As long as one keeps searching, the answers come.

-Joan Baez
ABSTRACT

For many psychiatric disorders there are still unmet needs for treatment. Using genetically modified mice provide means to study the systems that underlie these disorders as well as identifying potential novel targets for treatment. In this thesis we focused on mouse models of two different systems, using behavioral measures to study their relationship with anxiety, depression, alcoholism and schizophrenia.

In the first part we investigated the anti anxiety-like effects of neuropeptide Y (NPY) in mice. Administration of NPY into the lateral ventricle produced an anxiolytic-like behavior in regular C57BL/6J mice which was not evident in mice lacking the NPY Y1 receptor, confirming that the anxiolytic-like response of NPY is dependent on the NPY Y1 receptor. In addition, the NPY Y1 receptor mutant mice showed a depressant-like behavior on two different paradigms. Both the antidepressant effect of fluoxetine, and its action to stimulate hippocampal neurogenesis were intact in the NPY Y1 receptor mutant mice.

Increased levels of glutamate, which could potentially be caused by a deficiency in glutamate transporters has been implicated in a wide variety of psychiatric disorders. In the second part of this work we started with examining the glutamate aspartate transporter (GLAST) mutant mice for behavioral stress responses and alcohol addiction. Lack of GLAST did not alter anxiety-like behavior or the anxiety-related response to stress, and quite contrary to what was predicted, GLAST mutant mice demonstrated a decrease of alcohol consumption and conditioned place preference for alcohol. Furthermore, in an examination of the consequences of this gene deletion in schizophrenia-like behavior the GLAST mutants exhibited phenotypic features associated with the positive, negative and cognitive symptoms of schizophrenia.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid</td>
</tr>
<tr>
<td>CPP</td>
<td>Conditioned place preference</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin releasing hormone</td>
</tr>
<tr>
<td>EAAT</td>
<td>Excitatory amino acid transporter</td>
</tr>
<tr>
<td>ECT</td>
<td>Electroconvulsive therapy</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GLAST</td>
<td>Glutamate-aspartate transporter</td>
</tr>
<tr>
<td>GLT-1</td>
<td>Glutamate transporter 1</td>
</tr>
<tr>
<td>i.c.v</td>
<td>Intracerebroventricular</td>
</tr>
<tr>
<td>mGlu</td>
<td>Metabotropic glutamate</td>
</tr>
<tr>
<td>MK-801</td>
<td>(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (Dizocilpine)</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
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<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NAcc</td>
<td>Nucleus Accumbens</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>PCP</td>
<td>Phencyclidine</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>PPI</td>
<td>Prepulse inhibition of startle</td>
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<tr>
<td>PTSD</td>
<td>Post-traumatic stress disorder</td>
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<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitor</td>
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</table>
1 INTRODUCTION

Mental health problems remain the subject of prejudice and taboo, despite an estimated 450 million people suffering from mental or behavioral disorders\(^1\). Of the major neuropsychiatric disorders, it is estimated that about 70 million suffer from alcohol dependence and 24 million of schizophrenia worldwide\(^1\). In a given year, about 40 million adults in the US alone are affected by anxiety disorders\(^2\) and the World Health Organization estimates 121 million people worldwide to suffer from depression\(^1\). Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year.

These figures show the extent of suffering directly caused by psychiatric disorders and to this should be added suffering caused to family members and significant others. In developing countries, mental health services are not widely available, while in the developed world, access to treatment is less limited, but still insufficient. Even when treatment is available, in too many cases the treatment strategies are not sufficiently effective, and often have serious side effects. Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, and in doing so identifying novel mechanisms that can be targeted by more effective pharmacotherapies. The work presented here has been carried out with this ultimate objective in mind.

The studies in this thesis focus on some of the most common psychiatric disorders, where great unmet medical needs remain: depression and anxiety, alcoholism and schizophrenia. These disorders are commonly comorbid, and are also thought to be associated with common etiological factors related to stress, suggesting overlapping pathophysiological factors. The present studies focus on mouse models of two potential neurochemical / molecular factors that had been implicated in regulation of behavioral stress responses and addiction: neuropeptide
Y/neuropeptide Y1 receptor and glutamate/GLAST transporter. Unexpected findings made during the course of this work pointed to a relevance of the latter among these molecular targets, GLAST, for the pathophysiology of schizophrenia, and these leads were followed up here.

In the following sections, the major diagnostic characteristics (and the main current drug treatment options) of depression and anxiety, alcoholism and schizophrenia will be summarized. Next, the rationale and utility for using the mouse as a model species to study these disorders will be discussed, along with a description of the mouse behavioral tests commonly used to do so. Lastly, the literature on the potential role of neuropeptide Y, and glutamate and its transporter GLAST, in these disorders will be briefly introduced.

1.1 ANXIETY AND DEPRESSION

There are several forms of anxiety and depression disorders (see Table 1). Although each anxiety disorder has different symptoms they all cluster around excessive, irrational fear and dread. Common depressive symptoms include persistent sadness, feelings of hopelessness, guilt, irritability, loss of interest in activities that once were pleasurable, abnormalities in appetite and sleep, and thoughts of suicide. The severity, frequency and duration of these symptoms vary between individuals and comorbidity is high between these two disorders. In addition, both anxiety and depression frequently occur together with other mental and physical conditions such as substance abuse, heart disease, stroke, cancer, diabetes and dementia where the symptoms of anxiety and depression can be masked or, in other cases, worsened by these diseases.
Table 1. DSM-IV categorization of the major sub-disorders of anxiety and depression.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Characteristics of the disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td></td>
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<tr>
<td>Panic disorder</td>
<td>Sudden attacks of terror, accompanied by a pounding heart, sweatiness, weakness, faintness, or dizziness.</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>Excessive fear of unfamiliar environments and situations where escape might be difficult.</td>
</tr>
<tr>
<td>Post-traumatic stress disorder</td>
<td>Persistent re-experiencing of traumatic event, avoidance of stimuli associated with the trauma, increased general arousal.</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>Excessive worry and rumination about multiple issues.</td>
</tr>
<tr>
<td>Social anxiety disorder</td>
<td>Excessive fear about interacting with people.</td>
</tr>
<tr>
<td>Obsessive compulsive disorder</td>
<td>Persistent thoughts (obsessions) and use of rituals (compulsions) to control the anxiety that are produced by these thoughts.</td>
</tr>
<tr>
<td>Specific phobias</td>
<td>Marked and persistent fear and avoidance of a specific object or situation.</td>
</tr>
<tr>
<td>Depression</td>
<td></td>
</tr>
<tr>
<td>Major depressive disorder</td>
<td>Pervasive low mood, low self-esteem, and loss of interest or pleasure in usual activities.</td>
</tr>
<tr>
<td>Dysthymic disorder</td>
<td>Mild form of major depressive disorder that persists for at least two years.</td>
</tr>
<tr>
<td>Psychotic depression</td>
<td>Severe major depressive disorder accompanied by some form of psychosis.</td>
</tr>
<tr>
<td>Postpartum depression</td>
<td>New mother develops major depressive episode within a month after delivery.</td>
</tr>
<tr>
<td>Seasonal affective disorder</td>
<td>Depressive illness during the winter months.</td>
</tr>
</tbody>
</table>
Pharmacological treatment of anxiety and depression is one of the great success stories of modern neuropsychopharmacology. The use of modern antidepressants alone has been shown to save countless lives by preventing suicides. However, the current treatments continue to have limited efficacy in a considerable number of patients with mood disorders and are not always well tolerated. Compounds that target the GABA<sub>A</sub> receptors, such as the benzodiazepines, have been most commonly used in the treatment of anxiety disorders; however these can potentially cause sedation, cognitive impairment and dependence. Today’s most prescribed drugs for both anxiety and depression belong to the family of selective serotonin reuptake inhibitors (SSRIs), which inhibit the uptake of serotonin (5-HT) from the synapse. Although this class has a better tolerability than the monoamine oxidase inhibitors and tricyclic antidepressants, they still have limited efficacy, slow onset of action, and side effects such as sexual dysfunction, nausea and sleep disturbances. In addition, about one third of patients with mood disorders treated with SSRIs are non-responders. Thus, new therapeutics targets that would improve the treatment of anxiety and depressive disorders are needed.

A long held dogma maintained that no new neurons were produced in the adult mammalian brain. However, stem cells with potential to generate new neurons are found in the hippocampal dentate gyrus and the subventricular zone of the adult mammalian brain. Neurogenesis is the process through which neurons are created from these pluripotent stem cells. This process, first recognized to occur in rodents, but whose presence in humans was long questioned, does indeed occur in our species. In rodents, exposure to stress (a risk-factor for depression), suppresses the formation of new neurons in rodents while treatment with antidepressants increase neurogenesis. The depression-related efficacy of chronic antidepressant treatment has been shown to be dependent on hippocampal neurogenesis; although not all findings have consistently shown this. A possible involvement of
neurogenesis in mediating antidepressant actions could potentially explain the delayed onset of these medications. Hippocampal neurogenesis therefore provides an interesting measure related to depression.

1.2 ALCOHOL ADDICTION

Alcohol addiction, or alcoholism for short, is a chronic, relapsing disorder characterized by compulsive alcohol seeking and use despite adverse consequences. The DSM-IV, which in its current version uses the term “alcohol dependence” defines it as a cluster of cognitive, behavioral and physiological symptoms (Table 2).

Behavioral treatments such as cognitive behavioral therapy can improve outcomes in alcoholism. However, behavioral treatments have limited effect sizes and classical long-term studies of alcohol dependent men show that full remission is rare, while morbidity and mortality are high.

The first approved pharmacological treatment for alcoholism was disulfiram (Antabuse), an alcohol dehydrogenase inhibitor that blocks the metabolism of alcohol. This drug provides a deterrent against alcohol use, as intake will lead to an accumulation of acetaldehyde, resulting in highly aversive (and potentially dangerous) flushing and tachycardia. The efficacy of this treatment strategy has been debated and clinical trials have given inconsistent results. Of more recent drug treatments, naltrexone, an opiate antagonist, is effective in treating alcoholism by reducing alcohol reward, and may be particularly effective in patients who are family history positive for alcoholism and / or carry a particular variant of the mu-opioid receptor gene. Finally, acamprosate, a drug that acts as a functional glutamate antagonist, has relatively consistently
Table 2. Diagnostic criteria for alcohol dependence according to DSM-IV.

A maladaptive pattern of substance abuse, leading to clinical impairment or distress, as manifested by three (or more) of the following criteria, occurring at any time in the same 12-month period:

- **Tolerance** – a significant increase in amount of alcohol necessary to achieve the same intoxicating effect.
- **Withdrawal** – increased anxiety and sympathetic nervous system activity after alcohol has been discontinued.
- Alcohol is consumed in larger amounts or over a longer period of time than intended.
- A great deal of time is spent to acquire and consume alcohol or to recover from its effects.
- Important social, occupational and recreational activities are given up in favor of the alcohol drinking.
- Persistent desire or unsuccessful efforts to cut down or control alcohol drinking.
- Alcohol consumption is continued despite knowledge of having persistent recurrent physical and psychological problem that is likely to have been caused or worsened by the alcohol.

been shown to reduce craving and relapse in European trials\textsuperscript{24} – although it has failed to show efficacy in two major US studies\textsuperscript{25, 26}.

1.3 **SCHIZOPHRENIA**

Schizophrenia is diagnosed based on the presence of a certain combination of symptoms for at least a six-month period\textsuperscript{27}. These symptoms may include delusions, hallucinations, and disordered speech and thinking, or what is commonly referred as to the ‘positive’ or ‘productive’ symptoms of the disorder. On the other hand, ‘negative’ symptoms are associated with poor
emotional expression and reaction, social withdrawal, and lack of motivation and interest in activities that are normally considered to induce pleasure. A third set of symptoms is considered to be a core feature of the illness and may be present before the onset of fulminant disorder, or be ‘prodromal’. These, the cognitive deficits, involve problems with attention, working memory, learning, verbal fluency, motor speed and executive functions, important for the ability to plan and organize.

Pharmacological treatment for schizophrenia was introduced in the 1950’s when it was serendipitously discovered that chlorpromazine was effective in reducing psychotic symptoms such as hallucination, delusions and agitation\textsuperscript{28}. This drug is an antagonist of dopamine D2 receptors, prompting the development of more potent dopamine antagonists such as haloperidol. D2 antagonists, commonly referred to as ‘antipsychotics’ (or today, ‘conventional’ or ‘typical’ antipsychotics, to distinguish them from the newer ‘atypical’ drugs) are effective in suppressing productive symptoms of schizophrenia, but also cause side effects such as Parkinsonism, tremor, rigidity, dystonia and dyskinesia\textsuperscript{29}.

However, first generation, typical antipsychotics, are not effective for the negative or cognitive symptoms of the disease\textsuperscript{30-34}. The so-called second generation, or ‘atypical’ antipsychotics (e.g., clozapine or olanzapine), have effects on D2 receptors as well as other systems, notably 5-HT receptors. They are somewhat more effective for treating negative/cognitive symptoms and avoid some of the more serious side effects\textsuperscript{35, 36}, but can still produce weight gain, hyperlipidimia and type II diabetes.
1.4 ANIMAL MODELS OF PSYCHIATRIC DISORDERS

An understanding of brain processes underlying psychiatric disorders faces considerable challenges. Although progress in studying the living human brain has been made in the last few decades by applying technologies such as functional magnetic resonance imaging (fMRI), there are still great limitations. Our ability to directly study neurochemical processes and signaling events in the human living brain, and to be confident that these are causally linked to behavior, continues to be limited. Studies in experimental animals can help provide some of the answers.

1.4.1 Ethical issues in animal research

The work described in this thesis involves experimentation in living animals. When animals are used for the benefit of humans, such as is the case in research, it is crucial that this is done in the most humane manner possible. On the other hand, while we may be able to survive without eating animals, using their fur, using them for transportation purposes, sports and even their company, we are unlikely to understand devastating mental disorders without the use of experimental animals. Research with laboratory animals has already produced important discoveries, and helped develop treatments for mental disorders. However, unmet medical needs in this area continue to be extensive, and are unlikely to be addressed without research that uses experimental animals.

Both scientists and governments in many countries promote best practice in animal experimentation. The ‘three Rs’, which were first described by Russell and Burch\textsuperscript{37}, form the basis of the ethical laws in many countries:

**Reduce** the number of animals tested, or obtain more information from the same animal.
Replace the animal models with alternative methods whenever possible (e.g., computer models, cell cultures) to achieve same scientific aim.

Refine the methods used in order to reduce or eliminate discomfort and distress for the animals.

1.4.2 Utility and validity of mouse models

Brain structure and function vary along the phylogenetic scale and one of the most obvious differences is the increasing degree of corticalization from rodents to primates. Nevertheless, much of the fundamental neurocircuitry is conserved between species, allowing insights to be gained from experiments using rodents. For decades rats have been the species of choice in preclinical neuropsychopharmacology research. This is in part due to the reliability and relative ease with which invasive techniques, such as site-directed brain microinjections, can be applied to this species, in combination with a wide range of well established behavioral models. In the last decade, however, the use of mice in neuropsychiatric research has increased markedly. This has been spurred by the development and application of molecular techniques such as gene targeting, which offer the opportunity to study the role of specific gene products in a living animal within a controlled experimental environment. The use of mice has also practical and economic advantages with regard to breeding and housing.

Of course, the mouse is not a miniature human. It is impossible to fully model the complexity of human neuropsychiatric disease in experimental animals. We can not ask a mouse how it feels or what it is thinking. Therefore, the utility and validity of mouse models must be evaluated based on objective criteria. The following criteria for the validity of approaches to modeling...
human behavior in experimental animals have been proposed by Robbins and Sahakian\textsuperscript{38}:

1. **Face validity**: A model is said to have face validity if the behavior being measured "looks like" the human symptoms being modeled.

2. **Construct validity**: Refers to how well the theoretical underpinnings of the model corresponds to those of the human condition. This cannot be established in a single experiment but rather accumulates with the result from many studies over time.

3. **Predictive validity**: The extent to which a particular measure is able to predict future observations on some other dimension. The most relevant case of predictive validity in research using animal models is pharmacological validity, i.e. the ability of a drug effect in an animal model to predict the same effect in humans.

### 1.4.3 Mouse behavioral tests for psychiatric disorders

The early days of using genetically modified mice in behavioral work brought the experience that targeted mutations or overexpression models may affect behavior in non-specific ways. It has therefore become the consensus of the field that more than one task should be used before concluding specific effects of genetic modifications on a specific behavioral domain such as for instance anxiety- or depression-like phenotypes as these task also reflect different aspects of mood\textsuperscript{39}. 
1.4.4 Anxiety- and fear-related behaviors

Direct exposure to threatening stimuli elicits coordinated and evolutionarily conserved patterns of responses that are conveniently grouped under the construct of fear. In contrast, anxiety is defined as the activation of fear in the absence of an actual threat, and is the hallmark of anxiety disorders in humans. It is, however, commonly thought that the same neurobiological systems mediate both normal fear and pathological anxiety, and rodent animal models of anxiety are often based upon what is known about the natural behavioral pattern of rodents. For instance, presumably because of a strong selection pressure on small prey animals to avoid exposure to predators, mice have an innate aversion to exposed and well-lit spaces. On the other hand, foraging for food requires exploration of the environment.

Exploration-based tasks take advantage of the conflicting tendencies to approach versus avoid an area that could potentially be dangerous. The aversive area can take different forms in different tests: the central area of a brightly lit open field (novel open field test), open, elevated arms (elevated plus-maze), open, elevated quadrants (elevated zero-maze), a brightly lit compartment (light-dark exploration test, dark/light emergence test), a mirrored arena (mirrored chamber test), or a staircase (staircase test). High levels of exploration in an aversive environment are generally interpreted as low levels of anxiety-like behavior. As many anxiety disorders are characterized by an avoidance of perceived threat, these tests show face validity. More importantly, several of them have an extensively documented predictive validity as these avoidance behaviors can be reversed with common anxiolytics, such as benzodiazepines, while they are typically unaffected by psychotropic drugs that do not modulate anxiety in humans.

These approach-avoidance based tests can however be confounded by defects of normal sensory, neurological and
motor functions. A gene mutation can impair tactile and visual perception and thereby confound performance on these types of tasks. Measures such as general motor activity in any given task, for instance entries into the closed arms in the elevated plus-maze, or in a familiar environment, like the home cage, can provide useful information on how to interpret the outcome of these paradigms.

Other alternatives to the approach-avoidance tests have been developed and commonly referred to as punishment-based conflict paradigms. The most frequently used task is the Vogel conflict test that was initially developed for rats. Here water-deprived animals are provided with water from a spout which occasionally will also deliver a mild shock. Common anxiolytics, like diazepam, will attenuate the shock-induced suppression of drinking. This task has been successfully adapted to mice, but its use in this species has not yet been widely reported.

As exploration-based tasks can be confounded by effects on locomotion, tasks that are independent of locomotor activity can also be a useful complement in assessing anxiety-like behavior in mice. An activity-independent task, such as stress-induced hyperthermia, which measures innate responses in mice, is based on the increase in core body temperature and autonomic arousal after exposure to a mild stressor, for example the insertion of a rectal temperature probe.

The tasks listed above all rely on unconditioned behaviors. The fear conditioning paradigm is based on the classic work of Pavlov from early 1900’s. It measures fear-related behavior induced by exposure to a previously neutral stimulus, such as a tone, that has become associated with an aversive stimulus, i.e. foot shock. The dependent outcome measure in this model is generally the amount of freezing, a species specific fear response in mice and rats. This paradigm has been critical for mapping out the circuitry that mediates fear learning and memory.
1.4.5 Depression-related behaviors

Inability to cope with stress has been suggested to be one of the manifestations of major depression. Several behavioral models of depression have therefore been based on the involvement of exposure to inescapable stress. The forced swim test (also known as Porsolt's test) is probably the most widely used screening test for antidepressant activity. This test is based on the concept of behavioral despair when an animal is lowered into a confined space with tepid water. Initially, animals will vigorously try to escape. However, following variable intervals of time, these attempts will cease, and will be followed by immobility. This is typically interpreted as behavioral despair, and reduction of immobility time is observed following treatment with antidepressants. A related test is the tail suspension test, in which mice are hung upside-down by their tail, and also exhibit passive immobility after minutes of struggling. Both of these tests have been shown to have predictive validity with common antidepressants. A limitation is that these tests are sensitive to acute antidepressant administration, whereas chronic treatment is required for therapeutic efficacy in humans. Novelty-induced hypophagia refers to the increased latency to eat a familiar food in a novel environment. Although primarily a model of anxiety, Dulawa and colleagues have shown this paradigm to be sensitive to chronic but not acute or subchronic antidepressant treatment suggesting that it may be a useful test for depression-related behavior.

A test that has increased in popularity as a model of depression is the chronic mild stress paradigm, which is based on the exposure to repeated unpredictable stressors, such as wet bedding, constant lighting and food deprivation. This procedure has been shown to reduce hippocampal neurogenesis, induce immunological, neurochemical and behavioral alterations in mice which resemble many of those observed in depressed patients. As many of these
behaviors can be rescued with chronic, but not acute, antidepressant treatment this model has been suggested to have face validity, predictive validity as well as construct validity\textsuperscript{75}.

### 1.4.6 Ethanol-related behaviors

Voluntary consumption and self-administration paradigms are commonly used in the assessment of rewarding ethanol effects in rodents. Mice will spontaneously consume ethanol, although the amount consumed varies significantly between strains. A simple method to assess voluntary drinking is the two-bottle-free-choice paradigm, where one bottle contains water and the other alcohol of various concentrations\textsuperscript{76}. Under these conditions, consumption at higher ethanol concentrations can be driven by the pharmacological effects of ethanol, while consumption at lower concentrations can be driven by taste or calories\textsuperscript{77}.

Mice will also self-administer ethanol under operant conditions, again depending on strain. Operant paradigms require a behavioral response such as a lever-press or a nose-poke reinforced by ethanol, and are thought to more directly gauge motivation to obtain the drug than two-bottle drinking\textsuperscript{78}. Conditioned place preference (CPP) is another reward-related paradigm where rodents are exposed to an apparatus that has two distinct environments. Mice are exposed to one distinct environment following ethanol treatment, while the other environment is paired with vehicle pretreatment. After a number of such conditioning sessions, preference for the ethanol-associated environment is assessed under drug-free conditions as a measure of learned reward\textsuperscript{79}.

In humans, reduced sensitivity to acute ataxic and sedative effects of ethanol has considerable heritability, and has been linked to genetic risk for developing alcoholism\textsuperscript{80}. A variety of
tests measure the sensitivity to the acute effects of ethanol in mice. These effects differ markedly depending upon dose. For example, and again depending on mouse strain, low doses (e.g., 1 g/kg body weight) stimulate locomotor activity\(^1\), whereas higher doses induced ataxia (e.g., 2 g/kg body weight), and still higher doses (e.g., 3-4 g/kg body weight) produce sedation/sleep\(^2\). Potential differences in ethanol metabolism can affect the outcome on these and reward-related measures and should be examined separately to help interpret the pharmacodynamic effects of ethanol.

1.4.7 Schizophrenia-related behaviors

Schizophrenia is a uniquely human disorder, and it is impossible to model its full phenotypic spectrum in a mouse or a rat. For instance, subjective symptoms such as delusions and hallucinations can clearly not be modeled. Nonetheless, certain symptoms from each of the three main symptom categories can be modeled in rodents (Table 3).

Among positive symptoms, a subset of schizophrenic patients exhibit psychomotor agitation - hyperactivity or increased stereotypic movements\(^3\). Locomotor hyperactivity is readily assayed in rodents. Drugs such as ketamine, phencyclidine (PCP) and amphetamine can induce a psychotic state in healthy subjects and provoke relapse in patients with schizophrenia\(^4; 5\). Since these drugs can also induce locomotor hyperactivity in both humans, rats and mice, ability of candidate medications to inhibit PCP- or amphetamine-induced hyperlocomotion by them has been widely used to screen for antipsychotic activity of candidate medications\(^6\).

Among negative symptoms, social withdrawal can be measured in mice via tests of social interaction\(^6\). Here the test mouse is
### Table 3. Rodent behavioral measures relevant to symptoms of schizophrenia.

<table>
<thead>
<tr>
<th>Category</th>
<th>Clinical Manifestation</th>
<th>Behavioral measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive symptoms</td>
<td>Psychomotor agitation</td>
<td>Increased locomotor activity in response to novelty or stress.</td>
</tr>
<tr>
<td></td>
<td>Increased sensitivity to psychostimulants</td>
<td>Increased locomotor activity in response to NMDA receptor antagonists (such as MK-801, PCP, ketamine).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased locomotor activity in response to amphetamine.</td>
</tr>
<tr>
<td>Negative symptoms</td>
<td>Social withdrawal</td>
<td>Decreased interaction with conspecific.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased preference for caged social stimulus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased preference for novel social stimulus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Altered social dominance.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased nesting behavior.</td>
</tr>
<tr>
<td>Anhedonia</td>
<td></td>
<td>Decreased reinforcing properties of drugs of abuse or natural reward.</td>
</tr>
<tr>
<td>Cognitive symptom</td>
<td>Memory deficit</td>
<td>Decreased spatial learning in Morris water maze, 8-arm radial maze.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impairment in non-spatial operant learning.</td>
</tr>
<tr>
<td></td>
<td>Attentional deficit</td>
<td>Deficit in pre-attentive processes such as prepulse inhibition.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased latent inhibition.</td>
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<tr>
<td></td>
<td></td>
<td>Deficit in 5-choice serial reaction time test (5-CSRTT).</td>
</tr>
<tr>
<td>Executive function</td>
<td></td>
<td>Impaired extra-dimensional set-shifting.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased perseveration to stimuli in reversal learning.</td>
</tr>
</tbody>
</table>
allowed to interact with an unfamiliar mouse that is either freely moving \cite{87} or trapped in a wire-bar cage \cite{88, 89}. When a sheet of cotton is introduced to the home cage mice typically shred the material to build a nest \cite{90, 91}. This is considered to be a cooperative and "social" activity, and thus a measure of social behavior \cite{92}. Reduced nesting behavior has therefore been another proposed index of social behavior in rodents. Finally, loss of pleasure in normally pleasurable activities (anhedonia) is commonly measured by loss of the normal preference for sucrose-containing solutions in mice \cite{91}.

Rodent tests for the cognitive/executive component of schizophrenia include a wide variety of maze- and operant-based assays for working memory, reversal learning and extradimensional set-shifting and attention in the 5-choice serial reaction time task \cite{86}. Sensorimotor gating, as measured by prepulse inhibition of startle, can also be included in this category \cite{93}.

### 1.5 NEUROPEPTIDE Y IN DEPRESSION AND ANXIETY

Neuropeptides are short-chained amino acid neurotransmitters that are commonly co-localized with amino acids and biogenic amines. Neuropeptides including neuropeptide Y (NPY), galanin, vasopressin, substance S, and corticotropin-releasing hormone (CRH) have been studied for their potential involvement in the pathophysiology of mood and anxiety disorders \cite{94}. This interest has stemmed from their expression in brain regions involved in emotional processing, interesting modulatory action on neurotransmission and the fact that certain neuropeptides, including NPY and galanin, are preferentially released at high firing frequencies, suggesting a role as ‘alarm systems’ under conditions of stress \cite{95}.
One of the most abundant neuropeptides in the brain is NPY, a 36-amino acid peptide belonging to the family of pancreatic peptides. NPY is evolutionarily highly conserved\textsuperscript{96}, implying an important functional role. It is expressed throughout the peripheral and central nervous system\textsuperscript{97} and can be found at high concentrations in brain areas such as the hypothalamus, basal ganglia, hippocampus, amygdala, and thalamus\textsuperscript{98; 99} where it exerts important roles in feeding, seizure activity, cognition and emotion\textsuperscript{100; 101}. To date, four G-protein coupled NPY receptors have been cloned in mammals (Y1, Y2, Y4, Y5), all with a unique profile and distribution\textsuperscript{102}. While Y4 is found mostly in peripheral tissue, Y1, Y2 and Y5 receptor subtypes mediate the central nervous system (CNS) effects of NPY. Y5 receptors have a distribution and functional profile that overlap with those of Y1 receptors while Y2 receptor mRNA is distinct from that of Y1 and Y5\textsuperscript{102}. Y2 receptors are distinct in that they can act as presynaptic NPY autoreceptors, where blockade potentiates the release of endogenous NPY.

Administration of NPY has been demonstrated to decrease anxiety-like behavior in rats on a variety of tasks, including the elevated plus-maze, Vogel conflict test, fear potentiated startle, and fear conditioned responses in rats\textsuperscript{103-106}, while mutant mice lacking NPY exhibit increased anxiety-like behavior on some tests and under some conditions\textsuperscript{107; 108}. NPY is expressed and released following stress, and attenuates its behavioral consequences\textsuperscript{109; 110}, suggesting that NPY could be recruited to mitigate the effects of prolonged stress. In agreement with this notion, central overexpression of NPY suppresses the anxiogenic responses to stress in transgenic rats\textsuperscript{111}. The mechanism by which NPY exerts anti-stress effects remains unclear but might be related to inhibition of glutamate release\textsuperscript{112}, suppression of CRH release\textsuperscript{113} and potentiation of GABA-mediated neurotransmission\textsuperscript{114}.

The pronounced anti-anxiety actions of NPY have been suggested to be mediated via the Y1 receptor\textsuperscript{105; 115} although
activation of both Y1 and Y5 receptors in the amygdala was reported to produce an anxiolytic-like effect in rats\textsuperscript{105, 116}. So far only one study using Y1 receptor knockout mice has studied anxiety-like behavior, and reported results that were highly dependent on both task and circadian rhythm\textsuperscript{117}. Thus, when the studies presented here were initiated, the role of the Y1 receptor in anxiety remained to be established.

Human studies have reported decreased levels of NPY in the cerebrospinal fluid (CSF) of patients with major depression\textsuperscript{118-120}, which were elevated after chronic antidepressant treatment\textsuperscript{121} and electroconvulsive therapy (ECT)\textsuperscript{122}. Furthermore, an association study showed a haplotype block of the preproNPY gene carrying a functional -399T->C substitution to be associated with late onset alcoholism, the clinical subtype of alcoholism that is characterized by high trait anxiety\textsuperscript{20}. In a preliminary analysis, this variant was also found to represent a vulnerability factor for depression by the study cite above which reported decreased NPY levels in depressed patients. Finally, the functional importance of NPY and its genetic variation for stress responses in humans was elegantly demonstrated recently using a range of approaches including functional brain imaging\textsuperscript{123}.

In rodent studies, the Flinders Sensitive Line (FSL) and Fawn Hooded (FW) rat models of depression both exhibit significantly reduced baseline level of NPY in the hippocampus\textsuperscript{124-126}, compared to controls. The specificity of hippocampal NPY changes for depression-related behaviors in these lines was further supported by the potentiation of NPY levels in this structure after ECT\textsuperscript{124, 127} showing that increased NPY reversed the phenotype. Decreased Y1 receptor mRNA expression has also been found in the hippocampus of FSL rats\textsuperscript{125}. This is in line with pharmacological studies demonstrating that Y1 antagonism blocks the antidepressant-like effect of a Y1 agonist\textsuperscript{128, 129}. Finally, NPY Y1 receptors have been suggested to modulate hippocampal neurogenesis\textsuperscript{130, 131}.
These data could potentially link the role of the Y1 receptor in depression-related behavior with antidepressant drug effects.

1.6 GLUTAMATE AND THE GLUTAMATE TRANSPORTER, GLAST IN ANXIETY, ALCOHOLISM AND SCHIZOPHRENIA

Glutamate (glutamic acid) is one of the twenty naturally occurring amino acids, and is the major excitatory neurotransmitter in the brain. Glutamate exerts its action through ligand-gated ion channel (ionotropic) receptors, N-methyl-D-aspartate (NMDA), kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA): and through G-protein coupled metabotropic glutamate (mGlu) receptors 1-8. These are widely distributed throughout the brain although density is highest in the forebrain areas. Glutamate is neurotoxic at high concentrations, and its synaptic concentration is therefore tightly controlled. Following release, it is rapidly eliminated from the synaptic cleft by five different glutamate transporters (EAAT1-5). GLAST (EAAT1) and GLT-1 (EAAT2) appear to clear the majority of synaptic glutamate and are predominantly found in glia, whereas EAAC1 (EAAT3), EAAT4 and EAAT5 are expressed in neurons throughout the brain. Glutamate is a particularly important regulator of learning and memory but also implicated in the pathophysiology of major psychiatric disorders, including mood and anxiety disorders, alcoholism and schizophrenia.

In rodents, exposure to stress produces a robust increase in glutamate release in forebrain regions including the hippocampus and prefrontal cortex (PFC). While findings have not been consistent, elevated glutamate levels have also been found in patients with mood disorders. More specifically, there is broad glial cell loss and reduced expression of the astrocytic glutamate transporters EAAT1 and EAAT2 in frontal...
regions of individuals with major depression which could cause glutamatergic dysregulation. In agreement with this, riluzole, an antiglutamatergic compound known to enhance glutamate transporter expression and glutamate transporter activity, has also been demonstrated to have antidepressant-like effects in rodents and antidepressant activity in humans.

The natural history of alcoholism is characterized by years of cycles consisting of alcohol intoxication, withdrawal, abstinence, and relapse. In vivo microdialysis studies in rats exposed to repeated cycles of ethanol exposure demonstrate that this cycling leads to an increase in extracellular glutamate levels. This ‘hyperglutamatergic state’ has been hypothesized to be due in part to a decreased function of glutamate transporters. In support of the hyperglutamatergic model, a recent study found that mice with a targeted mutation of the clock gene Per2 had increased levels of glutamate in the ventral striatum, driven by impaired expression of GLAST. These mice exhibited increased voluntary ethanol consumption. Moreover, both the elevated glutamate levels and the excessive ethanol consumption were normalized by acamprosate administration. It has therefore been hypothesized that inhibition or downregulation of GLAST would mimic the hyperglutamatergic state seen after withdrawal from chronic cyclical ethanol exposure. However, GLAST is overexpressed in PFC of alcoholics. These seemingly paradoxical observations could possibly reflect that the hyperglutamatergic state caused by chronic ethanol exposure is driven by other mechanisms, and that GLAST expression is recruited in an attempt to buffer elevated extracellular glutamate levels.

Healthy people experience transient psychotic symptoms similar to those of schizophrenia after administration of a NMDA antagonist such as ketamine or phencyclidine (PCP), and patients with schizophrenia experience exacerbated psychosis after these treatments. This has supported a model of NMDAR receptor hypofunction in schizophrenia. Genetic
analysis in humans has suggested an association between some NMDA receptor gene polymorphisms and schizophrenia\textsuperscript{152; 153}, (but see\textsuperscript{154; 155}) and post-mortem studies has revealed abnormalities in both NMDA receptor subunits and proteins associated with the NMDA receptor, although results have been somewhat inconsistent\textsuperscript{156}.

Various rat and mouse models have demonstrated that deletion or blockade of NMDA receptors causes schizophrenia-related phenotypes\textsuperscript{157-159}. Moreover, recent studies have demonstrated that NMDA receptor blockade inhibits GABAergic interneurons in the PFC which in turn leads to disinhibition of glutamatergic pyramidal neurons and elevates glutamate levels in this region\textsuperscript{160-164}, and therefore increase glutamatergic output to projection areas such as the striatum. Interestingly, elevated glutamate levels in the PFC are attenuated by activation of the presynaptic mGlu2/3 receptors\textsuperscript{164}. This mechanism shows antipsychotic-like activity in that it reduces PCP-induced hyperlocomotion in animals\textsuperscript{164; 165}, and was recently demonstrated to be effective in treating human schizophrenia\textsuperscript{166}.

Increased glutamate levels could be due to a deficit in glutamate uptake by the one or several glutamate transporters. Post-mortem studies have shown inconsistencies with regard to expression and protein levels of glutamate transporters in the brains of patients with schizophrenia\textsuperscript{167-169}. However, a recent genetic study looking at rare structural variants in patients with schizophrenia revealed glutamate transporter GLAST (EAAT1) to be partially deleted\textsuperscript{170}. Beyond this the role of GLAST in schizophrenia is unclear.
2 AIMS OF THE THESIS

AIM I: To study the pharmacological effects of NPY, and role of the NPY Y1 receptor, in mediating anxiety- and depression-like behavior in mice.

SPECIFIC AIMS:

- To study the anxiolytic-like effects of intracerebroventricular administration of NPY and galanin in C57BL/6J mice (Paper I).
- To investigate the role of NPY Y1 receptor in mediating the anxiety- and depression-like effects of NPY, using NPY Y1 receptor knockout mice (Paper II).

AIM II: To study the role of the glutamate transporter, GLAST, in mice with focus on behaviors related to mood, schizophrenia and alcoholism.

SPECIFIC AIMS:

- To study the consequences of a constitutive loss of glutamate transporter GLAST for anxiety-like behaviors in mice (Preliminary data).
- To study the consequences of constitutive loss of glutamate transporter GLAST for ethanol-related behaviors (Paper III).
- To study the consequences of constitutive loss of glutamate transporter GLAST for schizophrenia-related behaviors in mice (Paper IV and V).
3 MATERIAL AND METHODS

Detailed procedures of methods can be found in Paper I-V.

3.1 ANIMAL EXPERIMENTS

Animals

For experiments conducted in Paper I and IV, male C57BL6/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME, USA.

NPY Y1R knockout mice used in Paper II were kindly contributed by Professor Patrik Ernfors at the Karolinska Institute, Stockholm, Sweden. These mice were generated as previously described by Naveilhan and colleagues\(^{171}\) and for the current study backcrossed into C57BL/6/Sca for 7 generations.

Glutamate-aspartate transporter (GLAST) knockout mice used in Papers III, IV and V were obtained from Dr Jeffrey Rothstein at the John Hopkins University, Baltimore, MD, USA. These mice were generated as previously described by Watase and colleagues\(^{172}\). The GLAST mutant mice have been backcrossed on a C57BL/6J background. Unfortunately, our collaborators do not know how many generations this mutation has been backcrossed into C57BL/6J.

To avoid phenotypic abnormalities resulting from potential genotypic differences in maternal behavior and early life environment\(^{173}\), knockout, heterozygous and wild-type mice were all generated from heterozygous × heterozygous matings. Ear punches were obtained for genotyping and mice were identified by tail- or toe-tattoos or had subcutaneously implanted microchips at 14 days of age. At weaning, mice were housed in groups of 1-4 per cage in a temperature- and humidity-controlled vivarium, under 12-hr light/dark cycle (lights on 0600 h), with *ad libitum* access to food and water. Both
males and females were used in the experiments unless otherwise stated.

**General behavioral procedures**

For most procedures, mice were acclimated to the test room for 1 hr prior to testing. In order to prevent outside noise from influencing behavior, a noise generator created a constant white background noise of 65dB. Apparatuses were cleaned with 70% ethanol after each subject. All experimental procedures were approved by the National Institute of Mental Health and National Institute of Alcohol Abuse and Alcoholism Animal Care and Use Committee and followed the NIH guidelines ‘Using Animals in Intramural Research.’

**Functional observational battery**

To ensure that NPY Y1 and GLAST mutations did not result in non-specific disruption of sensory or neurological functions, which could confound performance on some of the specific behavioral tests, mice were evaluated using a functional observational battery. A more detailed procedure can be found in Paper II and V.

**Fear- and anxiety-related behaviors**

Spontaneous anxiety-like behaviors were assessed in Paper I, II and in preliminary data for GLAST knockout mice. We used the novel open field, elevated plus-maze and light-dark exploration test. To test for learned fear we used Pavlovian fear conditioning.

Given the observation of locomotor hyperactivity in GLAST knockout mice (as discussed below) we also assessed anxiety-related behavior in these mice via stress-induced hypothermia. Briefly, mice were singly housed 24 h prior to testing. Testing was conducted at around 1400 hr, at which time rectal temperature was measured by inserting a Thermalert TH-5
thermometer (Physitemp, Clifton, NJ, USA) 2 cm into the rectum and a stable reading was given for 10 sec. Rectal temperature was measured to the nearest 0.1°C. Measurement was done twice in each mouse i.e., at t=0 (T₁) and t=+10 min (T₂). The difference in temperature (T₂-T₁) was taken as the index of stress-induced hypothermia.

Exposure to stress has been shown to increase extracellular glutamate in the rat forebrain\textsuperscript{135, 137, 174, 175}. We asked whether loss of extracellular glutamate reuptake in GLAST knockout mice would affect the consequences of chronic exposure to stress. Briefly, we used a chronic restraint stress regime in which mice were placed in ventilated 50 mL Falcon tubes for 2 hr/day (1000 – 1200 hr) for 10 consecutive days in a separate room from non-stressed controls (which remained undisturbed in the home cage). To provide a systemic measure of the effects of the stressor\textsuperscript{73, 176} body weight was measured before and after 10-day stress regime, and the stress-induced percent change in body weight normalized to the change in weight in non-stressed controls over the same period (change in non-stressed minus change in stressed).

One day after the final stress episode, stressed mice and controls were tested for anxiety-like behavior using the dark-light emergence test as previously described\textsuperscript{177, 178}. Briefly, the mouse was placed in an opaque black Plexiglas shelter (39 x 13 x 16 cm) that opened, via a 13 x 8 cm aperture at floor level, onto a larger white Plexiglas square arena (39 x 39 x 35 cm) illuminated to ~90 lux. The percent of time in a 15-min session spent in the open arena, and the number of shelter-arena transitions were recorded by video-tracking software (Ethovision, Noldus Information Technology, Leesburg, VA, USA).

**Depression-related behaviors**

Depression-related behavior was assessed using the forced swim test to evaluate the antidepressant-related effects of acute
fluoxetine treatment in Paper II. The novelty-induced hypophagia test was also used in this paper to assess the antidepressant-like effects of chronic fluoxetine treatment.

**Ethanol-related behaviors**

In Paper III, two-bottle free choice drinking and ethanol conditioned place preference for ethanol were used to assess ethanol reward in the GLAST knockout mice. Sensitivity to acute ethanol effects was also measured via ethanol-induced locomotor activity, ataxia, hypothermia and loss of righting reflex.

**Schizophrenia-related behaviors**

Behaviors relevant to the positive symptoms of schizophrenia (i.e., novelty- and psychotomimetic-induced locomotor hyperactivity) were assessed using the novel open field in Paper IV. Behaviors relevant to the negative and cognitive symptoms of schizophrenia were assessed in Paper V via the sociability and social novelty preference test and free social paradigm, and analysis of nesting behavior, anhedonia, prepulse inhibition of startle and operant-based reinforcement, extinction, discrimination, and reversal tests.

### 3.2 DRUGS

NPY administered into the periphery does not cross the blood-brain-barrier. In Papers I and II the effects of NPY on behavior were assessed via delivery directly to the lateral ventricles via an indwelling cannula. A more detailed description of this procedure can be found in Papers I and II. NPY (American Peptide, Sunnyvale, CA, USA) and galanin (American Peptide, Sunnyvale, CA, USA) were dissolved in deionized water and administered into the lateral ventricles in a volume of 0.5 µL.
Fluoxetine hydrochloride (LKT Laboratories Inc, St. Paul, MN, USA) acutely administered in Paper II was dissolved in 0.9% saline and injected intraperitoneally in a volume of 10 ml/kg body weight. For chronic treatment in the same paper, fluoxetine was made available ad libitum in the drinking water in bottles covered with aluminum foil to protect the drug from light. Drug concentration was determined from average daily water consumption and average body weight for each genotype to achieve the desired dose of 10 mg/kg/day, which produces brain concentrations in the clinical range. In Paper IV we used phencyclidine (Sigma, St. Louis, MO, USA), MK-801 (Sigma, St. Louis, MO, USA) and haloperidol hydrochloride (Sigma, St. Louis, MO, USA) which were dissolved in 0.9% saline and injected intraperitoneally in a volume of 10 ml/kg body weight. The mGlu2/3 agonist, LY379268 (ToCris, Ellisville, MI, USA), was used in Paper IV and V and dissolved in 0.9% saline (pH adjusted using 1M NaOH) and injected intraperitoneally in a volume of 10 ml/kg.

3.3 HIPPOCAMPAL NEUROGENESIS

In Paper II fluoxetine-induced hippocampal neurogenesis was assessed by injecting 5’-bromo-2’-deoxyuridine (BrdU, 10 mg/ml in 0.007 N NaOH/0.9% saline) intraperitoneally at a dose of 200 mg/kg body weight, on day 30 of fluoxetine treatment.

3.4 STATISTICAL ANALYSIS

Data were analyzed using analysis of variance (ANOVA) followed by Newman-Keuls post hoc tests, or using nonparametric Kruskal-Wallis ANOVA if data did not meet parametric assumptions. To determine the effects of sex, drug treatment, stress or social stimulus a two-way ANOVA was used
and the effects of time or trial were analyzed using repeated-measures ANOVA. Survival analysis was conducted using Mantel-Cox test.
4 RESULTS AND DISCUSSION

4.1 ANXIOLYTIC-LIKE ACTIONS OF CENTRALLY ADMINISTERED NPY IN C57BL6/J MICE (PAPER I)

Various stress- and anxiety-related behaviors have been shown to be mediated by administration of NPY and galanin\textsuperscript{103-106, 179, 180}. However, these data have been mainly based on studies in rats. Due to the extensive use of mutant mouse models in the study of these behaviors, the purpose of this study was to confirm these effects in C57BL/6J inbred strain of mice, as this is a commonly used background strain in behavioral studies.

The anxiolytic-like effects of NPY were consistent with what has previously been seen in rats\textsuperscript{103-106}. Two different doses of NPY, administered into the ventricles, produced a robust anxiolytic-like behavior in the elevated plus-maze and light↔dark exploration test. NPY did however not induce any change in anxiety-like behavior in the open field, presumably due to the relative insensitivity of this test to anxiolytic effects\textsuperscript{181}. When administered prior to training, NPY also reduced the fear response to a conditioned cue in the Pavlovian fear conditioning paradigm.

In contrast to anxiolytic-like response produced by NPY, intracerebroventricular (i.c.v) administration of galanin failed to alter anxiety-like behavior in mice under baseline conditions, which is consistent with what has been seen in transgenic mice overexpressing galanin\textsuperscript{182, 183}. 
4.2 ANXIOLYTIC-LIKE ACTIONS OF NPY ARE DEPENDENT ON THE NPY Y1 RECEPTOR (PAPER II)

Pharmacological studies have suggested that the anxiolytic- and depressant-like effect of NPY would be mediated through the NPY Y1 receptor\textsuperscript{105; 115; 128; 129}. However due to the limited selectivity of pharmacological NPY receptor agonists / antagonists this has remained an unresolved question. Mice deficient of the NPY Y1 receptor therefore offer an attractive tool for elucidating this question.

NPY Y1 knockout mice showed no overt baseline anxiety-like phenotype in the same tests which we had previously demonstrated sensitivity to the anxiolytic-like effects of exogenous NPY (Paper I). This is somewhat contradictory to previous findings which have shown altered anxiety-like behavior in NPY Y1 deficient mice, although those findings were dependent on task and time of testing within the circadian rhythm\textsuperscript{117}. More importantly, however, while central administration of NPY produced a robust decrease in anxiety-like behavior in wild-type controls, this was absent in the NPY Y1 receptor mutant mice (Figure 1), demonstrating that the NPY Y1 receptor is in fact necessary for the anxiolytic-like response to NPY.

Increased depression-like behavior has previously been shown after pharmacological blockade of NPY Y1 receptor\textsuperscript{128; 129} which was further supported by an increase in baseline depression-like behavior in NPY Y1 receptor mutant mice. The antidepressant effect of both acute and chronic fluoxetine administration was intact in the NPY Y1 receptor knockout mice. Thus, these treatments rescued the depression-like phenotype in both the forced swim test and novelty-induced hypophagia test respectively, indicating an intact sensitivity to antidepressants.
Figure 1. Anxiolytic-like effect of NPY is lacking in NPY Y1 receptor knockout mice. Administration of two doses of NPY into the lateral ventricle significantly increased percent open arm time in wild-type treated mice but not in NPY Y1 receptor knockout mice. *P<0.05, **P<0.01 vs. wild-type/vehicle. WT=wild-type, KO=knockout.

It has been suggested that hippocampal neurogenesis is required for the behavioral effects of chronic antidepressant treatment\textsuperscript{12}, and recent studies have suggested a possible involvement of NPY and NPY Y1 receptors in this mechanism\textsuperscript{130, 131}. However, we found that deletion of the NPY Y1 receptor did not disrupt either baseline dentate stem cell proliferation, or the increase in their dentate granule cell proliferation induced by chronic fluoxetine administration. This demonstrated that fluoxetine-induced hippocampal neurogenesis is independent of NPY Y1 receptor, and that antidepressant-like effects of the NPY are not mediated by modulation of hippocampal neurogenesis.

The unexpected lack of baseline increase in anxiety-like behavior in the NPY Y1 mutants could potentially be related to their C57BL/6 genetic background. Several comparative studies using inbred strains have demonstrated significant differences in emotionality-related behaviors\textsuperscript{184-187}. Mouse strains like C57BL/6J and FVB/NJ are low-anxiety-like strains whereas for instance 129Sv/J, BALB/CJ and DBA2/J mice are considered
to be high-anxiety-like strains. Neuropeptide systems are considered to act as “alarm systems” that are activated during conditions of high fear or chronic stress. It is therefore possible that in a low-anxiety-like strain, like the C57BL/6, our test conditions were not sufficient to trigger NPY release. As an important note in this context, low release of endogenous NPY in amygdala, a region highly relevant to anxiety, has been demonstrated in C57BL/6J compared to DBA/2J. This could also explain the discrepancy in baseline anxiety-like behavior with NPY Y1 knockout mice on a C57BL/6J x 129/SvJ mixed background strain used by Karl and colleagues.

4.3 NORMAL ANXIETY-LIKE BEHAVIOR IN GLUTamate TRANSPORTER (GLAST) KNOCKOUT MICE (PRELIMINARY DATA)

There is evidence of glutamatergic dysfunction in mood and anxiety disorders. For example, abnormal expression of glutamate, its receptors and transporters have been found in anxious and depressed patients. Modulating glutamate signaling by targeting glutamate transporters could therefore provide a mechanism for altering mood. In the current study, there were no significant differences in baseline anxiety-like behavior in glutamate transporter, GLAST, mutant mice when compared to WT controls (Table 4).

Exposure to stress elevates glutamate release in the rat forebrain. We therefore wondered whether effects of GLAST knockout would become apparent after exposure to stress. We subjected mice to a 10-day restraint stress regime, that has previously been shown to produce increases in corticosterone in C57BL/6J mice, and produce neuronal morphological changes in the hippocampus and basolateral amygdala of both rats and mice. The stress procedure did appear to affect the well-being of the mice as it significantly
Table 4. Baseline anxiety-related behaviors in GLAST KO mice. Anxiety-like behavior in the elevated plus-maze, light↔dark exploration test, dark/light emergence test and stress-induced hypothermia were no different between genotypes. n=11-19/genotype.

<table>
<thead>
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<th>Behavior</th>
<th>WT</th>
<th>HET</th>
<th>KO</th>
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</thead>
<tbody>
<tr>
<td>Elevated plus-maze</td>
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<tr>
<td>% open time</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% time in light compartment</td>
<td>32.1 ±2.2</td>
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<td>34.0 ±3.3</td>
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<tr>
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<tr>
<td>% time outside shelter</td>
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</tr>
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</table>

affected their body weights (Figure 2A). Overall GLAST knockout mice showed increased anxiety-like behavior in this test, although this was independent of stress (Figure 2B). Instead, a clear anxiolytic-like behavior was seen in mice that had undergone stress. This may seem somewhat paradoxical, but a recent study in our laboratory has shown that the anxiety-like effects of the same stress paradigm may be strain dependent (unpublished observations) where C57BL/6J strains respond
with decreased anxiety-like behavior compared to for instance DBA/2J, which show a robust anxiogenic-like behavior after stress. In conclusion, however, these data did not provide evidence that loss of GLAST increased anxiety-like behavior.

4.4 REDUCED VOLUNTARY ETHANOL CONSUMPTION AND ETHANOL REWARD IN GLUTAMATE TRANSPORTER (GLAST) KNOCKOUT MICE (PAPER III)

Based on literature reviewed in the Introduction, we had hypothesized that a deletion of GLAST would mimic the increase in glutamatergic tone thought to be an integral part of pathophysiological mechanisms in alcoholism. However, contrary to what was expected, the deletion of GLAST instead attenuated ethanol consumption and reward. Specifically,
GLAST knockout mice showed decreased ethanol consumption (A). Wild-type and heterozygous mice spent significantly more time on the ethanol-paired side during testing, whilst GLAST knockout mice did not show any side preference (B). **$P<0.01$. WT=wild-type, HET=heterozygous, KO=knockout

The failure to show CPP for ethanol can therefore not be accounted for by a deficit in associative learning. Increased locomotor activity during the test trial, which was also evident in the GLAST mutant mice, is another parameter that possibly could influence the expression of CPP\textsuperscript{198}. Low activity levels are...
often associated with stronger expression of place preference\textsuperscript{198-202}. However, GLAST mutants did not display this inverse negative correlation, indicating that a heightened activity was not contributing to the lack of place preference. It could be argued that a decreased sensitivity to the ethanol dose used in this study could explain this lack of association between activity and preference. Instead, however, the dose of ethanol used in the CPP in fact increase locomotor activity in the GLAST knockouts.

While an increased locomotor response to ethanol was found in the GLAST mutants, the sensitivity to the ataxic, hypothermic and sedative/hypnotic effects of ethanol was not different between genotypes.

In summary, these results were unexpected with regard to the main outcome measures, i.e., voluntary ethanol intake and CPP for ethanol. These findings are likely to be the result of compensatory changes in response to a loss of function during critical developmental periods\textsuperscript{203}. Identifying the underlying compensatory mechanisms could provide novel insight into a mechanism that mediates reduced ethanol reward, and will be the objective in future studies.

4.5 SCHIZOPHRENIA-RELATED BEHAVIORS IN GLUTAMATE TRANSPORTER (GLAST) KNOCKOUT MICE (PAPERS IV AND V)

While glutamatergic neurotransmission has been increasingly implicated in the pathophysiology of schizophrenia\textsuperscript{204}, little is known about the involvement of glutamate transporters in this disease. Due to the lack of selective pharmacological tools that block these transporters, there have been few studies using rodent models. Mice deficient in the glutamate transporter, GLAST, demonstrated a robust increase in locomotor activity in
the novel open field (Figure 4A), but not in a familiar home cage environment (Figure 4B), as well as an increased sensitivity to the NMDA antagonist MK-801 (Figure 4C). Interestingly, this increase in sensitivity was also seen after a low dose of ethanol both in the open field and in the ethanol-induced place preference conditioning (Paper III). This could perhaps be due to ethanol’s ability to inhibit the NMDA receptor\textsuperscript{205; 206} and is in line with reports of increases in the positive psychotic symptoms in schizophrenic patients receiving alcohol\textsuperscript{207}.

These findings demonstrate that GLAST knockout mice display phenotypic features relevant to the positive symptoms of schizophrenia. A possible mechanistic explanation for this stems from recent data from pharmacological and electrophysiological

![Figure 4. Locomotor hyperactivity in GLAST knockout mice. GLAST knockout mice show increased locomotor activity in the novel open field (A) but not in, a familiar environment, like the home-cage (B). Treatment with 0.2 mg/kg MK-801 produced a significantly greater increase in open field locomotor activity in GLAST knockout mice than wild-type controls (C). Treatment with 1 mg/kg mGlu2/3 agonist, LY379268, attenuated the hyperactivity in GLAST knockout mice (D). *P<0.05 vs. wild-type/same treatment. ##P<0.01 vs. wild-type/same treatment. WT=wild-type, HET=heterozygous, KO=knockout.](image-url)
studies in rats. These data suggest that the psychotomimetic effects of NMDA receptor antagonists result from blockade of NMDA receptors on GABAergic interneurons in the PFC. This causes disinhibition and results in overexcitation of glutamatergic pyramidal output neurons in this region\textsuperscript{160-162}. We hypothesized that loss of glutamate reuptake may have the same net effect. Our finding that LY379268, which increases presynaptic inhibition of glutamate release\textsuperscript{164}, rescues the GLAST knockout phenotype (Figure 4D) supports this hypothesis. We point out, however, that increased extracellular levels of glutamate in the GLAST knockout mice have not yet been confirmed.

Whereas typical antipsychotics, like haloperidol, have been shown to attenuate positive symptoms of schizophrenia, they have poor efficacy on the negative and cognitive domains of the disease. As more evidence has been provided that these behaviors are partially mediated through prefrontal areas\textsuperscript{208} it seems likely that glutamatergic neurotransmission could play a key role. Several studies have implicated glutamatergic function to be involved in social behavior\textsuperscript{209-211}, although the effects of the mGlu2/3 agonist on social behavior remains to be elucidated. Our studies showed GLAST mutant mice to have abnormal social behavior. This was evident both in terms of sociability, the lack of preference for social stimulus over an inanimate stimulus, and a lesser degree of social novelty preference in the GLAST mutants compared to wild-type controls. In addition, GLAST mutant mice also showed a poor nesting behavior which has commonly been interpreted as a model of self-neglect in schizophrenia\textsuperscript{92}.

Prepulse inhibition of startle response (PPI) is a neurological phenomenon thought to reflect the ability to filter out irrelevant information. PPI deficits are commonly found in schizophrenic patients (and their unaffected relatives)\textsuperscript{212, 213}. In the GLAST mutant mice, the PPI was normal although there was a significant impairment in the startle response. PPI and the
startle response are mediated through different neural systems and are dissociable on a behavioral level\textsuperscript{214}. It has been reported that GLAST knockouts develop hearing loss following exposure to loud noise\textsuperscript{215}, which could affect the outcome of this test. This is however unlikely given that PPI was not affected. Also of note, several studies have reported that treatments with mGlu2 or mGlu2/3 agonist does not rescue the effect of PCP induced PPI deficits\textsuperscript{216-218}, maybe indicating that the role of glutamate on PPI is less straightforward, and possible that the predictive validity of PPI for antipsychotic activity may be limited.

GLAST knockout mice were impaired on an instrumental visual discrimination paradigm. The impairment was partial as mice were normal on a simpler acquisition and extinction task. Given the ability of LY379268 to rescue the locomotor hyperactivity in GLAST knockout mice (Paper IV), we tested whether the drug could also improve discrimination learning in the knockouts, but found it ineffective (Paper V). There are a number of possible explanations for this negative finding. Previous studies have demonstrated tolerance to the ability of LY379268 to produce motor incoordination and reduce nicotine self-administration (but not prevent PCP-induced hyperactivity) following repeated treatment\textsuperscript{219, 220}. Therefore, chronic treatment with LY379268 in our experiment could have caused tolerance development that negated any beneficial effects the drug might have had. Although the neural circuits of our discrimination task remain to be identified, initial evidence suggests that performance is supported more by dorsal striatum than PFC (Jonathan L. Brigman, Personal Communication). A possible explanation for the failure of the mGlu2/3 agonist to rescue the discrimination deficit in the mutants could be due to the inability of the drug to target the circuitry mediating this behavior. The implication of a progressive recruitment of dorsal striatum in the discrimination task is particularly intriguing given the impaired cortico-striatal plasticity observed in the GLAST knockout mice (Louise Adermark, Personal Communication).
5 SUMMARY

Exogenous NPY was shown to exert an anxiolytic-like effect in the mouse, consistent with what previous studies have shown in rats. This decrease in anxiety-like behavior has been suggested to be mediated by the NPY Y1 receptor. Although NPY Y1 receptor knockout mice did not show an overt spontaneous anxiety-like response, the anxiolytic-like behavior seen after exogenous NPY in wild-type controls was absent in the NPY Y1 receptor knockout mice. This demonstrates that the NPY Y1 receptor is indeed required for the anxiety-like response of NPY. Furthermore, NPY Y1 receptor mutants had increased depression-like behavior, which was attenuated after both acute and chronic treatment of fluoxetine. Together these data show that the NPY Y1 receptor is required to mediate NPY effects on anxiety-like behavior, but not for behavioral or neurogenesis-stimulating actions of antidepressant drugs such as fluoxetine.

GLAST knockout mice did not display an altered anxiety-like phenotype. These mice also failed to show an abnormal anxiety-related response to chronic stress suggesting that the GLAST is not involved in the effects of stress. GLAST knockout mice showed an augmented locomotor response to ethanol, while ataxic and sedative effects of higher ethanol doses were normal. However, contrary to a priori prediction, GLAST knockout mice showed a decrease, rather than an increase, in the rewarding effects of ethanol on two separate measures. While the mechanisms behind this phenotype remains to be elucidated, preliminary data suggest that impaired ability to synthesis and / or release endocannabinoids may be involved.

GLAST knockout mice also showed phenotypic features relevant to positive, negative as well as cognitive symptoms of schizophrenia. While extracellular levels of glutamate have not been investigated in GLAST knockout mice, phenotypic rescue of locomotor hyperactivity by the mGlu2/3 receptor agonist,
LY379268, would be consistent with excessive glutamate signaling driving this phenotype.
6 CONCLUDING REMARKS

Psychiatric disorders are one of the leading causes of disability and suffering worldwide. There is a tremendous need to find better treatment and this can best be achieved with a greater understanding of the basic pathophysiology of these disorders. Molecular techniques, such as gene targeting, offer opportunities to study the role of specific gene products on neural mechanisms and behavioral outcomes in experimental animals. While it is impossible to model the full extent of a human mental disorder in a lower animal such as the mouse, mouse models are currently helping elucidate crucial brain mechanisms and finding new drugs to treat these devastating disorders. So the search goes on…
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