-related genome sequence, in one affected patient, but no controls among MZ discordant twin pairs (Deb-Rinker et al., 2002). Yet another study investigating DNA modification in \textit{DRD2}, a gene for which specific polymorphisms have been associated with SCZ, in two MZ twin pairs, one concordant and one discordant for SCZ, reported that the affected twin from the pair discordant for SCZ was epigenetically closer to the affected concordant twins than to his unaffected MZ co-twin (Petronis et al., 2003).
DNA methylation has also been implicated in MDD: One study investigating the expression of DNA methyltransferase (DNMT) mRNA in several brain regions of suicides who had been diagnosed with MDD found that $DNMT$ expression was altered in the frontopolar cortex, amygdala, and the paraventricular nucleus of the hypothalamus (Poulter et al., 2008). The study also found gene-specific aberrations in DNA methylation (hypermethylated cytosine/guanosine sites) in the GABA(A) receptor alpha1 subunit promoter region, the transcript of which is underexpressed in suicides/depressed brains relative to controls.

### 1.5.3.3 Epigenetic Control of $Disc1$, $Pde4b$ and $Bdnf$

With respect to our genes of interest, there is limited information available on the extent of epigenetic modifications to $Disc1$ and $Pde4b$. However, several recent studies have demonstrated the role of epigenetics in modulating $Bdnf$ expression. A study investigating the influence of a single immobilization stress on the levels of total $Bdnf$ mRNA in the rat hippocampus found that the stressor affected $Bdnf$ gene transcription via epigenetic regulation: significant decreases in the levels of acetylated histone H3 were found at the promoters of some of the exons (Fuchikami et al., 2009). Another study showed that early childhood maltreatment produces changes in $Bdnf$ methylation that causes altered $Bdnf$ expression in the adult rat prefrontal cortex (Roth et al., 2009). The heritability of methylation profiles was furthermore demonstrated in the study: the investigators observed altered $Bdnf$ DNA methylation in the offspring of females that had previously experienced the maltreatment regimen (Roth et al., 2009). Moreover, CSD stress has been shown to downregulate $Bdnf$ transcripts III and IV via increased repressive histone methylation at their corresponding promoters in mice (Tsankova et al., 2006).
1.5.3.4 Histone Deacetylases and Valproic Acid

Histone acetyltransferases and histone deacetylases (HDACs) are key elements in the dynamic regulation of gene expression. These two enzymes remodel chromatin to control gene expression without altering gene sequence (Wiech et al., 2009). Hyperacetylation of the N-terminal tails of histones H3 and H4 correlates with gene activation, and deacetylation mediates transcriptional repression (Krämer et al., 2003). Mammalian HDACs can be divided into three subclasses, class I, II, and III; Class I HDACs are constitutively nuclear (HDACs 1, 2, 3 and 8) (Krämer et al., 2003).

Many diseases are associated with changes in gene expression caused by mutations affecting transcription factors that are recruited by HDACs. Since HDACs repress transcriptional activity, HDAC inhibitors can reverse aberrant repression and lead to re-expression of genes (Krämer et al., 2003). Cancer has been the primary target for the clinical development of HDAC inhibitors as treatments so far, and the first HDAC inhibitor was approved for treatment of cutaneous T cell lymphoma (Wiech et al., 2009). Nevertheless, recent studies have started to reveal the potential of HDAC inhibitors for treatment of other diseases as well, including psychiatric conditions. For example, one animal study demonstrated that the HDAC inhibitor sodium butyrate exerts antidepressant-like effects in the mouse by decreasing immobility in the FST (improved behavioural despair) and increasing BDNF levels in the frontal cortex (Schroeder et al., 2007). One HDAC inhibitor of special interest in psychiatry is valproic acid (valproate in salt form, Figure 1.5), widely used clinically as an antiepileptic drug and also in the treatment of bipolar disorder and, rarely, in MDD and SCZ (Haddad et al., 2009). Valproate preferentially inhibits class I HDACs and has been shown to induce proteasomal degradation of HDAC 2 (Göttlicher, 2004). Levels of acetylated histone 3 (H3) have been shown to significantly increase upon
valproate treatment in patients diagnosed with SCZ or bipolar disorder (Sharma et al., 2006), and valproate-driven HDAC inhibition has been postulated to correct the neurochemical and behavioural aspects of SCZ in a methionine-induced epigenetic mouse model (Tremolizzo et al., 2005).

Figure 1.5. Sodium valproate, salt equivalent of valproic acid. The compound is used clinically in the treatment of bipolar disorder and sometimes in major depression and schizophrenia. It is a potent HDAC inhibitor.

1.6 Animal Models for Schizophrenia and Major Depressive Disorder

1.6.1 The Concept of Endophenotypes

Unlike in Mendelian diseases, where genotype is indicative of phenotype, genetic certainty attributable to discrete genes rarely exists in diseases with complex genetics, such as SCZ and MDD (Altshuler, Daly and Lander, 2008; Gottesman and Gould, 2003). Psychiatric diseases present a particular challenge to researchers because of this and also because of the heterogeneity of disease symptoms. Therefore, a necessary step in the investigation of diseases such as SCZ and MDD is defining endophenotypes, a type of biomarkers for disease (Gottesman and Gould, 2003).
The concept was first introduced in psychiatry by Gottesman and Shields, who define endophenotypes as “internal phenotypes discoverable by a “biochemical test or microscopic examination”” (Gottesman and Gould, 2003). Essentially, endophenotypes are discrete, quantifiable and heritable phenotypes that ideally have monogenic roots. Even if a given endophenotype is known to have a polygenic basis, the level of complexity should be such that it has the potential to assist in the genetic dissection of the psychiatric disease, i.e., it has a defined genetic aetiology (Gottesman and Gould, 2003). However, it is important to note that putative endophenotypes do not have to reflect exclusively genetic effects, but could also reflect environmental and epigenetic effects, or be multifactorial in origin (Gottesman and Gould, 2003). An endophenotype may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological phenotype (Gottesman and Gould, 2003). Table 1a summarizes useful criteria in selecting/accepting endophenotypes (adapted from Gottesman and Gould, 2003). The concept of endophenotypes is essential to the development of animal models of psychiatric illness as animals will never display symptoms such as guilty ruminations, suicidal thoughts, or rapid speech (Gottesman and Gould, 2003).

Two well-established endophenotypes in SCZ are sensory motor gating and working memory dysfunction (see section 1.6.3.1). An example of a suggested endophenotype in MDD is altered rapid eye movement (REM) sleep (Modell and Lauer, 2007; Dugovic et al., 2000; Vogel et al., 1990). Changes in REM sleep often persist after treatment and can be predictive of the recurrence of depression (Modell and Lauer, 2007). REM sleep patterns are mediated by noradrenergic, serotonergic, and cholinergic systems and are under strong genetic control (Modell and Lauer, 2007; McCarley, 2004). Recent studies have shown that REM sleep is
inhibited during ontogeny; this inhibition is important for brain maturation and a lack of inhibition may predispose an individual to depression (Modell and Lauer, 2007).

<table>
<thead>
<tr>
<th>Table 1a. Criteria for the identification of endophenotypes.</th>
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<tbody>
<tr>
<td>1. The endophenotype is associated with illness in the population.</td>
</tr>
<tr>
<td>2. The endophenotype is heritable.</td>
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<tr>
<td>3. The endophenotype is primarily state-independent (manifests in an individual whether or not illness is active).</td>
</tr>
<tr>
<td>4. Within families, endophenotype and illness co-segregate.</td>
</tr>
<tr>
<td>*5. The endophenotype found in affected family members is found in nonaffected family members at a higher rate than in the general population.</td>
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</tbody>
</table>

*Criterion for diseases that display complex inheritance patterns.

1.6.2 Animal Models in Schizophrenia

1.6.2.1 Traditional Models

It is of course not possible to model and measure some aspects of SCZ such as hallucinations and delusions in animals, and such symptoms are very likely to be specific to humans (Chubb et al., 2008). However, we can create animal models that appear to mimic some anatomical, cognitive and behavioural deficits also observed in human patients of SCZ (the endophenotype approach). Dissection of the genetic aetiology of such deficits/abnormalities can help us validate findings from human association and linkage studies, and allow for pharmacological interventions including testing novel drugs or elucidating the mechanisms of action of drugs already in use clinically.
The deficits in attention-related processes, information processing and filtering of environmental stimuli observed in schizophrenic patients is often modelled in animals using prepulse inhibition (PPI) of the acoustic startle response and working memory tests. Deficits in filtering are related to sensory motor gating function and have been observed in schizophrenia-spectrum patients (Quednow et al., 2006; Braff, Geyer, and Swerdlow, 2001). The reproducibility of the finding in SCZ (Gottesman and Gould, 2003) and the fact that there is considerable evidence supporting a high degree of homology between measures of PPI in rodents and humans (Braff, Geyer, and Swerdlow, 2001), makes abnormal PPI a promising candidate endophenotype in SCZ. Validation comes from studies where some antipsychotics have been demonstrated to improve PPI deficits in schizophrenic patients (Wynn et al., 2007; Quednow et al., 2006).

There are many advantages of the PPI animal model, including the fact that PPI measurements are easily adapted to mice and rats, and the underlying mechanisms regulating PPI appear to be similar in animals and humans (Braff, Geyer, and Swerdlow, 2001). Studies have also demonstrated that antipsychotics can rescue PPI deficits in rodents (Powell et al., 2008; Flood, Gasior and Marino, 2007). Dopamine agonists and antagonists have been shown to influence PPI levels in rats (Bubenikova-Valesova et al., 2009), confirming the role of dopamine, heavily implicated in SCZ, in the molecular pathways for this endophenotype. Moreover, PPI regulation in animals has been found to be influenced by genetic factors (Feldcamp et al., in preparation; Weber et al., 2008; Shilling et al., 2008). Considering all of this evidence, the PPI model in SCZ has face, construct, predictive, and genetic validity.

There is a large body of evidence suggesting working memory deficits may be relevant to the pathophysiology of SCZ: Working memory is compromised in schizophrenic patients
(Weinberger et al., 2001; Gottesman and Gould, 2003). Postmortem studies have shown
abnormalities in the dorsolateral prefrontal cortex of schizophrenics (Harrison, 1999), a brain
region that is involved in working memory (Weinberger et al., 2001). The heritability of working
memory deficits in SCZ has been shown (Cannon et al., 2000) and specific chromosomal regions
that appear to be involved in working memory have been identified (Gasperoni et al., 2003).
Most encouragingly, one region identified by Gasperoni et al., the region 1q41, has previously
been suggested in traditional linkage studies of SCZ (Ekelund et al., 2000; Millar et al., 2000; St.
Clair et al., 1990). Furthermore, performance on working memory tasks is reproducible
(Gottesman and Gould, 2003), making it a good candidate endophenotype for SCZ. Animal
models based on this endophenotype make use of behavioural tests such as an alternation task on
a T-maze, or the Morris water maze, or various tasks in operant chambers to make experimental
measurements (Dudchenko, 2004). Rodent models of working memory deficits usually involve
experimentally-induced prefrontal dysfunction and such models have successfully demonstrated
the significance of aberrant dopaminergic and glutamatergic signalling in the medial prefrontal
cortex for working memory (Castner, Goldman-Rakic and Williams, 2004).

Beyond the endophenotype approach, pharmacological animal models have been developed for
SCZ using hallucinogens or stimulants. Administration of the stimulant amphetamine to rodents
is a well-established model. Chronic administration leads to amphetamine sensitization and there
is good evidence supporting the use of this model for some of the positive symptoms of SCZ
(Featherstone, Kapur and Fletcher, 2007). There is also evidence of long-lasting cognitive
deficits related to attention and cognitive flexibility in amphetamine-sensitized rodents
(Featherstone, Kapur and Fletcher, 2007). Thus, despite the lack of face validity for this model,
an argument can be made for aetiological validity (Geyer and Markou, 2000). It is thought that
amphetamine sensitization influences behaviour by altering the mesolimbic dopamine system and prefrontal cortical function (Featherstone, Kapur and Fletcher, 2007).

Genetic mutant rodent models constitute the final major group of animal models in SCZ, and these models are of great interest in the context of the recent identification of important candidate genes for the disease. Some genetic mutant models include the catechol-O-methyltransferase gene (COMT) knockout and transgenic mice, the neuregulin-1 gene (NRG1) mutants, dopamine transporter mutants (including the chakragati mouse (Dawe and Ratty, 2007; Ratty et al., 1992)) and Disc1 mutants (Desbonnet, Waddington and O’Tuathaigh, 2009). Since these Disc1 mutants are the basis of the experiments in this thesis, they will be discussed below in detail (section 1.6.2.2).

1.6.2.2 Disc1 Mouse Models

Since the report of DISC1 in 2000 by Millar and coworkers, there have been several lines of Disc1 mutant mice created that display phenotypes that mimic some aspects of SCZ (Table 1b). The first Disc1 mutant line was created using a deletion variant of mouse Disc1 (mDisc1) found in the 129S6 Sv/Ev strain. This strain was found to carry a 25 bp deletion in exon 6 of Disc1 that introduces a termination codon at exon 7, resulting in a truncated Disc1 due to the frameshift mutation (Koike et al., 2006). When mDisc1 is transferred to mice of the C57Bl/6J genetic background, impairments in working memory are observed (Kvajo et al., 2008; Koike et al., 2006). It is suggested that alterations to the organization of newly formed and mature neurons as well as deficits in short-term plasticity contribute to the observed working memory impairments (Kvajo et al., 2008).
Besides the naturally-occurring mutated mouse *Disc1* found in the 129S6 Sv/Ev strain, several lines of mouse mutants have been developed that express some mutated form of human *DISC1*. One such transgenic mouse was developed by Hikida and coworkers (2007). They generated a transgenic mouse expressing a dominant-negative form of *DISC1* (DN-DISC1) under the α-Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMKII) promoter (Hikida *et al.*, 2007). These DN-DISC1 mutants display behavioural abnormalities such as hyperactivity, deficits in sensorimotor gating (as measured by PPI) and olfactory-associated behaviour, and an anhedonia/depression-like state. No deficits in working memory were found. In vivo MRI data from these mice also revealed an enlargement of the lateral ventricles, especially on the left side. This suggests a link to the asymmetrical change in anatomy found in brains of schizophrenics (Hikida *et al.*, 2007). Furthermore, lateral ventricular enlargement has been consistently found in human subjects with SCZ (Harrison and Weinberger, 2005; Freedman, 2003; Thaker and Carpenter, 2001), and has been reported in other mouse models of SCZ, such as the chakragati mouse (Dawe and Ratty, 2007; Torres *et al.*, 2005; Ratty *et al.*, 1992).

A second transgenic mouse expressing a *DISC1* C-terminal fragment (DISC1-cc), also under the control of the α-CaMKII promoter, which is active only in primary neurons of the forebrain, was developed by Li and coworkers (2007). DISC1-cc mice display some schizophrenia-related phenotypes when the induction is done in the early postnatal period, but not when done in adult mice. These mutants display reduced hippocampal dendritic complexity, depressive-like traits, deficits in spatial working memory, and reduced sociability. Li *et al.* were able to validate their model using human data obtained from a sample of MZ twins discordant for the disease: they found that individuals with a DISC1 haplotype associating with SCZ as well as working memory impairments and reduced gray matter density were more likely to show deficits in sociability.
than those without the haplotype (2007). Similar findings have of course been reported elsewhere (see section 1.5.1.1).

A third mutant line was developed by Pletnikov and coworkers that made use of the Tet-off double transgenic system under the regulation of the CAMKII promoter to generate transgenic mice with inducible expression of mutant human DISC1 (hDISC1) limited to forebrain regions, including the cerebral cortex, hippocampus and striatum (Pletnikov et al., 2008). These mice display some sex-specific behavioural deficits: spontaneous hyperactivity in the open field and reduced social interaction in males, and impaired spatial working memory in females. These mutants also have mild enlargement of the lateral ventricles and attenuation of neurite outgrowth in primary cortical neurons (Pletnikov et al., 2008).

In addition to the transgenic mutants described above, two mutant lines carrying single point-mutations in the endogenous mouse Disc1 have recently been developed (Clapcote et al., 2007). These mutants were created by chemical mutagenesis of exon 2 of Disc1 using N-ethyl-N-nitrosourea (ENU), which primarily causes simple DNA point mutations (Soewarto, Klaften and Rubio-Aliaga, 2009). Exon 2 was focused on as it is present in all known DISC1 isoforms and as it encodes most of the head domain of the DISC1 protein, which is the region known to interact with PDE4B in humans (Clapcote et al., 2007; Millar et al., 2005). One of these two mutant lines, the L100P homozygous mutant line, shows a phenotype that is consistent with many endophenotypes of SCZ and mimics many of the symptoms of the disease. L100P mice have impaired PPI and LI, appear more anxious in the open field (hyperlocomotion), have poorer performance in the T-maze test of working memory and have decreased brain volume (Clapcote et al., 2007). Even more encouragingly, some of the cognitive deficits were reversed by
antipsychotic treatment in this mutant line (Clapcote et al., 2007). The second of these two lines (the Q31L line) will be discussed in a later section (see section 1.6.3.2).

Table 1b. Disc1 genetic mouse mutants in schizophrenia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Alterations in behavioural phenotype</th>
<th>Brain morphological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koike et al., 2006;</td>
<td>✓ working memory deficit</td>
<td>✓ alterations to neuronal organization</td>
</tr>
<tr>
<td>Kvajo et al., 2008</td>
<td>✓ short term plasticity deficit</td>
<td>✓ mild decrease in PFC volume</td>
</tr>
<tr>
<td>Hikida et al., 2007</td>
<td>✓ hyperactivity</td>
<td>✓ enlargement of the lateral ventricles, especially on the left side</td>
</tr>
<tr>
<td></td>
<td>✓ deficits in sensorimotor gating</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ deficits in olfactory-associated behaviour</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ anhedonia/depression-like state</td>
<td></td>
</tr>
<tr>
<td>Li et al., 2007</td>
<td>✓ depressive-like traits</td>
<td>✓ decreased hippocampal dendritic complexity</td>
</tr>
<tr>
<td></td>
<td>✓ deficits in spatial working memory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ reduced sociability</td>
<td></td>
</tr>
<tr>
<td>Pletnikov et al., 2008</td>
<td>✓ spontaneous hyperactivity in males</td>
<td>✓ mild enlargement of the lateral ventricles</td>
</tr>
<tr>
<td></td>
<td>✓ reduced social interaction in males</td>
<td>✓ attenuation of neurite outgrowth in primary cortical neurons</td>
</tr>
<tr>
<td></td>
<td>✓ impaired spatial working memory in females</td>
<td></td>
</tr>
<tr>
<td>Clapcote et al., 2007</td>
<td>✓ impaired PPI</td>
<td>✓ decreased brain volume</td>
</tr>
<tr>
<td></td>
<td>✓ impaired LI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ more anxious in the open field</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ impaired working memory</td>
<td></td>
</tr>
</tbody>
</table>

1.6.3 Animal Models for Major Depressive Disorder

1.6.3.1 Stress Models of Depression

Among the most useful models for depressive disorders are those that involve stress-induced changes in behaviour (Henn and Vollmayr, 2005). Two well-established stress-related models that induce depression-like behavioural changes in animals are the learned helplessness model (LHM) and the chronic stress model (CSM). The LHM was developed based on the cognitive
theory of depression that suggests that stress outside the control of an organism, whether real or merely perceived as such, may lead to anxiety and behaviours of helplessness similar to those found in depression (Henn and Vollmayr, 2005). The LHM has good face and predictive validity: LHM animals show a loss of appetite and weight, and poor performance in both appetitively and aversively motivated tasks (Geyer and Markou, 2000). Moreover, many clinically used pharmacological treatments and electroconvulsive shock therapy are effective in reducing the behavioural and physiological abnormalities seen in LHM animals (Zazpe et al., 2007; Geyer and Markou, 2000). The behavioural despair model of depression is a variant of the LHM and consists of the forced swim test (FST, see section 1.8.4). The behavioural despair model has very good predictive validity with respect to the effects of antidepressants (Shieh et al., 2008; Thiébot, Martin and Puech, 1992).

The CSM was first suggested by Katz and Schmaltz (1980) and then modified by Willner (1997). The premise of the CSM is that chronic moderate stressors applied over a prolonged period of time will lead to the development of an anhedonic state in animals, which is a symptom often seen in depressed patients. CSM tends to increase immobility in the FST, decrease exploration in a novel environment, increase anxious behaviour and lead to a loss of body weight in rodents (Henn and Vollmayr, 2005).

1.6.3.2 Genetic Models
There are numerous animal models of depression that were developed on a genetic basis. Some examples are the Flinders Sensitive (FS) and Flinders Resistant (FR) lines of rats, congenitally learned helpless (cLH) and congenitally not learned helpless (cNLH) lines of rats, and mouse mutants with modifications to genes regulating the HPA axis (Müller and Holsboer, 2006). The
FS and FR lines were developed through selective breeding of rats exhibiting increased thermic responses to a muscarinic cholinergic agonist based on the hypothesis that depression involves a cholinergic-noradrenergic neurotransmitter imbalance (Geyer and Markou, 2000). FS rats display decreased sensitivity to rewarding stimuli after exposure to CMS (anhedonia) and REM sleep abnormalities (Henn and Vollmayr, 2005; Geyer and Markou, 2000), providing good construct validity for the model. cLH rats exhibit a helpless phenotype without exposure to stress such as uncontrollable shock, while cNLH rats are resistant to the effects of inescapable shock (Henn and Vollmayr, 2005). The cLH line also demonstrates features of anhedonia and anergia, indicative of chronic depression (Vollmayr et al., 2004). HPA-system mouse mutants are also good models as they not only demonstrate depression-like behavioural phenotypes, but also robust changes in corticotrophin releasing hormone and arginine vasopressin levels, both of which are altered in human patients of MDD (Müller and Holsboer, 2006). Furthermore, dopamine, serotonin, and norepinephrine transporter knockout mice that can provide a model for depression have been generated (Perona et al., 2008) and selective breeding of rats for their differential susceptibilities to stress-induced changes in FST performance has led to lines that are now used as genetic models of depression (Henn and Vollmayr, 2005).

The second line of the ENU-induced Disc1 mutants previously introduced, the Q31L line, displays a phenotype that is consistent with many aspects of MDD in human patients. Homozygous Q31L mice have small deficits in PPI and working memory, impaired LI, and a slight decrease in brain volume (Clapcote et al., 2007). However, most interestingly, this line displays increased immobility in the FST (behavioural despair), decreased sociability and decreased sucrose consumption, all behaviours indicative of anhedonia. Moreover, this line did not respond to antipsychotics, but the antidepressant bupropion improved the PPI deficit and
significantly decreased immobility in the FST selectively in the Q31L line (Clapcote et al., 2007). Figure 1.6 summarizes the behavioural phenotype of the two ENU Disc1 missense mutant lines.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>31L</th>
<th>31L/31L</th>
<th>31L/+</th>
<th>100P</th>
<th>100P/100P</th>
<th>100P/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety (elevated plus-maze)</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal activity</td>
<td>=</td>
<td>=</td>
<td>&gt;&gt;</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical activity</td>
<td>=</td>
<td>=</td>
<td>&gt;</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepulse inhibition (PPI)</td>
<td>&lt;</td>
<td>=</td>
<td>&lt;&lt;</td>
<td>&lt;&lt;</td>
<td>&lt;&lt;</td>
<td></td>
</tr>
<tr>
<td>Acoustic startle response</td>
<td>=</td>
<td>=</td>
<td>&lt;</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Startle reactivity</td>
<td>=</td>
<td>=</td>
<td>&lt;</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory brainstem response</td>
<td>n/a</td>
<td>n/a</td>
<td>=</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latent inhibition (LI)</td>
<td>&lt;&lt;</td>
<td>=</td>
<td>&lt;&lt;</td>
<td>&lt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working memory (T’ maze)</td>
<td>&lt;</td>
<td>n/a</td>
<td>&lt;&lt;</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial learning and memory (Morris water maze)</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forced swim immobility (FST)</td>
<td>&gt;</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sociability and social novelty</td>
<td>&lt;</td>
<td>&lt;</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose consumption</td>
<td>&lt;</td>
<td>n/a</td>
<td>=</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory function</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taste sensitivity</td>
<td>=</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain volume</td>
<td>&lt;</td>
<td>&lt;</td>
<td>&lt;&lt;</td>
<td>&lt;&lt;</td>
<td>&lt;&lt;</td>
<td></td>
</tr>
<tr>
<td>PDE4B activity</td>
<td>&lt;&lt;</td>
<td>n/a</td>
<td>=</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug Treatment</td>
<td>31L/31L</td>
<td>31L/+</td>
<td>100P/100P</td>
<td>100P/+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPI</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haloperidol</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rolipram</td>
<td>=</td>
<td>=</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bupropion</td>
<td>+++</td>
<td>=</td>
<td>=</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LI</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>=</td>
<td>=</td>
<td>+</td>
<td>=</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal activity</td>
<td>Clozapine</td>
<td>++</td>
<td>n/a</td>
<td>+</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>FST</td>
<td>Bupropion</td>
<td>++</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Rolipram</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

= no significant difference; < lower than +/-; << much lower than +/-; > greater than +/-; >> much greater than +/-; +, ++, +++ positive effect (p < 0.05, p < 0.01, and p < 0.001, respectively); n/a not applied.

Figure 1.6. Behavioural phenotype of ENU Disc1 missense mutant mouse lines. Reproduced from Clapcote et al., 2007. L100P/L100P mice display a psychosis-like phenotype while Q31L/Q31L mice show depression-like behaviour. Heterozygous mice in both lines show minimal differences from wildtype.
1.7 The Chronic Social Defeat Stress Paradigm in Rodents

1.7.1 Chronic Social Defeat (CSD)

The chronic social defeat (CSD) paradigm consists of the test subject being exposed to an aggressive partner over an extended period of time. It is based on the resident-intruder paradigm (Kinn et al., 2008). Rodents exposed to CSD stress tend to exhibit behaviours indicative of a state of anhedonia, and the paradigm is therefore believed to effectively model depression in animals. Table 1c contains a summary of behavioural changes reported by various groups using CSD. Most of the behavioural tests probe depression-like symptoms, making this paradigm most suitable for animal models of depression.

<table>
<thead>
<tr>
<th>Species</th>
<th>FST</th>
<th>EPM</th>
<th>PT</th>
<th>SC</th>
<th>OF</th>
<th>Social Interaction</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>↑immobility</td>
<td>↑anxiety</td>
<td>↑anxiety</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>Kudryavtseva et al., 1991 Avgustinovich et al., 1997 Kudryavtseva and Avgustinovich, 1998</td>
</tr>
<tr>
<td>Rat</td>
<td>↑immobility</td>
<td>-</td>
<td>N/A</td>
<td>↓</td>
<td>↓</td>
<td>N/A</td>
<td>Rygula et al., 2008 Kinn et al., 2008</td>
</tr>
</tbody>
</table>

Although the CSD paradigm is primarily used in animal models of depression, chronic social stress is also an important precipitating factor in schizophrenic patients, and it is even suggested that the risk for SCZ may be associated with social environmental stress in a dose-response pattern (Selten and Cantor-Graae, 2005; Rubino et al., 2009). One hypothesis is that chronic experience of social defeat may lead to increased risk for SCZ via increased sensitization or baseline activity of the mesolimbic dopamine system (Selten and Cantor-Graae, 2005). In fact, an experiment utilizing CSD has displayed selective dopaminergic hyperactivity in the
mesocorticolimbic system in defeated male Long-Evans rats (Tidey and Miczek, 1996), and, of course, it is now widely accepted that a hyperdopaminergic state is present in schizophrenic patients (Laruelle et al., 1999).

The effects of social defeat are heavily influenced by the specific environmental context; specifically, the housing conditions of the defeated animals after defeat exposure affect the long-term outcome greatly. Housing familiar male rats together after individual defeat exposure (one session) reduced the long-term adverse behavioural (exaggerated reaction to mild stressor, reduced mobility in the open field, increased anxiety in the EPM) and physiological (reduced body growth, increased HPA activity) effects of social defeat in one study (Ruis et al., 1999). Supportive findings were made in another study where rats that were isolated after defeat showed impaired social memory and decreased social interaction for up to 3 months after defeat, but rats that were housed socially showed no such impairments (Von Frijtag et al., 2000). Yet another study involving a single defeat session in male rats found that isolation after defeat amplified the reduction in the number of striatal DA transporter binding sites, but the density of striatal DA transporter binding sites was not affected in group-housed animals (Isovich et al., 2001). Housing environment also appears to be a great influence on the effects of social defeat in mice: One study found that defeated mice exhibited spatial learning impairments that persisted for weeks after defeat when the mice were housed alone (Fitchett et al., 2005). The study also found that pair housing reduced abnormally high levels of urinary corticosterone, which accompanied subordination (Fitchett et al., 2005). Another study demonstrated that previous social experience influences the susceptibility to the effects of future social stress in mice (Avitsur et al., 2003).
1.7.2 Chronic Social Defeat and Our Genes of Interest

The application of CSD to rodents elicits many physiological and behavioural changes as briefly discussed above. Initially, there is an immediate stress response when the first attack occurs. These transient changes in hormone levels and other physiological markers often do not persist, especially if the animal is returned to an enriched cage with other non-threatening, familiar conspecifics (see section 1.7.1). However, over an extended period of repeated defeat exposure (chronic defeat), more permanent changes take place in the animal’s neurocircuitry that influence behaviour and physiological changes at the molecular level.

BDNF is of particular interest to us in the context of social defeat because chronic social stress in rodents has been demonstrated to influence Bdnf expression in distinct brain regions such as the hippocampus and the nucleus accumbens. In the hippocampus, local infusion of Bdnf produces antidepressant-like effects in rodent behavioural models of depression, suggesting a protective effect of BDNF in the hippocampus (Shirayama et al., 2002). Conversely, chronic defeat stress has been shown to induce lasting downregulation of some Bdnf transcripts (III and IV) and increased repressive histone methylation at their corresponding promoters in another study (Tsankova et al., 2006). These findings are supported by another study where male Bdnf+/- mice exhibited increased immobility in the FST after chronic mild stress (Advani, Koek and Hensler, 2009). In the nucleus accumbens of mice, CSD has been shown to increase Bdnf protein levels and local deletion of Bdnf demonstrated to have an antidepressant-like effect (Berton et al., 2006).

There is limited evidence directly linking social stress and DISC1 or PDE4B. However, one study has shown that overexpression of DISC1 induced the assembly of eIF3- and TIA-1-
positive stress granules in vivo (in human neuroblastoma SH-SY5Y cells) and that DISC1 was not recruited to stress granules under oxidative stress or energy deprivation (Ogawa, Kasai and Akiyama, 2005). Furthermore, protein synthesis in cells is regulated in part by growth factors via eIF2B activation and in part by eIF2-alpha kinases that are engaged by long-term potentiation and repressed by long-term depression mediated by NMDA and metabotropic glutamate receptors (Carter, 2007). DISC1 appears to be a part of these networks either as a downstream signalling component or partner of BDNF and other growth factors (Carter, 2007; Millar et al., 2000). These findings indicate that aberrant expression of DISC1 may cause cellular stress and that DISC1 is involved in the translational regulation required for stress response (Ogawa, Kasai and Akiyama, 2005). The direct impact of CSD on Pde4b has not been investigated to our knowledge. However, Pde4b is a regulator of cAMP, a second messenger implicated in learning, memory, and mood (Millar et al., 2005), all processes subject to the influence of CSD.

1.8 Behavioural Phenotyping for Animal Models

Animal models are crucial in the study of disease because specific hypotheses can be tested under highly controlled conditions using research methods that are not possible in humans for ethical or practical reasons. They are important tools in the neuropsychiatric field as psychiatric diseases manifest themselves into measurable behavioural deficiencies (negative symptoms) or altered behaviours (positive symptoms) in patients. Mimicking endophenotypes consisting of complex behaviour is a necessary criterion in any useful animal model for a psychiatric disease. Some widely-used behavioural tests for phenotyping rodent models in SCZ and MDD, and the endophenotypes they measure are listed in Table 1d.
Table 1d. Behavioural tests for phenotyping rodent models.

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latent inhibition</td>
<td>Measure of information-processing in schizophrenia.</td>
</tr>
<tr>
<td>Elevated plus maze</td>
<td>Used to measure anxiety levels. Anxiety syndromes are recognized to be prevalent in both schizophrenia and depression.</td>
</tr>
<tr>
<td>Locomotion in an open field</td>
<td>Paradigm can be used to measure some or all of locomotor activity, exploratory behaviour, and anxiety.</td>
</tr>
<tr>
<td>Forced swim test</td>
<td>Used as a measure of behavioural despair; a screen for antidepressant activity (decrease in immobility time often observed with antidepressant treatment).</td>
</tr>
<tr>
<td>Social interaction (Sociability/social novelty)</td>
<td>Test quantifies social behaviours; reduced sociability and interest in social novelty is often associated with depression.</td>
</tr>
<tr>
<td>Partition test</td>
<td>Used to quantify sociability/social avoidance as well as anxiety.</td>
</tr>
</tbody>
</table>

1.8.1 Latent Inhibition

Latent inhibition (LI) refers to decreased salience of a conditioned stimulus (CS) when it is paired with an unconditioned stimulus (US) after prior exposure to the CS (Clapcote et al., 2007). After CS pre-exposure, a weaker CS-US association is formed when the CS is paired with the US and less attention is paid to the CS during CS-US training (Gould and Wehner, 1999). LI is believed to be a measure of decremental attention, which is the ability to decrease attention to irrelevant stimuli (Gould and Wehner, 1999). This ability to ignore irrelevant stimuli prevents the formation of unnecessary associations and, consequently, prevents the unnecessary expenditure of energy. Some studies have reported schizophrenic patients to exhibit abnormal LI when they are experiencing psychotic episodes (Lubow, 2005). For example, in one study, reduced LI was shown in acute schizophrenics that were naive to treatment (Williams et al.,
In another, LI was found to correlate with duration of illness: Patients with a relatively short duration of illness (<12 months) showed reversed or absent LI, whereas patients with longer duration of illness (>12 months) showed intact LI (Gray et al., 1995). The authors suggest that the restoration of LI in schizophrenic patients over time is due to factors intrinsic to the evolution of the disease.

The phenomenon of LI has been demonstrated to be under genetic influence in animals: The development of LI was examined in a number of inbred strains of mice, and it was found that 129/Svev, C57BL/6, BALB/cByJ, AKR, and DBA/2 mice develop LI, but 129/SvJ, CBA, A, and C3H mice do not (Gould and Wehner, 1999). The authors of this study also suggest that while the between-strain variation in LI supports a role for genetic factors, the within-strain variation demonstrates that environmental factors also influence LI.

Neural pathways implicated in LI include the hippocampus, nucleus accumbens and entorhinal cortex. The hippocampus is involved in attention-related processes and is necessary for learning and discriminating (Harrell and Allan, 2003). Thus, the hippocampus plays a crucial role in inhibiting the formation of unnecessary associations and allowing for the formation of meaningful or predictive associations (Corcoran and Maren, 2001). In particular, the hippocampal ventral subicular region has been shown to regulate LI-related behavioural and dopamine responses in the rat (Peterschmitt, Hoeltzel and Louilot, 2005). The nucleus accumbens has been implicated in LI-related process in various studies centered on dopamine functioning. For example, one study found that lesions of dopamine terminals in the nucleus accumbens resulted in potentiation of LI while LI was attenuated by increasing dopamine function in the nucleus accumbens (Joseph et al., 2000). Finally, the entorhinal cortex has been
demonstrated to have significant control in LI-related processes. It has been shown that the left entorhinal cortex exerts a crucial influence over LI-related dopamine responses in the left dorsomedial shell of the nucleus accumbens and the left anterior striatum (Jeanblanc et al., 2004), and that neonatal disconnection of the entorhinal cortex disrupts LI in adult rats (Peterschmitt, Meyer and Louilot, 2007). LI testing in animals has excellent predictive validity in SCZ because LI is attenuated by dopamine agonists and increased by dopamine antagonists in both rats and humans (Lubow, 2005).

### 1.8.2 Elevated Plus Maze

Anxiety syndromes are quite prevalent in both SCZ and MDD. Up to 62% of schizophrenic patients suffer from at least one comorbid anxiety disorder (Labrie, Clapcote and Roder, 2009) and symptoms of depression and anxiety frequently appear together in patients (Liebowitz, 1993; Lydiard and Brawman-Mintzer, 1998). The elevated plus maze (EPM) is a well-established test for anxiety in animal models. The test is based on the conflicting tendencies of mice to investigate novel environments and to avoid brightly-lit, open, elevated areas (Labrie, Clapcote and Roder, 2009; Hinojosa et al., 2006). The subject mouse is presented with equal access to open spaces and areas that are enclosed (Figure 1.7). A higher proportion of time spent in the enclosed arms is believed to be indicative of higher levels of anxiety as such areas are less threatening to mice than the well-lit open areas. Baseline anxiety levels in mice are genetically-influenced and vary among strains (Avgustinovich et al., 2000; Conti et al., 2004). Quantitative trait loci (QTL) for anxiety in mice have been suggested on chromosomes 1, 12, 15 (Flint et al., 1995) and 5 (Cohen, Kang and Gulick, 2001) using EPM to differentiate phenotypes.
Anxiolytic drugs tend to increase time spent in open arms and anxiogenic drugs tend to decrease this measure in rodents, providing strong support for the face and predictive validity of this paradigm. For example, early attempts at validating the EPM paradigm found that the anxiolytics chlordiazepoxide, diazepam and phenobarbitone, sodium pentobarbital and ethanol increased time on open arms, while yohimbine, pentylентetrazole, caffeine and amphetamine, agents that cause anxiety in man, significantly reduced time spent on open arms in rats and NIH Swiss mice (Pellow et al., 1985; Lister, 1987).

Several pathways are known to influence EPM performance. Benzodiazepines work via GABA\textsubscript{A} receptors, the most prevalent inhibitory receptor within the brain. The glutamate system is also a prime candidate for influencing EPM performance as it has been implicated in the pathophysiology of anxiety disorders (Labrie, Clapcote and Roder, 2009). Indeed, glutamatergic activity in the nucleus accumbens was demonstrated to incite novelty-seeking behaviour in the rat (Alvarez and Ruarte, 2001) and several recent studies have demonstrated the involvement of
the glutamate pathway in EPM activity in both mice and rats using glutamine receptor ligands as anxiolytics to increase time on open arms (Spooren and Gasparini, 2004; Palucha et al., 2004; Poleszak et al., 2008). Genetically modified mice with altered glutamatergic neurotransmission have also been shown to have altered EPM activity (Basu et al., 2009).

1.8.3 Locomotion in an Open Field

Locomotion in an open field (OF) is used to obtain measurements of general locomotor activity, activity in the horizontal dimension, and exploratory behaviour, activity in the vertical dimension. The open field can also be used to measure anxiety as mice are naturally averse to well-lit open spaces (Labrie, Clapcote and Roder, 2009); higher levels of anxiety should correlate with decreased time spent in the central area of the field. Finally, the test can also be used to measure risk assessment behaviour as done in a recent study by quantifying the stretched attend posture (Marques et al., 2008).

Drug-induced hyperactivity in the OF is a well-established rodent model of SCZ as locomotor activity is quite responsive to pharmacological manipulation. The inhibition of hyperlocomotion induced by amphetamine or phencyclidine has been widely used as a screening tool for antipsychotic activity of drug candidates for some time (Sun, Hu and Li, 2009). OF activity is also often used in drug sensitivity studies as an initial convenient method of probing pharmacological efficacy (Gill and Boyle, 2008).

Locomotor activity is thought to be influenced by dopamine receptor activation in the nucleus accumbens (Bast et al., 2001) and increases in extracellular dopamine levels in the medial prefrontal cortex (mechanism of action by amphetamines) (Ago et al., 2009; Heidbreder et al., 2009).
Locomotor activity varies across strains, indicating a genetic component. In addition, environmental influences that confer a psychiatric disorder-like phenotype in rodents affect locomotor activity. Isolation rearing (psychosocial deprivation) and maternal immune activation are two examples of paradigms where hyperlocomotion has been observed in animals: Isolation-reared rats display increased locomotor activity and decreased resting time in the OF (Levine et al., 2007), and respiratory infection of pregnant mice with the human influenza virus yields offspring that display deficiencies in exploratory behaviour in the OF (Shi et al., 2003). Antipsychotics have been demonstrated to restore activity in the OF to basal levels in some cases of environmentally-induced abnormalities (Shi et al., 2003; Heidbreder et al., 2001).

1.8.4 Forced Swim Test

This paradigm is a measure of behavioural despair (Porsolt et al., 1977). Rodents are forced to choose between swimming and floating in a tank of water from which they cannot escape. Generally, animals swim vigorously at first, and then, over time, become immobile. Although the construct validity of this model is questionable as it seems reasonable that animals may become immobile to conserve energy, the model has very high predictive validity in identifying antidepressant activity, especially after chronic administration (Willner, 1991). In addition, the rescue effect observed with antidepressants is very specific: anxiolytic drugs do not decrease immobility in the FST (Porsolt et al., 1977), so the paradigm specifically measures “depression” and not the related emotional behaviour of anxiety.

Since Porsolt’s original report on the development of this test (Porsolt et al., 1977), numerous antidepressant agents have been shown to decrease immobility time in the FST in rodents. One example is magnesium administration (Poleszak et al., 2005). Moreover, the identification of
gene candidates in depression and related psychiatric disorders have allowed for mutant mice to be tested in the FST for a convenient method of measure of the relationship of a gene to antidepressant activity, and thus, depression, in animals. For example, male dopamine receptor D5 null mutants display lower levels of immobility in the FST (Holmes et al., 2001), implicating dopamine in depression. This agrees well with other work that suggests disturbances in the dopamine system are linked to the cause of depression (Cabib & Puglisi-Allegra, 1994). There is also a fundamental inter-strain difference of response in the FST; in fact findings in one study suggest that strain is one of the most important parameters to consider (Petit-Demouliere et al., 2005). Environmental stressors and paradigms that are used to induce a depression-like state in rodents increase immobility in the FST too. Both the CSD paradigm (Henn and Vollmayr, 2005; Kudryavtseva, Bakshantovskaya and Koryakina, 1991) and the chronic mild stress paradigm (Kompagne et al., 2008) have been effective in producing increased immobility in the FST.

1.8.5 Social Interaction: Sociability and Social Novelty

Among the many negative symptoms that appear in schizophrenics, social dysfunction is an important one that has debilitating effects in patients’ lives. Social dysfunction has been reported to be correlated with deficits in attention, spatial organization and performance on visual spatial tasks (Dickerson et al., 1996; Cornblatt and Keilp, 1994). The paradigm here consists of two phases: a sociability phase that investigates social approach- and avoidance-related motivation and a social novelty phase that assesses social memory and the ability to discriminate a socially novel stimulus (Labrie, Lipina and Roder, 2008; Moy et al., 2004; Figure 1.8).
The presence of a genetic component in behaviours related to sociability and social novelty was demonstrated in the original study that established this paradigm through the utilization of five inbred strains of mice (Moy et al., 2004). Four strains, including the C57BL/6J strain (genetic background of the ENU Disc1 mutants), showed high levels of sociability and preference for social novelty. Sex and age had no significant effect on strain differences (Moy et al., 2004).

![Diagram](image)

**Figure 1.8. Sociability and social novelty paradigm.** (a) **Sociability.** An unfamiliar conspecific is placed inside a transparent barrier in one chamber to allow for visual, auditory and olfactory contact (Chamber 2) while the other remains empty; the test subject is placed in the middle compartment and presented with the choice between the two chambers (measures social approach or avoidance). (b) **Social novelty.** The conspecific from the sociability phase remains (now familiar) and a new unfamiliar animal is placed in the previously empty chamber (Chamber 1); the test subject is now presented with a choice between the familiar animal or social novelty; social memory is assessed.

Pharmacological intervention can affect sociability and social novelty. A recent study found that D-serine was effective in reversing the social approach deficit in Grin1<sup>D481N</sup> mutant mice (Labrie, Lipina and Roder, 2008). Another study reports altered sociability and preference for social
novelty in C57BL/6J mice with subchronic treatment with PCP. The treatment did not significantly affect behaviour in either discrimination or reversal learning tasks; thus this mouse subchronic PCP treatment paradigm could potentially be a good model of the social withdrawal component of SCZ (Brigman et al., 2009). Yet another study employing this test paradigm on mutant mice with heterozygous deletion of transmembrane (TM)-domain neuregulin-1 (NRG1), a candidate susceptibility gene for SCZ, found that the NRG1 mutants lost the preference for social novelty displayed by wildtype mice (O'Tuathaigh et al., 2007). This disruption to social novelty preference in NRG1 mutants may involve deficits in aspects of social recognition memory (O'Tuathaigh et al., 2007).

1.8.6 Partition Test

The partition test (PT) is a variation of a social interaction test that measures behavioural reactivity of the subject to a neighbour, providing an index of anxiety in the subject (Avgustinovich et al., 2000). The test subject is placed across a partition that allows for visual, sound and olfactory, but not physical, contact with a neighbour. It is reasoned that approaching the partition is indicative of interest in the animal on the other side, and the test is therefore another way to evaluate sociability (Avgustinovich, Gorbach and Kudryavtseva, 1997), which is inversely associated with anxiety. In addition, through the use of first a familiar, then an unfamiliar mouse on the other side of the partition, preference for social novelty can also be measured using the PT paradigm.

Factors influencing anxiety levels in rodents should and do affect PT performance. For example, long-term chronic exposure to social defeat has been demonstrated to reduce communicative
behaviour in the PT in defeated subjects where elevated levels of anxiety were also demonstrated using the EPM (Kudryavtseva and Avgustinovich, 1998; Avgustinovich, Gorbach and Kudryavtseva, 1997; Kudryavtseva, Bakshatnovskaya and Koryakina, 1991). However, chronic treatment with the anxiolytic agents ipsapirone and buspirone of C57BL/6J mice exposed to CSD had no effect in the PT, but did increase time on open arms in the EPM (Avgustinovich, Alekseyenko and Koryakina, 2003). The investigators reason that this may be the case because PT is believed to measure trait anxiety, an enduring feature of the subject that is preferably influenced by genetic factors and EPM measures state anxiety, defined as the emotional reaction to a stressful stimulus in an unfamiliar environment (primarily affected by environmental factors) (Avgustinovich et al., 2000). The C57BL/6J strain is thought to have a high level of trait anxiety that masks any reduction in state anxiety in the PT (Avgustinovich, Alekseyenko and Koryakina, 2003).

1.9 Genetic Phenotyping: Gene Expression by Real Time RT-PCR

1.9.1 Real Time RT-PCR and the Standard Curve Method

Assays based on reverse transcription-PCR (RT-PCR) are the most common method for evaluating gene expression patterns and comparing mRNA levels in different samples (Bustin, 2002). The technique is based on reverse transcription of mRNA to cDNA, and consequent amplification of the cDNA transcript using traditional PCR methods. Despite the wide usage of RT-PCR, it is important to note that some studies have reported wide variability of results from RT-PCR assays, and it has been demonstrated to be unreliable as a clinical diagnostic tool (Bustin, 2002). Yet, there has been much work done to make improvements in the procedure.
such as better internal standards, more reliable references for data normalization and the introduction of new mathematical models for RT-PCR data analysis (Bustin, 2002).

*Real time* RT-PCR is becoming an increasingly important method, being preferentially used in high-throughput and accurate expression profiling of selected genes (Vandesompele et al., 2002). Because it measures the amount of PCR product formed during the course of the reaction by monitoring the fluorescence of dyes or probes present in the reaction mixture (Figure 1.9), it is possible to calculate the number of DNA molecules of the amplified sequence that were initially present in the sample (Kubista et al., 2006). The measure of interest is the number of amplification cycles required to obtain a particular amount of DNA molecules, the threshold cycle number, $C_T$.

![Figure 1.9. Basic mechanism of Taqman® probe-based real-time RT-PCR.](http://www3.appliedbiosystems.com/AB_Home/applicationstechnologies/Real-TimePCR/TaqManvsSYBRGreenChemistries/index.htm) Adapted from “TaqMan® vs. SYBR® Green Chemistries” on the Applied Biosystems Inc. website (http://www3.appliedbiosystems.com/AB_Home/applicationstechnologies/Real-TimePCR/TaqManvsSYBRGreenChemistries/index.htm), accessed May 23, 2009. (a) *Polymerization.* Fluorescent reporter dye R and quencher Q are at the 5’ and 3’ ends of the probe, respectively. (b) *Strand displacement.* As long as the probe is intact (R and Q both in place), emission of R is quenched. (c) *Cleavage.* DNA polymerase cleaves R from the probe during every extension cycle. (d) *Polymerization completed.* When R is liberated from the sequence attached to Q, fluorescent emission occurs and is detected.
Real time RT-PCR can be used for absolute or relative quantification; relative quantification consists of normalization of the transcript of interest to one or more endogenous control or housekeeping genes. The standard curve method is a relative quantification method. It uses a set of relative standards to obtain a linear relationship between $C_T$ and the dilution factor, which gives us the relative amount of cDNA (Figure 1.10).

![Figure 1.10. Example of a standard curve.](image)

The values obtained from the best-fit line are used to calculate relative amounts of the gene of interest in unknown samples as $RE=10^{\left(\frac{C_T-b}{m}\right)}$, where $RE = \text{relative quantity of gene of interest}$, $b = \text{intercept}$ and $m = \text{slope of the best-fit line}$.

### 1.9.2 Endogenous Control/Housekeeping Genes

Quantitative analyses using real time RT-PCR techniques requires the use of endogenous control genes (housekeeping genes) to control for variation in the amount of genetic material between samples, usually present due to unequal reaction efficiencies in the cDNA synthesis procedure (Romanowski et al., 2007). The expression of the gene of interest is normalized relative to the expression of the control gene, allowing for comparisons between different samples in different reaction wells. Control genes should show stable, unregulated expression in the tissue type from which the sample originates (Romanowski et al., 2007). Traditionally, genes such as $\beta$-actin,
glyceraldehyde-3-phosphate-dehydrogenase (Gapdh), cyclophilin, and 18S ribosomal RNA (rRNA) have been used in work with the murine brain, with Gapdh and β-actin being the most popular (Suzuki, Higgins and Crawford, 2000). However, recent evidence suggests these genes may not be suitable in every case as experimental treatments, aging and tissue type impacts their expression levels (Bas et al., 2004; Bustin, 2002).

Levels of Gapdh and β-actin have been shown to vary across some brain regions (Boda et al., 2009) and one study found that 18S rRNA and Gapdh should be used in mouse brain trauma injury, but that β-actin and β-microtubulin should be avoided due to non-stable expression (Rhinn et al., 2008). Studies in other mammals have also shown that both GAPDH and β-ACTIN are not suitable as internal control genes (Moe et al., 2001; Schmittgen and Zakrajsek, 2000). However, Gapdh and β-actin continue to be popular control/housekeeping genes, and some studies have found evidence in support of their continued use. For example, one study found that Gapdh and β-actin levels were similar in the cerebral cortex of mice independent of experimental treatment and sex (Sellayah et al., 2008). Due to many conflicting findings, it is suggested that endogenous control genes should be properly validated before used in a study (Dheda et al., 2004; Schmittgen and Zakrajsek, 2000). One approach to minimizing the effect of variation in control gene expression is using the geometric mean of multiple housekeeping genes (Jiang et al., 2009; Lipska et al., 2006; Vandesompele et al., 2002).
2 Materials and Methods

2.1 Animals

Male C57BL/J6 mice (wildtype) and two strains of heterozygous Disc1 mutant mice, Q39L -/+ and L100P -/+, also male, were used for all experiments. Wildtype mice were supplied either by Charles River Laboratories (CRL) or The Jackson Laboratory (TJL). Disc1 mutants were generated at Mount Sinai Hospital Animal Facilities and at the Toronto Centre for Phenogenomics (TCP) using ENU-induced mutation to exon 5 of mouse Disc1 (Clapcote et al., 2007). Mice were kept under a 12 hour artificial light/12 hour dark cycle (lights on at 07:00 h) in a room with constant temperature (23.3°C) and humidity. Food (standard laboratory rodent chow) and water was available ad libitum.

2.2 Social Defeat Paradigm

The CSD paradigm used was very similar to those previously described (Kudryavtseva et al., 1991; Kudryavtseva et al., 1995; Berton et al., 2006). The defeat boxes were designed and built in our lab: opaque galvanized steel boxes (25×29×16 cm) had drilled covers for light and ventilation. A perforated Plexiglas partition was used to keep each pair of mice physically separated. Pairs of mice were placed in the defeat boxes and allowed to acclimatize for three-five days before the start of each experiment. Defeat exposure began with a 5-minute activation period (steel cover removed and replaced by transparent Plexiglas cover for increased light), the perforated partition was removed to allow the pairs to physically interact. Pairs were allowed to interact for a period of 10 minutes, unless intervention was absolutely necessary due to visible physical injury to the defeated partner such as torn skin or blood. To maximize agonistic confrontation and minimize habituation, defeated mice were rotated every 24 hours after defeat.
exposure. Aggressors stayed in their home cages. Aggressors were used in several experiments if they exhibited good aggression and appeared healthy after the previous experiment(s). Control mice were housed with larger and older C57BL/J6 mice, but partitions were never removed (no physical contact) to avoid the establishment of a social order in control cages. Further specifics are given for each experiment in their respective sections.

2.3 Behavioural Assessment

2.3.1 Interaction Behaviour (IB)

This assessment quantified behaviours exhibited by animals during agonistic confrontations. Data were collected from sessions toward the end of the defeat exposure period (T9-10 or T19-20) when defeat behaviours should be well established and easier to quantify. The Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands) was used to collect and record data. The following behavioural parameters were recorded: (1) defeat-indicative behaviour: running away from aggressor, freezing near aggressor, freezing away from aggressor (usually in a corner), allowing aggressor to groom (frozen in position until aggressor done); (2) aggressive behaviour: fighting back, tail wagging, chasing aggressor (with or without provocation - reversal of roles); and (3) neutral behaviour: self-grooming, exploration (active movement not in response to aggressor’s movements, sniffing or digging of bedding).
2.3.2  Partition Test (PT)

The partition test has been previously described in detail (Kudryavtseva, 1994; Avgustinovich et al., 1997). A modified version was utilized here: defeated/control mice were observed in their native boxes (where they had been for at least 20 hours), thus an acclimatization period was not deemed necessary. Observation started as soon as the opaque steel cover was replaced with a transparent Plexiglas cover. The Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands) was used to collect and record data. The total observation period was 15 minutes; at the 10-minute mark, the native aggressor was removed and quickly replaced with a foreign aggressor. The parameters used to assess the behaviour of animals were as follows: (1) number of approaches to the partition; (2) duration of time spent near the partition (active sniffing or climbing of partition, digging near partition); (3) average time spent near the partition per approach; (4) freezing near partition (no movement for a minimum of 5 seconds); (5) freezing elsewhere within the cage (no movement for a minimum of 5 seconds, away from the partition); (6) duration of time spent on self-grooming; (7) number of exploration periods (active movement, sniffing of bedding or digging of bedding); and (8) duration of exploration periods.

2.3.3  Locomotion in a Novel Open-Field (OF)

The open field test was conducted using a modified version of those previously described (Labrie et al. 2009; Young et al., 2008) that only measured horizontal (motor) and vertical (exploratory) activity. Animals were habituated to the lighting (artificial, fluorescent) in the testing room for 0.5-1 hour before being placed in a 41×41×33 cm box with transparent Plexiglas walls and ceiling, a white floor and infrared beams to detect horizontal and vertical movements through beam breaks (7420/7430, Ugo Basile, Comerio, Italy). Mice were placed in the centre of the box.
and data collection started at this time. Total test session was 30 minutes, divided into six 5-minute intervals.

2.3.4 Forced Swim Test (FST)

Test was conducted as previously described (St. Clair et al. 2008; Clapcote et al, 2007 suppl.). The test animal was placed into a transparent plastic cylinder (25×18.5 cm) filled with tap water at a temperature of 24.5±0.5°C to a depth of 18 cm. Two minutes were allowed to elapse before the observation was started. The time spent swimming (or thrashing/climbing) and floating (no activity except what is required to keep head above water) was recorded for the next four minutes using The Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands). In the rare case that a mouse could not keep itself afloat (i.e. its head appears to dip below water surface), the animal was picked up and brought out of the water, and excluded from the test.

2.3.5 Elevated Plus Maze (EPM)

The EPM test was conducted as previously described (Labrie et al., 2009; Clapcote et al., 2007 suppl.). The maze consisted of two open arms (25×5 cm) and two closed arms (25×5×30 cm) that were raised from the floor by 50 cm. The room was dimly lit (open arms: 63-68 lx, closed arms: < 2 lx.). Mice were placed in the centre of the maze (central platform, 5×5 cm) facing an open arm at the start of observation. Observation period was 5 minutes for each mouse, during which time the behaviour of the animal was quantified using the following parameters: (1) duration of time spent in open arms; (2) duration of activity in the centre; (3) duration of time spent in closed arms; (4) passages between the closed arms across the centre; (5) risk assessment (peeking out of closed arms); (6) number of head-dips (peeking over side of open arms toward
the floor); (7) number of times mice reached the final third of any open arm; and (8) the total number of entries (into open and closed arms) for use as a measure of overall motor activity. Data was recorded using The Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands).

2.3.6 Latent Inhibition (LI)

Latent inhibition was assessed using a conditioned licking response paradigm in which white noise was used as the conditioned stimulus (CS) and a mild foot shock as the unconditioned stimulus (US). This paradigm has been described in detail elsewhere (Labrie et al., 2008; Clapcote et al., 2007 suppl.; Lipina et al., 2005). The test chambers used consisted of conditioning chambers equipped with bottles with metal sipper tubes enclosed in sound-attenuating chambers (ENV-022M) and connected to a lickometer (ENV-350CM; all from Med Associates, St. Albans, VT) to detect and count licks. The conditioning chambers had removable floors; one that consisted of metal rods was used on pre-exposure and conditioning days, another that was a flat piece of aluminum was used otherwise. The LI procedure consisted of a pre-training phase (days 1–4), a pre-exposure phase (day 5), a conditioning phase (day 6), a baseline/lick retraining phase (day 7) and a testing phase (day 8). In the pre-training phase, mice learned to obtain water by licking the metal sipper tube in the test chambers. This learning was facilitated by the removal of water from their native home cages 24 hours prior to the first pre-training session (increased salience in test chamber). The pre-training sessions consisted of a 5-minute acclimatization period (access to sipper tube blocked), followed by a 15-minute session during which latency to the first lick and the total number of licks were recorded. The pre-exposure phase consisted of only half of the animals (on average) receiving 40 white noise bursts (85 dB in intensity, 10 seconds in duration, 60 seconds interstimulus interval). The non-pre-
exposed (NPE) animals also underwent a session in the test chambers of identical duration, but received no stimulus. The conditioning phase consisted of the pairing of the CS (sound) to the US (shock of 0.37 mA intensity, 1 second duration). Mice received two noise-shock pairings, the first 5 minutes after the start of the session, and the second 5 minutes after that. The shock immediately followed noise termination and was administered via the metal rods of the grid floor wired to a shock generator (Med Associates, model ENV-414) via a scrambler. Mice were allowed access to water in their home cages for 15 minutes on pre-exposure and conditioning days as no water was available in the conditioning chambers. The lick retraining phase was identical to the pre-training phase and the same parameters recorded to ensure mice were still licking above threshold for LI testing (we set a minimum of 200 licks in the 15-minute period, although mice generally licked well above this number). Finally, the testing phase consisted of each mouse being placed in a chamber with access to the sipper tube as under pre-training conditions. After completion of the first 75 licks, the CS (noise) was presented and it lasted until lick 101. The time to first lick, time to complete licks 50-75 (period A) and the time to complete licks 76-101 (period B) were recorded. Drinking suppression was calculated as the suppression ratio A/(A+B). A higher suppression score is indicative of a lower suppression of drinking, and LI consists of lower suppression of drinking (higher suppression score) in the pre-exposed compared to the non-pre-exposed mice.

2.3.7 Sociability and Social Novelty (SSN)

The test was conducted in a similar manner to previously described protocols (Labrie et al., 2008; Clapcote et al., 2007 suppl.; Moy et al., 2004). A transparent Plexiglas box (53×25.6×23 cm) divided into three chambers connected by Plexiglas partitions with centrally placed openings (7.3×23 cm) was used for the paradigm. The outer chambers were identical (19.5 cm long each)
and each contained transparent Plexiglas cylindrical cages (13×8 cm diameter) that were perforated with holes of 1 cm diameter, placed at the centre of the chamber. These cages were used to contain the stranger mice, with the perforations allowing the test mouse to perceive auditory, visual, and olfactory cues. Containing the stranger mouse in the cylinder ensures that all social approach is initiated by the test mouse and is investigatory only, without direct physical contact (Clapcote et al., 2007 suppl.). At the beginning of each test session, the test mouse was placed in the central chamber and was allowed to freely explore for a period of 5 minutes. After the adaptation period, an unfamiliar mouse (male C57BL/6J, “stranger 1”) was placed inside one of the cylindrical cages in one of the side-chambers. Data collection started at this point, which designated the “sociability” phase: the amount of time spent in the chamber with the stranger mouse versus the amount of time spent in the chamber containing the empty cage. After a period of 10 minutes, another unfamiliar mouse (also male C57BL/J6, “stranger 2”) was placed inside the other cylinder in the opposite side-chamber. The activity of the test mouse was recorded for a further 10 min. This was the “social novelty” phase as the test mouse now had the option of exploring the central chamber, the side-chamber containing the initially stranger, now familiar, mouse, or the side-the chamber containing the novel stranger. Data collection was done using The Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands). The parameters recorded were: (1) the number of entries by the test mouse into each side-chamber; (2) time spent in each side-chamber; (3) latency to entry into each of the two side-chambers; (4) time spent being mobile by the test mouse; and (5) time spent being immobile (freezing). The test mouse was considered to be in a chamber if its head and two front paws had entered the chamber.
2.4 Chronic Social Defeat Experiments

2.4.1 Behavioural Testing Cohort: Twenty Days of Defeat in Disc1 Mutant Mice

Male Q31L -/+ and L100P -/+ mutant mice were used with littermate +/- control mice (Q31L -/+, n=6; L100P -/+, n =13; +/-, n=8; age 10-12 weeks). Male C57B/LJ6 mice, some pre-exposed to the social defeat paradigm, were used as aggressors in this experiment. Each pair was allowed to interact for 5 consecutive days to establish their defeated/aggressor status. Rotation into a foreign cage with a new aggressor was then started and continued every 24 hours after daily defeat exposure. After 17 days of defeat, mice were tested in the EPM. Testing in the OF was done between the 18th and 20th day of social defeat exposure. From the 19th to the 20th day, social activity of the defeated mice was tested using the S/SN paradigm. After twenty days of defeat, mice were subjected to the FST. Aggressors were then removed and defeated mice allowed to live individually in the defeat cages. LI testing was conducted over the span of a week with food available *ad libitum*, but no access to water in the home cages. After LI testing was complete, a second bottle with a 1 % sugar solution was added to each box for the SC test. Recordings were made for the subsequent week. Mice were then sacrificed by cervical dislocation followed by decapitation, and the following brain regions harvested: amygdala, nucleus accumbens, prefrontal cortex, hypothalamus, hippocampus, striatum and brainstem.

2.4.2 Behavioural Testing Cohort: Ten Days of Defeat in Wildtype Mice

Male C57BL/6J mice of age 9 weeks (n=13) were exposed to agonistic confrontation for ten consecutive days. Male C57BL/6J of age 5-6 months were used as aggressors. The control group (n=13) consisted of C57BL/6J mice (9 weeks) housed across older male C57s (5-6 months), but
not having any physical interaction with their respective partners. After 10 days of defeat, approximately half of the subjects from each group (losers: n=6, controls: n=6) were sacrificed by cervical dislocation followed by decapitation, and the following brain regions harvested: hypothalamus, PFC, amygdala, NAc, striatum, brainstem and hippocampus. The remaining mice were kept for behavioural assessment. Aggressors were removed from the social defeat boxes containing these mice for the duration of the testing period. The battery of behavioural tests consisted of the following: PT, OF, EPM, and FST. All tests were completed within two days after the last confrontation with aggressor mice. Upon completion of all behavioural tests, mice were sacrificed and brain regions harvested as before.

2.4.3 Molecular-Genetic Cohort: Twenty Days of Defeat in Wildtype Mice

Male C57BL/6 J mice of age 8-10 weeks (n=6) were exposed to agonistic confrontation for twenty consecutive days. A control group (n=6) consisted of mice housed across from aggressor mice, but with no physical contact with these mice (partition never removed). Older, larger C57BL/6 J males were used as aggressors. After 20 days of defeat, defeated and control subjects were sacrificed by cervical dislocation followed by decapitation, and the following brain regions obtained: amygdala, nucleus accumbens, prefrontal cortex, hypothalamus, hippocampus, striatum and brainstem.

2.4.4 Molecular-Genetic Cohort: Twenty Days of Defeat in L100P -/+ Mice

Male L100P -/+ mice of age 8-10 weeks (n=12) were exposed to agonistic confrontation for twenty consecutive days. A control group of n=6 was used. Older male C57B/LJ6 mice (12-16
weeks) were used as aggressors. Mice were sacrificed by cervical dislocation followed by decapitation 24 hours after the last day of defeat exposure, and brain regions harvested as before.

2.4.5 Molecular-Genetic Cohort: Twenty Days of Defeat in Q31L -/+ Mice

Male Q31L -/+ mice of age 10-12 weeks (n=5) were exposed to agonistic confrontation for twenty consecutive days. A control group of n=4 was utilized. Older male C57B/LJ6 mice were used as aggressors. Mice were sacrificed by cervical dislocation followed by decapitation 24 hours after the last day of defeat exposure, and brain regions harvested as before.

2.4.6 Twenty Days of Defeat in Wildtype Mice with Chronic Valproate Treatment

Male C57B/LJ6 mice were used (vehicle-defeat, n=8; valproate-defeat, n=8, vehicle-control, n=7; valproate-control, n=6; age 8-10 weeks). Male C57B/LJ6 mice slightly larger in size (on average 2-3 g heavier; age 12-14 weeks) were used as aggressors in this experiment. Rotation started after 5 consecutive days in the same cage to establish social status. Valproate and vehicle treatment started on the eighth day of defeat. Mice received valproate (249.3 mg/kg) or vehicle (0.9% saline) injections intraperitoneally twice daily at 10 am and 6 pm. After 22 days of defeat, subject mice were sacrificed by cervical dislocation followed by decapitation, and the following brain regions harvested (18 hours after last injection): amygdala, nucleus accumbens, prefrontal cortex, hypothalamus, hippocampus, striatum and brainstem. Prior to sacrifice, half of each treatment group and all of the control mice were tested in the following behavioural paradigms over the last three days of defeat (T20-T22), in order: PT, OF, SI and FST.
2.5 Molecular Analysis

2.5.1 Tissue Collection

After sacrifice by cervical dislocation, whole brains were obtained from animals and quickly washed using normal saline at 4°C. A gross dissection was then performed according to standard procedures for the desired brain regions on a cold plate at 5-10°C. Harvested samples were immediately snap-frozen using either liquid N\textsubscript{2} or dry ice. Samples were stored at -80°C until processing.

2.5.2 RNA Extraction and cDNA Synthesis

Tissue samples were mechanically homogenized and then lysed using Trizol\textsuperscript{®} Reagent (Invitrogen Life Technologies Corporation, Carlsbad, CA; 500-1000µL per sample, depending on sample size). Chloroform was then added (200 µL per 1000 µL of Trizol\textsuperscript{®}) to the samples and the samples centrifuged at 14 000 rpm and 4 °C for 15 min to separate the aqueous and organic phases. The aqueous phase was extracted and washed with 70% ethanol at 0°C to precipitate the RNA. Total RNA was purified from this solution using the Absolutely RNA Miniprep\textsuperscript{®} kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. Yield and quality of RNA obtained was estimated via optical density measurements. An A\textsubscript{260/280} value between 1.7 and 2.2 was considered relatively free of protein and suitable for RT-PCR work. The RNA solutions were then concentrated to 1.2 µg/µL using Microcron YM-10 centrifugal filter devices (Millipore Corporation, Bedford, MA). cDNA was prepared using the Omniscript Reverse Transcription kit by Qiagen (Qiagen Corporation, Valencia, CA) according to the manufacturer’s instructions.
### 2.5.3 Real-Time Reverse Transcription Polymerase Chain Reaction

RT-PCR was performed in quadruplicate, using a reaction volume of 50 µL (96-well setup), on the ABI PRISM 7000 sequence detection system (Applied Biosystems Inc.) and TaqMan® Assays-on-Demand™ Gene Expression assays (Applied Biosystems Inc., Foster City, CA). The RT-PCR cycle consisted of the following stages: (1) an activation step for AmpErase UNG (50 °C, 2 min), an enzyme that blocks reamplification of PCR products in subsequent amplification cycles, preventing false positives; (2) an activation step for Taq polymerase (95 °C, 10 min); (3) 40 cycles of denaturation (95 °C, 15 sec/cycle); and (4) an elongation step (60 °C, 1 min). Fluorescence was measured during the last step.

Gene expression was quantified using the standard curve method using *Gapdh* and *β-actin* expression levels for normalization. *Gapdh* was chosen as the endogenous control as it has been reported to display the least variation in expression levels across time points in the mouse PFC (Semeralul *et al.*, 2006), and *β-actin* added because it is preferred to use the geometric mean of multiple endogenous control genes for normalization (see section 1.9.2). Standard curves for rodent *Gapdh* (part number 4308313, Applied Biosystems Inc.), mouse *β-actin* (part number 4352341E, Applied Biosystems Inc.) and the three target genes were constructed using a series of known serial dilutions from a pooled sample of control mice to elicit the relationship between *Cₜ* values and cDNA content. All molecular-genetic data was analyzed using the same standard curves, allowing for comparisons between experiments. Assays for genes of interest used are listed below.
Gene | ABI Assay/Part No. | NCBI accession number
--- | --- | ---
Mouse *Disc1* | Mm00533313_m1 | NM_174853.2
 | | NM_174854.2
Mouse *Bdnf*, transcript III | Mm00432069_m1 | NM_001048141.1
Mouse *Pde4b* | Mm00480174_m1 | NM_019840.2

All mRNA expression levels are expressed as a *relative normalized expression* (RNE) value. The RNE was calculated as follows:

\[
RNE = \frac{\text{Relative expression of gene of interest (RE}_{GOI})}{\text{Relative expression of endogenous control (RE}_{EC})}
\]

where \( RE_{GOI} = 10^{\frac{C_f - \text{Intercept}}{\text{Slope}}} \) and \( RE_{EC} = \text{Geometric mean of } RE_{\text{Gapdh}} \text{ and } RE_{\beta\text{-actin}} \).

The values for the intercept and slope were obtained from the standard curve for the gene of interest. \( RE_{\text{Gapdh}} \) and \( RE_{\beta\text{-actin}} \) were calculated as \( RE_{GOI} \) using values from their respective standard curves.

### 2.6 Statistical Analysis

All data were analyzed for overall significant effects using ANOVA in Minitab 15 (Minitab Inc., State College, PA; ANOVA function where \( n \) values equal or the General Linear Model (GLM) function where \( n \) values not equal). Although MANOVA was an option in some cases, it was deemed to be overly conservative in detecting significance in the current work, and therefore, ANOVA was chosen as previously done (Clapcote *et al.*, 2007 suppl.). The Grubbs test
(maximum normed residual test) was used to identify and eliminate outliers for behavioural scores. The test compared individual values across all response parameters, and if a test animal had an unusual score in one category, it was eliminated from the dataset for all measurements.

Post-hoc analyses were performed subsequent to finding any significance using the Tukey-Kramer HSD method (or Tukey’s HSD where sample sizes were equal). The Tukey-Kramer (or Tukey’s) method was used here as the Bonferroni correction may be too stringent and may overestimate the probability of a false rejection (Perneger, 1998). The HSD has been shown to remain sufficiently conservative for comparing three means with unequal sample sizes (Cohen, 1969). For multiple comparisons, each behavioural response observed was regarded as a separate family unit and the overall family-wise error rate was set at 5%. Power analyses for statistical tests were performed using Cohen’s $d$ values to estimate effect sizes (Cohen, 1969). Powers were calculated using the smallest $n$ value for each test or assay.

LI data was analyzed using ANOVA with main factors of pre-exposure and genotype. Data for naive and defeated animals were analyzed separately. FST data was analyzed using genotype and defeat status as main factors, or defeat status and drug as main factors, or defeat duration as the main factor. OF data was analyzed using defeat status and genotype as main factors, or genotype as main factor, or defeat duration as main factor. EPM data was analyzed using genotype and defeat status as main factors. SSN data was analyzed with genotype, defeat status, chamber side and test condition as main factors. Gene expression data was subjected to ANOVA using genotype, defeat status and brain region as main factors, or defeat status and drug as main factors. Linear regression analysis using the least squares method was also used in the statistical evaluation of standard curves for RT-PCR data to obtain relative RNE values.
3 Results

3.1 The Effect of CSD Duration

In developing our CSD paradigm, an important variable tested was the length of the period of time mice should be exposed to CSD. We tested +/+ mice with 10 days of defeat (T10; n_1 = 7, n_2 = 6) and compared the group to +/+ mice that were exposed to CSD for twenty days (T20; n_1, n_2 = 8). The control group consisted of mice individually housed in social defeat boxes for 10 days (T0; n_1 = 7, n_2 = 6). We used an alpha level of 0.05 for significance in all statistical tests. All values reported are the arithmetic mean ± SEM. See section 2.6 for details.

3.1.1 Alterations in Affective Behaviours of Defeated T10- and T20-Wildtype Mice

3.1.1.1 Locomotion in a Novel Open Field

ANOVA analysis with defeat status as between-subjects factor and time period as within-subjects factor showed significant effects of both defeat status and time period on horizontal locomotor activity ($F_{(2,124)} = 12.25, p < 0.001$ and $F_{(5,124)} = 4.39, p = 0.001$, respectively) and a significant effect of defeat status only on vertical exploratory activity ($F_{(2,124)} = 16.99, p < 0.001$). T20 mice were significantly less active in the horizontal plane than both T0 and T10 mice ($t = 4.73, p < 0.001$ and $t = 3.49, p = 0.002$) across all time intervals (Figure 3.1). T10 mice did not differ significantly from T0 mice, but there was a trend towards slightly reduced locomotion. Horizontal activity also decreased over time in all mice ($p < 0.005$ for a difference between motion in the first 5 minutes versus motion in the last 5 minutes for all mice). T20 mice also displayed significantly reduced vertical activity across all time periods compared to both T0
mice (t = 5.57, p < 0.001) and T10 mice (t = 4.13, p < 0.001). There were no statistically significant differences between the T0 and T10 mice.

Figure 3.1. Locomotion in a novel open field. (a) *Horizontal motion*. Defeat status and time period had significant effects on horizontal activity ($F_{(2,124)} = 12.25, p < 0.001$ and $F_{(5,124)} = 4.39, p = 0.001$). T20 mice were less active than T0 and T10 mice (t = 4.73, p < 0.001 and t = 3.49, p = 0.002) across all time intervals. Activity decreased over time in all mice. (b) *Vertical motion*. Defeat status had a significant effect on vertical activity ($F_{(2,124)} = 16.99, p < 0.001$). T20 mice were less active than T0 and T10 mice (t = 5.57, p < 0.001; t = 4.13, p < 0.001). T0: n = 7, T10: n = 7, T20: n = 8.*T20 mice significantly differ from both T0 and T10 mice (time periods are considered independently for vertical motion; p < 0.05).
3.1.1.2 Forced Swim Test

One-way ANOVA analysis showed defeat status had a significant effect on the amount of time mice spent immobile (floating) in the forced swim test ($F_{(2,18)} = 20.10, p < 0.001$; Figure 3.2). Tukey’s HSD post hoc analyses showed T10 and T20 mice spent significantly more time immobile compared to control T0 mice ($t = 3.99, p = 0.002$ and $t = 6.26, p < 0.001$, respectively). Furthermore, there may be a dose-effect of the duration of CSD for immobility time: T20 mice displayed a trend of greater immobility than T10 mice, although this difference only neared statistical significance ($t = 2.27, p = 0.09$).

![Figure 3.2. Immobility in the forced swim test.](image)

**Figure 3.2. Immobility in the forced swim test.** Defeat status had a significant effect on time spent immobile ($F_{(2,18)} = 20.10, p < 0.001$). T10 and T20 mice spent more time immobile than T0 mice ($t = 3.99, p = 0.002$ and $t = 6.26, p < 0.001$). T0: n = 7, T10: n = 7, T20: n = 7.

3.1.2 Changes in Disc1, Bdnf(III) and Pde4b Expression in T10- and T20-Wildtypes

One-way ANOVA showed no significant effect of length of defeat period on Disc1, Bdnf transcript III or Pde4b expression in the hippocampus (Figure 3.3) or the hypothalamus (data not shown). No additional regions were analyzed.
3.2 Behavioural Changes Induced by CSD in *Disc1* Heterozygous Mutant Mice

Mice were tested in a selection of behavioural tests from the array used by Clapcote *et al.* in the initial phenotyping of *Disc1* ENU mutant mice (2007) after CSD exposure. CSD had genotype-dependent effects on affective and social behaviours. L100P -/+ mice displayed the largest behavioural changes.

### 3.2.1 Alterations in Affective Behaviours

#### 3.2.1.1 Elevated Plus Maze

ANOVA analysis with genotype and defeat status as main factors found a significant effect of the interaction of both on the fraction of entries into open arms, OA (F(2,65) = 3.43, p = 0.039), and a significant effect of defeat status on the fraction of entries onto the central platform (CP) (F(1,65) = 12.54, p = 0.001). *Post hoc* analyses using Tukey’s HSD showed that defeated
L100P-/+ mice entered OA significantly less than naive L100P -/+ mice (t = 3.49, p = 0.011), while there were no differences between naive and defeated mice within the Q31L -/+ or +/+ groups (Figure 3.4a). CSD also increased the number of entries onto the CP in L100P -/+ mice (t = 3.31, p = 0.018; Figure 3.4b). Neither main factor had a significant effect on the number of entries into closed arms (CA), the total number of entries, the number of passages, risk assessment, or the number of head dips (data not shown). The interaction term (genotype × defeat status) only neared significance for risk assessment (F(2,65) = 2.85, p = 0.065). Finally, defeat status had an effect on the number of times mice reached the end of OA (the final third of the length of OA; F(1,65) = 12.90, p = 0.001; data not shown). Post hoc analysis showed a significant difference for the L100P -/+ group only: no defeated subject reached the end of OA while most naive L100P -/+ mice did several times (11 of 15 mice did an average of 2.45 ± 0.29 times; t = 4.03, p = 0.002).

Figure 3.4. Performance in the elevated plus maze. (a) Number of entries into open arms from central platform or closed arms. # indicates a significant difference between defeated L100P -/+ mice and naive L100P -/+ mice (t = 3.49, p = 0.011). (b) Number of entries into central platform from open or closed arms. *CSD increased the number of entries into the central platform in L100P -/+ mice: t = 3.31, p = 0.018). +/+: naive n = 20, defeated n = 8; Q31L -/+: naive n = 10, defeated n = 6; L100P -/+: naive n = 15, defeated n = 12.
Moreover, genotype and defeat status had significant effects on time spent in CA ($F_{(2,65)} = 3.23$, $p = 0.046$ and $F_{(1,65)} = 54.28$, $p < 0.001$, respectively), while the interaction term only neared significance for time spent on OA ($F_{(2,65)} = 2.84$, $p = 0.066$). *Post hoc* analyses revealed a difference between naive and defeated L100P +/- mice for time on OA ($t = 2.99$, $p = 0.043$; Figure 3.5a). All defeated mice appeared to spend less time in CA than their naive counterparts (+/+: $t = 3.89$, $p = 0.003$; Q31L -/+: $t = 5.37$, $p < 0.001$; L100P +/-: $t = 3.27$, $p = 0.020$). Moreover, defeated L100P +/- mice spent more time in CA than defeated Q31L +/- mice ($t = 3.06$, $p = 0.036$; Figure 3.5b). There were no differences between groups for time spent on the CP (data not shown).

*Figure 3.5. Percentage time spent in various compartments in the EPM.* (a) *Open arms.* *Defeated L100P +/- mice spent less time on open arms than naive L100P/+ mice ($t = 2.99$, $p = 0.043$).* (b) *Closed arms.* Defeated mice spent less time in closed arms across all genotypes (+/+: $t = 3.89$, $p = 0.003$; Q31L -/+: $t = 5.37$, $p < 0.001$; L100P +/-: $t = 3.27$, $p = 0.020$). *Defeated L100P +/- mice also differed significantly from defeated Q31L +/- mice ($t = 3.06$, $p = 0.036$).

### 3.2.1.2 Forced Swim Test

ANOVA analysis with genotype and defeat status as main factors showed a significant effect of defeat status on immobility time ($F_{(1,42)} = 50.47$, $p < 0.001$). *Post hoc* analysis showed that defeated mice within each genotype displayed significantly greater immobility than their naive
counterparts (+/+: t = 3.89, p = 0.004; Q31L -/+: t = 3.37, p = 0.019; L100P -/+: t = 5.10, < 0.001). Although the interaction term was not statistically significant, it should be noted that the difference in immobility time between naive and defeated mice was largest for the L100P -/+ group (Figure 3.6).

![Figure 3.6](image)

**Figure 3.6. Performance in the forced swim test.** There was a significant effect of defeat status on immobility ($F_{(1,42)} = 50.47, p < 0.001$). Defeated mice displayed significantly greater immobility than their naive counterparts (+/+: $t = 3.89, p = 0.004$; Q31L -/+: $t = 3.37, p = 0.019$; L100P -/+: $t = 5.10, < 0.001$). +/+: n = 7, Q31L -/+: n = 6, L100P -/+: n = 6.

### 3.2.1.3 Locomotion in a Novel Open Field

ANOVA analysis of data from the first 5 minutes of the OF test using genotype and defeat status as the main factors showed that only defeat status had a significant effect on horizontal activity ($F_{(1,57)} = 6.84, p = 0.011$). Post hoc analyses did not yield statistically significant differences between naive and defeated mice within any genotype group, but there was a significant difference between all naive and all defeated mice ($t = 2.62, p = 0.011$; Figure 3.7). Genotype, defeat status and the interaction term had no effect on vertical activity for the first 5 minutes in a novel open field (data not shown).
Figure 3.7. Horizontal activity in a novel open field in naïve and defeated mice. Figure depicts first 5-minute period from the OF test. Defeated status had a significant effect on locomotion ($F_{(1,57)} = 6.84, p = 0.011$), but genotype had no effect. * denotes a significant difference between all naive mice and all defeated mice ($t = 2.62, p = 0.011$). +/-: $n = 8$, Q31L +/-: $n = 6$, L100P +/-: $n = 6$.

3.2.2 Alterations in Social Behaviour

3.2.2.1 Sociability and Social Novelty

Repeated measures ANOVA with genotype, defeat status, test condition and chamber side as factors (Moy et al., 2004) showed significant main effects of several of these terms. All significant factors are denoted in Table 3a along with their respective F and p values. Post hoc analyses using Tukey’s HSD showed significant differences between genotypes both pre- and post-CSD exposure. Naive L100P +/- mice spent less time with Stranger 1 compared to naive Q31L +/- mice ($t = 4.06, p = 0.002$) and naive +/- mice ($t = 3.83, p = 0.004$) when the sociability phase and the social novelty phase were considered together. In addition, CSD decreased the time spent with Stranger 1 across both test phases in Q31L +/- mice ($t = 3.07, p = 0.036$).
Table 3a. Significance levels of various factors on measures in the SSN test.

<table>
<thead>
<tr>
<th>Factor</th>
<th>% Chamber Time</th>
<th>Number of Chamber Entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>F(2,132) = 10.38, p &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Defeat status</td>
<td>F(1,132) = 9.13, p = 0.003</td>
<td>F(1,132) = 28.96, p &lt; 0.001</td>
</tr>
<tr>
<td>Test condition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chamber side</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Defeat status</td>
<td>F(2,132) = 10.36, p &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Test condition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Chamber side</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Defeat status × Test condition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Defeat status × Chamber side</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Test condition × Chamber side</td>
<td>F(1,132) = 33.16, p &lt; 0.001</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Defeat status × Test condition</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Defeat status × Chamber side</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Test condition × Chamber side</td>
<td>F(2,132) = 3.36, p = 0.038</td>
<td>-</td>
</tr>
<tr>
<td>Defeat status × Test condition × Chamber side</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Genotype × Defeat status × Test condition × Chamber side</td>
<td>F(2,132) = 3.97, p = 0.021</td>
<td>-</td>
</tr>
</tbody>
</table>
Within-group repeated measures ANOVA was used to detect side preference (chamber 1 or chamber 2, grouped by test condition). Significance was detected only for the social novelty test condition where “genotype × defeat status × chamber side” had a significant effect on time spent in a given chamber ($F_{(2,66)} = 3.28, p = 0.044$). In the sociability phase, naive L100P -/+ mice spent less time in the empty chamber compared to naive Q31L -/+ mice (Figure 3.8b; $t = 4.37, p = 0.003$) and less time in the chamber with Stranger 1 compared to naive +/+ mice ($t = 3.53, p = 0.034$). In the social novelty phase, both naive L100P -/+ mice and naive Q31L -/+ mice displayed low preference for social novelty as they spent less time with Stranger 2 than naive +/+ mice (Figure 3.9b; $t = 3.78, p = 0.016$ and $t = 3.59, p = 0.028$, respectively). Naive L100P -/+ mice also spent less time with Stanger 1 in this test phase compared to naive Q31L -/+ mice ($t = 3.49, p = 0.038$) and naive +/+ mice ($t = 3.23, p = 0.075$). Wildtype mice showed a general preference for social novelty as the introduction of a novel Stranger 2 resulted in a significant decrease in time spent with Stranger 1 (Figure 3.9b; $t = 3.56, p = 0.009$, vs. sociability phase) and a corresponding increase in time spent with Stranger 2 ($t = 3.56, p = 0.009$, vs. time spent with Stranger 1 in social novelty phase). Moreover, CSD decreased this preference for social novelty in +/+ mice as defeated +/+ mice spent less time with Stranger 2 compared to naive +/+ mice in the social novelty test phase ($t = 4.27, p = 0.003$). Finally, we also found significant effects of genotype and genotype × defeat status on time spent walking. Naive L100P -/+ mice spent more time walking than all naive and defeated +/+ and Q31L -/+ mice (vs. +/+ naive: $t = 7.32$; vs. +/+ defeated: $t = 4.92$; vs. Q31L -/+ naive: $t = 6.37$; vs. Q31L -/+ defeated: $t = 5.09$; $p < 0.001$ for all). While CSD did not have an effect on walking in +/+ or Q31L -/+ mice, defeated L100P -/+ mice spent less time walking than naive L100P -/+ mice (Figure 3.10; $t = 4.46, p < 0.001$).
Figure 3.8. Performance in the sociability phase of the SSN test. (a) Number of entries. (b) % Time. Naive L100P -/+ mice spent less time in the empty chamber compared to naive Q31L -/+ mice and less time in the chamber with Stranger 1 compared to +/+ mice. *p = 0.034; **p = 0.003. +/+: naive n = 6, defeated n = 8; Q31L -/+: naive n = 8, defeated n = 6; L100P -/+: naive n = 6, defeated n = 6.
Figure 3.9. Performance in the social novelty phase of the SSN test. (a) Number of entries. (b) % Time. Naive wildtype mice spent more time with Stranger 2 than Stranger 1. Defeated wildtype mice spent less time with Stranger 2 than naive wildtypes. Naive L100P +/- and Q31L +/- mice spent less time with Stranger 2 than naive wildtype mice. Naive L100P +/- mice also spent less time with Stranger 1 compared to naive Q31L +/- and wildtype mice. *p = 0.009; **p = 0.003 vs. naive wildtypes; "p = 0.028 vs. naive +/-; "p = 0.016 vs. naive +/-; "p = 0.038 vs. naive Q31L +/-. +/-: naive n = 6, defeated n = 8; Q31L +/-: naive n = 8, defeated n = 6; L100P +/-: naive n = 6, defeated n = 6.
Figure 3.10. Time spent walking in the sociability and social novelty test. Test condition had no effect on % time for walking, thus average time is reported. Naive L100P/+ mice spent more time walking than any other group (p < 0.001; * denotes significance). +/+: naive n = 6, defeated n = 8; Q31L -/+: naive n = 8, defeated n = 6; L100P -/+: naive n = 6, defeated n = 6.

3.2.3 Effect of CSD on Cognitive Function

3.2.3.1 Latent Inhibition

One-way ANOVA performed on data from training period for the defeated cohort with genotype, day and genotype × day as factors showed no effect of any factor on latency to first lick, but significant effects of both genotype (F(2,135) = 3.69, p = 0.028) and day (F(4,135) = 29.52, p < 0.001) on the total number of licks. Figure 3.11a shows that latency remained relatively constant within groups over all days and did not differ between groups. Figure 3.11b shows that all genotype groups had a similar starting level of licking (day 1 readings). Significant differences emerged between the number of licks made on day 1 and days 3, 4 and baseline day within each genotype (p generally < 0.001; Figure 3.11).
Figure 3.11. Characteristics from training period for LI testing for defeated mice. (a) Latency to first lick. Latency remained constant across sessions and did not differ between genotypes. (b) Number of licks. All mice learned to lick over time; the number of licks was greater on days 3, 4 and baseline day (T3, T4 and BL) compared to day 1 (T1) for each group. Significance when compared to T1 within genotype: *p < 0.001, **p = 0.005, ***p = 0.030. +/-: n = 8, Q31L +/- n = 9; L100P +/-: n = 13.

Analyses of suppression ratio data for naive and defeated animals were done separately due to differences in the design of the pretraining period. Naive animals were trained to lick for 5 days, while defeated for 4 only. Both sets of data were analyzed by two-way ANOVA with main factors of genotype and preexposure level (0, 40 dB). For naive animals, both genotype and
preexposure level had significant main effects, but their interaction term did not ($F_{(2,32)} = 3.37, p = 0.047, F_{(1,32)} = 7.34, p = 0.011$ and $F_{(2,32)} = 1.68, p = 0.203$, respectively). Post hoc analyses showed that Q31L -/+ mice differed from wildtype mice significantly ($t = 2.58, p = 0.038$) and all NPE mice differed significantly from all PE mice ($t = 2.71, p = 0.011$). Within each genotype group, the wildtype-PE mice neared significance for a difference relative to the wildtype-NPE mice ($t = 3.01, p = 0.052$). For the defeated cohort, only the preexposure level term had a significant effect ($F_{(1,32)} = 11.21, p = 0.003$), while the interaction term neared significance ($F_{(2,32)} = 3.37, p = 0.051$). The significant effect of genotype on suppression ratio observed in naive animals diminished in CSD-exposed animals. Post hoc analyses solidified the effect of preexposure level ($t = 3.35, p = 0.003$, for all PE mice versus all NPE mice). Normal LI was observed in wildtype-defeated mice ($t = 3.74, p = 0.011$), while both groups of heterozygous defeated mutant mice displayed abnormal LI.

Figure 3.12. Latent inhibition in wildtype and Disc1 ENU heterozygous mutants. (a) Naïve mice. (b) Defeated mice. A lower suppression ratio indicates a stronger suppression of drinking and ‘normal’ LI consists of a lower suppression ratio in the NPE group compared to the PE group. Naïve wildtype mice neared statistical significance for normal LI (p = 0.052, #), and defeated wildtype mice displayed normal LI with statistical significance (p = 0.011, *). LI could not be established in naïve nor defeated animals from either mutant line. +/+ naïve: PE n = 7, NPE n = 5; +/+ defeated: PE n = 4, NPE n = 4; Q31L -/+ naïve: PE n = 7, NPE n = 9; Q31L -/+ defeated: PE n = 4, NPE n = 5; L100P -/+ naïve: PE n = 6, NPE n = 4; L100P -/+ defeated: PE n = 6, NPE n = 7.
3.2.4 Gene-Environment Interaction

Figure 3.13 shows interaction plots for the main factors ‘genotype’ and ‘defeat status’ for a variety of behavioural measures. F tests show that the interaction term \((G \times E)\) was not significant for immobility in the FST or horizontal activity in the OF \((F_{(2,42)} = 0.66, p = 0.522\) and \(F_{(2,57)} = 0.21, p = 0.813\), respectively), neared significance for time spent on open arms in the EPM \((F_{(2,65)} = 2.84, p = 0.066\), and was significant for the number of entries onto open arms in the EPM as well as time spent in chamber 1 and time spent walking in the SSN \((F_{(2,65)} = 5.26, p = 0.039, F_{(2,65)} = 7.42 p = 0.001, F_{(2,66)} = 3.60, p = 0.033,\) and \(F_{(2,66)} = 14.62, p < 0.001\), respectively). As the main outcome of the LI test of interest to us is qualitative in nature (the presence or absence of LI), interaction plot analysis was not applied to LI data. Statistical significance is usually present where genotype lines vary in slopes and are not parallel (Figures 3.13c-d); when genotype lines are parallel (or near-parallel) as in Figures 3.13a-b, the \(G \times E\) term is not significant. An interesting observation is that the line for the L100P -/+ group deviates the most from the other two genotype lines in most of the plots, both in magnitude and direction of slopes.
(a) FST ($Y = \text{Mean \% immobility}$)

(b) OF ($Y = \text{Mean horizontal activity}$)

(c) EPM

($Y = \text{Mean \% time in OA}$)

($Y = \text{Mean number of entries onto OA}$)
Figure 3.13. **Gene-environment interaction plots.** (a) Immobility in the FST. $G \times E$ had no significant effect, but L100P/+ mice differ from wildtypes more than Q31L/+ mice. (b) Horizontal activity in the OF. $G \times E$ had no significant effect. (c) Time spent on open arms, number of entries onto open arms and number of entries into closed arms in the EPM. $G \times E$ neared statistical significance for time ($p = 0.066$), and was significant for number of entries onto open arms ($p = 0.008$) and number of entries into closed arms ($p = 0.001$). L100P/+ mice deviated from wildtypes more than Q31L/+ mice. (d) Time spent in chamber 1 and on walking in the SSN. $G \times E$ had a significant effect on time spent in chamber 1 when test condition was included in analysis ($p = 0.033$) and on time spent walking ($p < 0.001$). Naive L100P -/+ mice appeared to deviate more than Q31L -/+ mice from wildtype naives, with the slopes of the various lines going in the opposite direction of those for Q31L -/+ and wildtype mice.
3.3 Effect of CSD on *Disc1*, *Bdnf(III)* and *Pde4b* Expression

There were no significant effects of CSD or the interaction term (defeat status × genotype) on *Disc1*, *Bdnf(III)* and *Pde4b* mRNA expression, but there were significant effects of genotype and tissue source (brain region). There was a difference in *Bdnf(III)* mRNA expression in striatal tissue (L100P -/+ mice << Q31L -/+ mice), no differences for any genes between any genotype in prefrontal cortical tissue, and several significant differences among genotypes in expression of all three genes in hippocampal and hypothalamic tissues. In particular, L100P -/+ and Q31L -/+ mice were found to express higher levels of *Disc1* mRNA in the hippocampus compared to wildtype mice, L100P -/+ mice higher levels of *Bdnf(III)* in both hippocampus and hypothalamus compared to wildtype and Q31L -/+ mice, and L100P -/+ mice higher levels of *Pde4b* mRNA in the hippocampus and hypothalamus compared to wildtype mice (see section 3.3.2).

3.3.1 Method of Quantifying Gene Expression and Standard Curves

For the comparative Cₜ (ΔΔCₜ) method to yield valid results, primer efficiencies of the genes of interest and the control genes should be close enough for ΔCₜ (Cₜ (gene of interest) - Cₜ (control gene)) to remain relatively constant over a range of cDNA concentrations. A plot of ΔCₜ against the log of the dilution factor of a sample should therefore yield a slope of approximately zero (< 0.1 acceptable in practice). We found large differences in the primer efficiencies of our genes of interest and *Gapdh* (Table 3b), and the absolute value of the slopes of our validation curves for all three genes were >> 0.1 (Figure 3.14). The alternate method of quantifying gene expression, the relative standard curve method, was therefore used here.
Table 3b. Primer Efficiencies of Gene Assays (%)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Actin</td>
<td>95.03</td>
</tr>
<tr>
<td>Gapdh</td>
<td>70.50</td>
</tr>
<tr>
<td>Disc1</td>
<td>85.21</td>
</tr>
<tr>
<td>Pde4b</td>
<td>90.23</td>
</tr>
<tr>
<td>Bdnf(III)</td>
<td>88.33</td>
</tr>
</tbody>
</table>

Figure 3.14. Validation curves for Disc1, Bdnf(III) and Pde4b. (a) ΔC_T values were obtained relative to Gapdh. (b) All slopes > 0.1, which prohibits utilization of the ΔΔC_T method here. Although β-Actin had a better primer efficiency, inter-sample variability in its mRNA levels were too large and thus the use of β-Actin alone for endogenous control was deemed unreliable.
Figure 3.15 depicts all standard curves obtained for our control genes, \textit{Gapdh} and \textit{\( \beta \)-actin}, and our genes of interest, \textit{Disc1}, \textit{Bdnf(III)}, and \textit{Pde4b}. All standard curves were generated from the same cDNA sample (created from pooling four hippocampal template RNA samples from C57Bl/6J mice (wildtype background)). All data were analyzed using the values obtained from these curves (Table 3c). A standard curve was accepted when \( R^2 > 0.99 \) with a significant p-value (< 0.05).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3_15a.png}
\caption{(a) Standard curves for the endogenous control genes \textit{Gapdh} and \textit{\( \beta \)-actin}.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig3_15b.png}
\caption{(b) Standard curves for our genes of interest, \textit{Disc1}, \textit{Bdnf(III)} and \textit{Pde4b}.}
\end{figure}
### Table 3c. All Standard Curves

<table>
<thead>
<tr>
<th>Gene</th>
<th>Regression R²</th>
<th>Significance (P-value)</th>
<th>Intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Actin</td>
<td>0.9999</td>
<td>8.71 × 10⁻¹²</td>
<td>20.1076</td>
<td>3.4472</td>
</tr>
<tr>
<td>Gapdh</td>
<td>0.9994</td>
<td>2.96 × 10⁻⁹</td>
<td>22.1619</td>
<td>4.3155</td>
</tr>
<tr>
<td>Disc1</td>
<td>0.9970</td>
<td>3.46 × 10⁻⁶</td>
<td>31.0138</td>
<td>3.7360</td>
</tr>
<tr>
<td>Bdnf</td>
<td>0.9999</td>
<td>9.94 × 10⁻¹²</td>
<td>27.9739</td>
<td>3.5807</td>
</tr>
<tr>
<td>Pde4b</td>
<td>0.9991</td>
<td>8.64 × 10⁻⁹</td>
<td>24.0498</td>
<td>3.6374</td>
</tr>
</tbody>
</table>

#### 3.3.2 Disc1, Bdnf(III) and Pde4b Expression

ANOVA with genotype, tissue source and defeat status as main factors showed that only genotype and tissue source had significant main effects on Disc1, Bdnf(III) and Pde4b expression. Their interaction term also had a significant effect on gene expression (Table 3d). CSD had no significant main effect.

### Table 3d. F-test (ANOVA) Significant Factors and P-values

<table>
<thead>
<tr>
<th></th>
<th>Disc1</th>
<th>Bdnf(III)</th>
<th>Pde4b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F(2, 96) = 33.97, p &lt; 0.001</td>
<td>F(2, 96) = 36.76, p &lt; 0.001</td>
<td>F(2, 96) = 16.88, p &lt; 0.001</td>
</tr>
<tr>
<td>Tissue source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F(3, 96) = 45.85, p &lt; 0.001</td>
<td>F(3, 96) = 92.09, p &lt; 0.001</td>
<td>F(3, 96) = 43.32, p &lt; 0.001</td>
</tr>
<tr>
<td>Genotype × Tissue source</td>
<td>F(6, 96) = 13.78, p &lt; 0.001</td>
<td>F(6, 96) = 44.83, p &lt; 0.001</td>
<td>F(6, 96) = 9.38, p &lt; 0.001</td>
</tr>
</tbody>
</table>
Post hoc analyses showed significant differences in mRNA expression in the hippocampi and hypothalami of L100P -/+ and Q31L -/+ mice compared to +/+ mice within both naive and defeated groups (Figure 3.16). Disc1 mRNA expression in the hippocampus was found to be higher in both mutant lines versus wildtype mice (+/+ naive vs. L100P -/+ naive: t = 4.41, p = 0.006; vs. Q31L -/+ naive: t = 4.78, p = 0.002; +/+ defeated vs. L100P -/+ defeated: t = 6.33, p < 0.001; vs. Q31L -/+ defeated: t = 8.40, p < 0.001) and higher in the hypothalami of Q31L -/+ mice relative to L100P -/+ mice (naive: t = 4.01, p = 0.022; defeated: t = 4.38, p = 0.006).

Bdnf(III) mRNA levels were found to be significantly higher in the hippocampi and hypothalami of L100P -/+ mice relative to both wildtype and Q31L -/+ mice, and significantly lower in the striata of L100P -/+ mice relative to Q31L -/+ mice (for hippocampus: naive L100P -/+ vs. naive +/+: t = 8.88; vs. naive Q31L -/+: t = 8.14; defeated L100P -/+ vs. defeated +/+: t = 12.29; vs. defeated Q31L -/+: t = 11.23; p < 0.001 for all; for hypothalamus: naive L100P -/+ vs. naive +/+: t = 4.38, p = 0.007; vs. naive Q31L -/+: t = 4.68, p = 0.002; defeated L100P -/+ vs. defeated +/+: t = 3.97, p = 0.025; vs. defeated Q31L -/+: t = 4.34, p = 0.007; for striatum: t = 3.61, p = 0.024).

Finally, Pde4b mRNA levels were found to be higher in the hippocampi of L100P -/+ mice relative to the hippocampi of wildtype mice in both naive and defeated groups, and also higher in the hypothalami of L100P -/+ mice relative to wildtype mice when defeat status was ignored (for hippocampus: naive: t = 5.21; defeated: t = 6.16; p < 0.001 for all; for hypothalamus: t = 4.33, p = 0.002).
Figure 3.16. *Disc1*, *Bdnf(III)* and *Pde4b* mRNA expression in hippocampal, hypothalamic, prefrontal-cortical and striatal tissue samples from naive and defeated wildtypes and *Disc1* ENU heterozygous mutants. RNE = relative normalized expression of gene of interest, normalized to the geometric mean of two endogenous controls, *Gapdh* and *β-actin*. Genotype, tissue source, and genotype × tissue source had significant main effects on gene expression (p < 0.001 for all; see Table 3d). Significant differences in *Disc1* expression: hippocampus: {L100P -/+ = Q31L -/+} > +/++; hypothalamus: L100P -/+ < Q31L -/+. Significant differences in *Bdnf(III)* expression: striatal tissue: L100P -/+ << Q31L -/+; hippocampus, hypothalamus: L100P -/+ >> {+/+ = Q31L -/+}. Significant differences in *Pde4b* expression: hippocampus, hypothalamus: L100P -/+ > +/+. *significance with or without including defeat status; **significance only when animals are grouped by genotype excluding defeat status. +/+: n = 6 naive, 6 defeated; Q31L -/+: n = 4 naive, 5 defeated; L100P -/+: n = 5 naive, 4 defeated
3.4 Effect of Chronic Valproate Treatment in +/- Mice

3.4.1 Interaction with an Aggressive Conspecific (Aggressor)

The 19th defeat session was quantified using specific behaviours that are typically observed in social defeat: running away from aggressor, chasing aggressor, freezing near aggressor, freezing in a corner, fighting back, exploration (neutral behaviour), and allowing grooming by aggressor (Figure 3.17). ‘Total defeated behaviour’ was defined as the sum of running away from aggressor, freezing near aggressor, freezing in a corner, and allowing grooming by aggressor, all behaviours typically displayed by defeated mice (Kudryavtseva et al., 1995). Chasing the aggressor and fighting back are aggressive behaviours that are typically not seen in defeated animals and are indicative of resistance to a subordinate social position. A late defeat session was chosen because the social order should be established by then. There were no statistically significant differences between the vehicle- and valproate-treated animals in the time spent on specific behaviours nor the number of times specific behaviours were exhibited. However, there may be a trend toward decreased defeated behaviour in valproate-treated animals (for ‘Total Defeated Behaviour’, t = 1.49, p = 0.159).

3.4.2 Effect of Valproate Treatment on Affective Behaviours

3.4.2.1 Forced Swim Test

ANOVA with defeat status, drug treatment and the interaction factor was used to analyze data. Neither defeat status nor drug treatment had a significant effect on immobility in the FST, but the interaction factor did (F(1,17) = 8.14, p = 0.011). Post hoc analysis using Tukey’s HSD revealed a statistically significant difference only between the vehicle-naive and valproate-naive groups (t =
3.77, p = 0.008, Figure 3.18). No significant effect of CSD on immobility was seen in this experiment.

Figure 3.17. Interaction with aggressor during social defeat. (a) Number of occurrences of specific behaviours. (b) Time spent on specific behaviours. There were no significant differences between the vehicle- and valproate-treated groups. Vehicle-defeated, valproate-defeated: n = 8.
3.4.2.2 Locomotion in a Novel Open Field

ANOVA analysis with defeat status, drug treatment and time period as main factors showed a significant effect of defeat status for both horizontal and vertical activity ($F_{(1,150)} = 9.33, p = 0.003$ and $F_{(1,150)} = 14.68, p < 0.001$, respectively). Drug treatment and the interaction factors had no effect, while time period only neared a significant effect on horizontal and vertical activity ($F_{(5,150)} = 2.03, p = 0.078$, $F_{(5,150)} = 1.99, p = 0.083$, respectively). Post hoc analyses showed that vehicle-treated defeated animals had higher total horizontal activity than naive animals from both vehicle- and valproate-treated groups (Figure 3.19a; $t = 2.96, p = 0.019$ and $t = 2.99, p = 0.017$, respectively). Naive and defeated animals within the valproate group did not differ in horizontal activity ($t = 1.40, p = 0.504$). For activity in the vertical direction, defeated animals within the valproate-treated group were less active than their naive counterparts (Figure 3.19b; $t = 3.34, p =$...
0.006) as well as vehicle-naives (t = 2.61, p = 0.048). Furthermore, vehicle-treated defeated animals were less active than valproate-naives (t = 2.80, p = 0.029). There was no significant difference between naive and defeated mice within the vehicle-treated group.

Figure 3.19. Activity in the OF in vehicle- and valproate-treated +/+ animals. (a) Horizontal activity. The defeated-valproate group was less active than the naive-valproate group (p = 0.019) and the naive-vehicle group (p = 0.017). (b) Vertical activity. The defeated-valproate group was less active than the naive-valproate group (p = 0.006) and the naive-vehicle group (p = 0.048). The naive-valproate group was also more active than the defeated-vehicle group (p = 0.029). * denotes significance vs. both defeated groups; # denotes significance vs. both defeated groups. Defeated-vehicle, defeated-valproate: n = 8; vehicle-naive: n = 7; valproate-naive: n = 8.
3.4.2.3 Partition Test

ANOVA analysis showed a significant effect of defeat status on the number of times the subject approached the partition in an active manner ($F_{(1,25)} = 13.75, p = 0.001$) and a significant effect of defeat status × drug identity on the number of times subjects froze near the partition ($F_{(1,25)} = 7.07, p = 0.013$). Tukey’s HSD showed that the naive-vehicle group did not differ in the number of times the partition was approached (active) from any other group, but the naive-valproate group approached the partition significantly less than both defeated-valproate and defeated-vehicle groups ($t = 3.45, p = 0.010$ and $t = 3.23, p = 0.017$, respectively; Figure 3.20a). Defeat status neared significance for the number of times subjects froze anywhere within the cage except near the partition ($F_{(1,25)} = 3.83, p = 0.062$), and had a significant effect on the time spent near the partition, both actively and freezing, and the time spent on exploration within the cage ($F_{(1,25)} = 4.71, p = 0.040$, $F_{(1,25)} = 6.67, p = 0.016$ and $F_{(1,25)} = 11.48, p = 0.002$, respectively).

Post hoc analyses showed no significant differences between individual groups in active time near the partition, but there was a significant difference between all defeated animals and all naive animals, ignoring drug treatment ($t = 2.17, p = 0.040$). The defeated-valproate group also tended to approach the partition (active) more than the naive-valproate group, but this was not quite statistically significant ($t = 2.62, p = 0.066$). For time spent freezing near the partition, defeated and naive animals differed significantly within the vehicle group ($t = 2.99, p = 0.030$; Figure 3.20b), while defeated mice did not differ significantly from naive mice within the valproate group. All defeated animals spent significantly more time on neutral activities, grouped as ‘exploration’, compared to naives ($t = 3.39, p = 0.002$). In particular, significant differences were observed between naive and control mice within the vehicle-treated group ($t = 2.82, p = 0.043$) and defeated-vehicle and valproate-naive ($t = 3.06, p = 0.025$).
Figure 3.20. Performance in the partition test in vehicle- and valproate-treated +/+ animals. (a) Number of occurrences of specific behavioural events. The defeated-valproate group approached the partition more than the naive-valproate group ($p = 0.010$). (b) Time spent on various behaviours. Defeated-vehicle animals froze near the partition significantly more than naive-vehicle animals ($p = 0.030$). Naive animals from both vehicle and valproate groups spent less time on neutral behaviours (exploration) than defeated-vehicle animals ($p = 0.043$ and $p = 0.025$, respectively). Defeated-vehicle, defeated-valproate: $n = 8$; vehicle-naive: $n = 7$; valproate-naive: $n = 8$.

3.4.3 Effect of Valproate Treatment on Disc1, Bdnf(III), and Pde4b mRNA Expression

Two-way ANOVA showed that neither defeat status nor drug treatment had a significant effect on the expression of any of our genes of interest in hippocampal samples (data not shown). The interaction term was also not significant. No other brain regions were analyzed.
4 Discussion

Human monozygotic twins show great variability in disease phenotype in mental illness such as SCZ or MDD, despite having identical DNA sequence. Moreover, environmental factors have been shown to affect disease risk (Fava and Kendler, 2000; Sullivan et al., 2000; Oh and Petronis, 2008; Krabbendam and van Os, 2005; Moghaddam and Jackson, 2004). Most traditional animal models in SCZ and MDD have focused on either genetic or environmental components in isolation. However, this kind of approach does not address gene-environment interactions and their effect on molecular mechanisms controlling disease development and progression (van Zelst, 2008). Gene-environment interactions ($G \times E$) have become increasingly important in the investigation of disease aetiology in SCZ and MDD (van Zelst, 2008). The objective of this thesis was to model putative interactions between mutations in the mouse Disc1 gene (genetic component) and the stressor CSD to simulate the social stress associated with precipitating psychotic and depressive episodes in humans (environmental component).

Behavioural phenotyping of two lines of heterozygous Disc1 mutant mice with different ENU-induced, non-synonomous point mutations in Disc1 compared with wildtype mice with and without CSD exposure (defeated and naïve, respectively), demonstrated that the effect of CSD does vary with genotype. L100P -/+ mice were most sensitive to the effects of CSD. This finding suggests that the two lines of Disc1 mutant mice are not prone to exclusively psychosis-like or exclusively depression-like behavioural deficits upon stress exposure as suggested by the behavioural profiles of their respective homozygous counterparts. Rather, it appears that the L100P mutation renders mice more vulnerable to CSD stress. No significant effect of CSD on Disc1, Bdnf(III) or Pde4b mRNA expression was found, but significant differences in the basal levels of expression of all three genes in the hippocampi and hypothalami of the mutant mice.
were found. L100P/+ mice also expressed lower levels of \textit{Bdnf(III)} in the striatum. A clear relationship between behavioural alterations and molecular-genetic changes was not found in the current work, but a significant limitation of the study is the number of genes investigated. The following sections will discuss further limitations of our work, as well as possible interpretations and implications of our findings. Our data suggest that psychosis and depression-related behaviours may reflect quantitative differences in the severity of response to a stressor, rather than resulting from categorically different types of aetiology, as is traditionally assumed (DSM-IV-TR).

4.1 Limitations

4.1.1 Technical Limitations

Potential technical sources of variation in this study included different animal handlers and batches of animals generated and manipulated at different times. Behavioural data for naive mice were obtained by Tatiana Lipina, while behavioural data for defeated mice was obtained by FNH. Frequency and type of animal handling is known to influence rodent anxiety levels (Hogg, 1996), and since there are bound to be innate differences in the way different experimenters handle animals, there is likely to be some variability introduced this way. However, all measured behaviours were strictly defined and all criteria for recording observations were set by the same person (T.L.) to minimize any observer effects (see Appendix A). The behavioural data for naive and defeated mice were also obtained a year apart. This may have introduced genetic or epigenetic drift as a confounding factor in this work. In line with this concern, it is important to note that due to a limited number of social defeat boxes available, animals also underwent CSD in batches. To minimize any confounding effect due to the temporal variation, environmental
conditions (temperature, lighting, humidity, air circulation, testing equipment, cleaning agents and frequency of bedding changes) were kept constant, and defeat exposure quantified where possible.

Another potential source of concern that applies to the behavioural work only is that naive mice were group-housed in native home cages, while defeated mice stayed in isolation in social defeat boxes for the entire duration of behavioural testing. Thus, most likely, the effect of CSD is confounded here with the effect of another type of psychosocial stress, isolation. An ideal approach to isolate the specific effect of CSD would be to have four groups: group-housed naives, naives housed in isolation, and defeated mice group-housed or housed in isolation. However, we are not too particularly interested in the type of psychosocial stress, but rather just the presence or absence of it. Moreover, the effects of defeat stress are known to be reduced when animals are group-housed post-defeat (Fitchett et al., 2005; Von Frijtag et al., 2000), and long-term isolation is known to cause depression-like symptoms in rodents (Matsumoto et al., 2005), making the current approach perhaps necessary to detect a significant difference in socially-stressed animals for further investigation on a molecular level.

One of the greatest challenges in this work was the consistent application of CSD. It is difficult to quantify defeat and impossible to dose the amount of defeat experienced by a subject mouse. This problem was minimized by strictly defining the duration of exposure to aggressor mice, by exposing subject animals to different aggressors (averaging effect), by carrying out CSD at the same time of day each session, and by defining criteria for intervention and applying it objectively. Nonetheless, the number and severity of attacks varied with aggressor-loser pair and day (same aggressor reacted differently to different defeated subjects or on different days).
Furthermore, aggressors sometimes simply appeared uninterested in interacting with the subject mouse. Mice exposed to CSD also varied greatly in the period of time required to produce submissive behaviours, and sometimes “defeated” mice spontaneously became aggressive, even after consistent defeat behaviour had previously been shown.

4.1.2 Selection of Behavioural Tests

Although the objective of this project requires behavioural tests that measure both depression-like and psychosis-like states in mice, the tests presented here are skewed toward only measuring depression-like symptoms. Most of the tests measure affective behaviours (EPM, FST, OF), and only one tests social behaviour and one cognitive function. This is in part due to some technical issues with the testing equipment. PPI, one of the most widely-used psychosis-related tests, was originally obtained, but is not presented here as there is some doubt in the validity of the data due to improper calibration discovered after data was obtained and animals sacrificed. Moreover, two very important aspects of psychosis are impaired working memory (see section 1.6.3.1) and learning deficits (Weinberger et al., 2001; Gottesman and Gould, 2003). We did not test either, but should have included an alternation task on a T-maze or similar behavioural tests for this purpose (Dudchenko, 2004). Current work is being done in our group to include the novel object test and the Morris water maze, tests that measure memory integrity and attention, and spatial learning and memory, respectively. The Morris water maze can also be a measure of working memory (Dudchenko, 2004).

Other limitations to behavioural testing equipment included leaking bottles in the sucrose consumption test, which invalidated consumption measurements. A great limitation in the OF test was that the instrument used to record motion only recorded horizontal and vertical beam breaks.
OF tests can produce much more information if the pattern of motion is also recorded as the amount of time and motion in the central area of the field is thought to be highly correlated with anxiety levels (Labrie, Clapcote and Roder, 2009). Also, latencies and speed of motion can be useful for discounting other underlying causes that may exist and confound or obscure any true differences in motion between groups due to CSD. Finally, as all mice were not tested at the same time, it was imperative for all behavioural tests to utilize the same protocol and equipment. This holds true for all tests except LI, where naive animals underwent five days of training while defeated animals four days only. However, identical training paradigms were used, and LI is compared here between naive and defeated groups only qualitatively – whether LI was present or not.

4.1.3 Limitations in Gene Expression Assays and Gene-Behaviour Correlations

Complex behaviours are rarely determined by a single or a few genes; instead, most are regulated by a network of hundreds or thousands of genes (Cuccato, Della-Gatta and di Bernardo, 2009). The current work only investigated three very specific gene transcripts due to their suggested involvement in mental disease and social defeat in previous studies (Ogawa, Kasai and Akiyama, 2005; Berton et al., 2006; Tsankova et al., 2006). This limits our ability to find candidate genes for gene-behaviour relationships in the effects of CSD. High-throughput techniques such as microarrays that can probe for many thousands of gene transcripts may readily identify such gene candidates. Such work is currently underway in our laboratory. Moreover, as post-transcriptional modifications may occur, and, as complex behaviour is the product of the interaction of many gene products, it is likely an oversimplification to assume that gene mRNA levels correlate well with their translated product levels or in vivo functional effects. This issue
can perhaps be addressed by protein assays of not just our genes of interest, but also candidate interacting partners in a network analysis-type investigation (Kestler et al., 2008). Such work will likely follow in our laboratory in the near future.

The quantities of mRNA produced from experimental tissues were usually quite low due to the small tissue samples available. In fact, for several regions, such as the amygdala and the nucleus accumbens, insufficient quantities of total mRNA prevented quantification. However, these regions are of great interest to us as Bdnf levels in the mouse nucleus accumbens have been shown to be directly affected by CSD (Berton et al., 2006) and the amygdala is heavily implicated in fear memory (Ehrlich et al., 2009). Another concern is the cellular heterogeneity of brain tissue. Studies have shown differences in transcript expression patterns between individual neurons in morphologically similar regions of the CNS (Gong et al, 2003). Our method of homogenization of grossly-dissected brain regions may obscure small, but important changes in expression patterns in specific neuronal subtypes that may be linked to a particular behaviour.

All transcriptomic data reported in the current work was normalized to Gapdh and β-actin mRNA levels. The validity of choosing these transcripts depends on the assumption that our treatments did not affect the expression of these control genes and that they are relatively stable in their expression patterns over time in all animals. However, it is known that Gapdh and β-actin are expressed with significant differences in their expression levels in all tissues (Kouadjo et al., 2007). GAPDH mRNA levels have been shown to not be constant (Zhu et al., 2001) and to vary among cellular subpopulations of the same pathological origin (Goidin et al., 2001). The fact that Gapdh is implicated in cellular functions such as nuclear RNA export, DNA replication, DNA repair, exocytotic membrane fusion, cytoskeletal organization and phosphotransferase
activity (Sirover, 1999), strongly suggests that its expression would be influenced by a stressor such as CSD. Gapdh has also been pathologically implicated in apoptosis and neurodegenerative disease (Tatton et al., 2000), further making its use as an endogenous control gene in the current work questionable. Moreover, the use of $\beta$-actin is questionable due to findings of unstable expression (Rhinn et al., 2008) and variation across brain regions (Boda et al., 2009). Some of the doubt in our endogenous controls could have been alleviated by doing a study first to find the most stably-expressed genes to use as endogenous controls in the RT-PCR work. However, constraints in time and sample availability made this not possible at this time, and the practice is widely accepted in the scientific community in spite of its limitations (Bustin, 2002).

### 4.1.4 Retrospective Power Analysis of Data

Power analysis allows us to measure the probability of making a Type II error as the Type II error rate is not fixed and harder to detect than the Type I error rate, the $\alpha$ level (we have fixed it here at 0.05; Cohen and Lea, 2004, p. 123). Unfortunately, post hoc power analyses reveal that the sample sizes reported in this thesis are sometimes too small, leaving the possibility that Type II errors exist in some of the data reported. One way to increase the power of our data is of course to increase sample sizes and this is currently being done in our laboratory. We expect our ongoing work to produce more reliable data as well as possibly more significant differences between genotypes as Type II error consists of false negatives, which means we may be failing to observe a difference between groups when in fact there is one.

The effect size, ES, is “the degree to which the phenomenon is present in the population” (Cohen, 1969, p. 9) and one estimate of the ES that is widely used is related to the term Cohen’s $d$ given as Equation 4.1a (Cohen, 1969, p. 269). Once $d$ is known, the estimate of the ES, $f$, and
the power of a given F test in ANOVA at a level $\alpha$ with $n$ number of subjects can be calculated (Cohen, 1969, p. 14). In general, the smaller the ES is, the larger $n$ needs to be. Calculation of $f$ is conditional upon the distribution of the other group means as only the maximum and minimum means are actually used in calculating $d$ (Cohen, 1969, p. 270). The distribution in our data was decided to most aptly fall in the ‘intermediate variability’ category (Cohen, 1969, p. 270), and thus $f$ is calculated as Equation 4.1c. Table 4a lists post hoc power levels obtained for most data in the current report.

$$d = \frac{m_{\text{max}} - m_{\text{min}}}{\sigma} \quad \text{Equation 4.1a}$$

$\sigma$ can be estimated as a pooled standard deviation, $s$, according to Equation 4.1b:

$$s = \sqrt{\frac{(n_2-1)s_2^2 + (n_2-1)s_2^2}{n_1 + n_2}} \quad \text{Equation 4.1b}$$

$$f = \frac{d}{\sqrt\frac{k+1}{2(n-1)}} \quad \text{Equation 4.1c}$$

As seen in Table 4a, a number of our behavioural tests are quite low in statistical power, with only the EPM and LI for defeated animals being acceptably powerful. This means that in the SSN, the OF, the FST and LI (naive) tests, there could be actual differences between genotypes and genotype × CSD treatments that we are not detecting due to insufficient sample sizes. In the valproate experiment, power levels are very low for all tests, both behavioural and molecular, indicating a need for larger sample sizes. Of course, our analysis is retrospective and the ES values are estimated from the very data for which power is calculated. Though this gives us an indication of the Type II error rate, an ideal approach would be to obtain $f$ values from different data sets. Unfortunately, no comparable data was available to us at the time of analysis as no pilot experiments were performed.
### Table 4a. Power analysis of behavioural and molecular data at the $\alpha = 0.05$ level.

<table>
<thead>
<tr>
<th>Measure of interest</th>
<th>ES estimate, $f$</th>
<th>$n_{\text{min}}$</th>
<th>Power of $F$ test in ANOVA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>OA entries in the EPM</em></td>
<td>0.83</td>
<td>16</td>
<td>84.0</td>
</tr>
<tr>
<td>% <em>Time walking in the SSN</em></td>
<td>0.91</td>
<td>12</td>
<td>79.3</td>
</tr>
<tr>
<td><em>Sociability in the SSN</em></td>
<td>0.72</td>
<td>12</td>
<td>57.9</td>
</tr>
<tr>
<td><em>Social novelty in the SSN</em></td>
<td>0.84</td>
<td>12</td>
<td>72.2</td>
</tr>
<tr>
<td><em>Immobility in the FST</em></td>
<td>0.82</td>
<td>14</td>
<td>77.3</td>
</tr>
<tr>
<td><em>Horizontal activity on the OF</em></td>
<td>0.32</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td><em>Vertical activity on the OF</em></td>
<td>0.65</td>
<td>19</td>
<td>70</td>
</tr>
<tr>
<td><em>LI Naive</em></td>
<td>0.74*</td>
<td>10</td>
<td>51.8</td>
</tr>
<tr>
<td><em>LI Defeated</em></td>
<td>3.14*</td>
<td>8</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hippocampal Disc1</em></td>
<td>2.07</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hippocampal Bdnf</em></td>
<td>2.16</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hippocampal Pde4b</em></td>
<td>1.99</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hypothalamic Disc1</em></td>
<td>1.10</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hypothalamic Bdnf</em></td>
<td>2.37</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Hypothalamic Pde4b</em></td>
<td>0.87</td>
<td>9</td>
<td>98.5</td>
</tr>
<tr>
<td><em>Prefrontal cortical Disc1</em></td>
<td>1.24</td>
<td>9</td>
<td>99.9</td>
</tr>
<tr>
<td><em>Prefrontal cortical Bdnf</em></td>
<td>0.64</td>
<td>9</td>
<td>84.6</td>
</tr>
<tr>
<td><em>Prefrontal cortical Pde4b</em></td>
<td>0.48</td>
<td>9</td>
<td>59.1</td>
</tr>
<tr>
<td><em>Striatal Disc1</em></td>
<td>0.84</td>
<td>9</td>
<td>97.8</td>
</tr>
<tr>
<td><em>Striatal Bdnf</em></td>
<td>0.90</td>
<td>9</td>
<td>99.0</td>
</tr>
<tr>
<td><em>Striatal Pde4b</em></td>
<td>0.65</td>
<td>9</td>
<td>85.5</td>
</tr>
<tr>
<td><em>Valproate Experiment</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Immobility in the FST</em></td>
<td>0.75</td>
<td>8</td>
<td>53.5</td>
</tr>
<tr>
<td><em>Partition time, active</em></td>
<td>0.56</td>
<td>13</td>
<td>50</td>
</tr>
<tr>
<td><em>Partition time, inactive</em></td>
<td>0.49</td>
<td>13</td>
<td>40</td>
</tr>
<tr>
<td><em>Interaction behaviour</em></td>
<td>0.13</td>
<td>8</td>
<td>6.4</td>
</tr>
<tr>
<td><em>Gene expression, Disc1</em></td>
<td>0.50</td>
<td>13</td>
<td>43</td>
</tr>
<tr>
<td><em>Gene expression, Bdnf</em></td>
<td>0.11</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td><em>Gene expression, Pde4b</em></td>
<td>0.20</td>
<td>13</td>
<td>11</td>
</tr>
</tbody>
</table>

*Effect size estimate $f$ calculated in wildtype animals.
4.1.5 Additional Limitations

Time constraints and availability of heterozygous mutant mice prevented an ideal experimental approach for the behavioural work presented here. Ideally, control mice would be housed in isolation in social defeat boxes as done with the molecular cohorts to control for isolation effects. Unfortunately, the animal facility where this work was carried out, the Toronto Centre for Phenogenomics, experienced a severe pinworm outbreak lasting from mid-2008 to early-2009. This resulted in closing of the behavioural testing facilities for a couple of months for decontamination as well as the euthanasia of a large number of Disc1 mutant mice.

4.2 Synopsis of Results

4.2.1 Effect of CSD Duration

CSD for ten days (T10) produced no significant changes in locomotor and exploratory behaviour in C57Bl6J mice, but did increase behavioural despair as measured by immobility in the FST. CSD for twenty days (T20) also increased immobility in the FST, and although the difference relative to T10 was not statistically significant, it is slightly higher. Most interestingly, whereas deficiencies were not detected in T10 mice in the OF, T20 mice were significantly less active on both the horizontal and vertical planes relative to naive (T0) mice and T10 mice. A decrease in locomotion and exploratory behaviour has previously been shown to occur with CSD in mice, and it is suggested to be indicative of an anhedonia-like state (Kudryavtseva et al., 1991; Avgustinovich et al., 1997; Kudryavtseva and Avgustinovich, 1998). Consequently, as ten days of CSD produced only a deficit in one of our measured behaviours while twenty days produced deficits in both, it is proposed that the length of the period of CSD has a dose response-like effect for producing a state of anhedonia in mice. This also suggests that the FST is more sensitive in
detecting CSD-induced behavioural alterations than the OF. CSD duration had no effect on
Disc1, Bdnf(III) or Pde4b mRNA expression. However, their involvement in CSD stress
response cannot be excluded (see section 4.1.3). A more thorough study may include assays of
gene-products and other interacting partners (network analysis). Also, expression changes may
be subtle and require larger sample sizes for detection by transcript assays.

The observation that the duration of CSD has a dose response-like effect on certain behaviours is
consistent with the stress sensitization hypothesis (section 1.5.3.3). The hypothesis suggests that
exposure to psychosocial or environmental stress progressively increases the behavioural and
biological response to subsequent exposures due to a dysregulation of the HPA axis (van Winkel,
Stefanis and Myin-Germeys, 2008; Yuii, Suzuki and Kurachi, 2007). It follows then that T20
CSD would cause more behavioural changes than T10 CSD. One potential pitfall is that duration
of CSD may be confounded by other factors during the CSD procedure, such as physical stress
or injury.

4.2.2 Behavioural Changes Induced by CSD in Disc1 Mutant Mice

As Disc1 Q31L -/- mutant mice show more abnormalities in depression-related behavioural tests,
and Disc1 L100P -/- mutant mice show more abnormalities in psychosis-related behavioural tests
(Clapcote et al., 2007), it was postulated that CSD would exacerbate depression-like behaviours
in Disc1 Q31L -/+ mutant mice and psychosis-like behaviours in Disc1 L100P -/+ mice.

Moreover, it was hypothesized that both heterozygous mutant lines would be more vulnerable to
the behavioural effects of CSD compared to wildtype mice, and that Q31L -/+ mice would be
more sensitive than L100P -/+ mice since the effect of social and psychological stressors is
greater in MDD than in SCZ (Myin-Germeys et al., 2003; Paykel, 1978). However, our data do
not support these hypotheses. Q31L -/+ mice do not appear to be specifically sensitive to CSD; in fact, L100P -/+ mice showed the greatest post-CSD behavioural changes overall. Table 4b summarizes the results of the behavioural assays and these results are discussed in greater detail below.

<table>
<thead>
<tr>
<th>Table 4b. Synopsis of Results</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Naive Q31L/+</strong></td>
</tr>
<tr>
<td>EPM</td>
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<td></td>
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<td></td>
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<tr>
<td>FST</td>
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<td></td>
</tr>
<tr>
<td>OF</td>
</tr>
<tr>
<td>SSN</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>LI</td>
</tr>
</tbody>
</table>

CSD appeared to increase anxiety in the OF in all mice and in the EPM in L100P -/+ mice only. All defeated animals showed decreased horizontal motion in the OF (Figure 3.7), and genotype or the interaction term had no effect. This observation indicates increased anxiety in defeated animals as motion in the first few minutes in a novel environment is greatly affected by anxiety,
and mice are naturally averse to well-lit open spaces (Labrie, Clapcote and Roder, 2009).

However, the power of our test was only 21%, and more reliable results require larger sample
sizes. In the EPM, defeated L100P -/+ mice made fewer entries onto open arms (Figure 3.4a) and
spent less time there (Figure 3.5a) relative to naive L100P -/+ mice, whereas no such differences
existed between defeated and naive mice in the +/+ or Q31L -/+ groups. Defeated L100P -/+ mice
also ceased to reach the end of open arms (section 3.2.1.1), further indicative of the higher
state of anxiety in the defeated cohort. Moreover, defeated L100P -/+ mice spent more time in
closed arms compared to defeated Q31L -/+ mice (Figure 3.5b). These findings suggest
increased vulnerability to CSD in mice with the L100P mutation.

CSD also increased behavioural despair in the FST in all mice significantly (Figure 3.6). No
effect of genotype or any interaction was found, but L100P -/+ mice displayed the greatest
absolute change in immobility (281% increase from naive state). This suggests greater
vulnerability in L100P -/+ mice, that may be detected in statistical analyses by increasing the
sample size (power of our test was only 77%). In addition, the strong effect of CSD on
immobility time may have produced a ceiling effect inhibiting the detection of the influence of
genotype. Moreover, several L100P -/+ mice had to be rescued from the water during the
experiment because they started sinking (4 mice; eliminated from analysis), while no +/+ or
Q31L -/+ mice displayed such behaviour. This observation is inconsistent with the hypothesis of
energy conservation being the motivation for immobility in the FST (West, 1990). Clearly,
conserved energy is of no utility if the animal dies. Behavioural despair alone does not explain
our observation either as mice should not be “suicidal” and a minimum attempt to float would be
expected. The prolonged period of CSD exposure may have rendered the animals in poor
physical condition, unable to attempt to swim/float.
CSD had no effect on sociability and preference for social novelty in either Disc1 mutant line, but reduced preference for social novelty in +/+ mice (Figure 3.9b). Both Disc1 lines displayed lower sociability and preference for social novelty in the SSN in the naive state, making this test unsuitable for this study as a “floor” effect may exist where appetite for social novelty and sociability is already so low that CSD cannot reduce it any further. L100P -/+ mutants spent less time with Stranger 1 in the sociability phase, and both mutant lines spent less time with Stranger 2 in the social novelty phase than +/+ mice (Figures 3.8b, 3.9b). These abnormal levels of sociability and/or appetite for social novelty may be due to higher basal anxiety levels in social contexts or due to deficits in aspects of social recognition memory (O'Tuathaigh et al., 2007). In a recent study, a mutant mouse line with heterozygous deletion of NRG1, a candidate susceptibility gene for SCZ, displayed lower preference for social novelty, while both spatial learning and working memory processes appeared intact (O'Tuathaigh et al., 2007). This suggests neural pathways involved in social behaviour may be more sensitive to genetic defects, and may explain why deficits are present in our naive heterozygous mutants in the SSN.

Lastly, CSD also had no effect on LI as LI persisted in defeated +/+ mice (Figure 3.12b), indicating CSD has minimal impact on associative learning, and, in particular, on decremental attention (Gould and Wehner, 1999). This is consistent with the fact that as CSD is a psychosocial stressor, it should – intuitively - have a smaller effect on cognitive processes than affective behaviours. We originally chose LI as one of our behavioural tests because normal LI was observed in Q31L -/+ mice by Clapcote et al. (2007). However, we did not reproduce that here, most likely due to low statistical power of the LI test in the naive group (only 52%). This also likely contributed to the p-value for LI in naive +/+ mice only nearing significance (p = 0.052). We accept this value, however, as LI has previously been shown to develop in C57Bl6J
mice (Singer, Feldon and Yee, 2009; Gould and Wehner, 1999). We are not aware of any other work measuring the effect of CSD on LI, but other environmental insults have been shown to affect LI (Gould and Wehner, 1999). For example, repeated restraint stress has been shown to enhance LI in drug-naive stressed C57Bl6 mice (Mongeau et al., 2007).

4.2.3 Gene Expression and Gene-Behaviour Correlation in Disc1 Mutant Mice

Stress due to an environmental insult such as CSD is known to influence gene expression via epigenetic modifications to DNA (Roth et al., 2009; Tsankova et al., 2006; Petronis et al., 2003). Therefore, we expected CSD to alter Disc1, Bdnf(III) and Pde4b mRNA levels in our mice, and this alteration to be influenced by genotype. However, we found that CSD did not affect the mRNA levels of our genes of interest, but that there were significant differences in the basal levels of some of these genes in some brain regions between the mutant lines and between each line and wildtype mice. L100P -/+ mice displayed the greatest differences from +/+ mice, having higher levels of Disc1 and Pde4b in the hippocampus, higher levels of Bdnf(III) in the hippocampus and hypothalamus, and higher levels of Pde4b in the hypothalamus (Table 4c). Q31L -/+ mice showed higher levels of Disc1 in the hippocampus compared to +/+ mice. L100P -/+ mice had higher Bdnf(III) levels in the hippocampus and hypothalamus, and lower Bdnf(III) in the striatum compared to Q31L -/+ mice. There were no differences for any genes between any genotypes in prefrontal cortical tissue samples.
It is clear that L100P -/+ mice also differ more from +/- mice than Q31L -/+ mice at the transcriptional level, in addition to being more abnormal in behaviour. The higher levels of all three genes of interest in the hippocampi and of Disc1 and Pde4b in the hypothalami of L100P -/+ mice suggests the L100P mutation causes aberrant gene expression that may produce downstream effects that make these mice more vulnerable to CSD stress. However, mRNA levels do of course not always predict protein expression or biological activity accurately. For example, Clapcote et al. reported that the L100P mutation significantly decreased Disc1-Pde4b protein binding in the homozygous mutants (Clapcote et al., 2007). Thus, even though hippocampal and hypothalamic Pde4b mRNA levels are elevated in L100P -/+ mice, other biological mechanisms may be activated to compensate for this aberration as the observed phenotype of these mice is relatively normal. Essentially, mRNA levels alone provide only one measure of many possible changes.
One of our main findings is the significant increase of $Bdnf(III)$ in L100P -/+ hippocampi and hypothalami. This is inconsistent with the BDNF hypothesis of depression as L100P -/+ mice were more sensitive to CSD and displayed greater behavioural deficits in spite of the higher levels of $Bdnf(III)$ (see section 4.3.3). A recent study in rats showed that environmental enrichment (EE), which is known to upregulate Bdnf in the rodent hippocampus (Zajac et al., 2009; Zhu et al., 2006), decreased Bdnf levels in the rat striatum (Angelucci et al., 2009 (abstract)). As we found decreased levels of $Bdnf(III)$ in the striatum of L100P -/+ mice, it may be that some of the pathways affected by stimuli such as EE are also activated to compensate for the L100P -/+ mutation, resulting in a similar basal Bdnf profile. However, EE is known to decrease immobility time in the FST (Zambrana et al., 2007), which does not predict an increased vulnerability in L100P -/+ mice to CSD.

### 4.2.4 Effect of Valproate Treatment in Wildtype Mice

Valproate is a weak antidepressant and is primarily used in psychiatry to treat mania as a mood stabilizer (Haddad et al., 2009). However, it is also a potent HDAC inhibitor and is used in epigenetic studies to reverse aberrant repression and lead to re-expression of genes (Krämer et al., 2003). Furthermore, HDAC inhibition with valproate has been shown to correct the neurochemical and behavioural aspects of SCZ in a methionine-induced mouse model (Tremolizzo et al., 2005). Thus, as we believed CSD stress would repress gene transcription, we hypothesized that chronic treatment with valproate concurrent to CSD could have a protective effect by inhibiting transcription repression and result in less severe behavioural deficits. However, we found limited evidence in support of our hypothesis. The FST and OF results suggest that these behavioural measures may be inappropriate in this study design. The valproate-naive animals had significantly higher immobility time relative to vehicle-naive
animals in the FST (Figure 3.18). This is likely due to the sedative effect of valproate, which is well known in human clinical contexts (Schwarz et al., 2009). Valproate-naive animals displayed lower horizontal activity than vehicle-defeated animals in the OF; this could also be due to the sedating effect of valproate. Moreover, the effect size of CSD is likely too small to detect in the OF using the sample sizes we used here as no effect of CSD was observed. In fact, vehicle-treated defeated animals had significantly higher total horizontal activity than their naive counterparts (Figure 3.19a). This is believed to be a Type I error. For activity in the vertical direction, defeated animals within the valproate-treated group were less active than their naive counterparts as well as vehicle-naives (Figure 3.19b). Moreover, vehicle-treated defeated animals were less active than valproate-naives. These observations suggest that CSD decreases exploratory behaviour and that valproate does not improve this deficit.

There is some evidence warranting further investigation from the PT: while there was no difference between naive and defeated animals within the vehicle group, defeated animals treated with valproate actively approached the partition significantly more than their naive counterparts (Figure 3.20a). However, valproate-defeated mice did not differ from either vehicle group. This may mean that although valproate increased sociability in defeated animals when compared to valproate-control animals, this is not due to decreased anxiety, but rather due to valproate decreasing sociability in the valproate-control group, likely because of its sedative effect. Moreover, vehicle-control mice spent significantly more inactive time near the partition than vehicle-defeated mice (Figure 3.20b), indicating CSD increased anxiety. The fact that this difference did not exist between control and defeated mice within the valproate group may suggest that valproate treatment resisted increased anxiety in defeated mice.
Stress due to an external environmental insult is known to influence gene expression via epigenetic modifications to DNA (Roth et al., 2009; Tsankova et al., 2006; Petronis et al., 2003). As HDACs play pivotal roles in regulating gene transcription, it was expected that valproate, a potent HDAC inhibitor, would suppress any transcription repression and increase gene expression. However, we did not detect any significant effect of CSD or valproate treatment on Disc1, Bdnf(III) or Pde4b expression. There are two possibilities for this: either there is no direct effect of valproate on these particular transcripts, or there is an effect that remains undetected due to limitations in our study design. One such limitation to our study is that there may be an elevated level of stress in control animals due to the regimen of twice-daily injections. Indeed, saline injection has been used in other chronic stress models such as the CSM in rodents as a stress-inducing element (Izumi et al., 1997). This limitation also applies to our behavioural data. Elevated stress levels in control animals would obscure any true changes in defeated animals due to increased immobility in the FST, decreased mobility in the OF, and increased anxiety in the PT. In fact, although CSD is known to increase immobility in the FST, and has been shown to do so in our other experiments as well, there was no such increase in this experiment. This failure to replicate the increased immobility here indicates higher levels of behavioural despair in the control group. An improved study design would include control animals that were naive to both CSD and the injection process.

4.3 Conclusions

4.3.1 Gene × Environment Interaction

We have demonstrated a gene-environment interaction between mutations in the mouse Disc1 gene and CSD. Our results show that mice with one copy of the Disc1-L100P mutation are more
vulnerable to CSD stress than mice with one copy of the *Disc1*-Q31L mutation and wildtype control mice. In our experiments, CSD did not influence LI development in any group and exhibited the strongest influence on affective behaviours, mainly anxiety levels, consistent with the hypothesis that the effect of social and psychological stressors is greater in MDD than in SCZ (Myin-Germeys et al., 2003; Paykel, 1978). The $G \times E$ term had a significant effect on the number of entries onto OA and neared significance for risk assessment and time spent on OA in the EPM, had a significant effect in the SSN when test condition and chamber side were taken into consideration, and a significant effect on walking time in the SSN. The interaction plots for the EPM and SSN show disordinal interaction (Figure 3.13c, d), where slopes are in opposite directions (Cohen, 1969, p. 154). This shows that the amount of change in behaviour due to CSD among the genotypes varied not only in magnitude, but also in direction. Our gene-environment interaction model may be useful in identifying genetic risk factors for MDD as the genetic differences are clearly correlated with differential vulnerability to CSD between the two heterozygous mutants. Further work will seek to identify molecular mechanisms that mediate the downstream effect of the different mutations on molecular pathways also affected by CSD (section 4.4). The $G \times E$ term was not significant in the FST and the OF, but we believe this is a Type II error due to low power of our statistical tests. The FST and OF interaction plots show some interaction as the genotype lines are not parallel. However, it is clear that the interaction effect, which is the effect size ($f$), is small. This is likely why we did not obtain statistical significance for the $G \times E$ term in these tests using our sample sizes.

### 4.3.2 The *Disc1*-L100P Mutation

We have obtained strong evidence supporting the hypothesis that *Disc1* is involved in mental disease. As our experiments show, even one copy of the mutated gene can cause extremely
different phenotypes. The L100P mutation results in a very different transcriptional profile in addition to increased sensitivity to CSD. This finding complements findings from other experiments with these mutant lines done in our group. The first experiment involved the maternal inflammation model, which is based on the observation that maternal viral infection such as influenza during pregnancy is a risk factor for SCZ (Nawa and Takei, 2006). Polyinosinic:polycytidylic acid (polyI:C) is often used to elicit an inflammation response similar to those produced by viral infections such as elevated cytokine levels in animal models (Meyer et al., 2005). We therefore hypothesized that maternal immune activation using polyI:C during pregnancy would exacerbate the SCZ phenotype in L100P -/+ offspring. Indeed, L100P -/+ offspring of mothers treated with 2.5 mg/kg PolyI:C demonstrated increased locomotion in the OF, exacerbated PPI deficits, and impaired associative memory in the NPE group in LI (unpublished data). Q31L -/+ offspring, even at a higher PolyI:C dose of 5 mg/kg, were phenotypically identical to wildtype offspring. This demonstrates that the L100P -/+ line is also more sensitive to another environmental stressor – in this case, a prenatal stressor.

In the second experiment, L100P -/- homozygous mutant mice, which show abnormal PPI and hyperlocomotion, were treated with valproate for 15 days and displayed significant normalization of PPI and locomotor activity (Lipina, Haque et al., in preparation). A microarray study was also done, and we found several promising gene candidates that were validated with real-time RT-PCR (6 of 10 candidates had correlation values > 0.8 with p-values < 0.01). As previously mentioned, valproate is primarily used to treat patients diagnosed with bipolar disorder (BP), and low PPI and hyperactivity are also observed in BP patients during acute mania and in animal models of mania (Perry et al., 2001; Franks et al., 1983; Wolf and Wagner, 2003; Decker et al., 2000). Thus, it can be argued that L100P -/- mice also represent an animal model
with symptoms of mania. Considering the data presented in the current thesis in the context of these findings suggests that the L100P mutation in the Disc1 gene is not linked to an exclusively SCZ-like phenotype. Rather, the homozygous condition could represent a psychopathophysiologica unit that encompasses symptoms we classify as SCZ-like (psychosis) or BP-like (mood disorder), and the heterozygous condition renders an increased vulnerability to environmental stressors where the resulting phenotype is a consequence of the type of stressor (Figure 4.1).

Figure 4.1. Disc1-L100P heterozygous mutant mice exposed to two different types of stressors produce two different behavioural phenotypes. Adapted from an original scheme prepared by Albert Wong. The L100P -/+ mutant line was hypothesized to be predisposed to a SCZ-like phenotype while the Q31L -/+ mutant line to a depression-like phenotype. Accordingly, we expected both maternal inflammation and social defeat to exacerbate symptoms of SCZ in L100P -/+ mice and depression in Q31L -/+ mice. However, we found that Q31L -/+ mice are relatively resistant to both stressors, mostly exhibiting phenotypic changes in a similar fashion to wildtype mice. L100P -/+ mice, on the other hand, are sensitive to both stressors, and produce behavioural changes that are SCZ-like in the maternal inflammation model and depression-like in the social defeat model. This suggests the L100P mutation in the Disc1 gene is not linked to SCZ itself, but only influences the level of vulnerability of the animal to external stressors.
4.3.3 The Role of Bdnf in Depression

BDNF is heavily implicated in depression in published literature (see section 1.5.1.3). In short, local infusion of Bdnf produces antidepressant-like effects in the rodent hippocampus in behavioural models of depression (Shirayama et al., 2002), hippocampal BDNF levels are known to increase with antidepressant treatment (Shieh et al., 2008), and CSD has been shown to induce lasting downregulation of Bdnf(III) in the mouse hippocampus (Tsankova et al., 2006). These observations suggest that increased BDNF in the hippocampus is sufficient to induce antidepressant effects. However, our findings suggest that pre-existing elevations in BDNF may not protect against the depressant effects of CSD. L100P -/+ mice had significantly elevated levels of Bdnf(III) in the hippocampus and hypothalamus, but also displayed the greatest behavioural deficits. Recent studies in other groups also contradict earlier reports that low BDNF expression is correlated with depression (Groves, 2007). For example, one study has shown increased hippocampal Bdnf protein levels in mice that display higher anxiety in the EPM and OF, and higher behavioural despair in the FST following communal nesting (Branchi et al., 2006). Moreover, the antidepressant fluoxetine has been shown to decrease Bdnf mRNA in the rat hippocampus (Miro et al., 2002). It is likely that the relationship between Bdnf levels and depression is complex and correlated in only some contexts.

4.3.4 Valproate Treatment in Social Defeat Model

Although no solid evidence of an effect of chronic valproate treatment was found in the current work, it could be due to our selection of behavioural tests. As CSD induces behaviour that is in some ways similar to a sedative-induced lethargic state (such as increased floating in the FST and decreased activity in the OF and PT), these tests seem not compatible with the sedating effects of valproate. However, several studies have shown beneficial effects of valproate in other
animals models such as the methionine-induced epigenetic mouse model for SCZ (Tremolizzo et al., 2005) and a D-amphetamine/chlordiazepoxide-induced hyperactive rat model of mania (Arban et al., 2005). Therefore, treatment with a HDAC inhibitor is still of great interest for the CSD model, and other HDAC inhibitors such as trichostatin or sodium butyrate may be better suited for a factorial study design with CSD.

4.3.5 Implications for Clinical Psychiatry

Two findings of this study are of relevance to clinical psychiatry. The first pertains to the observation that the duration of CSD had a dose-response like effect on the severity of behavioural deficits, supporting the stress sensitization hypothesis. This finding provides experimental evidence for clinical approaches that seek to provide early intervention or preventive strategies in patients experiencing chronic stress. There is clinical evidence that early intervention in MDD improves treatment outcomes and reduces the number of depressive episodes (Callan and Howland, 2009; Harrington and Clark, 1998).

An ongoing debate in psychiatry centres on the correct classification of diagnoses, specifically, whether psychosis and mood disorders should be considered distinct conditions or different degrees of severity on a spectrum of the same type of condition (Esterberg and Compton, 2009; Peralta and Cuesta, 2008; Lake and Hurwitz, 2007; Lake and Hurwitz, 2006). The second main finding of this study is that L100P -/+ mice are more sensitive to CSD, in the context of L100P -/- mice having more psychosis-related behaviours. These two observations, and the observation that maternal inflammation produces a SCZ-like state in L100P -/+ mice (section 4.3.2), suggest that different behavioural deficits in L100P and Q31L homozygous mutants may reflect differences in the severity of behavioural consequences, rather than categorically different types
of brain abnormality. In other words, psychosis and depression are not necessarily the result of distinct disease processes, but are different levels of disease severity on the same spectrum (Figure 4.2).

**Figure 4.2. Proposed model for contribution of genetic component and CSD to observed behavioural phenotype.** We suggest that symptoms of depression and psychosis in our model appear when certain “thresholds” of neurological abnormality are crossed. There is a synergistic effect of the gene-environmental insult interaction factor and the L100P +/- heterozygous mutant is more vulnerable to CSD due to its genetic predisposition. Our model implies that depression and psychosis are outcomes of different degrees of the same neuropathy.

The above suggests that a dimensional, rather than traditionally categorical classification system may be more appropriate for psychiatric diseases. There is a growing body of evidence in published literature that suggests this as well (Peralta and Cuesta, 2008; Lake and Hurwitz, 2007; Lake and Hurwitz, 2006), and psychosis and mood disorders often occur together. For example, post-traumatic stress disorder and postpartum depression (triggered by environmental stress/hormonal changes) often present comorbid symptoms of psychosis (Marrs *et al.*, 2009; Hamner and Robert, 2005), and up to 50% of patients diagnosed with schizophrenia also show symptoms of comorbid depression (Furtado, Srihari and Kumar, 2009). Moreover, many drugs
are known to be effective in patients presenting symptoms of both psychosis and a mood disorder. For example, atypical antipsychotics are used in the treatment of post-traumatic stress disorder (Hamner and Robert, 2005) and the mood stabilizer valproate is sometimes used in the treatment of SCZ. This suggests these drugs may be working on pathways that are common to SCZ and MDD. In short, our data is convergent with current clinical thinking about the need for both categorical and dimensional elements in psychiatric disease classifications, an idea which is currently being debated in DSM-V committees (Dr. Ken Zucker, personal communication).

4.4 Future Prospects

4.4.1 Expanding Behavioural Phenotyping and Microarray Studies

The selection of behavioural tests reported in this thesis probe a range of important behaviours, but the array is quite limited. The EPM, FST and OF all probe affective behaviours, the SSN probes social behaviour and social memory, and the LI probes cognitive function. This ignores several other important behavioural/cognitive measures. In particular, PPI is an important measure for cognitive function (sensorimotor gating) that could reveal significant differences between groups. PPI performance has been demonstrated to be sensitive to environmental insults (Weiss and Feldon, 2001), and could be useful in detecting abnormalities in the Q31L/+ mice (naive L100P +/- mice show impairment). Furthermore, spatial learning and memory in the Morris watermaze, working memory in the T-maze and olfactory function assessment would be interesting to probe. Naive Q31L +/- and L100P +/- mice have been reported to have normal olfactory function and Morris water maze performance, and CSD is very likely to affect these behaviours/cognitive functions. Thus, the presence of any G × E interactions should be detected
in these tests. Moreover, working memory is an important model in SCZ, and has been demonstrated to be affected by environmental stressors (Gottesman and Gould, 2003).

The scope of this project with respect to molecular-genetic work was very limited. Although no clear relationship of Disc1, Bdnf(III) and Pde4b mRNA expression levels to CSD-induced behavioural alterations were found, a more extensive study at the genomic level may find promising gene candidates for further investigation. Behaviour is likely to be controlled at a macroscopic level such as neural circuits that involve numerous genes and gene products. Therefore, microarray studies to probe changes in gene expression could identify genes that correlate with CSD-induced behavioural alterations and/or code for products that are interacting partners of Disc1, Bdnf or Pde4b. We are currently undertaking such a study in our laboratory.

4.4.2 Pharmacological (Predictive) Validation of Model

Although most of the behavioural tests utilized in the current work probe behavioural traits that can intuitively be grouped into psychosis- or depressive-like states, it is impossible to categorically discern the mental state of a mouse. Thus, although construct and face validity may exist for this G × E interaction mouse model, predictive validation is most desirable. Therefore, future directions will include pharmacological validation of the model using known drug treatments. Experimental designs should be constructed so that both lines of mutants are treated with both antipsychotics and antidepressants. If antidepressants rescue all mice in the appropriate tests, but antipsychotics do not, it can reasonably be deduced that CSD only produces depression-like effects in L100P -/+ mice.
4.4.3 Mechanistic Studies: Epigenetics

The findings of the initial experiment reported here suggest epigenetic involvement cannot be excluded as valproate treatment did have minor improvement on CSD-induced behavioural deficits. However, the sedative side effects of valproate make this drug not a prime candidate for our study design. Future experiments should include other drugs known to work via epigenetic modifications such as sodium butyrate and trichostatin (Monneret, 2005). Beyond drug-treatment experiments, another avenue that could produce interesting epigenetic data would be ChIP arrays (Wilkinson et al., 2009).
References


Psychopharmacology (Berl) 156, 234-258.


Feighner, J. P. (1999). Overview of antidepressants currently used to treat anxiety disorders. J
Clin Psychiatry 60 Suppl 22, 18-22.


Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonak, J., Lind, K., Sindelka, R.,


test for antidepressants. Arch Int Pharmacodyn Ther 229, 327-336.


Biol Psychiatry 57, 500-509.


Zucker, K. (2009). Personal communication to Albert Wong; communicated to FNH.

## Appendix A. Definitions and Recording Criteria for Behavioural Tests

### Forced Swim Test

<table>
<thead>
<tr>
<th>Observation Term</th>
<th>Definition/Criteria for Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming</td>
<td>active with all four or three paws</td>
</tr>
<tr>
<td>Floating</td>
<td>floating in an upright position without additional activity other than that necessary for the animal to keep its head above water</td>
</tr>
<tr>
<td>Medium</td>
<td>active with two paws only; there is some directional movement through the water</td>
</tr>
</tbody>
</table>

### Elevated Plus Maze

<table>
<thead>
<tr>
<th>Observation Term</th>
<th>Definition/Criteria for Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open arms</td>
<td>all four paws on either open arm</td>
</tr>
<tr>
<td>Center platform</td>
<td>all four paws on platform</td>
</tr>
<tr>
<td>Closed arms</td>
<td>all four paws inside either closed arm</td>
</tr>
<tr>
<td>Passages</td>
<td>running from one closed arm to the other without any hesitation in the centre</td>
</tr>
<tr>
<td>Risk assessment</td>
<td>head outside closed arms, minimum two paws (hind legs) inside closed arms</td>
</tr>
<tr>
<td>Head-dips</td>
<td>bending head down over open arms to peek at the floor</td>
</tr>
<tr>
<td>End of open arms</td>
<td>at the final third of the length of either open arm</td>
</tr>
</tbody>
</table>
### Sociability and Social Novelty

<table>
<thead>
<tr>
<th>Observation Term</th>
<th>Definition/Criteria for Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chamber 1/2</td>
<td>all four paws inside respective chamber</td>
</tr>
<tr>
<td>Freezing</td>
<td>no movement for a minimum of 5 seconds</td>
</tr>
<tr>
<td>Walking</td>
<td>movement between chambers</td>
</tr>
</tbody>
</table>

### Partition Test

<table>
<thead>
<tr>
<th>Observation Term</th>
<th>Definition/Criteria for Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partition, active</td>
<td>subject actively approaches the partition and shows interest in the conspecific on the other side (sniffs and climbs partition wall, digs at the bottom)</td>
</tr>
<tr>
<td>Partition, freezing</td>
<td>freezing near partition; no movement for a minimum of 5 seconds</td>
</tr>
<tr>
<td>General freezing</td>
<td>freezing elsewhere within the cage; no movement for a minimum of 5 seconds; away from the partition</td>
</tr>
<tr>
<td>Grooming</td>
<td>self-grooming</td>
</tr>
<tr>
<td>Exploration</td>
<td>neutral behaviours; does not show interest in conspecific on other side; sniffing of bedding or digging of bedding</td>
</tr>
</tbody>
</table>

### Interaction Behaviour

<table>
<thead>
<tr>
<th>Observation Term</th>
<th>Definition/Criteria for Recording</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running away</td>
<td>actively seeking escape from aggressor</td>
</tr>
<tr>
<td>Chasing aggressor</td>
<td>actively chasing aggressor</td>
</tr>
<tr>
<td>Freezing near aggressor</td>
<td>no movement for minimum 5 sec when aggressor is nearby</td>
</tr>
<tr>
<td>Freezing in corner</td>
<td>no movement for minimum 5 sec in any corner of defeat box; aggressor is not nearby</td>
</tr>
<tr>
<td>Behaviour</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Fighting back</td>
<td>Actively attacking aggressor when attacked first</td>
</tr>
<tr>
<td>Exploring</td>
<td>Neutral behaviours; no interaction with/interest in aggressor;</td>
</tr>
<tr>
<td></td>
<td>Sniffing bedding, eating etc.</td>
</tr>
<tr>
<td>Groomed by aggressor</td>
<td>Aggressor grooming subject; subject shows no resistance</td>
</tr>
<tr>
<td>Total defeated behaviour</td>
<td>Running away + Freezing near aggressor + Freezing in corner +</td>
</tr>
<tr>
<td></td>
<td>Groomed by aggressor</td>
</tr>
</tbody>
</table>
# Appendix B. Factors and Conditions for ANOVA Analyses

<table>
<thead>
<tr>
<th>Test</th>
<th>Type of ANOVA/GLM</th>
<th>Main effect factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FST – Defeat duration</strong></td>
<td>One-way ANOVA</td>
<td>Defeat duration; three levels (T0, T10, T20)</td>
</tr>
<tr>
<td><strong>FST – Gene-environment</strong></td>
<td>Two-way ANOVA</td>
<td>Genotype; three levels (+/+, L100P -/+, Q31L -/+); Defeat status; two levels (naive, defeated)</td>
</tr>
<tr>
<td><strong>FST – Valproate treatment</strong></td>
<td>Two-way ANOVA</td>
<td>Defeat status; two levels (naive, defeated) Drug identity; two levels (vehicle, valproate)</td>
</tr>
<tr>
<td><strong>OF – Defeat duration</strong></td>
<td>One-way repeated measures ANOVA</td>
<td>Defeat duration; three levels (T0, T10, T20) (Time intervals repeated measures factor)</td>
</tr>
<tr>
<td><strong>OF – Gene-environment</strong></td>
<td>Two-way ANOVA</td>
<td>Genotype; three levels (+/+, L100P -/+, Q31L -/+); Defeat status; two levels (naive, defeated)</td>
</tr>
<tr>
<td><strong>OF – Valproate treatment</strong></td>
<td>Two-way repeated measures ANOVA</td>
<td>Defeat status; two levels (naive, defeated) Drug identity; two levels (vehicle, valproate) (Time intervals repeated measures factor)</td>
</tr>
<tr>
<td>Experiment</td>
<td>Design</td>
<td>Factors</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>EPM</td>
<td>Two-way ANOVA</td>
<td>Genotype; three levels (+/+, L100P -/+, Q31L -/+)&lt;br&gt;Defeat status; two levels (naive, defeated)</td>
</tr>
<tr>
<td>LI</td>
<td>Two-way ANOVA</td>
<td>Genotype; three levels (+/+, L100P -/+, Q31L -/+)&lt;br&gt;Preexposure level; two levels (0 dB, 40 dB)</td>
</tr>
<tr>
<td>PT</td>
<td>Two-way ANOVA</td>
<td>Defeat status; two levels (naive, defeated)&lt;br&gt;Drug identity; two levels (vehicle, valproate)</td>
</tr>
<tr>
<td>IB</td>
<td>One-way ANOVA</td>
<td>Drug identity; two levels (vehicle, valproate)</td>
</tr>
<tr>
<td>SSN</td>
<td>Four-way GLM</td>
<td>Genotype; three levels (+/+, L100P -/+, Q31L -/+)&lt;br&gt;Defeat status; two levels (naive, defeated)&lt;br&gt;Test condition; two levels (sociability, social novelty)&lt;br&gt;Chamber side; two levels (chamber 1, chamber 2)</td>
</tr>
</tbody>
</table>