DEVELOPING BEHAVIORAL ASSAYS TO STUDY DOPAMINE-RELATED DISORDERS IN *ZEBRAFISH (DANIO RERIO)*

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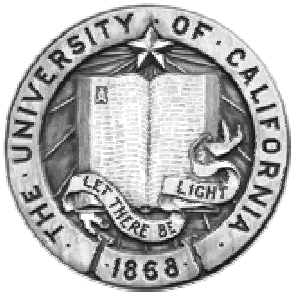

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. Purpose of the study…… 6

1.2. Genetics on traditional model organisms…… 6

1.3. The zebrafish as a model – genetic screens…… 7

1.4. A model for human disease…….. 11

1.5. The dopaminergic system in higher vertebrates…….. 13

1.6. Human diseases involving dopaminergic pathways……... 14

1.6.1. Parkinson's disease (PD)…… 14

1.6.2. Drug and alcohol addiction…… 17

1.6.3. Schizophrenia…… 22

1.6.4. Anxiety and depression…… 23

1.6.5. Attention deficit hyperactivity disorder (ADHD)…… 23

1.7. The zebrafish dopaminergic system and the too few (tfu) mutant…… 24

2. BEHAVIORAL ASSAYS AND RESULTS

2.1. Outline of the study…….. 26

2.2. Establishing a locomotor assay – materials and methods…….. 28

2.2.1. Video record setup…… 28

2.2.2. Data analysis…… 28

2.2.3. The experimental animals and variability: developing a protocol… 30

2.2.4. Protocol for experiments on seven-day old fry…… 31

2.3. Basal locomotor activity of different wt fry and the tfu mutant…… 32

2.3.1. Fish backgrounds…… 32

2.3.2. Protocol…… 32

2.3.3. Results…… 33

2.4. Screening of potentially interesting drugs…….. 34

2.4.1. Ethanol…… 34

2.4.2. Amphetamine…… 39

2.4.3. Cocaine…… 42

2.4.4. Morphine…… 44

2.4.5. MPTP…… 45

2.4.6. Fluphenazine…… 46
2.4.7. The combination of fluphenazine and ethanol…… 47
2.4.8. Levodopa…… 49
2.4.9. Apomorphine…… 51
2.4.10. Reserpine…… 51
2.5. Tolerance…… 52
2.5.1. Introduction…… 52
2.5.2. Rapid tolerance…… 53
2.5.3. Chronic tolerance…… 54
2.6. Open field assays…… 56
2.6.1. Development of an open field assay…… 56
2.6.2. Ethanol…… 56
2.6.3. Amphetamine, cocaine and morphine …… 56
2.7. Light/dark preference…… 60
2.7.1. Development of a light/dark preference assay…… 60
2.7.2. Results…… 61
2.8. Pigment response…… 62
2.9. Measurement of the alcohol concentration in seven-day old fry…….. 62

3. DISCUSSION

3.1. Rationale for the study – perspective…… 64
3.2. Locomotor activity assays…….. 65
3.2.1. Parkinson modeling – the too few mutant and wild type…… 65
*3.2.2. Modeling addiction…… **66***
3.2.3. Other drugs and aspects…… 67
3.3. Open field and light/dark assays……... 68
3.3.1. Anxiety models…… 68
3.3.2. Genetic screening employing anxiety models…… 68
3.4. Summary of assay procedures that can be used for genetic screens…… 70
3.5. Conclusion……... 71

4. REFERENCES…… 73

5. APPENDIX: Excel® data sheets showing the individual values of the charts presented in section 2……. 85

5.1. Basal locomotor activity of seven-day old ABC SG013

5.2. Basal locomotor activity of seven-day old ABC SG035

5.3. Basal locomotor activity of seven-day old ABC tfu1x47 x tfu1x-45 (wt)

5.4. Basal locomotor activity of seven-day old wild type SG019

5.5. Basal locomotor activity of seven-day old tfu mutant SG018

5.6. Basal locomotor activity of seven-day old wild type SG023

5.7. Basal locomotor activity of seven-day old WIK SG003

5.8. EtOH induced locomotor activity of seven-day old ABC SG013

5.9. EtOH induced locomotor activity of seven-day old ABC SG035

5.10. EtOH induced locomotor activity of seven-day old tfu1x47 x tfu1x-45 (wt)

5.11. EtOH induced locomotor activity of seven-day old wild type SG019

5.12. EtOH induced locomotor activity of seven-day old tfu mutant SG018

5.13. EtOH induced locomotor activity of seven-day old wild type SG023

5.14. EtOH induced locomotor activity of seven-day old WIK SG003

5.15. AMPH induced locomotor activity of seven-day old ABC SG013

5.16. AMPH induced locomotor activity of seven-day old wild type SG019

5.17. AMPH induced locomotor activity of seven-day old tfu mutant SG018

5.18. Locomotor activity of 14-day old ABC SG013 treated with cocaine

5.19. Locomotor activity of 14-day old ABC SG013 treated with morphine

5.20. Locomotor activity of seven-day old ABC SG013 treated with MPTP 1-5dpf

5.21. EtOH induced hyperactivity after fluphenazine pretreatment of seven-day old ABC

5.22. SG018 and SG019 locomotor activities on 14-day old fry given L-DOPA 1-13dpf

5.23. Rapid EtOH tolerance of seven-day old ABC SG013

5.24. Chronic EtOH tolerance of seven-day old ABC SG013

5.25. Seven-day old ABC SG013 thigmotaxis given 1.5% EtOH

5.26. Seven-day old WIK SG003 thigmotaxis given 1.5% EtOH

5.27. Seven-day old SG018 and AG019 thigmotaxis given 1.5% EtOH

5.28. Thigmotaxis of seven-day old ABC SG013 given AMPH

5.29. Thigmotaxis of seven-day old wild type SG019 given AMPH

5.30. Thigmotaxis of seven-day old tfu mutant SG018 given AMPH

5.31. Light/dark preference results of ABC SG013, SG018 and SG019

1. INTRODUCTION

1.1. Purpose of the study

Dopamine related neurological disorders in human include Parkinson's disease, drug and alcohol addiction, anxiety, depression, and schizophrenia. To understand the molecular and cellular basis of these disorders, it is highly desirable to establish appropriate animal models, especially in organisms that are amenable to genetic study. The overall goal of this laboratory project is to use the zebrafish, *Danio* (*Brachydanio*) *rerio* as a vertebrate genetic model to identify genes and pathways that are important for the etiology as well as pathogenesis of these human disorders. The specific goal of my study was to develop simple and robust behavioral assays that model some aspects of these human disorders. These assays can be used for future genetic screens to reveal the genetic causes of these human disorders Furthermore, having the dopamine-deficient zebrafish mutant, *too few* in hand, these assays could also be used to determine the functional roles of dopaminergic pathways in fish.

1.2. Genetics on traditional model organisms

The classical model systems such as *Saccaromyces cerevisiae* (yeast), *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (fruit fly) and *Mus musculus* (house mouse) have been of great importance to our understanding of biological processes in general (Guo et al., 1999a and references therein). Two genetic approaches, designated forward and reverse, have commonly been used in genetic studies. The goal of the forward genetic approach is to identify new genes by performing random, genome-wide mutagenesis to isolate mutants with specific phenotypes. This method has been successfully applied to invertebrates such as *Drosophila* (Nüsslein-Volhard and Wieschaus, 1980). As a result, many genes involved in development and nervous system function have been identified and characterized. However, many of these genes turned out to be generic (Roush, 1996). One objection against the use of invertebrates as models for humans is that they yield limited information about the development and function of structures unique to vertebrates. The reverse genetic approach involves ablation or overexpression of single genes to study their functions (Malicki et al., 2001). Functions of vertebrate genes, many identified by their homology to *C. elegans* or *Drosophila* counterparts, have been studied in mice using this approach (Capecchi, 1989). It has proven difficult to identify novel genes in vertebrates by forward genetic means. One

reason is that early developmental processes are less accessible in mice because they occur *in utero*. Second, space requirements are high and maintenance and breeding are very expensive (Dooley and Zon, 2000).

1.3. The zebrafish as a model – genetic screens

Over the past decade the zebrafish has emerged as a vertebrate model system for forward genetic studies, although it was recognized as early as 1981 as a system where such approaches would be feasible (Streisinger et al., 1981). The zebrafish is a tropical freshwater teleost originally found in slow streams and rice paddies and in the Ganges River in east India and Burma. No single model is perfect, but the zebrafish has features that make it easy to maintain, manipulate and observe in a laboratory (Driever et al., 1994). Fully grown fishes are small, only 3-5 cm long (fig 1.3). They do well in many environments and can be kept together in large numbers. This makes zebrafish (zf) husbandry economical and in a large zf laboratory the cost is as low as $3 per fish per year. Zf reach sexual maturity at three months and the females can lay 0-200 eggs on a weekly basis. Eggs are fertilized externally they develop rapidly and are completely transparent, making it easy to follow the development of every individual cell. For example one can see cell movements during gastrulation and formation of domains in the brain. At 24 hours post fertilization (hpf), the embryo already looks like a vertebrate (fig.1.1) with a well-structured central nervous system, visible eyes and a beating heart, whereas it takes 21 days in mice. Zf embryos hatch two days after fertilization, and fiveday old fry possess many patterns of behavior; for instance, swimming, feeding and complex escape reactions (Guo et al., 1999a). These characteristics, especially the easy access to a large quantity of transparent embryos, have made zf an excellent model system for studying vertebrate development and as further reviewed, genetic manipulation.

After some groundwork to determine the most effective method to perform large-scale saturation mutagenesis in zf, it became clear that chemical mutagenesis with *N*-ethyl-*N*-nitrosourea (ENU) would be the way of choice (Solnica-Krezel et al., 1994; Mullins et al., 1994). ENU makes it relatively easy to recover point mutations, which are effectively induced by alkylation of oxygen atoms of guanine and thymine in DNA (Knapik, 2000). A milestone in the history of studying zebrafish is the screenings performed by the laboratories of Nüsslein-Volhard in Tübingen, Germany and Driever in Boston, USA (Haffter et al., 1996,

Driever et al., 1996). These screens involved two generations of breeding to drive a recessive mutation to homozygosity (fig 1.2) and reveal its phenotype. G_0 males were mutagenized and

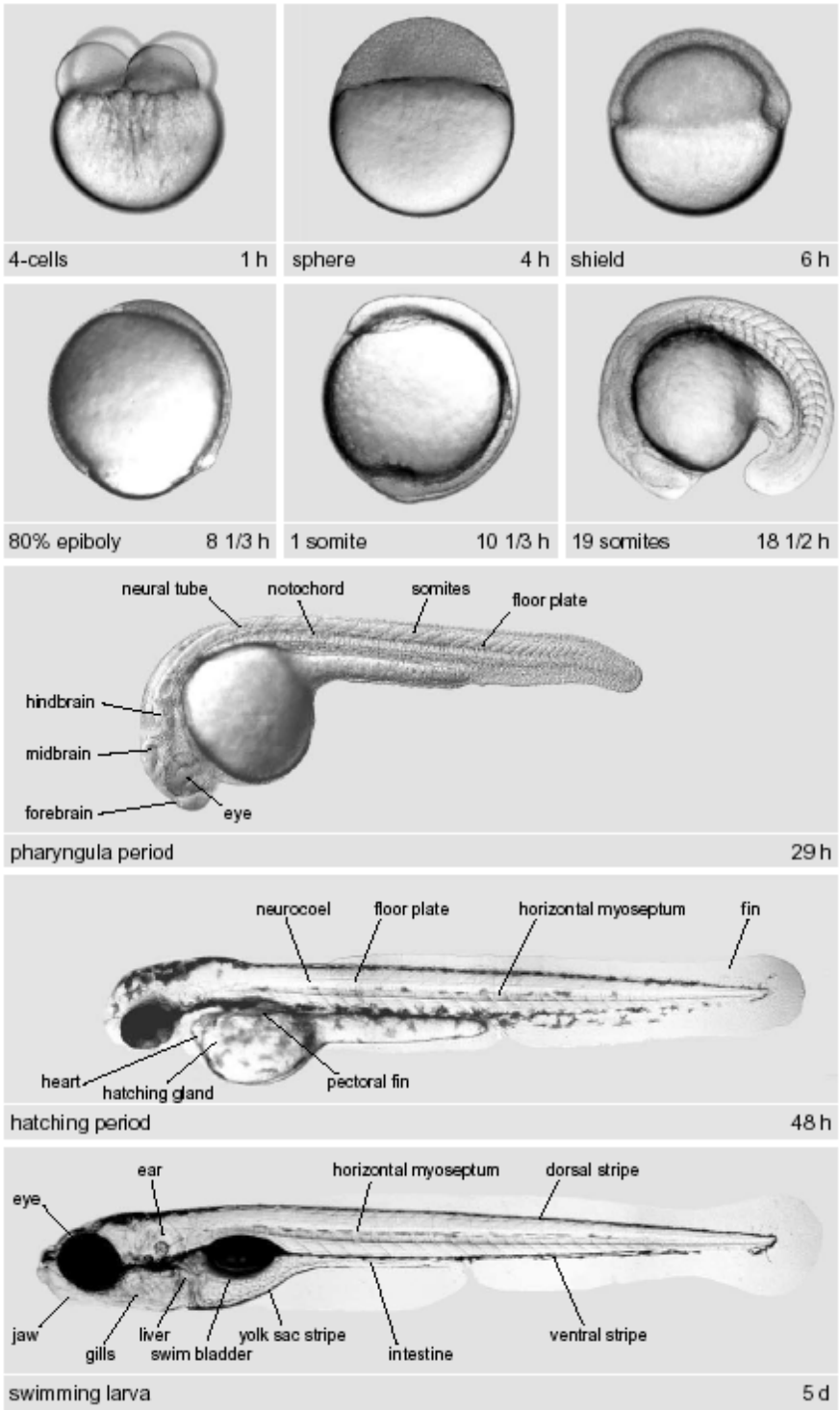

Fig. 1. 1. Embryos and larvae 1hpf to 5dpf. From Haffter et al., 1996.

founder F_1 fish established. From these F_1 founders, F_2 families were raised, half of which would be heterozygous carriers for the induced mutations, and sibling crosses among the F_2s were done to identify mutant phenotypes by visual inspection of the F_3 embryos with a dissecting microscope. Together, these screens resulted in the isolation and initial characterization of almost 2000 mutations affecting many aspects of embryonic development, from development of the embryonic axes, organogenesis and neural development to locomotion behavior (December issue, *Development*, 1996). The approach used for these screens is the dysmorphology method, which requires a visual phenotype. Zf offspring remain optically clear throughout the first 120 hours of development and can be observed hour after hour as the phenotype develops. Coupled with the ease of mutational analysis, this is the primary reason for the success of zf as a model system. However, many mutations are not linked to obvious dysmorphologies, but rather subtle alterations in gene expression or protein function. To deal with these, focused screens can be undertaken (Knapik, 2000). In these screens, a broad range of mutant phenotypes are not sought, but specific questions are asked; for instance, how catecholaminergic (CA) neurons are specified (Guo et al., 1999b). Focused screens can be classified as functional screens or marker-assisted screens. In functional screens, physiological functions of the larvae are looked at, such as abnormal heartbeat similar to human arrhythmias or abnormal behavior responses. For many zf structures and organs, there are only a few cells to look at. To circumvent this difficulty, marker-assisted screens can be done which implies *in situ* hybridization, immunohistochemistry, chemical dyes, or enzymatic reactions. Until now, the majority of these screens have utilized RNA probes that are available for cloned genes. However, the *in situ* procedure is laborious, and to maintain high throughput of marker-assisted screens, highly specific monoclonal antibodies would be an ideal tool. The recent speed with which zf transgenic technology is processing will allow many sorts of genetic manipulations to be done in zf (Fetcho, 1998). Transgenic fish with green fluorescent protein (GFP) as a reporter gene could also be made to visualize specific cells with interest in screens for mutants.

Recently, insertional mutagenesis has also been used to mutagenize zf (Golling et al, 2002). Retroviral vectors are used as mutagen. The virus enters reproductive cells of parent fish and inserts itself into the genome, sometimes disrupting a gene. Insertional mutagenesis is not as effective as chemically induced mutagenesis, but the affected genes are easier to track down, which is an advantage.

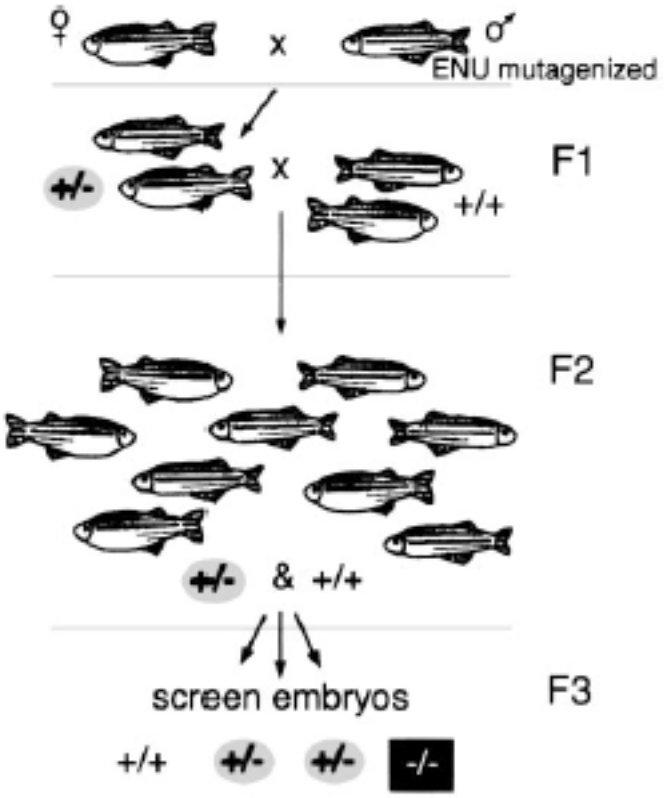

Fig. 1.2. Schematic outline of zf F_2 mutagenesis screens. ENU is used to mutagenize spermatogonia of G_0 males. Crosses are performed with wild type females to produce the F_1 generation, each fish possessing a unique set of mutations. Sibling matings create an F_2 generation, and the mutations are driven to homozygosity in the F_3 embryos. From Warren and Fishman, 1998.

With all these mutations in hand, there is hope that there will be new, unknown genes that will provide a better understanding of both developmental mechanisms, gene functions and disease-related pathways. The final step in mutational analysis is thus the identification of all the involved genes revealed by the mutations (Eisen, 1996). Three strategies for cloning are currently being used: the candidate gene approach, the positional-candidate gene approach and positional cloning (Knapik, 2000). The entire zf genome has been extensively mapped and will be completely sequenced by the end of 2003. A detailed gene map will facilitate the identification of genes disrupted by mutations and can suggest functions for human genes known only by sequence (Postlethwait et al., 1998).

1.4. A model for human disease

Initially, skeptics to the use of zf questioned the legitimacy of using a lowly teleost as a model for man, but the growing realization that all animals develop along remarkably similar lines has done much to undermine this objection (Concordet and Ingham, 1994). Much work has been done at earlier stages in zf development, and comparative embryology has demonstrated the relationship between the development of zf and other vertebrates, which provides a basis for genetic comparisons (Driever et al., 1994). On the other hand, there are clearly species-specific differences, so a full understanding of the mechanisms underlying vertebrate development will only be acquired by complementary studies in a variety of vertebrate species.

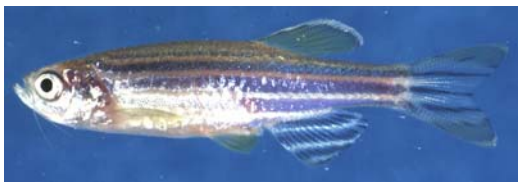

Fig. 1.3. The adult zebrafish.

Many of the zf mutant phenotypes identified in genetic screens are representative of known forms of human genetic diseases, providing a powerful approach for growing insight to the corresponding pathophysiology (Dooley and Zon, 2000). Zf is particularly amenable to the study of hematopoiesis (Amatruda and Zon, 1999). Blood circulation begins at 24hpf and the number and morphology of circulating cells are visible under a microscope. More than 50 mutants with defects in hematopoiesis have been identified, representing all stages of hematopoietic development (Weinstein et al., 1996; Ransom et al., 1996). The validity of using zf as a model for human disease is clearly illustrated by this research. For instance, the *sau* mutant results in a particular kind of anemia. By positional cloning, the mutant gene was found to encode an enzyme (ALAS2) required for the first step in heme biosynthesis. In humans, this mutation results in congenital anemia, and zf is thus the first animal model for this disease. Zf is also particularly amenable to studies of cardiovascular disorders. The heart resembles the human heart and starts beating at 22hpf. Mutations have been recovered that affect various aspects of cardiac development, form and function (Stainier et al., 1996). In addition, numerous other vertebrate-specific and clinically relevant processes are being investigated using zf; for example, kidney diseases, diabetes, blindness, deafness, neural disorders, cancer and addictions (Penberthy et al., 2002).

As mentioned previously, to fully benefit from the vast amount of information that the zf can provide, it is necessary to clone the genes that are responsible for the various phenotypes. As the human zf genome projects as well as those of rat and mouse approach completion, the cloning of the genes responsible for the mutant phenotypes will be much faster. Once a gene is identified, the rapidly expanding repertoire of molecular techniques can be applied to define function and elucidate complex biological pathways such as the genetic etiology of human disease. This deciphering is what Cowley refers to as "physiological genomics" (Cowley, 1999). The success of morpholino antisense technology in zf (Heasman, 2002), which basically means inhibiting gene function by blocking mRNA translation, provides a relatively simple and rapid approach to study gene function and potentially opens the door for modelling almost any inherited developmental defect (Penberthy et al., 2002).

The zf is uniquely positioned to bridge the gap between its vertebrate and invertebrate counterparts in studies in development and genetics (Dooley and Zon, 2000). There is no doubt this small vertebrate will help shed light on clinically relevant disorders and on various aspects of development. The full potential of zf as a model system has only begun to be realized.

1.5. The dopaminergic system in higher vertebrates

The monoamines were the first CNS transmitters to be discovered and comprise dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) (Rang et al., 1999). Many of the currently used psychotropic drugs owe their effects to mechanisms related to these mediators. They are localised in particular neurons or tracts and are associated with high-level behaviors, rather than overall synaptic excitation or inhibition. The synthesis of DA follows the same route as that of adrenaline, as outlined in fig. 1.4, but DAergic neurons lack DA β-hydroxylase (DBH). Dopaminergic neurons form three main systems. About 75% of the dopamine in the brain occurs in the nigrostriatal pathway with cell bodies in the midbrain *substantia nigra*, the axons running through the medial forebrain bundle and terminating in the *corpus striatum*. The second important system is the mesolimbic/mesocortical pathway, whose cell bodies are found in the midbrain *ventral tegmental area* (VTA), with fibers projecting, also via the medial forebrain bundle, to parts of the limbic system including the *nucleus accumbens* (NAc) and the *amygdaloid nucleus* (Am), and to the frontal cortex. Finally, the tuberohypophyseal system is a group of short neurons running from the hypothalamus to the pituitary, the hormone secretions of which they regulate. There are also many local DAergic interneurons in the retina, the olfactory cortex and in the medulla. Very simplified, the nigrostriatal system is involved in motor control, and the mesolimbic/mesocortical pathway is involved in the control of motivation, emotion, cognition and other behavioral effects. In the periphery, DA and DA receptors are present in the kidney, heart and vasculature where mainly sodium homeostasis and cardiovascular function is regulated (Missale et al, 1998). The DA receptors are members of the G-protein coupled receptors and classified as D_1 or D_2, linked to activation and inhibition of adenylate cyclase, respectively. Molecular cloning has identified further subgroups, D_1 and D_5 comprising the D_1 family, and D_2, D_3, D_4 comprising the original D_2 family. In addition to coupling to the second

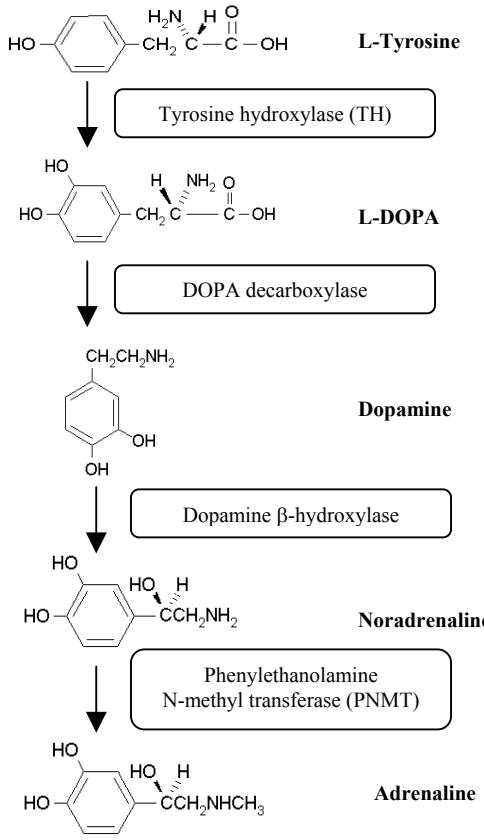

Fig. 1.4. Biosynthesis of catecholamines

messenger adenylate cyclase, they can also be linked to phospholipid hydrolysis as well as control of Ca^{2+} and K^{+} channels, regulation of ion transporters and arachidonic acid release. The receptor subtypes differ in their primary structure and show different affinities for DA agonists and antagonists as well as being expressed in distinct but overlapping areas in the brain. The D_1-class of receptors are most abundant and thought to be expressed postsynaptically to most DA nerve terminals while D_2-like receptors are located both pre- and postsynaptically. The anatomical and functional properties of the individual receptor subtypes have been reviewed extensively (Missale et al., 1998). In the past few years, studies on DA receptor knockout mice have provided a wealth of information about behavioral and molecular phenotypes associated with the inactivation of the individual subtypes of DA receptors (Glickstein and Schmauss, 2001), but there is still a vast amount of questions that remain unanswered

1.6. Human diseases involving dopaminergic pathways

1.6.1. Parkinson's disease (PD)

Parkinson's disease, first described by James Parkinson in 1817, is a chronically progressive neurodegenerative disorder with a prevalence of 1-2% in people over the age of 50 (Shastry, 2001). Fully developed PD comprises motor symptoms such as tremor, rigidity, brady- and hypokinesia. The hallmark of PD is degeneration of the DAergic nigrostriatal neurons and DA deficiency in the striatum, a pathway essential for motor function (fig 1.5). Another important pathological feature in PD is the presence of filamentous, cytoplasmic inclusions called Lewy bodies. Lewy bodies are present in DAergic neurons of the substantia nigra as well as in other

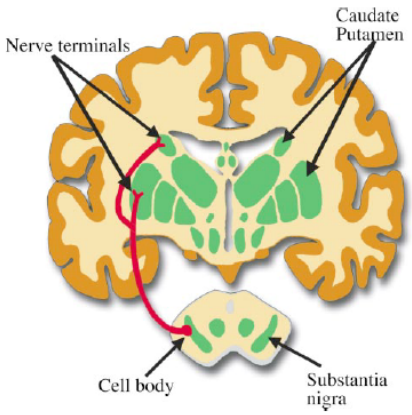

Fig. 1.5. Schematic cross section of the human brain, showing the nigrostriatal DAergic pathway, progressive degeneration of which leads to the major symptoms of PD. The caudate and the putamen constitutes the striatum. From Betarbet et al., 2002.

brain regions such as the cortex. Neuropathological damage to the amygdala, hippocampus, cholinergic cell bodies and other catecholaminergic cells is also common, resulting in a mild deficiency of NA and 5-HT (Hagan et al., 1997). In concert with DA depletion, these changes result in psychological and behavior symptoms including depression, memory and concentration difficulties, and sleep disturbances (Gancher, 2002). No PD cure currently exists, and medical treatment is directed towards alleviating symptoms (Clarke, 2002). Levodopa revolutionized the treatment of PD by relieving symptoms in most patients, largely displacing anticholinergic drugs of limited efficiency. Life expectancy is reduced by PD, and some studies suggest that L-DOPA treatment prolong patient survival by about five years, although this is now controversial (Hagan et al., 1997). Long-term use of L-DOPA is associated with motor complications such as dyskinesias along with a shortened response to each dose and an unpredictable "on-off" effect. A number of other drugs have been used: DA agonists, MAO B inhibitors or catechol-O-methyl transferase inhibitors, either alone or with reduced doses of L-DOPA, in an attempt to delay the onset of motor complications or to control complications once they have developed.

The causes of PD are unknown, but evidence suggests a multifactorial etiology involving genetic and environmental factors such as diet, toxins and exposure to drugs (Shastry, 2001). In rare genetic forms of PD, three proteins have been implicated: α-synuclein, a ubiquitin hydrolase (UCHL 1) and a ubiquitin ligase also known as parkin (Leroy et al., 1998; Kitada et al., 2000; Lucking et al., 2000). Apart from these, mutations in the mitochondrial complex I and mitochondrial t-RNA genes have been described in PD (Grasbon-Frodl et al., 1999). Two of the above proteins, UCHL1 and parkin, are involved in the ubiquitin proteosome pathway of abnormal protein degradation. The proteosome degrades proteins into small fragments and ultimately into amino acids. Thus, it is possible that mutations in the UCHL1 and parkin genes may lead to malfunction of the pathway, and damaged proteins are not degraded (Zhang et al., 2000). Instead, they lead to cell degeneration with an unknown mechanism. The third protein, α-synuclein, is a major component of the Lewy bodies found in PD (Goedert et al., 1998) and may be involved in synaptic function. It is an abundant brain protein localized to the nerve terminals, and it is degraded by the same ubiquitin mediated mechanism and its mutations may promote its aggregation or interfere with its degradation pathway, resulting in its abnormal accumulation.

Typical late onset PD is idiopathic and is likely determined by environmental factors (Langston, 1998). Epidemiological studies have suggested that exposure to household pesticides may contribute to the progressive degeneration of DAergic neurons. This suggestion has been reproduced in rats given rotenone (widely used as a household insecticide) (Betarbet et al., 2000). These animals showed Lewy body-like deposits containing α-synuclein and deterioration of nigrostriatal neurons. Interestingly, another neurotoxin, MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine) also causes irreversible PD-like symptoms in humans (Langston, 1985), and its derivative MPP+, after cytosolic MAO-B oxidation of MPTP, inhibits complex I of the electron transport chain, as does rotenone. Mitochondrial dysfunction, oxidative stress and accumulation of free radicals have been strongly implicated in PD pathogenesis, since oxidative stress related changes have been detected in the brain of PD patients (Jenner, 1998). These include elevated oxidative damage to DNA, proteins and

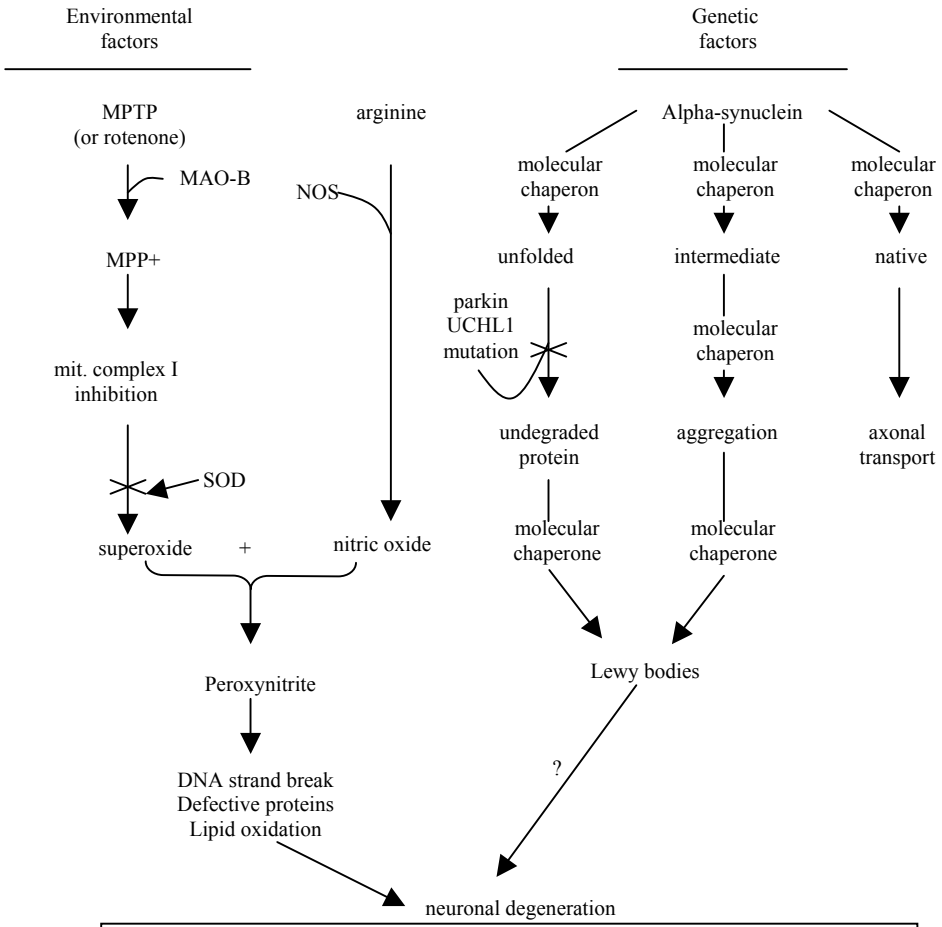

Fig. 1.6. A hypothetical scheme for neuronal degeneration in PD caused by environmental and genetic factors. Adapted from Shastry, 2001.

lipids, decreased levels of reduced glutathione and increased levels of superoxide dismutase (SOD). Reactive oxgen species (ROS) are formed under mitochondrial oxidative phosphorylation and DA metabolism - which produces H_2O_2 as a natural byproduct. Thus, DAergic neurons and their nerve terminals are believed to exist in a constant state of oxidative stress. Glutathione and SOD can to a large extent prevent ROS from damaging cellular and mitochondrial structures. However, partial inhibition of complex I in the electron transport chain, which is the case with MPTP and rotenone, greatly increases ROS production, which may overwhelm the protective mechanisms (Betarbet et al., 2002).

Many animal models have been employed for the study of PD: The reserpine model, the metamphetamine model, the 6-hydroxydopamine model, the MPTP model, the paraquat model, the rotenone model, the 3-nitrotyrosine model, and genetic models which have focused on the use of transgenic mice and *Drosophila*, which express the wild type or mutated α-synuclein (All reviewed by Betarbet et al., 2002). Despite years of research, however, the mechanisms responsible for chronic, progressive degeneration of nigral DAergic neurons remain elusive.

1.6.2. Drug and alcohol addiction

Addiction is increasingly seen as a neuropsychiatric disorder, a chronic, relapsing disease that results from the prolonged effects of drugs on the brain (Leshner, 1997). Drug addiction as defined by the American Psychiatric Association, is: “compulsive, drug-craving, and drug-seeking behavior and drug use, even in the face of negative consequences” (Betz et al., 2000). Prolonged drug use causes pervasive changes at many brain levels, cellular, structural and functional that persist long after the individual stops taking drugs. The addicted brain is distinctly different from the non-addicted brain, as seen by changes in brain metabolic activity, receptor and gene expression, and responsiveness to environmental cues. That is what makes addiction fundamentally a brain disease (Leshner, 1997). The use of drugs has well known and severe negative consequences for the individual as well as for society in general. Mental incapacity, loss of social and occupational function, higher susceptibility for infections such as HIV and hepatitis, over dosage and increased criminal behavior are some consequences associated with drugs. Drug addiction places an enormous burden on society, and time and money has been invested heavily towards a better understanding, treatment and prevention of addiction (Robbins and Everitt, 1999).

At first sight, the drugs of abuse form an extremely heterogenous pharmacological group. For instance heroin, amphetamine/cocaine, ethanol and nicotine influence several different chemical neurotransmitter systems in the brain. What links the drugs is an acute hedonic effect and the desire to repeat the drug administration, an action that reflects a commonality to all addictive drugs: activation of the mesolimbic DAergic pathway (fig.1.7). In 1988, Di Chiara and Imperato showed that drugs such as opiates, ethanol, nicotine, amphetamine and cocaine increases the extracellular DA concentration in the limbic NAc and to a lesser extent in the caudate in rats. These results, together with evidence that came from different sources (reviewed by Wise and Bozarth, 1987) provided biochemical evidence for the hypothesis that stimulation of DA transmission in the limbic system might be a fundamental property of drugs of abuse (Di Chiara and Imperato, 1988). However, many agents such as inhalants,

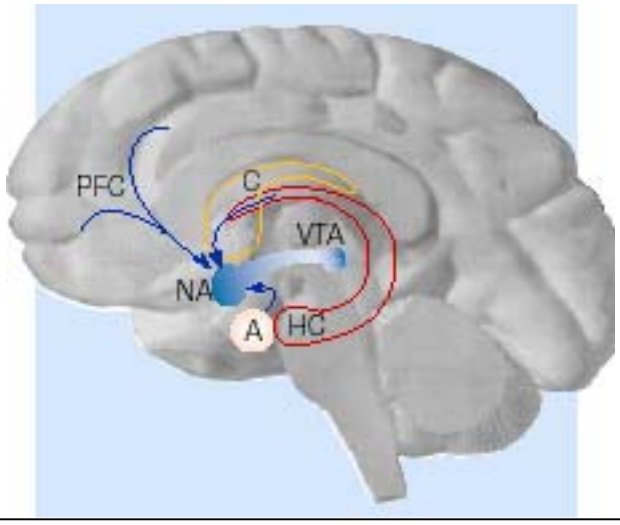

Fig. 1.7. Some of the brain structures affected by addictive drugs. The mesolimbic DA system originates in the VTA of the midbrain , and projects to the nucleus accumbens (here NA). The amygdala (A), hippocampus (HC) and prefrontal cortex (PFC) send excitatory projections to the nucleus accumbens. C is the caudate nucleus and equals the striatum. From Robbins and Everitt, 1999.

barbiturates or benzodiazepines do not mediate DA transmission consistently, despite the fact that these drugs have rewarding properties and are heavily abused (Spanagel and Weiss, 1999). The model is called "the dopamine hypothesis of reward" and is reviewed by Spanagel and Weiss, 1999. According to this model, DA innervation in the NAc is crucial in our reward system, and produces a sense of well-being. The DA system is thought to be activated by natural reinforcing stimuli such as food, water and sex, but also by brain stimulation and drugs. DA antagonists, transgenic or surgical interruption of DA neurotransmission, would attenuate the effects of the reinforcing stimuli. This model is widely

used as a theoretical framework for research and educational purposes, but has some empirical and conceptual problems and is critically reviewed by Salamone et al., 1997. However there is little doubt that the mesolimbic DA system is important in acquisition of reward and drug-seeking behavior. Other monoaminergic nuclei, such as those in the locus coeruleus (NA-containing cell bodies) and raphe (5-HT) are also believed to be important (Nutt, 1996), but multiple transmitters acting in multiple brain regions mediate the full diversity of drug effects. The stimulant drugs cocaine and amphetamine increase the concentration of synaptic monoamines. Cocaine increases DA by blocking the DA transporter (DAT), while amphetamine depletes presynaptic vesicles and reverses DAT in addition (Breiter et al., 1997; Jones et al., 1998). Opioid receptor agonists and THC (tetrahydrocannabinol) increase extracellular DA levels within the NAc by hyperpolarization of gamma-aminobutyric acid (GABA) interneurons in the VTA (Johnson and North, 1992; Tanda et al., 1997). After EtOH ingestion, extracellular DA is increased in a complex interplay between opioid, 5-HT_3, nicotinic acetylcholine, glutamate/NMDA (N-methyl-$_D$-aspartate) and GABA systems (reviewed by Radel and Goldman, 2001).

Table 1.1.

Neurobiological substrates for the acute reinforcing effects of drugs of abuse. From Leshner and Koob, 1998.

Drug of abuse	**Neurotransmitter**	**Sites**
Cocaine and amphetamines	dopamine serotonin	nucleus accumbens amygdala
Opiates	dopamine opioid peptides	ventral tegmental area nucleus accumbens
Nicotine	dopamine opioid peptides	ventral tegmental area nucleus accumbens amygdala
THC	dopamine opioid peptides	ventral tegmental area
Alcohol	dopamine opioid peptides serotonin GABA glutamate	ventral tegmental area nucleus accumbens amygdala

Psychostimulants acutely increase alertness and produce a sense of well-being in humans. In animal studies, the time spent quiescent is reduced while locomotor activity is increased. At higher doses, stereotypes are observed. If drugs are used repeatedly, some acute effects may diminish (tolerance), while others are enhanced (sensitization) (Berke and Hyman, 2000). These terms are defined as rightward and leftward shifts in the dose-response curve, respectively. In response to stimulation, homeostatic compensatory adaptations will act to maintain equilibrium by reducing drug effects. One example is internalization of D_1 receptors that can occur minutes after exposure to amphetamine (Dumartin et al., 1998), and results in a diminished cAMP response to subsequent D_1 stimulation, which is associated with a reduced drug effect. In the absence of drugs these neuronal adaptations are unmasked, and a subset of these may produce symptoms generally opposite to those of the drug, known as the withdrawal effect. Longer lasting changes in neurotransmission can be achieved through altered gene expression. For example, increased expression of dynorphin, which activates κ-opioid receptors on presynaptic DA neurons, causes decreased DA release (Cole et al., 1995) and may contribute to the dysphoria seen during withdrawal (Shippenberg and Rea, 1997). Some drugs give rise to clear physical symptoms, such as hypertension or abdominal cramps after opiate use. All addictive drugs can produce emotional withdrawal symptoms such as dysphoria, although such symptoms are not always observed (Markou and Koob, 1991). The set of withdrawal symptoms produced by a given addictive drug results from multiple homeostatic responses in multiple brain regions and can include differences in ion channels, enzymes and transcription factors (Rang et al., 1999). When drug administration stops, these neural systems gradually return to their normal sensitivity, taking from minutes to weeks, but none appears sufficiently long-lasting to be involved in the persistent tendency of addicted individuals to relapse (Berke and Hyman, 2000).

Berke and Hyman points out that recent investigations of the neurobiology of addiction have emphasized homeostatic adaptations (Koob and Le Moal, 1997; Leshner and Koob, 1998) and propose synaptic plasticity as a possible mechanism to drug addiction. They base this on several lines of evidence. First, rats will choose to spend more time in a location in which they have passively received an injection of addictive drug than in another location paired with saline injection, which is referred to as conditioned place preference (CPP) (Tzschentke, 1998). This has been compared with the fact that relapses among drug-addicted humans also involve associative learning, since relapse often occurs when drug addicts encounter people, places, or other cues associated with prior drug use (Shiffmann et al., 1996).

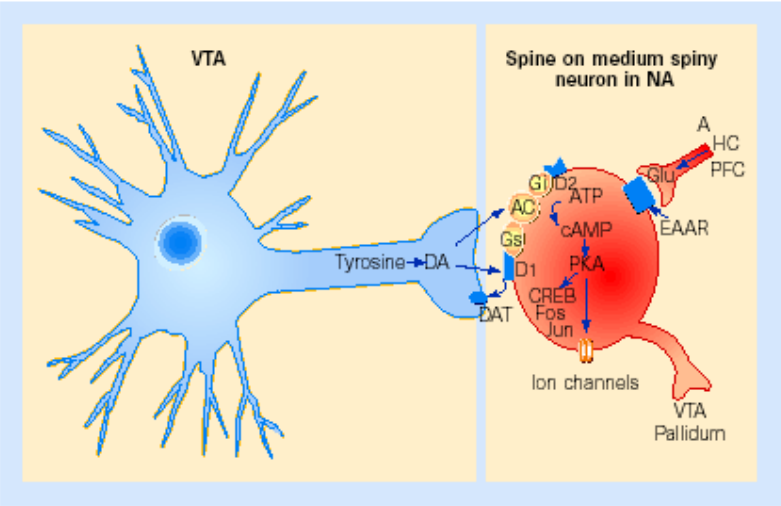

Fig. 1.8. A DA releasing neuron from VTA innervating one in NAc (here NA). Psychstimulants have their main site of action at DAT. DA acts at D_1 and D_2 receptors, which are coupled to G-proteins, components of the intracellular cAMP pathway. It also includes adenylyl cyclase (AC) and protein kinases (PKA). Possible substrates for the kinases include ion channels and nuclear transcription factors. Abbreviations: A, amygdala; HC, hippocampus; PFC, prefrontal cortex; EAAR, excitatory amino acid receptor; glu, glutamate. From Robbins and Everitt, 1999).

They also suggest a sensitization model: the drug *could* have an increased pharmacological effect as a result of an increased number of receptors or strengthening their coupling to effector proteins. Alternatively, an increased behavioral effect could be from the drug acting on neuronal circuits in which there are altered patterns of stored information, resulting from prior associative learning. Many experiments have demonstrated a role for associative learning in psychostimulant sensitization. One example is, if a rat is taken from its home cage to a novel test cage for intermittent AMPH injections, the sensitized locomotor response to a challenge dose is much greater if given in the test cage (purely drug associated environment) than if given in the home cage (Badiani et al., 1995). The conclusion so far is that this context-dependent sensitization and cue-conditioned human relapse suggest that the brain stores specific patterns of drug related information. Furthermore, it is shown that striatal DA assists consolidation of new behaviors. Parkinsonian patients, who are DA deficient, have specific deficits in habit or skill learning (Knowlton, 1996), while intrastriatal injections of psychostimulants can enhance learning of striatum-dependent tasks (Packard et al., 1994).

Consistent with the DA hypothesis of reward, unexpected rewards will transiently increase the firing of DA neurons. Firing remains unchanged by events that are as good as predicted, and they are depressed by events that are worse than predicted (Schultz, 1998). Given these properties, it has been suggested that such changes in DA release may be evoked when the animal's predictions of rewarding events turn out to be inaccurate and that DA is involved in adjusting those predictions. Transient changes in DA levels may correspond to the "error signal" found (Redgrave et al., 1999). Taken all this in hand, Berke and Hyman find it "striking" that D_1 receptors are coupled to the cAMP/PKA/CREB (cAMP response element binding protein) intracellular cascade (Konradi et al., 1994), a pathway implicated in long-term memory formation and synaptic change. D_1 receptors have been shown to have an important role in hippocampal long-term potentiation (LTP), the most influential current model for synaptic plasticity. The persistence of drug addiction may thus reflect the persistence of specific patterns of synaptic connectivity, as is thought to occur for normal memory formation. Berke and Hyman thereby conclude that the ability of addictive drugs to engage synaptic plasticity and thus to alter the functioning of neuronal circuits, is likely to be central to their ability to reinforce and thereby lead to addictive behaviors. However, despite much research on addiction, there undoubtedly still is a long way to go.

1.6.3. Schizophrenia

Schizophrenia is a chronic illness that afflicts approximately 1% of the population, and the disorder itself is characterized by a clinical manifestation of psychosis such as delusions and hallucinations, in addition to thought disorder and negative symptoms (Rang et al., 1999). All this results in a marked deterioration in social, personal and occupational functioning. Clinical symptoms of this neurodevelopmental disorder usually appear in young adulthood. The etiology remains unclear, but involves a combination of genetic and environmental factors, and changes in DA neurotransmission, as well as alterations in serotonergic, glutamatergic and GABAergic systems seem to somehow be involved (Egan and Weinberger, 1997). The hypothesis that the DAergic system is overactive in schizophrenic patients is based on the fact that antipsychotic drugs block DA receptors (Meltzer and Stahl, 1976). The major drawback with typical neuroleptics is adverse effects such as akinesia, muscular rigidty and irreversible tardive dyskinesia. It is believed that the antipsychotic effect is due to block of DA receptors in the mesolimbic/mesocortical system, whereas the side effects are thought to result from their actions on D_2 receptors in the striatum (Deutch et al., 1991). The discovery of atypical antipsychotic drugs, such as clozapine and olanzapine, which have high affinities to D_3 and

D_4 receptors, have made extrapyramidal side effects a smaller problem. Despite being a long-standing focus of psychiatric researchers, animal models have made relatively little impact in the field of schizophrenia, due to the difficulty of inducing and reproducing symptoms such as hallucinations and thought disorder (Kilts, 2001).

1.6.4. Anxiety and depression

Anxiety is an unspecific symptom and is often a reaction that is natural and necessary, but can sometimes be pathological (Holsten, 2001). Clinical conditions related to anxiety include phobic anxiety and panic disorder. In biological terms, anxiety may be regarded as a particular form of behavioral inhibition that occurs in response to novel, non-rewarding or punishing environments (Rang et al., 1999). Anxiety is a subjective human phenomenon and has no apparent counterpart in experimental animals, but behavioral models such as the elevated plus maze, conflict tests and open field test are applied with some success (Rang et al., 1999; Belzung and Griebel, 2001). There is substantial overlap between anxiety and depression, and the biological basis for both disorders are largely common, though not much is really known. The monoamine hypothesis has been proposed as a model for the causes of affective disorders, in which especially NA and 5-HT have central roles (Rang et al. 1999).

1.6.5. Attention deficit hyperactivity disorder (ADHD)

ADHD is an early onset, clinically heterogenous disorder of inattention, hyperactivity and impulsivity. Not much is known about the etiology, but studies have suggested both genetic and environmental causes that modify the developing brain, leading to structural and functional abnormalities (Faraone and Biederman, 1998 and references therein). Much data implicate frontolimbic brain dysfunction, parts that control attention and motor behavior. Molecular genetic studies have suggested that alterations in the D_2 receptor, D_4 receptor and DAT genes may increase susceptibility to ADHD. Psychostimulant drugs are used as symptomatic treatment.

1.7. The zebrafish dopaminergic system and the too few (tfu) mutant

In 1999, Guo et al. performed a systematic mutagenesis screen in zf in order to find new genes responsible for neuron development and differentiation (Guo et al., 1999b). They searched for subtle and specific mutations that change the number, morphology or identity of catecholaminergic (CA) neurons by using antibodies against different CA-synthesizing enzymes. In the developing zf, DAergic neurons were detected in the forebrain with the major populations located in the hypothalamus (HY) and minor groups in the telencephalon (olfactory bulb) and retina. The first few DAergic neurons were found in HY, as early as 1dpf. As the fish becomes a few days older, the TH staining intensifies and DA neurons are organized into discrete clusters (fig. 1.9). These results are consistent with findings in other teleost fishes such as the goldfish (Hornby and Piekut, 1990). While the retinal and the

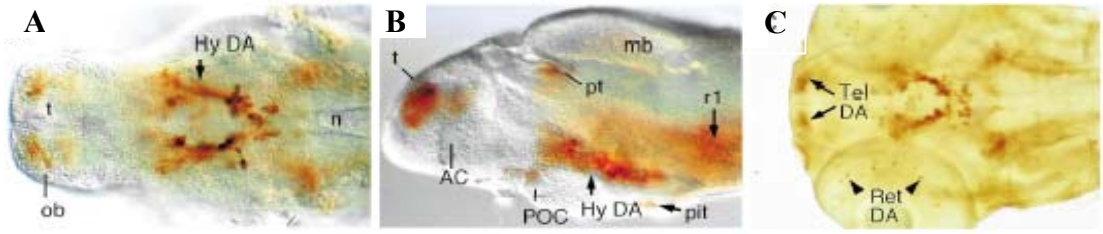

Fig. 1.9. CAergic neurons in the brain of zf embryos/larva stained with TH antibodies. Rostral to the left. (A) Ventral view of a 72hpf embryo, (B) lateral view of a 96 hpf fry, and (C) ventral view of a 72 hpf embryo embryo where the retinal DAergic neurons are visible. Abbreviations: AC, anterior commisure; mb, midbrain; n, notochord; ob, olfactory bulb; pit, pituitary; POC, postoptic commisure; pt, pretectum; ret, retina; r1, rhombomere 1 (locus coeruleus); t(el) telencephalon. From Guo et al., 1999b.

olfactory DA cells are conserved among all vertebrates, the HY and midbrain DA cells show variations among vertebrate species. In mammals and birds DAergic neurons develop in the midbrain as well as the forebrain, but only forebrain DA neurons are found in teleosts, including the zf (Manso et al, 1993; Guo et al., 1999b). Some of these DA neurons may be functionally analogous to the mammalian midbrain substantia nigra and VTA neurons in mammals (Puelles and Verney, 1998). The findings that ablation of DAergic neurons by MPTP treatment in goldfish (Poli et al, 1990; Pollard et al., 1992) and zf (Bretaud and Guo, unpublished data) leads to bradykinesia, is consistent with this hypothesis. Guo et al. (1999b) found mutations at five genetic loci, affecting DAergic neurons, four of which were embryonic lethal. One of the five, which was named *too few* (tfu), appeared to affect DAergic neurons specifically and have fewer DAergic HY neurons than wild type (wt) siblings (fig.

1.10). The remaining HY neurons in *tfu* embryos express TH at a normal level and seem to have normal morphology. In a 5dpf larva there are only 10-30% HY DA neurons compared to the wt sibling. Despite these defects, the mutant embryo and fry are indistinguishable from the wt, and most of them develop to adulthood.

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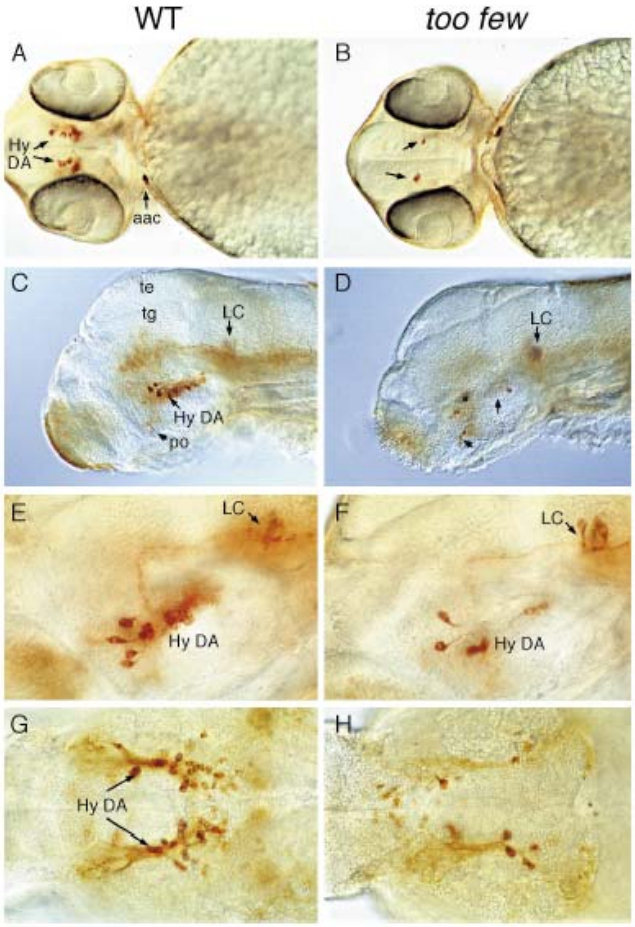

Fig. 1.10. TH antibody staining of the *too few* (*tfu*) mutant compared to wt sibling. (A-F) Two days old embryos showing a reduced number of HY DA neurons. (G-H) 4dpf fry showing that the reduction persists, so the deficits are not due to a delay in neuronal development. (A, B and G,H) Ventral views and (C-F) lateral views. Abbreviations: aac, arch assiciated catecholaminergic cells; LC, Locus coeruleus; po, postoptic region; te, tectum; tg, tegmentum. From Guo et al., 1999b.

2. BEHAVIOR ASSAYS AND RESULTS

2.1. Outline of the study

As stated in chapter 1.1, the specific goal of my project was to develop behavior assays that model some aspects of the human disorders in question and could be subsequently used in genetic screens. It was determined that seven-day old zf fry should be examined primarily, since they are easily obtained in large numbers, are already free-living and are able to escape from predators as described earlier and have developed an intricate DAergic nervous system. All these features make the juvenile zf a good candidate for high throughput genetic as well as drug screens.

The major characteristic of PD is bradykinesia. Some addictive drugs such as ethanol (EtOH), amphetamine (AMPH), cocaine, morphine and nicotine induce hyper locomotor activity in rodents (O'Neill and Shaw, 1999; Miller et al., 2001; Vetulani et al., 2001), and this locomotor stimulation is used as an animal model of human euphoria (although conditioned place preference experiments are more widely applied for such studies). Hence, measuring zf fish swim speeds is a potential approach for modeling aspects of PD and drug addiction including tolerance and sensitization, as well as a potential way to screen for mutants with altered swim properties.

After establishing a method to quantitatively measure locomotion and raising zf fry, the next step was to examine fry treated with different drugs. In addition, the effect of drugs on the *tfu* mutant, which has a reduction in brain DA neurons, was assessed. Most experiments were repeated multiple times to obtain a large sample size and be able to evaluate the significance of the findings. During the experiments, secondary behaviors to swim speed were observed, which led to development of an open field assay and a light/dark preference assay. In addition, pigment responses were noticed and photographed.

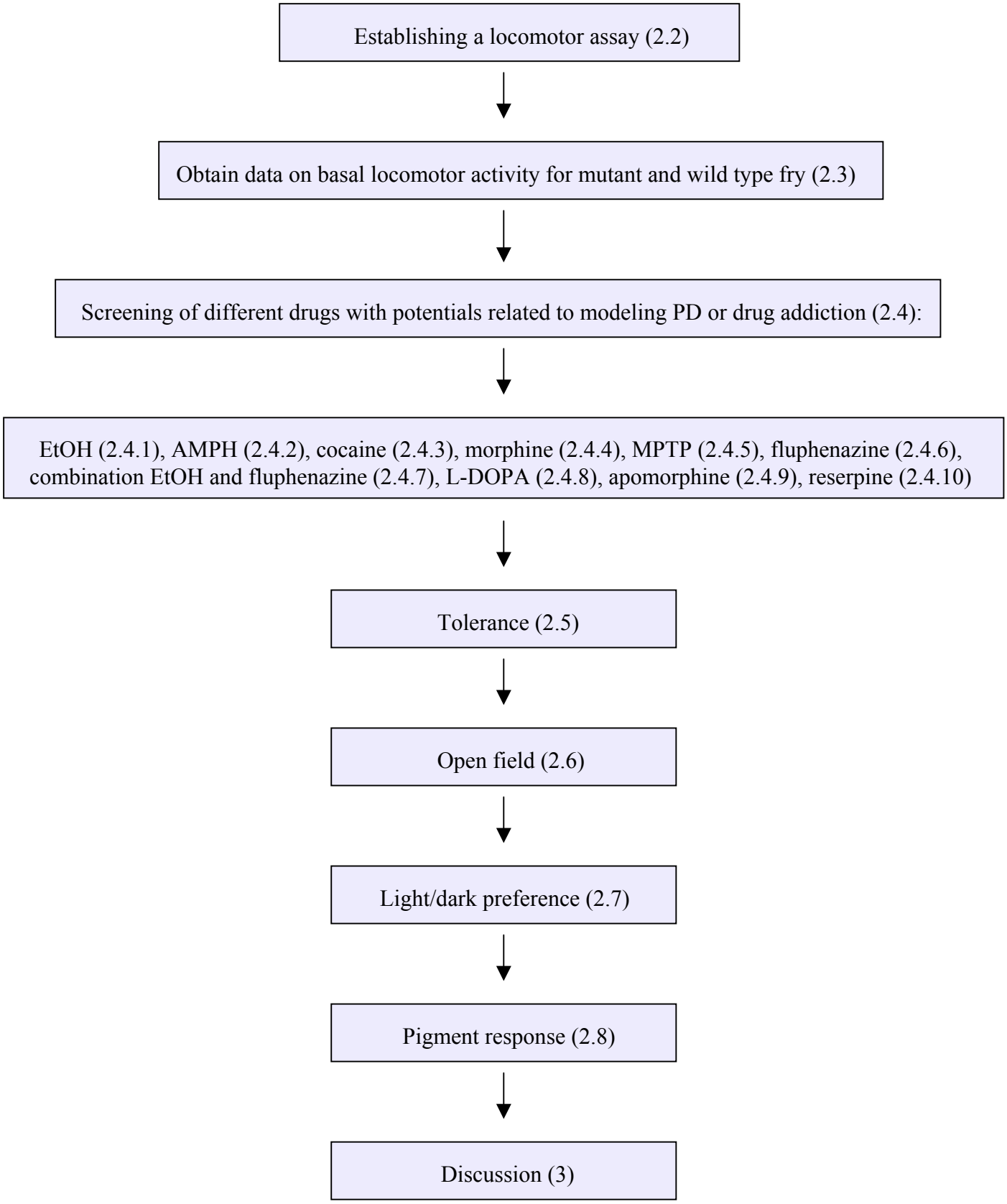

Fig. 2.1. Flowchart of the study

2.2. Establishing a locomotor assay – materials and methods

2.2.1. Video record setup

As mentioned in section 2.1, we wanted to develop an assay in which fry locomotor activity could be quantified. One way of accomplishing this is to count the number of times an animal crosses given borders in its environment. However, earlier in this laboratory, adult zf swim speeds have been successfully obtained by recording the fish in a tank with subsequent analysis of the resulting video, suggesting that the same approach may be feasible for fry as well. After considering the size of seven-day old fry, approximately 5mm long, an appropriate plexi glass view tray (Aladin Enterprises, Inc.) was found in which experiments on 10 fry could take place simultaneously. Internal measures were 6.0 x 8.0cm with a height of 2.0cm. Upon adding 20mL water, the fry was able to swim freely around in a more or less two-dimensional manner. A Sony handycam DCR-TRV 900 digital video camera recorder was placed on a tripod (see fig. 2.1) and connected to a Macintosh® G4 computer. Using the software Adobe Premiere® 5.1, it was possible to capture movies of the fry activity in the view tray and save it on the computer hard disk. The recording frame rate was 10 per second.

Fig. 2.1. The video record setup, including the plexi glass view tray on a light box. All housed in a large cardboard box in order to avoid the fry getting disturbed by external activities.

2.2.2. Data analysis

Having fish movies on the hard disk, application of the Dynamic image analysis system, DIAS® 3.1 (Solltec Inc., Oakdale, Iowa) enabled me to track the individual fish in the view tray and thereby calculating their speed. Due to pharmacokinetic reasons, it is obvious that the fry locomotor activity will change with time, at least for drugs that acutely affect it. Consequently, I decided to measure the swim speeds at several different time points during a

20-minute period. 20 minutes was suggested to be sufficient since zf fry are highly permeable to exogenous chemicals (Gerlai et al, 2000). After initial experiments, it was decided to measure the mean swim speed at 0, 1, 3, 5, 7, 10, 13, 16 and 20 minutes. Initially, 60 seconds of the movie was analyzed for each time point, but this approach turned out to be extremely time-consuming. Subsequent analysis of 20-second movie clips for each time point introduced too much variation compared to the 60-second clips, resulting in a very "sensitive" speed curve. 30-second movie clips became the choice because of the close resemblance to the 60-second clip results (data not shown). Minute 0 equals the first 30 seconds of the movie, minute 1 the time from 1:00 to 1:30 and so on, except for minute 20, which is the time from 19:30 to 20:00. The first analyzing step in the DIAS® software was thus to split up the Adobe Premiere® movie into distinct clips corresponding to the interesting parts and save them as nine small DIAS® movies. Subsequently, each fry in each movie was traced to obtain swimming paths. Successful DIAS® tracing on these small, transparent animals can be a difficult task and demands careful quality assurance. The right illumination to get the right contrast, enabling DIAS® to "see" and then trace the fry is crucial in obtaining valid results. Appropriate conditions would not have been achieved without a light box on which the view tray was placed (fig. 2.1). The camera settings, like program (AEA) and exposure (F11), are important. Optimal settings were found after some trying and failing. Furthermore the camera illumination had to be adjusted for each experimental day and the accurate position of the view tray for each recording. However, after obtaining the swim paths, DIAS® was able to calculate swim speeds in a new step. The exported file became readable to Excel® (Microsoft office® 2001). Employing macros made by Kayta Kobayashi, I was able to obtain the mean swim speed and some other features of the 10 fry for one specific time point. To make an Excel® chart showing swim speeds as a function of time, the procedure was repeated for each time point. As several repetitions were done for the same experiments, Excel® was also used to calculate the averages, standard deviations and errors in addition to making composite curves. Stata® 6.0 was employed for statistical analysis of the data, which applies two-sample, two-sided student's *t* tests with equal variances. The significance level was set to be 5% on all analysis.

2.2.3. The experimental animals and variability: developing a protocol

Zf were maintained according to well-established protocols (Westerfield, 1993). Adult fish of different genetic backgrounds or carrying the *tfu* mutation were crossed to obtain embryos. The progeny was kept in blue egg water in petri dishes in a 28°C incubator without a set light/dark circle until seven days old and mostly used for experiments at that time. Fry, on which experimental procedures were to be done at day 14, were transferred to 2-liter tanks containing fry water on day seven, kept in a 28°C room with a 14/10 hours light/dark circle and fed FryFeedKyowaA (Kyowa Co., Ltd., Japan) twice daily.

Table 2.1. Contents of egg water, blue egg water and fry water

	Egg water	Blue egg water	Fry water
$CaSO_4$ (EM® Science)	0.12g	0.12g	-
Instant ocean salt (Aquarium systems®)	0.20g	0.20g	3.0g
Methylene blue (LabChem® Inc.)	-	20µL 2% sol.	-
Millipore water (Nanopure Diamond, Barnstead®)	Ad 1.0L	Ad 1.0L	Ad 1.0L

Initially, on day seven, the experimental day, petridishes were left on the laboratory bench in the morning, fry were picked up with a Samco® plastic transfer pipette and transferred to egg water in a 50mL Falcon® tube with subsequent addition of drug and egg water, alternatively only egg water to a final volume of 20mL prior to emptying it all into the view tray for recording right away. This approach led to variability in drug response. Since behavioral assays are in general extremely sensitive to environmental factors, the most likely reason for the reproducibility issues was the protocol, which needed a higher degree of standardization. Some sources to the variation might have been the amount of egg water and fry in each dish during development. Also abnormally developing fry, eggshells and pollutants need to be removed from the dish to maintain a healthy environment. Furthermore, temperature changes in the water, changing from 28°C in the incubator to room temperature to 25°C shortly after placing the view tray on the light box might have been another reason. The physical handling with the pipette, the time they spent in the cylindrical Falcon® tube before recording, and how they were emptied into the view tray might have affected them. In addition, it may matter at what time of the day the experiments are done, due to the effect of circadian rhythms on locomotor activity. In addition, I asked the following questions: Do they need a specific

light/dark circle? Were equal amounts of drug added each time? Are there any genetic differences between the fish that can account for the response differences? As partly mentioned in section 2.2.2, the computer software/video record setup has its weaknesses. Can these be recognized and to some degree controlled? These are the most important issues concerning the reproducibility issue, and a series of recordings were undertaken with placebo and EtOH under several different experimental conditions. As a result, I ended up with a stricter protocol that led to a higher degree of standardization, and that hopefully led to less variability. Still, variability has been considerable, meaning in most cases that several repetitions of an experiment needed to be done to obtain significant results.

2.2.4. Protocol for experiments on seven-day old fry

After crossing and obtaining eggs on day zero, 25 mL blue egg water was measured up in a suitable amount of petridishes. ≈ 50 eggs, using a Fisherbrand® 5¾" glass pipette were then transferred to each petridish. The dishes were marked for the identity of fry and day of birth and put in a 28°C incubator. Every day, each dish was controlled; making sure the embryos/fry lived in a healthy environment by removing all individuals developing abnormally, eggshells and other pollutants. On day seven, "weeding" was done, and each petridish placed on a white surface on the lab bench for 1½-2 hours before recording the first group of 10 fry from the dish in question. The video camera was set up, adjusted and a short test with an accompanying DIAS®-trace was done to ascertain that good camera settings were found. The view tray was thoroughly cleaned, especially the edges and corners, with Kimberly-Clark® Delicate task wipers. 15.0mL egg water was measured up in a 50mL Falcon® tube and poured into the view tray. Then 10 fry were transferred with a glass pipette to the tray, letting the fry swim in to avoid volume changes. It was placed on the light box for 5 minutes, letting the fry habituate to the new, illuminated environment. The applicable drug or placebo solution was mixed with egg water to a total of 5.0 mL in the Flacon® tube. This solution was gently added to the view tray from one short end to the other and the view tray tilted gently five times in order to get a uniform drug solution without upsetting the fry too much. The experimental subjects were recorded for 20 minutes and the resulting movie saved for analysis.

2.3. Basal loco motor activity of different wt fry and the tfu mutant

2.3.1. Fish backgrounds

There are several different zf wt strains (http://zfin.org), each possessing different characteristics. The fish used in these experiments originate from three different backgrounds. The EK line is originally from Ekkwill Breeders in Florida and has a mixed background. The AB line comes from the A and the B strain bought in a pet shop in Oregon and is more or less inbred in a complicated manner since the 1970's. The ABC strain is descended from AB. The WIK strain comes from Germany, but was caught wild in India. Other strains have been obtained on expeditions, or from fish dealers in, for instance Hong Kong, Singapore and Indonesia. The *tfu* mutant and its wt siblings have a mixed AB/EK/WIK background.

Table 2.2. Summary of fish backgrounds

Fish	Background
SG003, WIK (wt)	WIK x WIK
SG013, ABC (wt) *	AB x AB
SG018, *too few* homozygote mutant *	AB/EK x AB/EK/WIK
SG019, *too few* wt sibling *	AB/EK x AB/EK
SG023, *too few* wt sibling	AB/EK x AB/EK
SG026, *too few* wt sibling	AB/EK x AB/EK
SG035, ABC (wt)	Progeny of SG013
*Tfu*1x47(wt) x *tfu*1x45(wt)	EK x EK

* These are the fish, the progeny of which was mostly applied in the experiments

Footnote: When fish is referred to as SG0XX, it is the progeny of SG0XX it is pointed at.

2.3.2. Protocol

A protocol similar to the one described in section 2.2.4 was applied, except for the progeny of *tfu*1x47 x *tfu*1x45, which was treated like initially described in section 2.2.3. This distinction is a very plausible explanation for the difference seen compared to the other wt fry during the first minutes, since that treatment is slightly rougher on the fry. WIK was recorded for 30 instead of 20 minutes on some occasions.

2.3.3. Results

WIK fry show a higher basal locomotor activity than AB and AB/EK fry and is significantly faster than SG013 at the 5.0% level of significance starting at minute 5 (fig 2.2). Sample sizes of SG023 and SG035 are small, 4 and 3 respectively, but should resemble the curves of SG019 and SG013, which have large sample sizes. One of the data sets for SG035 show particularly high speeds, contributing to the discrepancy to SG013. However, there is far from sufficient statistical evidence to claim that SG023 and SG035 are significantly different from SG019 and SG013 respectively. Thus, the conclusion so far is that all fry with an AB or AB/EK background swim equally fast.

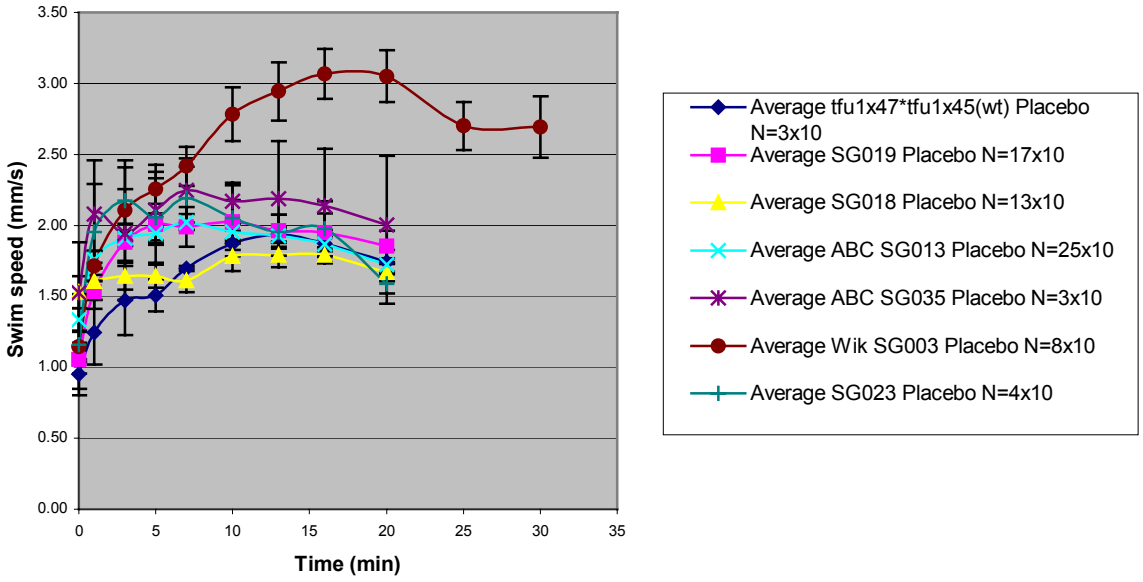

Fig. 2.2. Basal locomotor activity of seven-day old fry for different genetic backgrounds. The WIK fry have a much higher basal locomotor activity than AB or AB/EK fry. N=17x10 means 17 experiments where 10 fry were recorded each time. Error bars are ± standard error (SE).

Focusing on the fry with larger sample sizes, *too few* wild type (SG019) and ABC (SG013) have equal basal locomotor activities, resulting in significance probabilities close to 1 for almost the whole 20-minute period (fig. 2.3). Interestingly, the DA-deficient *tfu* homozygous mutant swims slower than its wt sibling during a large part of the first 10 minutes, but the difference is only significant at the 5% level, at minute 5 and 7, p-values equaling 0.048 and 0.038 respectively. Employing the protocol initially described in section 2.2.3, which is a little rougher to the fry, no difference was observed (data not shown). The sample sizes were also smaller.

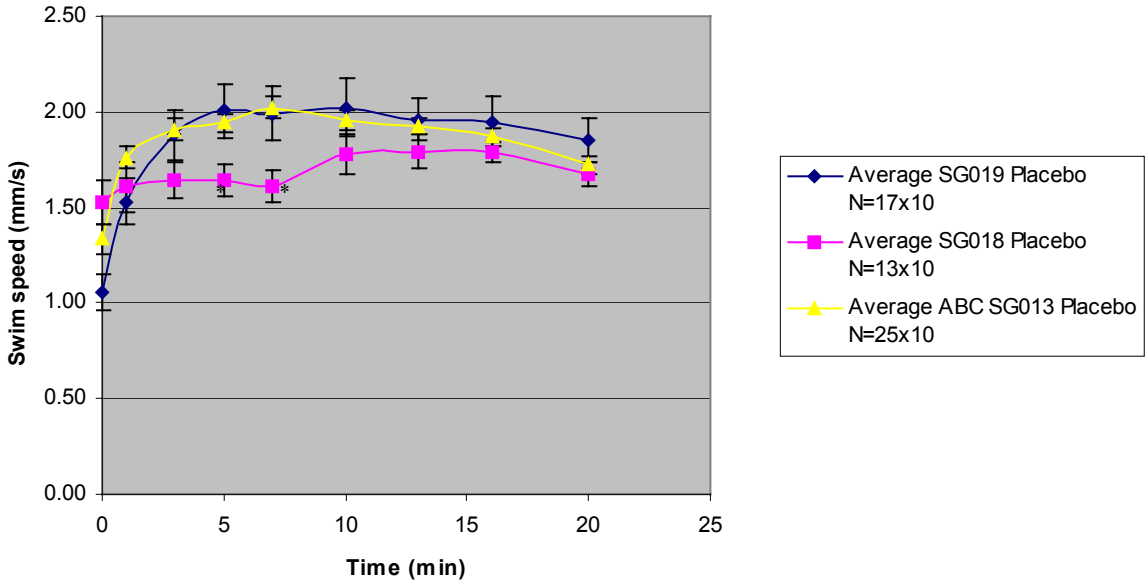

Fig. 2.3. Mutant basal locomotor activity compared to wild type. The *tfu* mutant is initially slower than sibling wt fry, while ABC and SG019 exhibit similar basal locomotor activity.

2.4. Screening of potentially interesting drugs

2.4.1. Ethanol

The protocol is as described in section 2.2.4, but to make it complete, Gold shield® ethyl alcohol (Gold shield chemical co.) stored in a -20°C refrigerator was taken out in an appropriate amount and transferred to a 15mL Falcon® tube with a screw cap and put on the laboratory bench to adjust to RT before use. The applicable volume was taken out with a p200 or p1000 pipette (Pipetman, Gilson®), for instance, 300µL for a final solution of 1.5% (V/V) EtOH in 20.0mL egg water. The cap was screwed back on, and the alcohol was pipetted into egg water in the 50mL Falcon® tube to a total of 5.0mL. Shortly after, this drug solution was added to the view tray on the light box containing 15.0mL egg water, and the fry recorded as described in section 2.2.4.

Results:

1.5% EtOH induces hyperlocomotor activity in seven-day old fry, different genetic backgrounds showing different sensitivities (fig. 2.4). SG019 shows approximately a ½-fold increase in activity, while ABC shows an impressive 3-fold change, despite similar basal swim speeds. Again, the SG035 curve would be expected to superimpose the SG013 curve but does not. The SG035 sample size is relatively small, and statistically there is far from

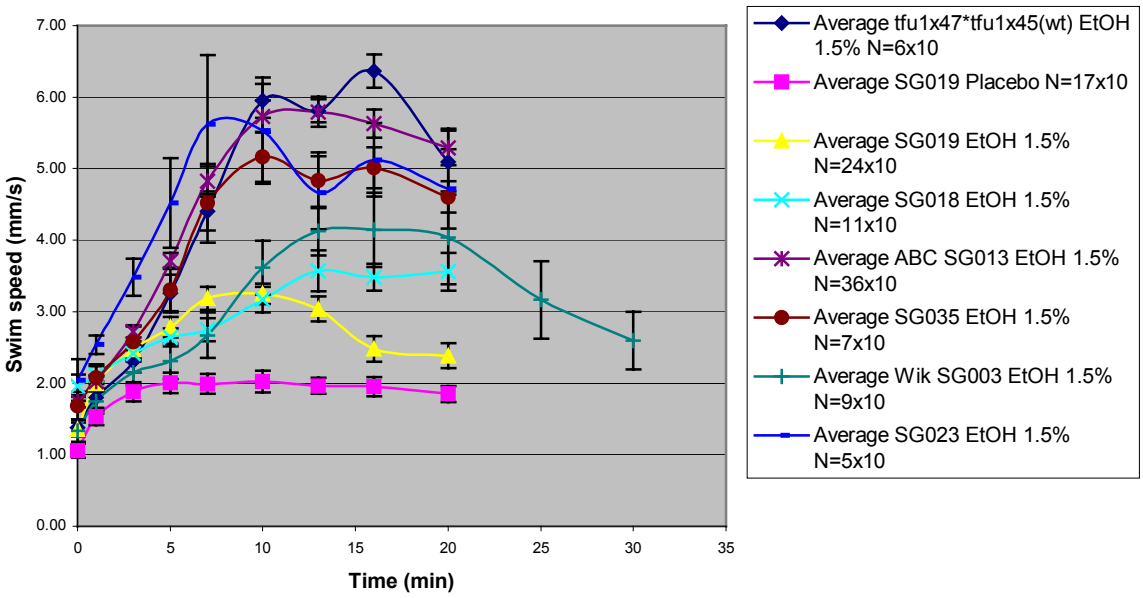

Fig. 2.4. Swimming in a 1.5% (V/V) ethanol solution induces hyperactivity in seven-day old fry. Different genetic backgrounds show different susceptibilities to the alcohol effect. The bottom SG019 curve shows a typical placebo curve for comparison.

enough evidence to claim that the two curves are different. The few SG035 experiments were mostly done during summer when the general tendency suggested a lower EtOH response, and is probably the reason for the discrepancy. It is harder, however, to explain the consistent, big difference between SG019 and SG023. The *tfu* mutant does not show a dramatically increased locomotor activity and is in general similar to its wild type sibling SG019, though there are some differences. The mutant is also this time somewhat slower in the beginning, however, not significantly. At minute 16 and 20 the picture changes, and *tfu* swims significantly faster than SG019 at minute 16 and 20. (p-values are 0.002 and 0.001, respectively). Making a chart of the relative speed increase (fig. 2.5), there is no big difference between SG018 and SG019 the first 10 minutes, but SG018 swims faster compared to the basal activity during the latter part.

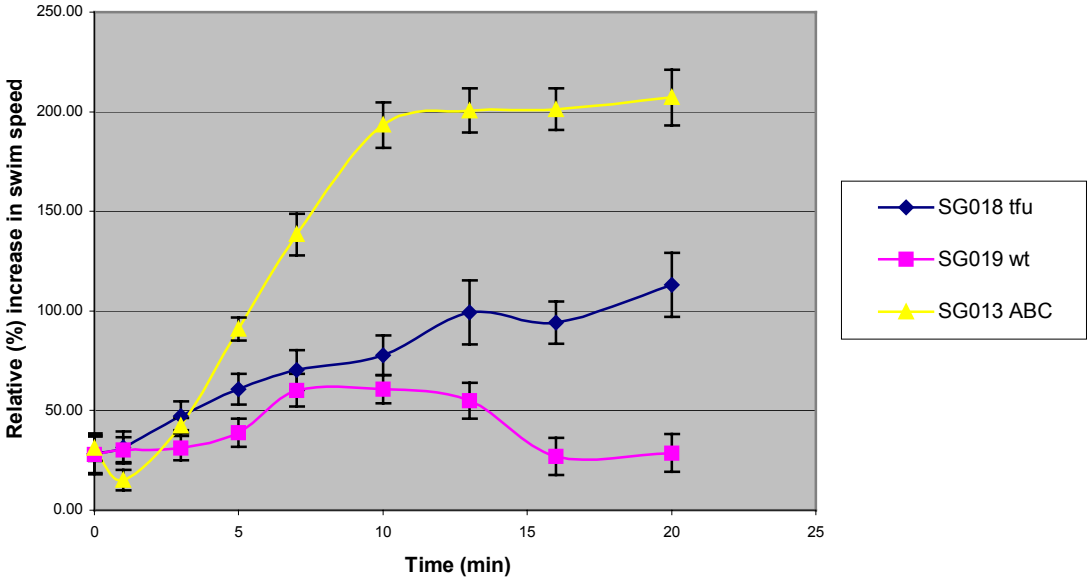

Fig. 2.5. Relative speed increase for SG013, SG018 and SG019 given 1.5% EtOH.

Treating ABC SG013 fry with several different alcohol concentrations, the "time-response" curves in fig. 2.6 are obtained. These curves suggest that there is a particular blood concentration that leads to the highest hyperactivity. Somewhat surprisingly, 0.5% EtOH does not affect the fry much acutely while 1.0% slowly induces movement to a significant extent. 1.5% is the optimal of the doses investigated and is used for many of the later experiments. The higher dose curves increasingly exhibit a small leftward shift earlier and earlier in the 20-minute period, however, clearly after a short while the fry get overwhelmed by the high concentration and swim slower as the sedative or intoxicating effect appears. After a while in 4.0% or at the end of a 3.0%-trial the fry stand still for all practical purposes. Some lose control and tilt forward or in extreme cases start floating around. One peculiar hallmark that was often observed during the individual experiments is the ability to recover for a short period after an initial knockout, being challenged with a high EtOH dose, visualized at minute 10 and 7 for 3.0 and 4.0% respectively. Upon transfer from EtOH solution to egg water, the fry recovered within a few minutes.

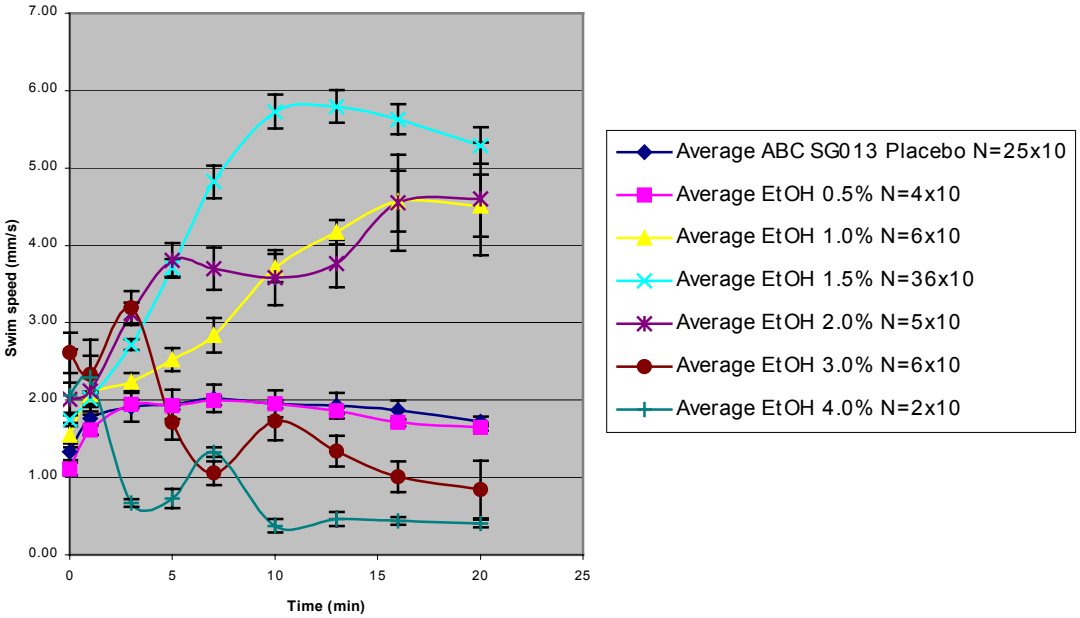

Fig. 2.6. ABC fry given increasingly higher EtOH doses. 1.5% (V/V) induces the greatest hyperactivity response, while higher doses cause hypoactivity.

The same experiment was done on SG019 (fig. 2.7) and SG018 *tfu* fry (fig. 2.8). As for 1.5%, SG019 show a weaker response to the locomotor stimulant effect of other concentrations as well, compared to SG013.

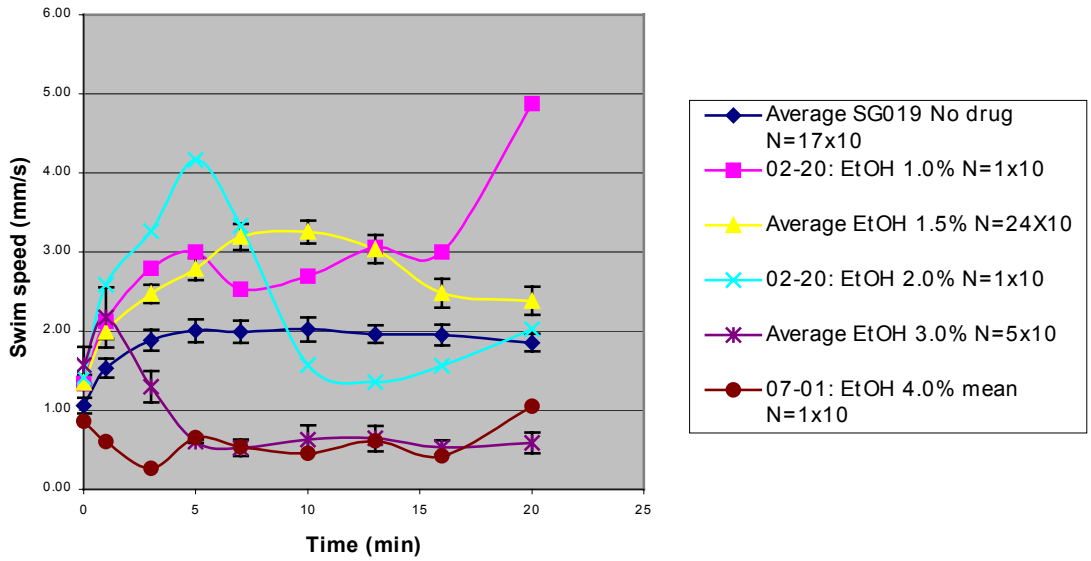

Fig. 2.7. SG019 Seven-day old fry treated with different EtOH doses. As expected, the same pattern as with ABC fry is seen, but the hyperactivity response is much smaller. SG019 appear more sensitive to the high alcohol doses and are more easily sedated. Notice that for 1.0, 2.0 and 4.0% the sample size is only 10 fry, resulting in huge error bars (not shown). However the curves give an idea of the drug response.

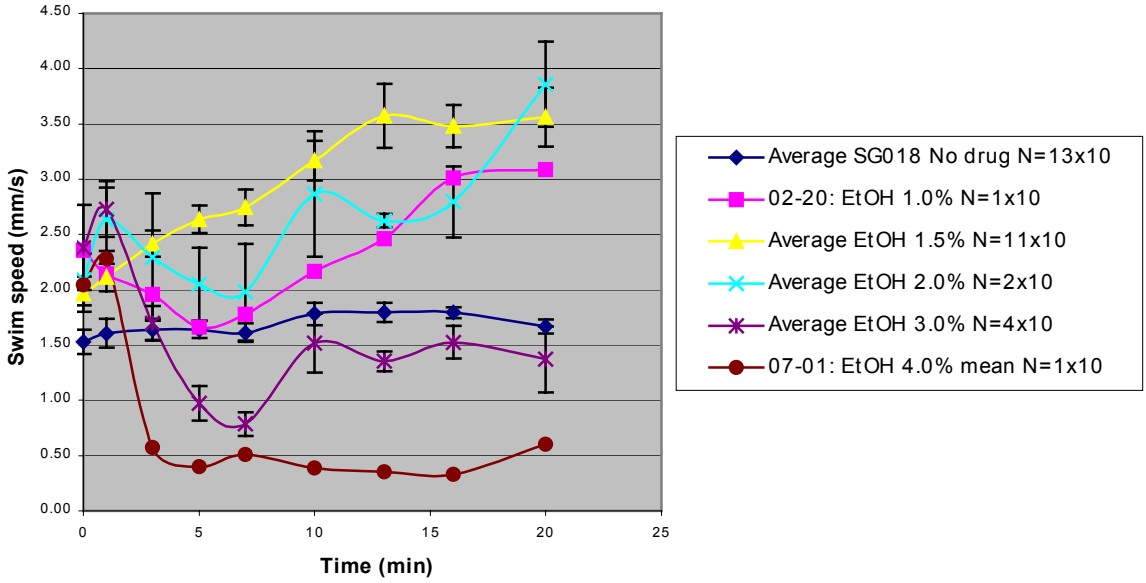

Fig. 2.8. Various EtOH concentrations on *tfu* mutant fry. Again, some of the sample sizes are small, but still the curves give some idea of the fry locomotor activity regarding the dose in question.

Comparing fry after the 3.0% challenge (fig. 2.9), ABC as mentioned earlier, gets significantly hyperactive in the beginning with a gradual decline in speed to swimming significantly slower than the control after minute 10 (p-values approaching zero). SG019 appears more sensitive to the sedative/intoxicating effect and after a short period of hyperactivity around minute one (p=0.046), these fry become severely hypoactive after five minutes. –They almost do not move at all, with corresponding p-values very close to zero compared to the placebo control. The *tfu* mutant also shows initial hyperactivity and subsequent hypo activity, but to a much lesser extent than SG019. In fact, 3.0% EtOH on SG018 is only statistically different from placebo at minute 5, 7 and 13 at the 5% level of significance, and resembles more the shape of the ABC 3.0% curve. Even the ABC fry seem to loose the battle against alcohol before the mutant does, even though the differences are not significant at minute 16 and 20.

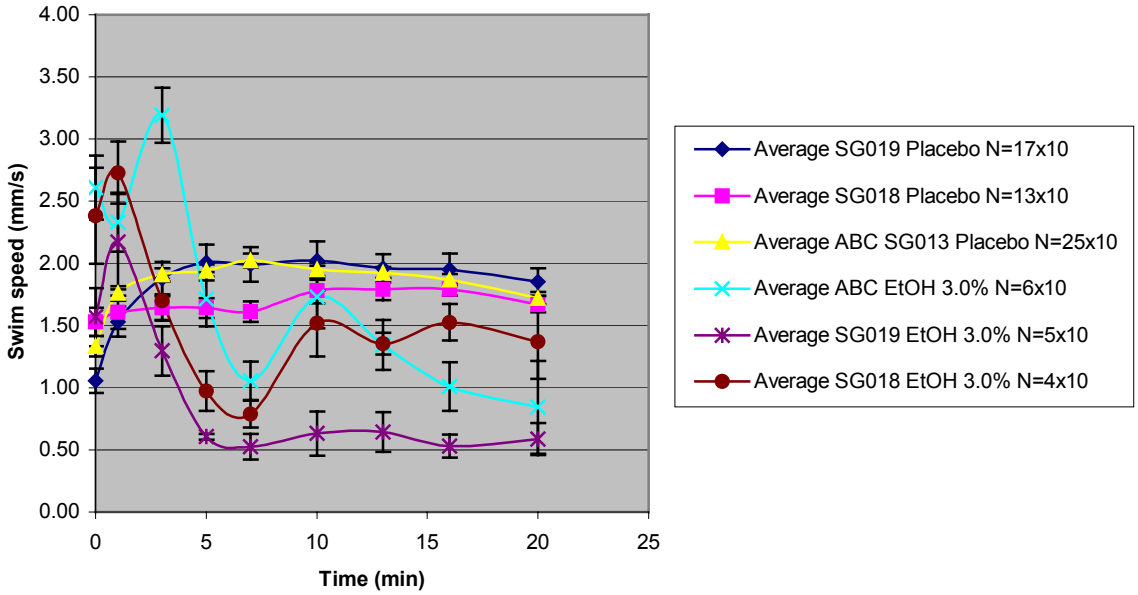

Fig. 2.9. ABC, *tfu* mutant and wt sibling, EtOH 3.0% vs. placebo. SG019 practically stops swimming after a few minutes, while the SG018 mutant appears more resistant to the alcohol effect.

These results taken together, suggest that the *tfu* mutant SG018 is more resistant to EtOH than the wt sibling SG019 is, since SG019 is knocked out much faster than SG019 by both 1.5% and 3.0%.

2.4.2. Amphetamine

Protocol:

The protocol is as described in section 2.2.4. 1mg D-amphetamine sulphate (Sigma®) was weighed on a Mettler Toledo® analysis weight and was flushed out of the weigh dish with 1.00mL dH_2O into a 1mL Eppendorf® tube. 100µL of the 1mg/mL solution was mixed with 900µL dH_2O in a new tube. Appropriate amounts of AMPH solution was taken out with a pipette to be mixed in a total volume of 5.0 mL egg water in a Falcon® tube and emptied into the view tray for recording as described earlier. 100µL, 400µL AMPH 0.1mg/mL and 200µL AMPH 1mg/mL was used for final AMPH concentrations of 0.5mg/L, 2mg/L and 10mg /L in the view tray, respectively.

Results:

AMPH causes an initial increase in locomotor activity, but it is far from as pronounced as the EtOH-increase. Still, all AMPH concentrations make the fry swim faster than untreated fry at minute three and five with p-values in the range from 0.01 to 0.04 (fig. 2.10). The further fate of the curves depends on the AMPH concentration. A low dose like 0.5mg/L makes seven days old ABC fry swim significantly faster than placebo-treated fry from three minutes throughout the whole 20-minute period. 2mg/L also leads to significantly higher swim speeds, but only through minute 13. There are only significant differences between 0.5 and 2.0mg/L at two time points. On the other hand, 10mg/L results in a significant swim speed decrease after minute 13.

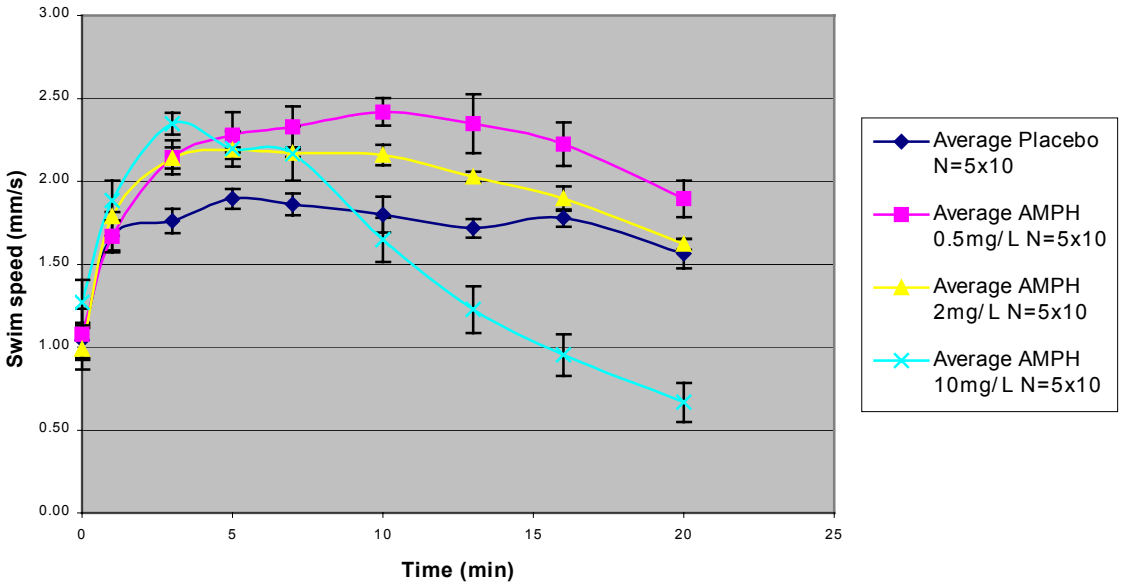

Fig. 2.10. ABC fry given different AMPH concentrations. Low doses cause hyperactivity while higher doses cause an initial hyperactivity followed by hypoactivity. Extending the curves beyond 20 minutes, hypoactivity would eventually most probably be seen for the lower doses as well. The placebo curve was obtained only with data from the same days that AMPH recordings were carried out.

SG019 wt fry show the same properties compared to ABC as to the response to EtOH (fig 2.11). The initial hyperlocomotor activity can barely be seen, and there are no significant differences. However, the same hypoactivity tendency can also be seen with SG019, and 10mg/L is significantly slower than placebo at minute 16 and 20.

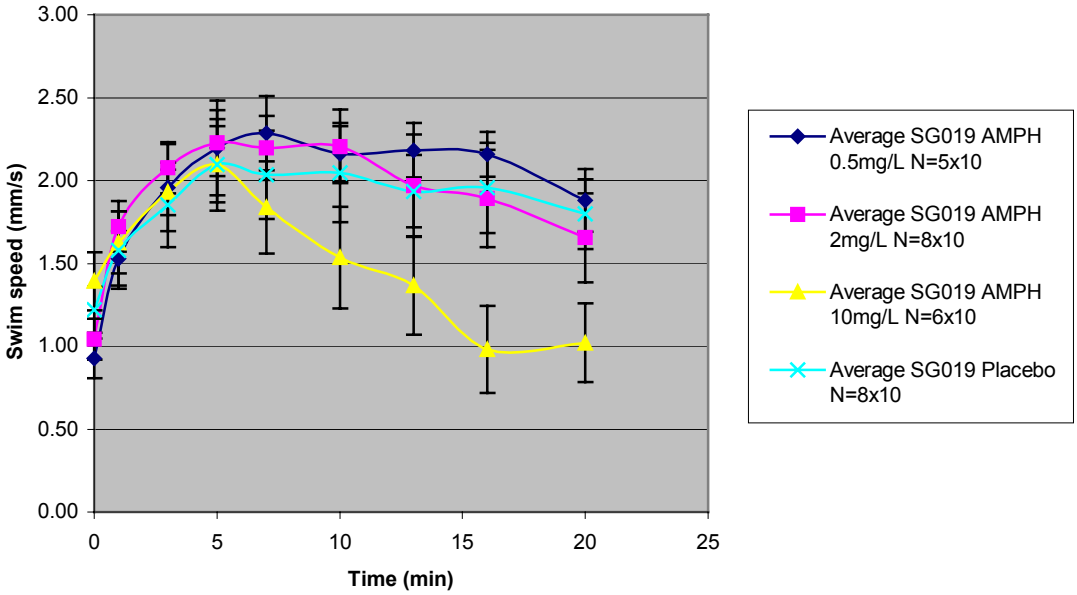

Fig. 2.11. SG019 wt fry given different AMPH concentrations. SG019 show no hyperactivity but share a common feature with ABC, namely swimming slower after a few minutes given a high dose AMPH. SG019 show a greater variability than ABC, which results in larger error bars. The placebo curve is obtained with data only from days AMPH recordings were done.

The SG018 mutant also has the same features as wt fry, but in contrast to its wt sibling shows initial hyperactivity and is "rescued" by AMPH to swim speeds very close to SG019 swim speeds (fig.2.12), meaning that there are no statistical differences between AMPH treated SG018 and SG019 fry. The *tfu* AMPH 0.5mg/L curve is significantly different from placebo from minute one through seven while 2mg/L is through minute five only. 10mg/L is significantly slower from minute ten and throughout the 20-minute period.

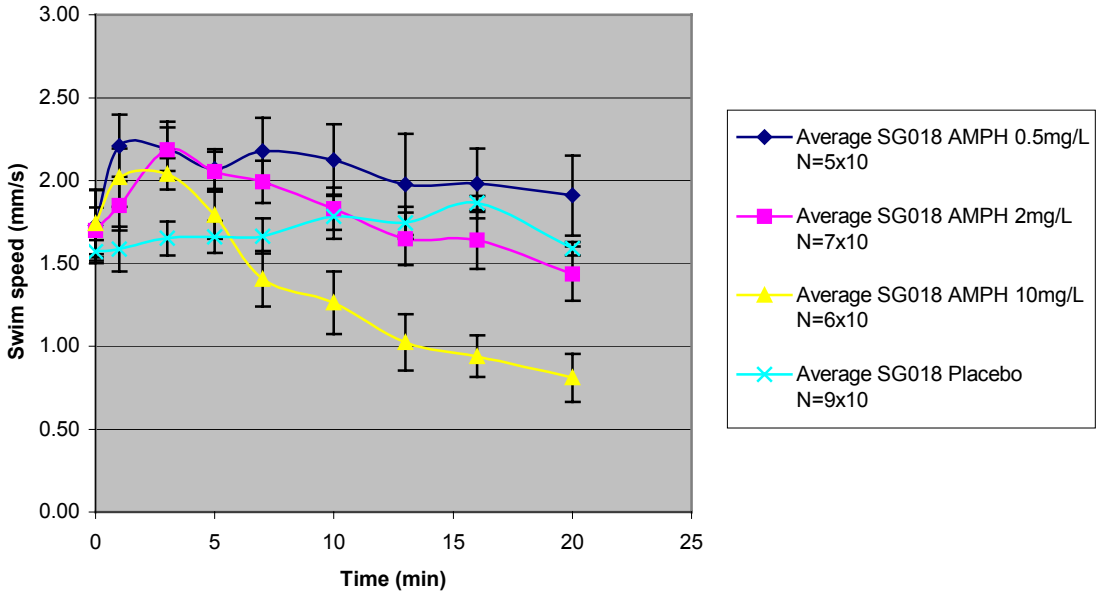

Fig. 2.12. SG018 *tfu* mutant given different AMPH concentrations. The SG018 curves resemble more those of ABC in terms of the AMPH effect in comparison to no drug, but the absolute swim speed values resemble those of its wt sibling, being "rescued" up to "normal" or even faster at minute one and partly minute three. The placebo curve is obtained only with data from days AMPH recordings were carried out.

AMPH experiments were also carried out with the progeny of *tfu*1x-47 x *tfu*1x-45 (wt) employing the protocol initially described in section 2.2.3. AMPH concentrations 0.5, 1, 2, 3, 4, 5 and 10mg/L with sample sizes from 1x10 to 4x10 resulted in higher swim speeds than ABC (data not shown) and with a characteristic stepwise decrease in swim speeds at later time points the higher the AMPH dose. Re-treatment after four drug-free hours with 2mg/L gave substantially lower swim speeds than placebo during the whole second 20-minute period (data not shown). Is the late phase hypoactivity due to depletion of DA stores?

In addition, two adult fish were recorded with 5mg/L AMPH and showed about a 20% locomotor activity increase for 30-40 minutes (data not shown).

2.4.3. Cocaine

Using the protocol initially described in section 2.2.3 with the progeny of *tfu*1x-47 x *tfu*1x-45 (wt), the response to cocaine hydrochloride (Sigma®) was examined. Experiments with doses of 0.5, 1, 2, 3, 4, 5 and 10 mg/L was carried out on 10 fry each (data not shown), but no interesting results found. The experiment was repeated two-three months later, this time with

SG019 fry and the final protocol as described in section 2.2.4, but fewer concentrations over a longer time-span. A 5mg/L concentration resulted initially in swim speeds identical to placebo (data not shown). At later time points hypoactivity was exhibited, reaching swim speeds around 1mm/s at approximately minute 40. A high concentration like 40mg/L led to severe hypoactivity already at three minutes. A possible anaesthetic effect?

14-day old ABC SG013 fry were also investigated with concentrations of 0.5, 2, 5 and 10mg/L. The fry were raised to day 14 as described early in section 2.2.3, fed in the morning of day 14 and placed on the laboratory bench. One hour before each individual recording took place, ten fry were transferred to 25mL fry water in a petridish. A cocaine HCl solution of the right concentration was prepared equally to as described for AMPH in section 2.4.2, and the rest of the experiment done as described for seven days old fry in section 2.2.4, except for the fact that fry water was applied as a swim medium instead of egg water. The results are shown in fig. 2.13. The expected hyperactivity was not observed this time either and further cocaine experiments were not implemented.

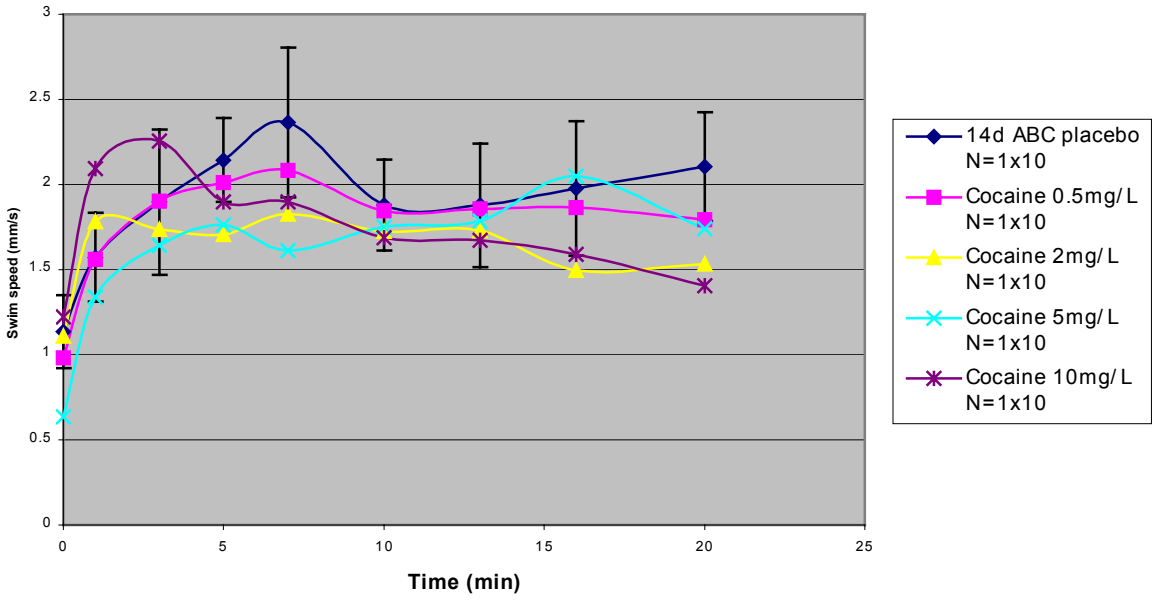

Fig. 2.13. 14-day old ABC fry given various cocaine concentrations. No distinction is seen compared to placebo for any dose. One might argue that 10mg/L exhibits some initial hyperactivity, but it would demand a much larger sample size to determine if that is the case. Error bars are obtained by direct calculations of the mean swim speeds of each single fry instead of the mean of 10 fry as for the previous charts. Only error bars for the placebo curve are shown.

2.4.4. Morphine

As for cocaine, initial morphine hydrochloride trihydrate (Sigma®) experiments with the progeny of *tfu*1x-47 x *tfu*1x-45 (wt) and SG019 seven-day old fry gave no positive results, concentrations ranging from 1.0-10mg/L (data not shown). Two subsequent morphine hemi[sulphate pentahydrate] (Sigma®) experiments using 14 days old SG023 and ABC fry were carried out only to confirm the same results and to optimize the protocol for experiments on 14-day old fry (data not shown). The third morphine experiment, having a seemingly good protocol in hand, showed no difference compared to placebo curves either (fig. 2.14). A 100mg/L morphine hemi[sulphatepentahydrate] stock solution was made by weighing 10mg morphine sulphate (Sigma®) on the Mettler Toledeo® analysis weight and adding 100.0mL dH_2O to it in a 100.0mL volumetric flask. The stock solution was transferred to 50mL Falcon® tubes and frozen. On the experimental day, one tube was taken to RT and thawed in good time before the experiments were carried out. The 14-day old ABC fry were handled as described for cocaine in section 2.4.3, and appropriate volumes of the morphine stock solution were taken out and mixed with 5.0mL fry water in a Falcon tube® for correct final concentrations in the 20.0mL view tray.

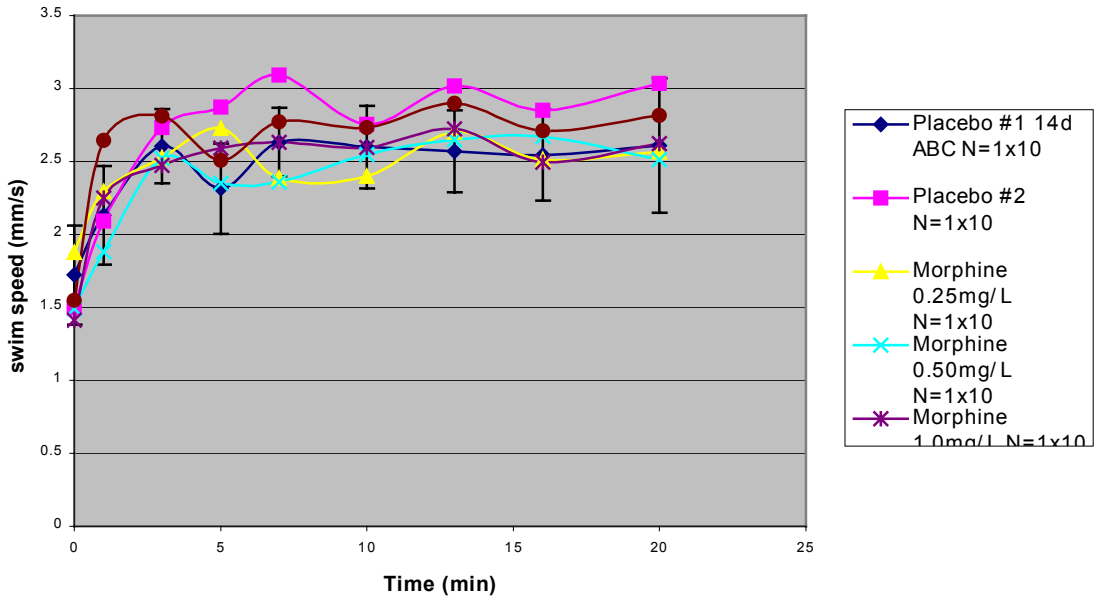

Fig. 2.14. 14-day old ABCfry given various concentrations of morphine. As is the case with cocaine, no obvious swim speed differences compared to placebo is observed for these concentrations. Error bars show the variety between mean speeds of single fry in the view tray, rather than variety between averages from several recprdings. Only error bars for placebo #1 are shown.

2.4.5. MPTP

The neurotoxin MPTP can induce a parkinsonian syndrome in humans and other primates (Langston, 1985), and primate MPTP models have thus drawn considerable attention as a system to search for anti-PD drugs. It has been shown that MPTP also causes a parkinsonian syndrome in the common goldfish (Pollard et al., 1992), seen as bradykinesia paralleled by a loss of DA and NA from the forebrain and midbrain. To investigate if similar features can be seen in zf fry and thus be applied as an MPTP-PD model, ABC fry were exposed to 43 and 215µM MPTP from 24hpf to 5dpf.

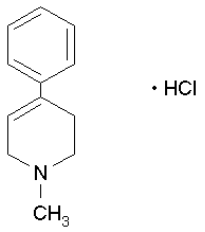

Fig. 2.15. MPTP HCl, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine hydrochloride.

Protocol:

A 2.0mg/mL stock solution was made from MPTP HCl (Sigma®) and kept frozen at -70ºC. Upon thawing, 9 and 45µL was taken out for final concentrations of 43 and 215 µM MPTP, respectively. These volumes were pipeted into two wells each to a total of 2.00mL in a 12 well plate (Fisher Scientific®). Two wells were filled with 2.00mL egg water for control. 24hpf embryos were dechorionated in 25mL 0.72mg/mL pronase solution (Sigma®), rinsed in egg water five times and placed seven and seven into each of the total of six occupied wells in the 12 well plate. The plate was kept in a 28°C room with a 14/10 hours light/dark circle. Each new day, the embryos/fry were transferred to a new 12 well plate with control and MPTP solutions, but finally taken out of drug at 5dpf. At day seven, ten control fry, ten 43µM and ten 215µM MPTP fry were transferred to 15.0mL egg water in the view tray and put on the light box for five minutes prior to adding another 5.0mL and recorded as described in section 2.2.4. However, the duration of the recordings was only five minutes, recorded with a frame rate of two per second. Data analysis was carried out according to section 2.2.3. MPTP was handled according to the Sigma material safety data sheet for MPTP (www.sigmaaldrich.com), and all materials in contact with the neurotoxin was bleached using a 1% sodium hypochlorite solution (Startbright®) for ten minutes.

Results:

A significant reduction in locomotor activity was observed (fig. 2.16) for the seven-day old fry, p-values being 0.03 and 0.001 compared to placebo control for 43µM and 215µM, respectively. There was no significant difference between the two MPTP-treated groups (p=0.13). To determine the underlying cellular basis of the locomotor hypoactivity, DAergic neurons were examined by in situ hybridization with an RNA probe against TH. A reduction in HY DAergic neurons was observed in the MPTP-treated fry, while NAergic neurons were normal (Bretaud and Guo, unpublished data not shown). These data indicate that MPTP can selectively reduce the number of DAergic neurons and lead to decreased locomotor activity in zf fry, and thus is a potential model for the study of PD.

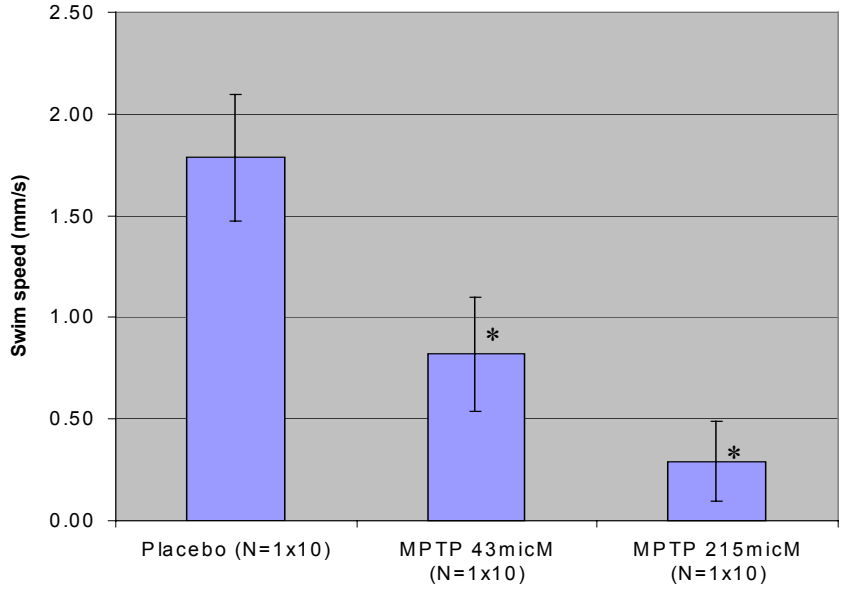

Fig. 2.16. Locomotor activity of seven-day old ABC fry exposed to MPTP for four days. MPTP-treatment results in a significant decrease in locomotor acivity. The swim speed is the mean of the mean of ten individual fry recorded for five minutes continuously. Error bars reflect the difference between the means of the individual ten fry.

2.4.6. Fluphenazine

Fluphenazine is a phenotiazine antipsychotic drug, which resembles chlorpromazine in many regards (Martindale, 1999). Its main drawbacks are extrapyramidal side effects as a result of D_2 receptor antagonism. It has been shown earlier in the lab that fluphenazine-treated fry at a concentration of 0.5mg/L for six hours leads to bradykinesia, a more jerky swimming pattern and loss of balance (unpublished results). Using the protocol briefly described early in section 2.2.3, treatment with doses of 0.5, 4.0 and 8.0mg/L for 20 minutes did not alter the swim speed of seven days old *tfu*1x-47 x *tfu*1x-45 (wt) fry (data not shown). High concentrations,

16, 32 and 64mg/L, which are very high for a potent drug like fluphenazine, induced hyperactivity the first ten minutes as compared to placebo-treated fry (data not shown). The experiment was repeated for 2.0 and 8.0mg/L applying the section 2.2.4 protocol and pre-treating ABC fry for two hours before recording (data not shown). But the sample size was low, and the fact that the placebo curve exhibited low swim speeds and that the drug curves showed unusually much variability, makes the data hard to interpret. The chance that 2.0 and 8.0mg/L fluphenazine induce hypolocomotor activity after a two-hour treatment should not be excluded.

2.4.7. The combination of fluphenazine and ethanol

In order to investigate if DA receptor antagonism would attenuate the stimulant effect of EtOH, fluphenazine was administered prior to and concomitantly with alcohol.

Protocol:

The protocol was followed as for seven-day old fry as described in section 2.2.4 with the following exception: In the morning of day seven, 10 and 10 fry were placed in individual petridishes containing 25mL blue egg water and put back into the 28°C incubator. Two hours before a specific recording, the matching set of ten fry was taken out and placed on the laboratory bench and either transferred to egg water or to a new petridish containing fluphenazine. An 8.0mg/L fluphenazine stock solution was made by weighing 8.0mg fluphenazine dihydrochloride (Sigma®) and adding it to one L of egg water. The fry transferred to egg water, were after two hours transferred again to 15.0ml fluphenazine solution of the appropriate concentration in the view tray. After five minutes on the light box, 300µL EtOH in 5.0mL egg water was added and the fry recorded as described earlier. The resulting curves are referred to as “fluphenazine acutely” in fig. 2.16. The corresponding curves of the experiments where fry were transferred directly into a fluphenazine solution are referred to as “fluphenazine pretreated”. These fry were handled exactly the same way as “fluphenazine acutely” except for the fact that they were done familiar with fluphenazine two hours earlier. The experiments were carried out using ABC fry, SG013 as well as SG035 for some recordings.

Results:

The fry pretreated for two hours with 2.0 and 8.0mg/L fluphenazine, show some initial hyperactivity, but far from as much as the EtOH 1.5% control (fig 2.17). At seven minutes and later, swim speeds equal or are slightly higher or lower than basal locomotor activity. This means, despite a small sample size, that fluphenazine pretreated fry at these concentrations move significantly slower than the EtOH control. Fry given a low fluphenazine dose like 0.10 or 0.50mg/L do not exhibit any dramatic differences. 2.0 and 8.0mg/L given just before EtOH somewhat surprisingly show a sensitized leftward shift and attain relatively high swim speeds at early time points, being significantly faster than EtOH control at minute three and five for 8.0 and 2.0mg/L fluphenazine, respectively. At later time points 2.0 mg/L is only significantly different at minute 13, while 8.0mg/L is different from minute ten and throughout the 20-minute period. It is also surprising that treatment with the latter concentration and 1.5% EtOH results in such a severe hypoactivity while simple treatment with 8.0mg/L fluphenazine for two hours most probably would lead to swim speeds between 1 and 2mm/s. One weakness about this experiment is that fluphenazine affects several neurotransmitters in addition to DA, thus it is hard to draw any conclusions on how important the DAergic system is in mediating the stimulant effect on fry locomotor activity.

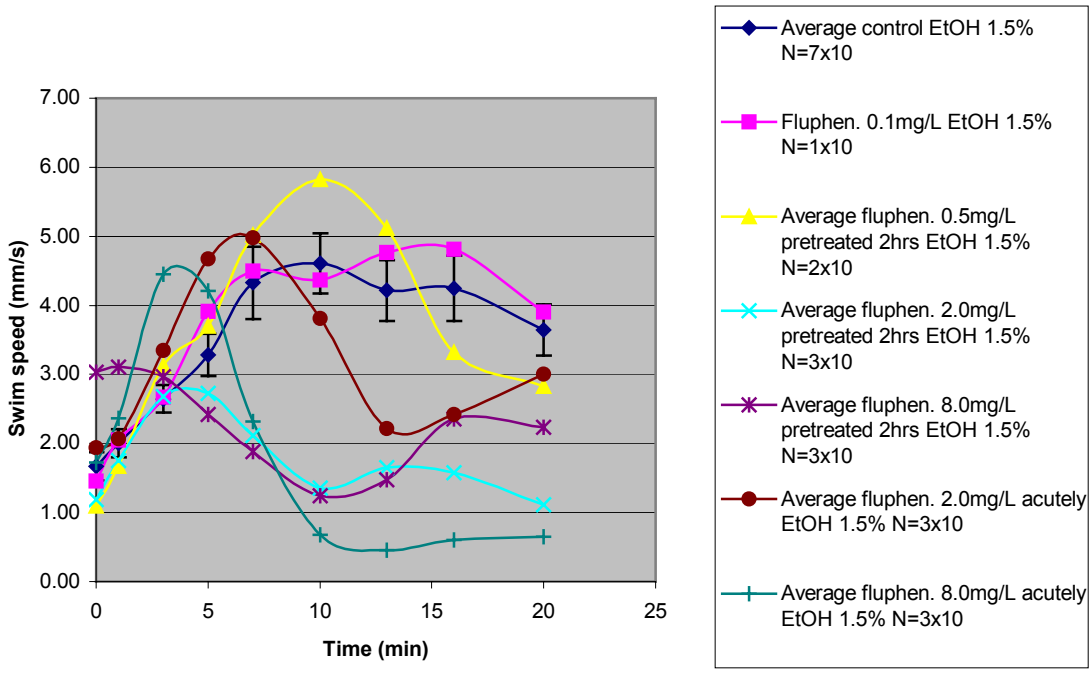

Fig. 2.17. Seven-day old fry given the DA receptor antagonist fluphenazine and then EtOH. Fry pretreated for two hours do not show much hyperactivity when given EtOH, while acute fluphenazine treatment results in a leftward shift for the time-speed curve with an accompanying decrease in swim speeds at later time points.

2.4.8. Levodopa

L-DOPA solutions of 5.0 and 20mg/L was given to seven-day old SG019 wt and SG018 mutant fry applying the protocol described in section 2.2.4, however, locomotor activity was identical to placebo activity. This is consistent with the fact that L-DOPA is not a stimulatory drug.

Hypothesis:

We hypothesized that by letting *tfu* fry grow up in a L-DOPA solution, it would supply the fry HY with DA after being converted by DOPA decarboxylase and compensate for the relative lack of DAergic neurons. Upon L-DOPA withdrawal, DA would only be supplied by production from L-tyrosine in the few remaining neurons, leading to a sudden decrease in DA with an accompanying reduction in locomotor activity.

Protocol:

10 250mg Larodopa® tablets (Roche®) were weighed to determine their relative L-DOPA content. Then one tablet was pulverized in a mortar with a pestle and 28mg powder was measured up and transferred to 1.0L of blue egg water to obtain a 20mg/L L-DOPA solution. The same procedure was carried out with 500mg vitamin C tablets (Rugby®), and an amount of tablet powder added to the L-DOPA solution to obtain a concentration of 20mg/L ascorbic acid (aa) as well. 1dpf SG018 and SG019 embryos were transferred to 25mL 5.0 and 20mg/L L-DOPA / 20mg/L aa solution in petridishes and kept in the incubator. For the 5.0mg/L L-DOPA solution, the L-DOPA stock solution was diluted with blue egg water containing an appropriate amount aa. Control fry were given 20mg/L aa. Ascorbic acid was added to delay the oxidation of L-DOPA (Pappert et al., 1996) but still, a new L-DOPA solution was made each 72 hours and the fry transferred to it in new petridishes/tanks. At 7dpf, the fry were transferred to 2L-tanks containing 1.0L fry water and the above mentioned concentrations of L-DOPA and aa. They were taken out of the incubator, placed in the 28°C room with a 14/10 hours light/dark circle and fed twice daily. 13dpf fry were transferred to plain fry water. 14dpf, tanks were put on the laboratory bench, transferred to petridishes containing fry water and recorded as described earlier.

Results:

As fig. 2.18 suggests, there is no difference between SG018 and SG019 14days old fry treated with L-DOPA from 1 to 13dpf with the described dose regime compared to placebo, and this evidence thus does not support our hypothesis. There is a surprisingly big locomotor activity difference between SG018 and SG019, and for 14 days old fry the swim speeds would in general be expected to be somewhat higher. Feeding induces more inter-fry variability and *tfu* fry appeared slightly bigger than wt in general. Ascorbic acid may adversely affect the fry and lead to retarded development and thus altered locomotor activity, but this possibility was never investigated further. 20mg/L aa changed the pH from 7 to about 6.5 using pH indicator paper (Whatman®), and an alternative approach would be to employ ascorbate as antioxidant or change the L-DOPA solution more frequently. Furthermore it is not known whether all L-DOPA went into solution, since it is "slightly" soluble (Martindale, 1999). Particles were observed in the drug solution, but were probably other hydrophobic tablet ingredients. Tablet ingredients could possibly also affect the fry and using pure L-DOPA from Sigma® would have been an alternative. Other reasons for not observing any difference could be the drug concentrations and the time aspects chosen for any step of the experimental process. No further experiments were done.

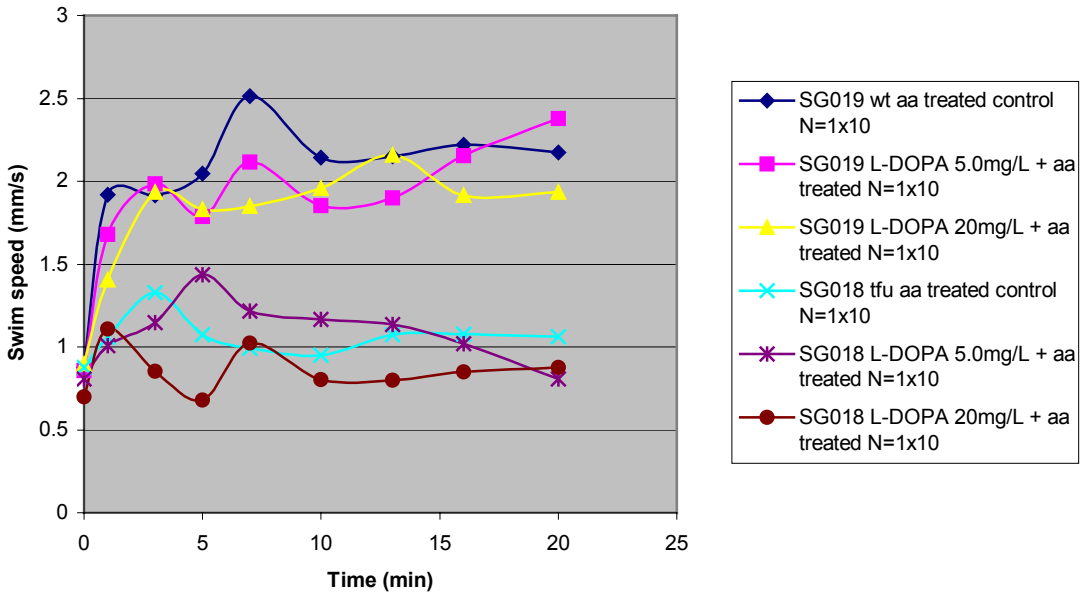

Fig. 2.18. 14-day old *tfu* mutant and wt treated with L-DOPA 1 to 13dpf do not exhibit altered locomotor activity compared to aa control. See text for details. Error bars not shown.

The above experiment was done earlier, keeping and feeding fry in petridishes until 14dpf without the addition of aa. L-DOPA treated wt showed hyperactivity compared to control. Tfu as hypothesized, a slight hypolocomotor activity, but the validity of these results were questioned since there were large differences in fry size even in the same petridish, and the small petridish environment after the start of feeding seemed detrimental to the fry.

2.4.9. Apomorphine

Apomorphine is a nonselective DA receptor agonist that induces hyperlocomotion in rodents, and intermittent administration results in behavioral sensitization (Mattingly et al., 1997). In order to investigate if the drug has similar properties in fry, 10mg apomorphine hydrochloride hemihydrate (Sigma®) was weighed and transferred to 1.00mL dH_2O in an Eppendorf® tube covered with aluminum foil. Subsequently it was diluted 10-fold as was done with AMPH. Seven days old SG019 fry were employed and recorded as described earlier. Apomorphine concentrations of 0.50, 4.0 and 16mg/L, showed a more or less progressive reduction in locomotion (data not shown). The drawback of this experiment was that the placebo-fry also swam very slow, meaning that the true effect of the drug is not revealed compared to placebo. The experiment was also done earlier on eight days old progeny of both *tfu*1x-47 x *tfu*1x-45 and *tfu* mutant using the protocol initially described in section 2.2.3 with the above mentioned apomorphine concentrations in addition to 2.0 and 8.0mg/L. Unfortunately, the same problem turned up, but the same tendencies were seen for both wt and mutant. A second problem involving apomorphine is its light sensitivity (Martindale, 1999), which leads to rapid oxidation and degradation of the drug. This is a problematic issue regarding recordings on a light box since the drug is exposed to a strong source of light during the experiment. How relevant this decomposition is, remains to be investigated. No further apomorphine experiments were undertaken, and sensitization was not addressed.

2.4.10. Reserpine

Monoamines are transported into vesicles in the presynaptic nerve terminal by vesicular monoamine transporters (VMAT), using a proton electrochemical gradient (Henry et al., 1994). Vesicular transport and up-concentration is inhibited by reserpine, meaning that the monoamines are degraded in cytocol. Reserpine was used earlier as a means to lower blood pressure, but is now used for research purposes where depletion of monoamines is desired. It has been reported that reserpine and the TH inhibitor α-methyl-p-tyrosine inhibit locomotor activity in adult *Drosophila* and that this decrease in activity could be prevented by

concomitant administration of L-DOPA. (Pendleton et al., 2000). We wanted to examine if the same was possible in zf fry by adminstration of reserpine (Sigma®) into the egg water. A concentration of 30μM was suggested. One obstacle was that reserpine is practically insoluble in water (Martindale, 1999). However, there was hope that 30μM would be low enough for it to dissolve, or at least lead to some saturated solution even though the concentration would be less than 30μM. Contrary to our optimistic hopes, practically nothing seemed to dissolve. Still, the experiments were carried out. ABC SG035 fry were raised as described in section 2.2.4. 5dpf, two times ten fry were transferred to petridishes containing reserpine, two times ten fry to petridishes containing reserpine and 20mg/L L-DOPA, and finally two times ten were kept as controls. After 48 hours, recordings were carried out as described earlier. Ten control fry were recorded for basal locomotor activity and ten control fry recorded with 1.5% EtOH. The same recordings were repeated for the reserpine and reserpine/L-DOPA fry. The resulting curves showed no striking differences for the three ethanol-treated groups of fry (data not shown). The placebo curve and reserpine/L-DOPA reflecting basal loco motor activity were identical, while the reserpine-treated fry exhibited swim speeds 0.5-1mm/s lower, as tentatively expected (data not shown). However, rather than the reserpine fry being especially slow, the controls were unusually fast, making it again hard to say if there really could be a difference.

Reserpine can be freely dissolved in acetic acid (Martindale, 1999), but that would probably not be an ideal swim medium for the fry. The above experiment was repeated, but 1%(V/V) dimethylsulfoxide (DMSO) was added hoping it would aid dissolution. This time, however, no difference at all was seen between the reserpine-treated and the other fry (data not shown). DMSO concentrations of 0.5 to 4%(V/V) were examined for reserpine solubility, but still, the drug would not dissolve. No further reserpine experiments were done.

2.5. Tolerance

2.5.1. Introduction

EtOH intake leads to pleasurable effects such as euphoria as well as aversive effects such as loss of motor coordination (reviewed by Fadda and Rosetti, 1998). Alcohol abuse is facilitated by the development of tolerance, in humans thought to develop rapidly to the aversive EtOH effects and to a lesser extent to its pleasurable properties. This imbalance has been suggested to encourage increased intake, which over time can lead to addiction.

Different forms of tolerance can be induced with protocols that vary the dose and frequency of EtOH administration. Tolerance that develops after a single short-term exposure is referred to as rapid tolerance, whereas tolerance acquired after repeated or prolonged exposures is referred to as chronic tolerance. The physiological basis for tolerance includes pharmacokinetic tolerance, which is the result of an altered drug concentration at the site of action, and pharmacodynamic tolerance, which is achieved by adaptive CNS changes. Rodents have proven good animal models in which studies of alcohol tolerance and dependence can be undertaken (Crabbe et al., 1994). It has been shown that *Drosophila* develops pharmacodynamic tolerance to the sedating and motor-impairing effects of EtOH (Scholz et al., 2000). Furthermore, EtOH tolerance also develops in adult goldfish (Greizerstein and Smith, 1974). Therefore, it would be interesting to investigate if tolerance can be observed in zf fry and thereby be used as a model for tolerance. Remembering the salient zf features regarding carrying out mutagenesis and screens, important genes responsible for tolerance and addiction could potentially be found in the future.

2.5.2. Rapid tolerance

Protocol:

ABC SG013 fry was raised as described in section 2.2.4. 6dpf, ten healthy fry were transferred to a new petridish containing approximately 19.7mL egg water and 300µL EtOH was added, equaling 1.5%(V/V) in the dish. Concomitantly, another ten fry were transferred to a petridish containing only egg water. The fry were exposed to drug and placebo for one hour before transferred back to blue egg water and put back in the 28°C incubator. On day seven, the individual dishes were taken out of the incubator and placed on the lab bench for two hours before recording. The recording was done exactly as described earlier with a final solution of 1.5%(V/V) EtOH in the view tray.

Results:

Applying this protocol, no tolerance was seen (fig. 2.19). The swim speed curves are more or less identical and statistical differences at the 5% level of significance cannot be found. Comparing the curves, they would rather suggest a tiny sensitization, but again the differences and sample sizes are far too small for suggesting otherwise than there is no difference.

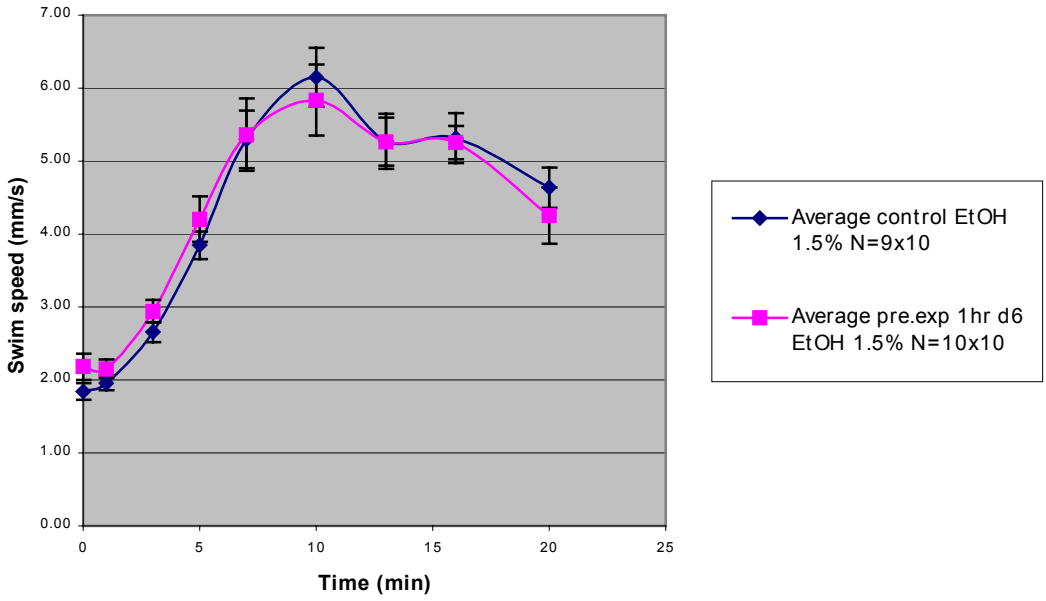

Fig. 2.19. ABC fry do not develop tolerance or sensitization using the protocol described.

The same experiment was done twice using 3.0% EtOH, but much variability/no logical pattern was seen.

2.5.3. Chronic tolerance

Protocol:

ABC SG013 fry was raised as described in section 2.2.4. 5 dpf, three sets of ten fry were transferred to three petridishes, one containing a placebo solution, one a 0.3% EtOH solution and one a 0.6% EtOH solution. The dishes were put back into the incubator for 44 hours, after which the fry were taken out of the drug solution and put into egg water for four hours to sober up before recording. The two first hours after the transfer were spent in the incubator before they were taken out and put on the lab bench for two hours before the experiment. Recording was done according to the protocol described in section 2.2.4 with EtOH 1.5% as drug solution.

Results:

Seven days old ABC fry do not show chronic tolerance either (fig. 2.20). The 0.3% pretreated curve has the shape expected for a tolerance curve, a rightward shift, but the sample size is too small for proving chronic tolerance. However, surprisingly the 0.6% curve resembles more

the control curve initially, but becomes very susceptible for EtOH's locomotor-impairing effects after minute 13. Upon visual inspection of the view tray, it was clearly observed that the 0.6% pretreated fry were more often "knocked out" than its control siblings, manifested by standing still or sometimes even by losing balance and tilting. The reason for this behavior is unknown, but one possibility is that all EtOH was not cleared from the fry system during the four-hour period before recording, and the fry would thus faster reach intoxicating blood alcohol concentrations than control fry. The fact that the 0.6% fry do not swim faster than controls initially, is evidence contradicting that suggestion, but 0.6% hyperactivity may be masked by an already developed tolerance. Again, the sample size is small and the variability is substantial, making it impossible to draw any conclusions.

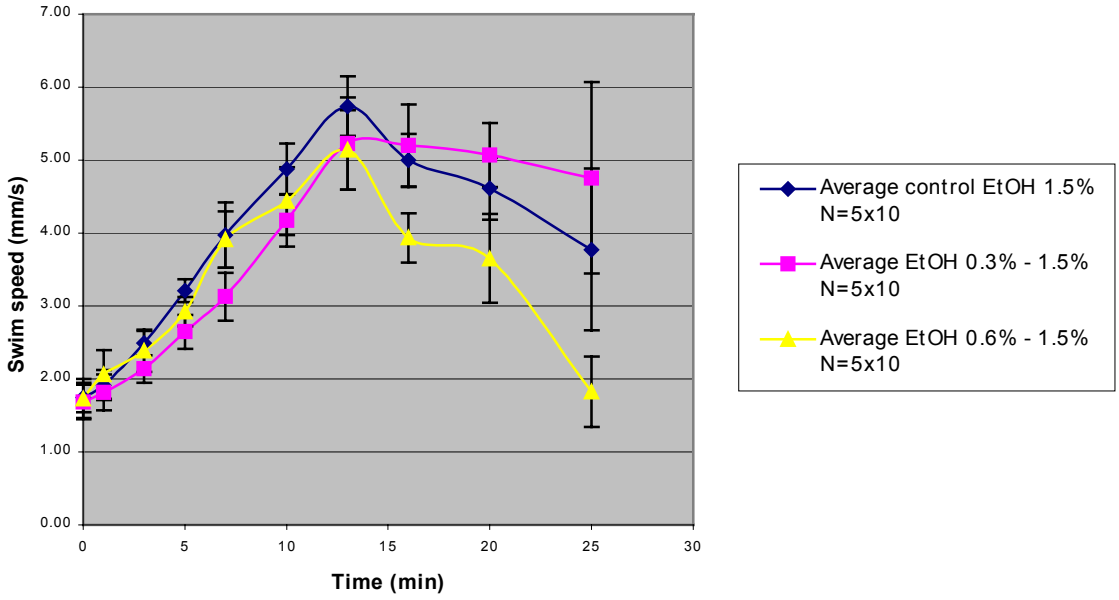

Fig. 2.20. ABC fry do not show chronic tolerance using the protocol described. The 0.3% pretreated curve exhibits the hallmarks of a tolerance-curve, but the sample size is too small to claim that tolerance is developed. For minute 25, the sample size is only 2x10.

The progeny of *tfu*1x-47 x *tfu*1x-45 (wt) was also used for tolerance experiments at an earlier point. Seven-day old fry were exposed to 1.5% EtOH for 20 minutes, transferred to egg water for fours hours, exposed to 1.5% EtOH for 20 minutes, transferred to egg water again for fours hours and recorded during the third exposure to 1.5% EtOH. Similar curves to the ones already shown were obtained, sometimes a little faster, sometimes a little slower than control. One common feature that is generally seen, is the fact that pre-exposed fry have larger standard deviations than control fry. There are only very few exceptions to the rule.

Despite that no tolerance or sensitization was observed, it does not mean there is none. Other protocols, using other EtOH concentrations, other time settings or environmental settings may be successful. Another possibility is that the seven days old zf still has not developed the CNS circuitry necessary for such delicate tasks.

2.6. Open field assays

2.6.1. Development of an open field assay

During analysis of the locomotion data, it was observed that EtOH-treated fry often prefers to swim close to the walls (thigmotaxis) in contrast to for instance AMPH. In order to investigate this interesting behavior further, Kayta Kobayashi helped make a new Excel® macro, enabling me to analyze the videos quantitatively in regard to fry position in the view tray. It was decided to divide the view tray into two compartments, one central part and one wall part. See fig. 2.21 for details. Analyzing videos, it was thus possible to calculate the fraction of fry in each compartment, making the basis for looking at another parameter in addition to swim speeds after drug treatment.

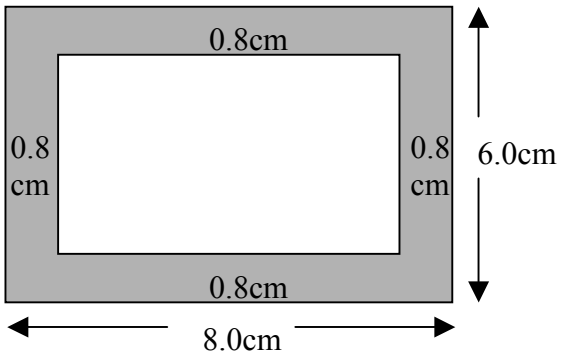

Fig. 2.21. The view tray with the compartments used in the open field assay. Using DIAS® and Excel®, we were able to construct imaginary compartments in the view tray, the computer keeping track of how many fry in each of the two compartments at any time point. In the results, the percentage of fry occupying the shaded wall area is given.

2.6.2. Ethanol

ABC SG013 fry were raised to 7dpf and recorded in a 1.5% EtOH solution as described earlier. The resulting videos were analyzed in DIAS® and Excel® in regard to fry positions in the view tray. Initially, EtOH-fry prefer the center compartment compared to placebo-treated

fry (fig. 2.22), although only statistically significant at three minutes (p=0.01). From minute ten, EtOH-treated fry prefer the wall area compared to placebo, p-values being very low.

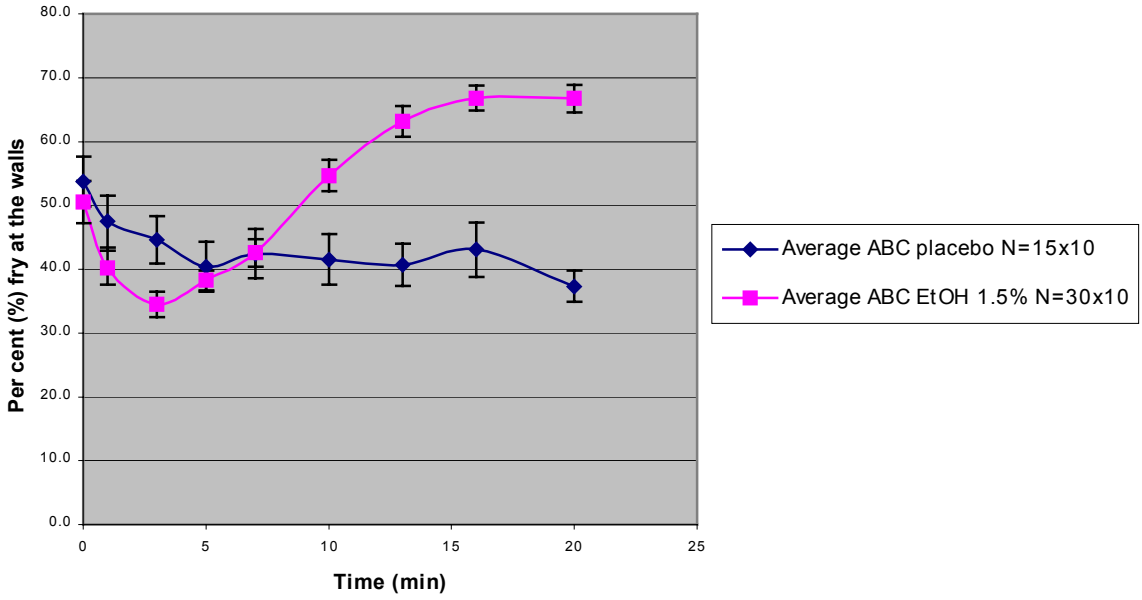

Fig. 2.22. Seven-day old ABC fry exhibit thigmotaxis when given EtOH.

The same analysis was also carried through on WIK, SG018 and SG019 fry. WIK (fig.2.23) show the same pattern as ABC, although there are no significant differences between placebo and EtOH in the early phase. From minute seven, EtOH treated fry show ”wall-hugging” compared to placebo. Notice that the percent values for WIK are lower than ABC, meaning that WIK in general swim more in the center of the view tray.

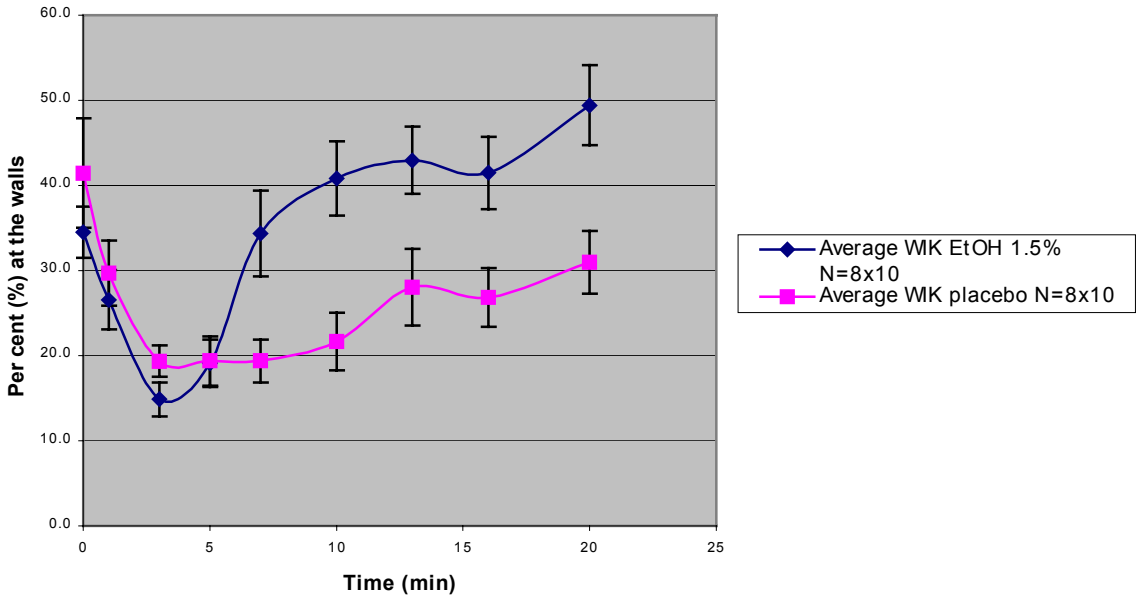

Fig. 2.23. WIK fry analyzed for place preference. The sample size is smaller than for that of ABC fry, but the same tendencies are observed. WIK starts to swim out to the walls sooner than ABC compared to the placebo curve, but in general WIK prefer to swim more in the center than ABC.

EtOH-treated SG019 fry are different from the above-mentioned wild types, being more or less similar to placebo all the way until minute 16 (fig.2.24). Nonetheless, "wall-hugging" is observed at the two late time points. Both the placebo and EtOH SG018 curves are similar to its wt sibling (fig. 2.24). However, although not statistically significant at the 5% level, there are obvious differences in the shape of the curves between SG018 and SG019. SG018 fry appear more sensitive to the alcohol effect at earlier stages, but still do not prefer the wall as much as wt during the last five minutes.

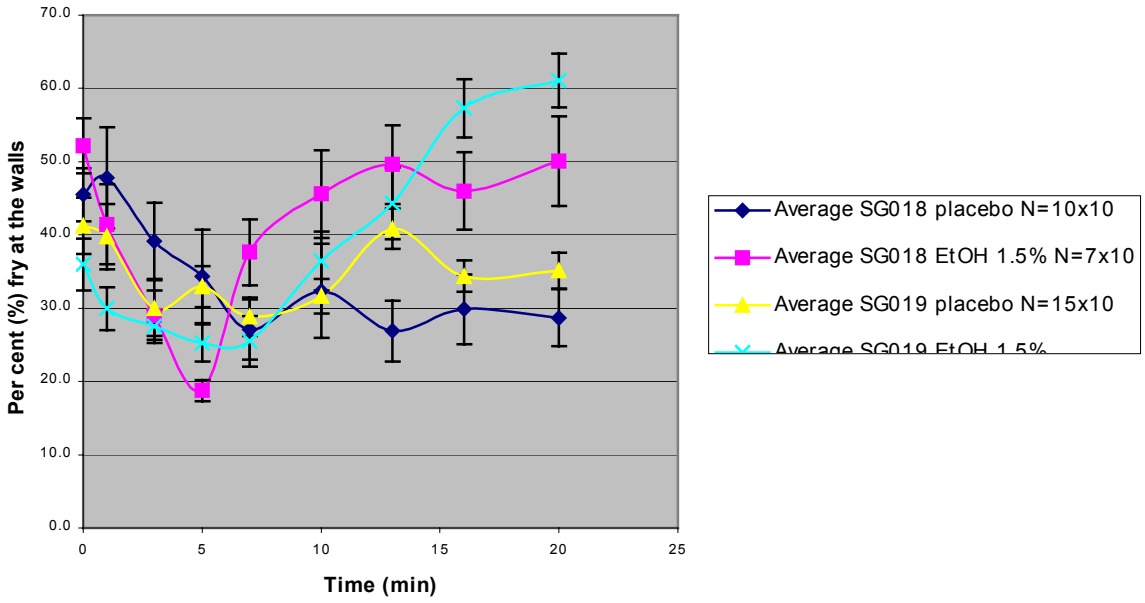

Fig. 2.24. Place preference of the *tfu* mutant and its wt sibling. The placebo-curves are similar, while EtOH-curves show some different properties in shape, although not significant.

Analyzing the ABC fry rapid tolerance movies, a leftward shift, though not significantly different from EtOH 1.5% control was found (data not shown). This is consistent with the small leftward change observed for swim speed (fig.2.19). Regarding chronic tolerance, the 0.3% curve showed a small rightward shift, also not significant (data not shown), but consistent with the corresponding swim speed data (fig. 2.20), while the 0.6% curve was shifted slightly to the left. The variability between individual recordings is large, and the fact that there is a substantial shift between the two *placebo* curves for rapid and chronic tolerance (data not shown), while the shapes are similar, raises doubt in how pronounced the thigmotaxis tolerance/sensitization shift really is, even for larger sample sizes.

2.6.3. Amphetamine, cocaine and morphine

Carrying out the same thigmotaxis analysis on ABC (data not shown), SG018 (fig. 2.25) and SG019 (data not shown) treated with AMPH, no difference compared to placebo is observed. Even though the sample sizes were low, no differences were seen for 14-day old ABC fry treated with morphine and cocaine either (data not shown).

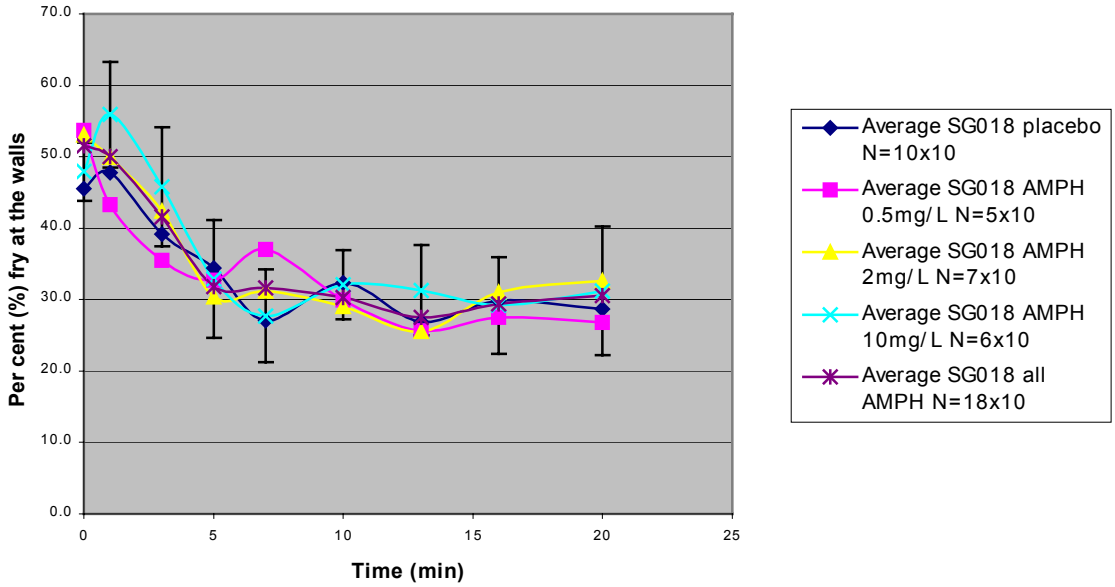

Fig. 2.25. Thigmotaxis analysis on *tfu* mutant given different doses AMPH. Significant deviations from placebo are not observed. Similar charts were obtained for ABC and SG019. Error bars for AMPH 10mg/L are shown.

2.7. Light/dark preference

2.7.1. Development of a light/dark preference assay

As the view tray illumination was slightly lower by the short edges due to the nature of the light box, it was suggested that fry might prefer a darker environment treated with EtOH, and thus that this could be a reason for the thigmotaxis observed. In order to investigate this further, a piece of paper just the size to cover 50% of the view tray was colored black and taped to the bottom of it (fig. 2.26). Fry were raised according to section 2.2.4, and 7dpf ABC, SG018 and SG019 were assessed when exposed to placebo and a 1.5% EtOH solution.

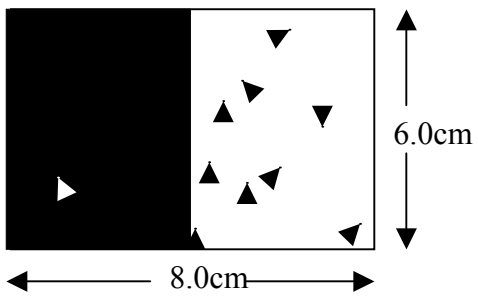

Fig. 2.26. The view tray as employed in the light/dark preference assay. Simply, a black piece of paper was taped to the bottom of the plexi glass view tray. Fry were placed into it, and the view tray was put on the light box for manual observation.

Instead of recording after the five-minute habituation period on the light box, fry in the light compartment were observed and counted manually each minute in the 20-minute period.

2.7.2. Results

The mean number of fry in the light compartment from minute five to 20 was calculated for each trial and multiplied with ten to obtain the relative portion of fry of the compartment in question. Given a placebo solution, the wildtypes ABC and SG019 showed no compartment preference, or a slight preference for the lighted area (fig. 2.27). The inter-wt placebo response was similar (p=0.54). A 1.5% EtOH solution resulted in a quick escape into the dark environment, significantly different than placebo, with p-values being 0.01 and 0.03 for ABC and SG019, respectively. The *tfu* mutant behaves differently, showing no specific pattern in the egg water solution. Sometimes, a large fraction swims in the light compartment and sometimes in the dark. However, when given EtOH, *tfu* show a preference for the dark area as wt, but not as pronounced. Statistically, there is no difference at the 5% level. *Tfu* placebo and EtOH cannot be shown to be different with such small sample sizes. Tfu EtOH is different from wt placebo (p=0.03-0.04).

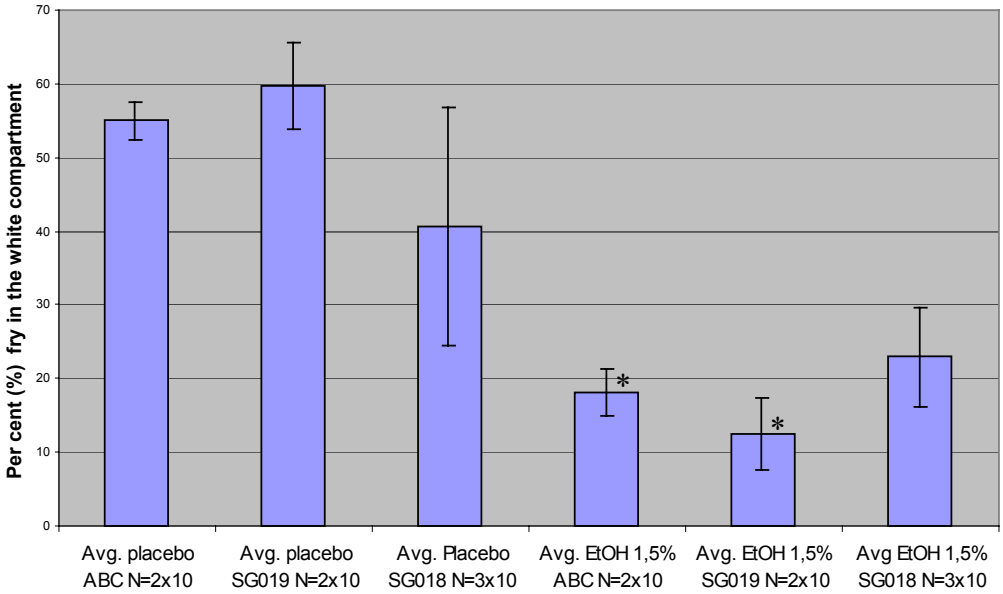

Fig. 2.27. Light/dark assay results. Given EtOH, seven-day old wt fry prefer a dark environment and is significantly different from untreated fry at the 5% level of significance. * refers to comparison to the same strain given placebo. The *tfu* mutant shows great variety given no drug and a preference for the dark part of the view tray given EtOH, as wt.

2.8. Pigment response

During EtOH experiments, it was observed that the fry became darker after a few minutes in solution. Sometimes, there was only a slight color difference compared to placebo, and sometimes the difference was substantial as showed in figure 2.28. In order to document the observation, 14-day old ABC fry were given 1.5% EtOH for 20 minutes in a petridish and then 200µL 0.16% tricaine methanesulfonate (Sigma®) solution was added to immobilize the fry. The fry were examined under a Leica® microscope and pictures were taken using an Axiocam digital camera (Zeiss®) and a PC with the software Axiovision (Zeiss®). The pictures suggest that the individual fry melanophores become larger upon EtOH treatment rather than that there is an increase in melanophore amount. Other EtOH concentrations gave the same result, and no other drugs induced any easily visible pigment change. The reason for the color change remains elusive, but one can speculate that EtOH has a direct effect on the melanophores, or alternatively that the change is mediated by ethanol's effect on the CNS.

Fig. 2.28. 14-day old ABC fry given EtOH. EtOH-treatment results in a pigment response, the melanophores becoming bigger. One placebo-treated fry (A) compared to fry given 1.5% EtOH for 20 minutes (B and C). B is caudally, while C is rostrally focused. Different batches of fry showed a considerable response variety.

2.9. Measurement of the alcohol concentration in seven-day old fry

In order to investigate pharmacokinetic properties of EtOH in fry, it would be desirable to measure the concentration in the fry. Does the fry concentration equal the solution concentration? Under tolerance experiments, is all alcohol cleared before subsequent EtOH exposures are executed?

In Moore et al., 1998, alcohol concentration was measured in *Drosophila*. Using their protocol as a basis, the EtOH concentration of seven days old fry was attempted measured without successful results. 300 fry were raised to 7dpf and exposed to EtOH 1.5% for 20

minutes in six different petridishes. The drug solution was emptied into a plastic funnel with a filter in the bottom, retaining the fry. 2x50mL 50mM TRIS-HCL (Sigma®) (pH 7.5) was poured in to rinse the fry for external EtOH. The filter was cut away with a razorblade and fry transferred to an Eppendorf® tube with 200µL TRIS-HCL, after which the tube was immediately frozen in liquid nitrogen. Upon thawing, the fry content was grinded using a plastic pistil just fitting the tube, and then centrifuged in 4°C at 14,000g for 15 minutes. 200µL of the supernatant was added to 3.0mL alcohol reagent 10 or 100 (Sigma®) in a Falcon® tube, reconstituted according to Alcohol procedure no. 333-UV (Sigma®). 200µL TRIS-HCL was used as control. Plain dH_2O was control for the control. Also a small volume standardized 0.08% (V/V) (Sigma®) solution in 200µL TRIS was used as alcohol control. After five minutes incubation of the EtOH with the alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide (NAD) solution, 100µL was taken out and the absorbance (A) measured with a UV spectrophotometer at 340nm, quantifying the newly formed NADH. The A increase at 340nm is directly proportional with the alcohol concentration in the sample.

It happened on a several occasions that the TRIS-HCl control solution had an absorbance above 0.50 of that of dH_2O control, resulting in invalid results (Alcohol procedure no. 333-UV). This is probably due to some pollutants, leading to reduction of NAD. Obtaining valid control results, calculated alcohol concentrations were 19 and 22%, numbers that appear somewhat high!! The plausible reason for this is that the supernatant contains soluble fry proteins that absorb at 340nm, thus resulting in too high alcohol concentrations. To correct for this in future measurements, trichloracetic acid could have been added, which would precipitate the proteins. Also, the supernatant of placebo treated fry could have been used as a control instead of TRIS-HCl. However, this procedure would have required at least 300 more fry, and the fish are not always co-operative about egg laying. Also protein measurements of the fry sample could have been carried out to determine the alcohol/protein ratio.

In order to calculate the percent alcohol concentration in fry, the volume of the fry had to be found. Noticing the volume of the fry suspension in the Eppendorf® tube after adding 200µL TRIS-HCl, the volume of one fry was estimated to equal 0.4-0.5µL.

Further alcohol concentration measurements were not carried out.

3. DISCUSSION

3.1. Rationale for the study - perspective

Understanding the molecular basis of complex disorders like addiction, PD and anxiety could be greatly aided by using forward genetic manipulation to lengthen the list of candidate genes involved. Several avenues of investigation have been taken to elucidate the genetic basis of these disorders. Selective inbreeding of mouse strains displaying differing degrees of anxiety and addiction-related behaviors has been used to correlate the behavior with particular genetic polymorphisms (for instance Crabbe et al., 1994; Knapp et al., 2000). A limited number of inbred strains and the time required to generate strains prevent characterization of more than a few genes of interest. Transgenic mouse have also been widely used to correlate specific behaviors with *already known* genes, extensively used for all the three above-mentioned diseases. Forward genetic methods, in which mutagenesis is undertaken, phenotypes are characterized and the genes subsequently cloned, on vertebrates displaying complex addiction-, PD- and anxious-related behavior would be ideal for expanding the list of candidate genes. As a result of factors discussed in section 1, zf is currently the vertebrate of choice in forward genetics, and the level of behavioral analysis possible in these animals is only now being explored. In regard to the addiction issue it has been shown that zf exhibit CPP to cocaine (Darland and Dowling, 2001) and morphine (Bretaud, unpublished results). The main aim of this study was to develop new assays where some aspects of dopamine-related disorders could be modeled. For instance, upon developing an assay that models addiction, one could carry through a mutagenesis screen looking for mutants that behave differently than wt in that paradigm, such as altered sensitivity to an addictive drug. Subsequent characterization of the involved gene(s) by cloning could enable us to discover new genes involved in the complex addiction process in the future. Further work could ultimately lead to the discovery of new drugs or novel therapies for the disease in question. Forward genetic screening of zf employing behavioral testing should be a promising way to uncover novel genes linked not only to disorders affecting the DAergic system but human diseases in general.

3.2. Locomotor activity assays

3.2.1. Parkinson modeling – the *too few* mutant and wild type

DA in mammals is involved in for instance locomotion, reward, and response to novelty/learning (as reviewed in section 1). There are three ways to find out if DA has a similar role in zf: Either by the employment of DA agonist and antagonist drugs or the use of mutant fish or transgenic fish. In order to assess the effect of pharmacological treatment or ablation/overexpression of genes, behavioral assays are needed either way. Having the *tfu* mutant in hand, which lacks DAergic neurons, the obvious approach would be to investigate if it could be employed as a PD model due to the nature of the disease. Lacking DAergic neurons, one could hypothesize that fish would exhibit bradykinesia, assessed in a behavior assay. As fig. 2.3 shows, this is only true for a couple of early time points in the swim speed assay. It has been established that there are no significant differences in the adult fish (Kobayashi, unpublished results) despite the significant loss of DA neurons (Guo et al., 1999b). *Tfu* mutant fry also showed that AMPH induces some hyperactivity (fig. 2.12), although the effect also could be mediated by other mediators such as NA and 5-HT. Explanations for these observations could be that the reduced DA function is compensated for, either by increased postsynaptic sensitivity as seen in human PD patients by denervation sensitization (Rang et al., 1999) or that other compensating neuronal circuits are made in the developing embryo.

As reported by Pollard et al., 1992 and as fig 2.16 suggests, MPTP neurotoxicity in gold- and zebrafish induces a syndrome that has many parallels to the parkinsonian state induced by this compound in humans and lower primates. The toxicity in both goldfish and primates appears to be related to the common mechanism of MPTP oxidation to MPP+ by MAO-B, and MAO inhibitors can function as neuroprotective agents (Pollard et al., 1992 and references therein). The MPTP toxicity observed in zf suggests that at least a subset of the DAergic HY neurons in the fish are functionally equivalent and correspond to the basal ganglia and *substantia nigra* in higher vertebrates, and that MPTP toxicity may mirror some aspects of idiopathic PD. Rodents are generally resistant to MPTP and the cost and scarcity of primates have limited their application (Pollard et al., and references therein). Thus zf have potential to be a simple and inexpensive way to study MPTP toxicity and may permit the zf to supplement primates for the purpose of searching and screening for neuroprotective drugs with relevance

to PD. A modification of the assay developed under section 2.4.5 being one potential way to do screens.

Although no acute effects were seen, the antipsychotic drug fluphenazine induced parkinsonian symptoms when zf fry were treated for six hours, acknowledged by speed measurements and observation under a microscope (unpublished data). This could potentially be taken advantage of in a mutagenesis screen, screening for mutant fry being resistant to the induced adverse effects. This could possibly lead to identification of genes involved in Parkinson's disease or genes involved in mediating side effects of the drug. Furthermore, a similar setup could be employed screening for antipsychotic drugs lacking extrapyramidal side effects. One obstacle for a drug screen may be solubility of the drug candidates, since some water solubility is required upon administration to fry. Modifications undertaken to increase solubility should not affect the fry. Furthermore, characterization of fry pharmacokinetic properties would be desirable. These two latter screen suggestions may not sound attractive but are possibilities.

3.2.2. Modeling addiction

Ethanol and amphetamine have a large number of measurable effects in human and model organisms. One of them, locomotor stimulation is employed as an animal model of human euphoria and has long been recognized (Phillips and Shen, 1996). An understanding of the spontaneous locomotor effect and stimulant effects of these drugs is beginning to emerge, while the basis of addiction is still largely unknown (section 1.7). During the little screening for drugs inducing hyperlocomotion in fry, EtOH and AMPH were the only ones found. ABC fry showed a consistent large increase in activity given EtOH, while the *tfu* and its wt sibling responded less (fig. 2.4). There are also differences between *tfu* and wt sibling, but it is not certain these can be accounted for by the relative lack of DA, since there is a genetic background difference between SG018 and SG019 (table 2.2). Furthermore we cannot be sure the stimulation was a result of the effect on CNS as expected. One caveat of pharmacologic manipulation is the concern about whether there is receptor homology to mammals in the model animals. The response to EtOH was large for some fry, but it is also possible that EtOH could have some aversive external effect, leading to the hyperactivity observed.

AMPH, EtOH and also cocaine induced hypoactivity in fry, leading to five potential ways to carry out a mutagenesis screen: screening for fry that react differently than those showing

hyperactivity to low doses of EtOH and AMPH, and screening for fry reacting differently to high, immobilizing doses of EtOH, AMPH and cocaine. Mutagenesis could be carried out using the efficient mutagen ENU as described in section 1.3. F_1 and F_2 generation mutant fish could be bred and the resulting F_3 fry screened using the corresponding protocols drawn up in section 2. Mutant fry found to have altered properties than wt could eventually be characterized by cloning and maybe novel genes executing the drug effects could be found. These simple behavioral assays acutely reflect swim speed and do not directly model addiction. However, even though not found to induce hyperactivity in seven- or 14-day old zf fry, addictive drugs in general lead to locomotor stimulation (see references in section 2.1). This means that upon finding interesting mutations in a simple locomotor assay, new more labor-intensive screens have to be undertaken to sort out genes that may be responsible for addiction and genes that have other effects. Self-administration assays in rodents or the conditioned place preference paradigm are behavior assay examples that more directly model drug addiction and drug preference and could be employed in the subsequent step. Self-administration briefly often involves rodents that press a lever for i.v. administration of drugs. In a CPP assay, application of a drug is paired to a second stimulus such as a particular set of visual cues. Upon further testing without the primary stimulus, the animal responds to the second stimulus alone with an approaching behavior. After identification of interesting phenotypes and cloning of the genes in question, new avenues in the understanding of addiction could be enlightened and ultimately novel therapies developed.

3.2.3. Other drugs and aspects

Experiments on EtOH tolerance and morphine did not give positive results, and the employed zf fry protocols are thus not suitable for mutagenesis screens. Tolerance and morphine- (and cocaine-) related behavior can potentially contribute to the understanding of addiction by using other behavior assays such as CPP.

Referring to fig. 2.17, it was observed that a two-hour treatment of fluphenazine before EtOH exposure more or less abolished the EtOH-effect. Fluphenazine is a general DA receptor antagonist. Thus, this suggests that the effect of ethanol on locomotor activity is mediated through the central nervous system and DA is important in mediating the stimulant effect. A single ten-fry experiment using 2.0mg/L fluphenazine pretreated for two hours alone, showed swim speeds close to placebo, implying that the reduced EtOH swim speed is not solely due to antipsychotic drug-induced PD. Surprisingly the acute treatment of fluphenazine

concomitantly with EtOH resulted in some sensitization compared to placebo. One explanation could be that in initial phases, fluphenazine acts as an antagonist at D_2 autoreceptors, increasing overall DA turnover. This could also be the explanation why high doses of plain fluphenazine induced hyperlocomotion, although this also could be a toxicity effect.

3.3. Open field and light/dark assays

3.3.1. Anxiety models

Strictly speaking, the swim speed assays in sections 2.3, 2.4 and 2.5 are also open field assays. These behavioral assays are commonly used to test both locomotor activity and anxiety in rodents (Bronikowski et al., 2000; Belzung and Griebel, 2001). Traditional parameters investigated are travel distance and number of fecal boli. Mice that move little, defecate often, or hurry to the wall zone and spend little time in the interior, are classified as less exploratory and more emotionally reactive or anxious. There is, to my knowledge, no standardized open field behavior assay, and rodent examples can be found in Parks et al., 1998; Ramesh et al., 1998; Köster et al., 1999. For anxiety research, the elevated plus-maze is one of the most popular anxiety models. Several knockout mice and drugs on wt mice have been investigated employing the plus-maze. EtOH in addition to other drugs has been found to be anxiolytic in mice, while AMPH failed to alter the indices of anxiety (Lister RG, 1987). A third behavior assay for measuring anxiety is the light/dark choice. Briefly, the test animal, often a rodent is placed in a test box consisting of a light and a dark compartment. The light compartment is illuminated with a lamp and is more aversive to the test animals than the dark area. The latency time to enter the dark compartment after being put in the light area, or the total time spent in the separate compartments are examples of measured parameters. Anxiolytic drugs like benzodiazepines result in a delay in the movement from dark to light in rodents. Some transgenic animals may show an increased preference for the dark compartment. Examples of light/dark behavioral assays can be found in Stork et al., 1999; Okuyama et al., 1999. A critical discussion of some mouse anxiety models can be found in Belzung and Griebel, 2001.

3.3.2. Genetic screening employing anxiety models

In section 2.6.2 and 2.7.2, it was found that EtOH treated fry exhibit thigmotaxis and flight into a dark compartment respectively. Analogy to rodent models suggests that this behavior is

anxiety-related. However, in rodents acutely administered EtOH is anxiolytic (Lister, 1987). Also, in non-alcoholic humans, alcohol administration results in an anxiolytic effect, but the assumption that anxiety reduction is a major factor in the etiology of drinking problems no longer appears to be plausible (Allan, 1995). Anxiety problems often occur in problem drinkers, and it is likely that anxiety is a consequence rather than a cause of excessive drinking. Alcohol withdrawal induces anxiety (Kiefer et al, 2002), but this is an effect seen later than at the acute intoxication phase right after administration and depends on secondary cellular responses to alcohol. These facts question the suggestion that the fry in the above-mentioned assays are anxious if the alcohol effect purely is mediated by the known CNS mechanisms. Do seven days old fry respond qualitatively different to EtOH in regard to anxiety than other model systems? Could one possible explanation for the "fear" observed, be an aversive external effect? To throw light on the nature of the "anxiety" observed, further investigations need to be carried out.

Based on the assumption that the open field and light/dark assay with seven-day old fry and EtOH are anxiety models, mutagenesis screens could be undertaken as described earlier, looking for fry behaving differently than wt. Screens both with and without EtOH could potentially be done for both of the assays. Regarding open field, placebo treated fry exhibiting thigmotaxis could carry mutations that make them more anxious with the potential identification of genes with a calming effect. An EtOH-based screen could be undertaken to identify mutations with a diminished sensitivity to EtOH-induced thigmotaxis, thus characterization of genes responsible for anxiety. Altered response in an EtOH based screen could also reflect mutations in genes, proteins of which are responsible for carrying out the effects of EtOH itself. Similar settings could be employed for the light/dark assay, fry preferring the light compartment being less anxious and fry preferring the dark compartment being more anxious. Given EtOH, no preference for the dark area could reflect mutations in genes responsible for mediating anxiety but also other alcohol effects, meaning that further investigations would need to take place to characterize the true nature of the phenotype. Upon cloning of genes responsible, new insight to the genetic basis and mechanisms of anxiety could be elucidated, ultimately resulting in novel therapies.

The *tfu* mutant shows altered response in the above-mentioned behavior assays, although not significantly different with such low sample sizes (fig 2.24 and 2.27). Still, it cannot be

excluded that the DA difference between *tfu* and wt is responsible for the behavior observed, since DA has a role in anxiety/stress as reviewed by Horger and Roth, 1996.

3.4. Summary of assay procedures that could be used for genetic screens

Carrying out a genetic screen is a huge amount of work. A large number of experimental animals need to be screened in order to have any hope finding mutations in interesting genes. Thus a genetic screen procedure should be as fast and simple as possible. Besides the obvious demand that there has to be a way to detect mutants, another main requirement is that the screen is robust. Much underlying variability leads to difficulties identifying mutants, and therefore it should be statistically easy to distinguish true mutants from wt. This will result in a lower number of false positives detected and false negatives; true mutants that failed to be discovered.

Generally, there was substantial variability in the locomotor activity assays. SG019 wt does not significantly respond to AMPH, and the alcohol response compared to other wildtype backgrounds is diminished. The *tfu* mutant having a slightly different background, it is hard to know if the differences seen really are due to the mutation or the general genotype. Carrying out a screen for locomotor activity mutants, employing a fish line such as ABC which exhibits a consistent and less variable swim speed increase would probably be a good idea. Since the difference between placebo and drug are bigger, mutants would be easier to part out. Regarding AMPH, the drug/placebo difference again is not considerable looking at hyperactivity, possibly making it difficult to identify mutants with high certainty. However, the hypoactivity shown by 10mg/L AMPH treated fry after 20 minutes is relatively consistent and may be a more robust assay for genetic screens, searching for mutants showing resistance to the AMPH induced locomotor changes.

Moving on to the open field assay, there was also some variability that would not make it easy always to judge from a single ten-fry curve whether it should be classified as a placebo- or EtOH curve. In general, however an EtOH curve is easily distinguished from a placebo curve, and the open field assay with EtOH on ABC fry would be a simple and relatively robust way for carrying out a genetic screen.

The light dark assay is much less labor-intensive than the open field assay because there is no need to carry out time-consuming DIAS® data analysis. In addition, this assay exhibits a seemingly consistent and not a very variable response both to placebo and EtOH, although sample sizes are regrettably small. The potential as a genetic screen tool looks very promising, but there is definitely a need to increase the sample sizes to clarify how consistent the results really are. It should be mentioned in the same sentence that another researcher in the lab obtained results similar to those obtained in this study, later.

3.5. Conclusion

As outlined in the opening, the overall goal of the study is to use zf as a vertebrate genetic model to identify genes and pathways that are important in the etiology and pathogenesis of DA-related disorders. In order to reach this goal, an array of smaller projects has to be carried out and put successfully together. One of the first steps in a mutagenesis screen and subsequent characterization of genes of interest would be the development of behavioral assays. Good assays are the foundation of successful mutagenesis screens. Employing a locomotor assay, several drugs were screened for interesting effects on zf fry. EtOH and AMPH turned out to induce hyperlocomotor activity at specific concentrations. The assays are simple and could be utilized in genetic screens looking for genes responsible for addiction-related behavior, although an AMPH screen would be much less robust than an EtOH screen because of a smaller drug response. At higher doses, hypoactivity could also be utilized for screening procedures. Several other drugs were tested in the locomotor assay, but none were found with consistent features significantly different from placebo like EtOH or amphetamine. Cocaine is a possible exception. Furthermore, an open field and a light/dark assay were developed. Investigation with EtOH suggests that these assays may model anxiety. Both assays have potential to be employed in mutagenesis screens, with or without drug, but the light/dark assay might be preferred because it seems to be relatively robust, although small sample sizes, and the especially quick nature of a screen. How successful a mutagenesis screen would be by employment of these behavioral assays remains to be elucidated, however genes involved in the etiology and pathogenesis of addiction and anxiety could eventually be discovered. The foundation of success rests on whether EtOH's effect really reflects CNS mediated behaviors and that analogy to rodent behavior can be drawn for the open field and light/dark assay. In addition to development of behavior assays, the characteristics of fry with different genetic backgrounds including the *tfu* mutant were assessed. Large response

differences were surprisingly seen between fry of different backgrounds, but no obvious clear differences were observed in the *tfu* mutant as a consequence of the DA-deficiency. Summarizing, the goals outlined for this specific study in section 1.1, have been reached, although less variability and a few more positive findings would have been desirable.

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5. APPENDIX: Excel® data sheets showing the individual values of the charts presented in section 2.

5.1. Basal locomotor activity of seven-day old ABC SG013

Time (min)	0	1	3	5	7	10	13	16	20
No drug met1, 02-14	1,59	1,86	1,61	1,74	1,70	1,81	1,84	1,75	1,59
No drug met2, 02-14	0,75	1,63	1,98	2,03	1,88	1,55	1,69	1,48	1,43
No drug 1, 02-16	1,11	1,52	2,14	1,70	2,34	2,20	1,86	2,34	1,68
No drug 2, 02-16	1,20	1,97	2,03	2,35	2,27	1,86	1,92	1,76	1,65
No drug 3, 02-16	0,95	1,52	1,72	1,70	1,81	1,67	1,88	1,71	1,84
No drug 4, 02-16	1,47	1,59	1,52	1,54	1,45	2,16	1,99	1,74	1,51
No drug 6, 02-16	0,61	1,63	1,92	2,14	1,60	1,91	1,94	1,69	2,10
No drug 8, 02-16	1,82	2,11	1,85	2,17	2,65	2,06	1,95	2,09	2,03
No drug 1 mean 05-23	2,05	1,76	2,15	2,09	2,00	1,75	2,01	1,85	1,74
No drug 2 mean 05-23	1,87	2,32	2,42	2,19	2,10	2,34	2,49	2,36	1,85
No drug 4 mean 05-23	1,51	2,00	2,25	2,14	2,18	2,28	2,19	2,03	1,73
No drug 5 mean 05-23	1,87	2,24	2,20	2,39	2,18	2,41	1,99	1,78	1,69
No drug 1 mean 05-23	1,19	1,80	1,71	2,04	1,98	2,02	1,92	1,72	1,67
No drug 2 mean 05-23	1,37	2,22	1,91	1,78	2,01	1,89	1,85	1,79	1,58
05-30: No drug cross2	1,18	1,47	1,69	2,14	2,15	1,95	2,18	1,75	1,24
05-30: No drug cross 3	1,24	1,77	2,16	1,91	2,41	2,30	2,20	1,78	1,93
06-21: No drug New curve	2,01	1,76	2,22	2,02	2,33	2,17	2,14	2,01	1,99
06-21: 0% Control	1,17	1,83	2,18	1,94	1,78	1,41	1,46	1,65	1,68
07-08: No drug #1,	1,13	1,19	1,53	1,56	1,73	1,75	2,02	2,14	2,25
07-08: No drug #2,	0,90	1,36	1,50	1,60	1,90	1,62	1,62	1,59	1,48
07-23: No drug 1	1,04	2,00	2,03	2,07	2,11	2,17	1,84	2,05	1,51
07-25: No drug 2	0,66	1,57	1,61	1,84	1,81	1,67	1,65	1,69	1,39
07-30: No drug 1	1,86	1,70	1,78	1,72	1,74	1,78	1,81	1,79	1,85
07-30: No drug 2	1,48	1,83	1,62	1,71	1,99	1,90	1,78	1,91	1,74
08-02: No drug	1,31	1,33	1,96	2,05	2,46	2,18	1,98	2,26	1,91
Average ABC SG013 Placebo N=25x10	1,33	1,76	1,91	1,94	2,02	1,95	1,93	1,87	1,72
Std dev	0,41	0,29	0,27	0,24	0,29	0,27	0,21	0,23	0,24
Std err	0,08	0,06	0,05	0,05	0,06	0,05	0,04	0,05	0,05

5.2. Basal locomotor activity of seven-day old ABC SG035

Time (min)	0	1	3	5	7	10	13	16	20
06-27: Control Placebo mean	2,23	2,52	2,87	2,52	2,64	2,37	2,53	2,73	2,68
07-04: No drug mean	1,19	1,61	1,52	1,73	1,85	1,91	1,39	1,37	1,06
07-18: Control No drug mean	1,16	2,10	1,43	2,07	2,25	2,21	2,65	2,31	2,27
Average ABC SG035 Placebo N=30	1,53	2,08	1,94	2,10	2,25	2,17	2,19	2,14	2,00
Std dev	0,61	0,45	0,81	0,40	0,39	0,23	0,70	0,70	0,84
Std err	0,35	0,26	0,47	0,23	0,23	0,13	0,40	0,40	0,49

5.3. Basal locomotor activity of seven-day old tfu1x-47 x tfu1x-45 (wt)

Time (min)	0	1	3	5	7	10	13	16	20
No drug 11-13	1,16	1,70	1,46	1,32	1,67	1,77	1,89	1,70	1,63
No drug 11-06-01	0,85	1,00	1,05	1,50	1,72	2,05	2,05	2,02	1,91
No drug 11-20-01	0,84	1,04	1,90	1,70	1,71	1,80	1,86	1,90	1,68
Average Tfu1x47*tfu1x45(wt) No drug N=30	0,95	1,25	1,47	1,51	1,70	1,87	1,93	1,87	1,74
Std dev	0,18	0,39	0,42	0,19	0,03	0,15	0,10	0,16	0,15
Std err	0,11	0,23	0,25	0,11	0,02	0,09	0,06	0,09	0,09

5.4. Basal locomotor activity of seven-day old wildtype SG019

Time (min)	0	1	3	5	7	10	13	16	20
02-20: No drug 1	1,33	2,36	2,57	2,89	2,82	2,45	2,05	2,77	2,21
02-20: No drug 2	1,31	0,99	0,82	0,79	0,86	0,61	0,99	0,95	0,97
02-27: No drug 3	1,09	1,15	1,51	1,83	2,09	2,06	2,07	1,97	1,57
02-27: No drug 4	0,94	1,21	1,56	1,39	1,44	1,31	1,85	1,52	1,72
02-28: No drug 1	0,67	1,25	1,81	2,06	2,45	2,53	2,63	2,67	2,58
02-28: No drug 2	1,53	1,95	2,60	2,50	2,22	2,32	2,07	1,75	2,27
03-06: No drug	0,64	1,27	1,81	1,93	1,81	2,09	1,57	1,74	1,81
04-18: No drug 1	1,10	1,56	1,52	1,66	1,48	1,23	1,52	1,45	1,75
04-18: No drug 2	0,83	1,46	1,71	1,80	1,83	1,65	1,55	1,31	1,30
05-10: Cr1 No drug mean	1,23	1,45	1,63	1,84	1,71	1,84	1,80	2,17	2,22
05-10: Cr2 No drug mean	0,71	1,53	2,02	1,97	2,02	1,82	1,96	1,84	1,80
07-01: No drug mean	0,73	0,75	1,62	1,80	1,72	2,10	1,84	1,45	1,28
07-09: No drug mean	2,02	2,68	3,66	3,31	3,15	3,28	2,97	2,88	2,53
07-16: No drug 1 mean	1,18	1,73	1,98	2,42	2,54	2,63	2,00	2,05	1,81
07-24: No drug 2 mean	0,37	1,97	2,28	2,26	2,28	2,30	2,29	2,17	1,76
07-24: No drug 1 mean	1,02	1,60	1,94	1,54	1,53	1,75	1,61	1,68	1,37
08-09: No drug	1,25	1,13	1,45	2,10	1,89	2,44	2,55	2,78	2,54
Average SG019 Placebo N=17x10	1,06	1,53	1,91	2,01	1,99	2,02	1,96	1,95	1,85
Std dev	0,40	0,50	0,63	0,59	0,57	0,63	0,46	0,54	0,46
Std err	0,10	0,12	0,15	0,14	0,14	0,15	0,11	0,13	0,11

5.5. Basal locomotor activity of seven-day old tfu mutant SG018

Time (min)	0	1	3	5	7	10	13	16	20
12-27: No drug (SG006)	1,25	0,92	1,10	1,55	1,51	1,49	1,74	1,77	1,60
02-04: No drug	1,82	1,40	1,31	1,45	2,00	2,07	2,27	1,68	1,67
02-14: No drug	0,59	1,06	1,42	1,57	1,55	2,33	2,26	2,01	2,34
02-21: No drug	1,11	1,60	1,68	1,34	1,15	1,65	1,70	1,44	1,79
02-28: No drug	1,64	0,94	1,32	1,31	1,10	1,71	1,37	1,97	1,38
05-10: No drug mean	2,05	2,21	1,80	1,36	1,40	1,36	1,38	1,49	1,59
07-01: No drug 1 mean	1,78	2,21	1,62	1,71	1,62	0,91	1,35	1,95	1,61
07-01: No drug 2 mean	1,39	1,44	1,50	1,81	1,78	2,05	2,10	1,89	1,70
07-09: No drug mean	1,46	1,53	1,44	1,38	1,86	1,73	1,89	1,93	1,74
07-31: No drug 2mean	1,44	1,59	1,73	2,04	1,74	2,08	1,78	1,75	1,59
07-31: No drug 3 mean	1,45	1,68	2,05	1,68	1,70	1,96	1,89	1,95	1,64
08-10: No drug 1	1,93	1,94	2,11	1,94	1,43	1,93	1,60	1,63	1,59
08-10: No drug 2	1,97	2,36	2,24	2,21	2,11	1,89	1,96	1,84	1,47
Average SG018 No drug N=13x10	1,53	1,61	1,64	1,64	1,61	1,78	1,79	1,79	1,67
Std dev	0,40	0,48	0,34	0,29	0,30	0,37	0,31	0,19	0,23
Std err	0,11	0,13	0,09	0,08	0,08	0,10	0,09	0,05	0,06

5.6. Basal locomotor activity of seven-day old wildtype SG023

Time (min)	0	1	3	5	7	10	13	16	20
03-22: No drug 1 hf mean	1,89	2,75	3,01	2,99	2,82	2,57	2,19	2,39	1,88
03-22: No drug 2 hf mean	0,46	1,89	1,80	1,57	1,79	1,46	1,79	1,59	1,23
07-01: No drug mean	0,64	1,10	1,86	1,73	2,04	2,09	2,12	2,21	1,72
08-09: No drug mean	1,64	2,06	2,02	1,95	2,11	2,09	1,71	1,71	1,52
Average SG023 No drug N=40	1,16	1,95	2,17	2,06	2,19	2,05	1,95	1,98	1,59
Std dev	0,71	0,68	0,57	0,64	0,44	0,45	0,24	0,39	0,28
Std err	0,36	0,34	0,28	0,32	0,22	0,23	0,12	0,19	0,14

5.7. Basal locomotor activity of seven-day old WIK SG003

Time (min)	0	1	3	5	7	10	13	16	20	25	30
05-21: No drug 1 mean	1,51	2,14	2,70	2,92	3,06	3,50	3,86	3,90	3,89		
05-21: No drug 2 mean	1,70	2,04	2,71	3,00	2,85	3,36	3,65	3,54	3,63	2,86	3,12
05-28: No drug #1 mean	1,02	1,33	1,82	2,18	2,37	2,64	3,01	3,09	2,88	2,81	3,06
05-28: No drug #2 mean	1,06	1,55	1,73	2,19	2,22	2,22	2,12	2,75	2,76	2,95	2,33
05-28: No drug #3 mean	0,99	1,75	2,15	2,11	2,51	2,61	2,79	2,85	3,10	2,19	2,37
05-28: No drug #4 mean	1,24	1,96	2,26	2,27	2,33	3,31	3,03	3,03	2,95	2,57	3,03
06-05: No drug 1 mean	0,78	1,45	1,88	1,70	2,12	2,48	2,60	3,15	2,98	3,47	3,33
06-05: No drug 2 mean	0,88	1,49	1,59	1,68	1,86	2,14	2,51	2,24	2,22	2,05	1,60
Average Wik SG003 No drug N=80	1,15	1,71	2,11	2,26	2,42	2,78	2,95	3,07	3,05	2,70	2,69
Std dev	0,32	0,30	0,43	0,49	0,39	0,54	0,58	0,50	0,52	0,48	0,61
Std err	0,11	0,11	0,15	0,17	0,14	0,19	0,21	0,18	0,18	0,17	0,22

5.8. EtOH induced locomotor activity of seven-day old ABC SG013

Time (min)	0	1	3	5	7	10	13	16	20
07-08: EtOH 0,5% #1 mean	0,93	1,19	1,60	1,48	1,61	1,46	1,57	1,57	1,58
07-08: EtOH 0,5% #2 mean	1,10	1,50	2,03	1,74	2,08	2,06	1,59	1,49	1,53
08-02: EtOH 0,5% #1 mean	1,42	2,21	2,43	2,28	2,43	2,27	2,18	2,05	1,81
08-02: EtOH 0,5% #2 mean	0,98	1,55	1,73	2,21	1,84	2,01	2,12	1,74	1,67
Average EtOH 0.5% N=4x10	1,11	1,61	1,95	1,93	1,99	1,95	1,86	1,71	1,65
Std dev	0,22	0,43	0,37	0,38	0,35	0,35	0,33	0,25	0,12
Std err	0,11	0,21	0,18	0,19	0,17	0,17	0,16	0,12	0,06
07-08: EtOH 1,0% #1 mean	1,36	1,43	1,74	1,87	2,04	3,47	4,56	5,97	4,83
07-08: EtOH 1,0% #2 mean	1,16	1,68	2,28	2,53	3,71	4,56	4,66	5,44	6,05
07-26: EtOH 1,0% mean	2,02	2,60	2,40	2,85	3,00	3,81	4,03	3,35	3,02
07-30: EtOH 1,0% mean	1,28	1,71	2,09	2,54	2,71	3,41	4,22	4,39	4,66
08-02: EtOH 1,0% #1 mean	1,42	2,43	2,31	2,52	2,68	3,55	3,76	3,98	4,11
08-02: EtOH 1,0% #2 mean	2,06	2,56	2,58	2,83	2,90	3,43	3,78	4,29	4,40
Average EtOH 1.0% N=6x10	1,55	2,07	2,23	2,52	2,84	3,70	4,17	4,57	4,51
Std dev	0,39	0,52	0,29	0,36	0,54	0,44	0,39	0,96	0,99
Std err	0,16	0,21	0,12	0,15	0,22	0,18	0,16	0,39	0,40
EtOH 1,5% Met 1, 02-07	1,25	1,18	3,13	3,67	4,98	5,13	4,50	4,73	4,70
EtOH 1,5% Met 2, 02-07	1,34	1,35	2,71	3,01	3,86	3,27	6,16	6,63	6,63
Incub-10pm-met1	1,49	3,35	3,77	3,32	4,26	4,51	5,61	5,14	4,21
Incub-10pm-met2	1,14	2,25	2,82	4,19	5,33	7,01	5,47	6,18	3,26
Incub-8am-met1	1,60	2,28	2,42	3,38	3,45	5,21	5,61	5,91	6,85
Incub-8am-met2	2,79	3,33	2,67	3,22	3,19	4,00	5,72	5,70	7,55
EtOH 1,5% 02-28	1,26	2,43	3,00	3,93	4,21	5,60	6,77	6,83	7,03
EtOH 1,5% #1 mean 05-23	2,74	2,88	3,44	4,80	7,02	7,39	7,33	5,15	5,85
EtOH 1,5% #2 mean	2,02	2,20	3,24	4,53	7,16	7,08	6,15	5,63	5,47
EtOH 1,5% #4 mean	2,29	2,11	2,21	2,45	3,54	4,79	6,39	5,97	6,63
EtOH 1,5% #5 mean	3,04	2,86	2,68	3,67	4,43	5,97	6,71	7,20	7,61
EtOH 1,5% Incubator #1 mean	1,42	1,53	2,60	3,69	4,52	4,65	5,79	6,69	6,37
EtOH 1,5% Incubator #2 mean	2,30	2,27	3,13	4,64	6,94	6,72	7,70	7,24	3,43
05-30: EtOH 1,5% Cross 2	1,51	2,10	3,34	4,76	6,01	7,47	7,42	7,05	6,66
05-30: EtOH 1,5% Cross 3	1,28	1,53	2,34	4,51	4,79	4,92	3,58	3,60	3,51
05-30: EtOH 1,5% Cr 2	1,04	1,74	3,45	5,11	6,80	8,15	7,92	5,97	6,94

05-30: Tol No drug Cr3 (EtOH 1,5%)	1,53	1,97	2,23	3,17	4,15	5,54	7,02	7,23	6,16
05-30: Tol No drug Cr2 (EtOH 1,5%)	1,41	1,58	2,77	3,48	5,29	7,72	7,66	7,85	7,15
06-21: EtOH 1,5% New curve	1,59	2,53	2,94	3,40	3,69	5,03	5,17	6,16	5,77
06-21: EtOH 1,5% prev untr.	1,88	1,43	2,38	3,01	2,97	4,86	3,88	4,49	4,21
07-08: EtOH 1,5% #1,	1,46	1,98	2,32	4,09	5,96	5,47	5,32	5,72	4,91
07-08: EtOH 1,5% #2,	2,07	1,73	2,82	3,74	5,26	7,66	7,94	6,08	7,17
07-16: Control #1 mean	1,83	2,19	2,59	4,47	6,39	8,40	5,64	5,29	5,00
07-16: Control #2 mean	2,34	2,41	3,24	3,92	6,92	6,87	5,69	7,02	4,56
07-19: Control #1 EtOH 1,5% mean	2,05	2,16	2,65	3,43	4,01	4,80	5,84	4,05	3,27
07-19: Control #2 EtOH 1,5% mean	2,30	2,35	2,58	3,32	4,01	5,17	6,50	5,41	5,84
07-26: Control #1 mean	2,08	1,86	2,76	4,05	5,93	6,66	5,26	5,18	5,98
07-26: Control #2 3hr mean	2,24	2,32	2,88	4,63	6,62	6,23	4,44	4,77	4,39
07-30: Control #1 mean	1,53	1,81	2,82	3,50	3,81	5,23	5,28	4,53	4,37
07-30: Control #2 mean	1,52	1,66	2,46	2,77	4,08	4,86	5,10	5,45	3,19
07-30: Control #3 mean	1,33	1,68	2,27	3,36	4,30	5,76	7,81	6,76	5,18
08-02: Control #1 mean	1,83	1,67	1,88	3,98	4,65	4,50	4,09	5,03	5,20
08-02: Control #2 mean	1,92	2,07	2,97	3,94	5,01	6,85	4,15	3,82	3,86
08-06: Flu 0mg EtOH 1,5% mean	0,98	1,23	2,05	2,72	3,71	3,83	3,43	2,63	1,90
08-06: Control #1 mean	1,36	1,49	2,04	2,75	3,53	5,29	4,95	5,22	4,53
08-06: Control #2 mean	1,23	1,36	2,22	2,94	2,79	3,55	4,67	4,29	5,06
Average EtOH 1.5% N=36x10	1,75	2,02	2,72	3,71	4,82	5,73	5,80	5,63	5,29
Std dev	0,51	0,53	0,44	0,66	1,26	1,31	1,26	1,16	1,42
Std err	0,08	0,09	0,07	0,11	0,21	0,22	0,21	0,19	0,24
07-08: EtOH 2,0% #1 mean	1,38	1,45	2,80	3,64	3,79	4,61	4,80	5,97	6,31
07-08: EtOH 2,0% #2 mean	2,04	2,49	3,51	4,35	4,48	3,82	3,98	6,15	6,45
07-30: EtOH 2,0% mean	1,89	1,85	2,83	3,04	3,29	3,47	3,55	3,58	3,27
08-02: EtOH 2,0% #1 mean	2,04	2,23	3,32	4,07	2,91	2,40	2,95	3,27	3,43
08-02: EtOH 2,0% #2 mean	2,71	2,56	3,15	3,93	4,03	3,62	3,53	3,79	3,54
Average EtOH 2.0% N=5x10	2,01	2,12	3,12	3,81	3,70	3,59	3,76	4,55	4,60
Std dev	0,47	0,46	0,31	0,50	0,61	0,79	0,69	1,39	1,63
Std err	0,21	0,21	0,14	0,22	0,27	0,36	0,31	0,62	0,73
07-08: EtOH 3,0% #1 mean	1,72	1,42	2,66	1,48	1,13	1,42	1,93	1,45	0,97
07-08: EtOH 3,0% #2 mean	1,98	2,57	3,21	1,89	1,50	2,32	1,43	0,46	0,27
07-26: EtOH 3,0% mean	3,23	2,30	3,61	0,91	1,22	2,63	0,52	0,47	0,31
07-30: EtOH 3,0% mean	2,69	3,01	3,31	2,51	1,29	1,56	1,52	1,45	0,46
08-02: EtOH 3,0% #1 mean	3,18	2,76	3,89	1,92	0,63	1,42	1,59	0,85	0,41
08-02: EtOH 3,0% #2 mean	2,87	1,93	2,47	1,56	0,57	1,02	1,06	1,39	2,62
Average EtOH 3.0% N=6x10	2,61	2,33	3,19	1,71	1,06	1,73	1,34	1,01	0,84
Std dev	0,63	0,58	0,54	0,54	0,38	0,61	0,49	0,48	0,91
Std err	0,26	0,24	0,22	0,22	0,15	0,25	0,20	0,20	0,37
07-08: EtOH 4,0% #1 mean	1,46	1,81	0,72	0,61	1,27	0,29	0,56	0,49	0,36
07-08: EtOH 4,0% #2 mean	2,66	2,78	0,62	0,85	1,39	0,46	0,37	0,39	0,45
Average EtOH 4.0% N=2x10	2,06	2,29	0,67	0,73	1,33	0,38	0,46	0,44	0,40
Std dev	0,85	0,69	0,07	0,18	0,09	0,12	0,13	0,07	0,07
Std err	0,60	0,48	0,05	0,12	0,06	0,09	0,09	0,05	0,05

5.9. EtOH induced locomotor activity of seven-day old ABC SG035

Time (min)	0	1	3	5	7	10	13	16	20
06-19: Control 1,5% EtOH mean	1,77	2,23	2,96	3,62	5,53	5,57	6,75	6,01	4,34
06-27: Control EtOH 1,5% mean	1,36	1,45	2,19	3,28	4,20	5,74	4,73	4,41	4,17
07-04: EtOH 1,5% mean	1,43	1,79	2,31	2,89	3,89	4,65	3,88	4,74	5,26
07-18: Control EtOH 1,5% mean	1,22	1,69	1,94	2,51	3,34	4,27	4,09	5,21	5,34
07-25: Control #1 (EtOH 1,5%) mean	1,73	2,21	2,46	3,03	3,15	4,98	5,69	5,84	4,99
07-25: Control #2 (EtOH 1,5%) mean	1,51	2,19	2,40	2,81	4,14	4,20	4,20	4,96	3,93
08-02: 0mg flu - EtOH 1,5% mean	2,75	2,98	3,79	5,01	7,35	6,74	4,54	3,91	4,19
Average SG035 EtOH 1,5% N=70	1,68	2,08	2,58	3,31	4,52	5,17	4,84	5,01	4,60
Std dev	0,51	0,50	0,62	0,83	1,47	0,92	1,03	0,75	0,58
Std err	0,19	0,19	0,23	0,31	0,55	0,35	0,39	0,28	0,22

5.10. EtOH induced locomotor activity of seven-day old tfu1x-47 x tfu1x-45 (wt)

Time (min)	0	1	3	5	7	10	13	16	20
EtOH 1,5% 01-30 Test 2	1,33	1,47	2,05	2,80	4,11	5,50	5,73	6,77	4,56
EtOH 1,5% 01-30 Test 4	1,32	1,87	2,42	2,62	4,11	5,37	5,95	7,00	6,07
EtOH 1,5% 01-30 Test 6	1,69	2,59	2,53	3,33	3,72	6,17	6,09	6,01	4,89
EtOH 1,5% 01-30 Test 7	1,51	2,11	2,49	3,67	4,18	6,30	5,06	5,41	3,22
EtOH1,5% 11-06	0,91	0,95	1,64	2,88	4,74	5,52	6,15	6,60	5,57
EtOH1,5% 11-13	1,50	1,81	2,71	4,27	5,59	6,83	5,86	6,40	6,27
Average Tfu1x47*tfu1x45(wt) EtOH 1,5% N=60	1,38	1,80	2,31	3,26	4,41	5,95	5,81	6,36	5,10
Std dev	0,27	0,56	0,39	0,62	0,66	0,58	0,40	0,58	1,13
Std err	0,11	0,23	0,16	0,26	0,27	0,24	0,16	0,23	0,46

5.11. EtOH induced locomotor activity of seven-day old wild type SG019

Time (min)	0	1	3	5	7	10	13	16	20
02-20: EtOH 1.0% N=1x10	1,34	2,12	2,79	3,00	2,53	2,69	3,06	3,00	4,88
Std dev	0,94	1,23	1,45	1,06	1,15	1,42	1,83	2,09	3,04
Std err	0,30	0,39	0,46	0,33	0,36	0,45	0,58	0,66	0,96
02-20: EtOH 1,5% 10pm Met1	2,36	2,74	3,21	3,39	3,92	3,55	3,22	2,89	1,56
02-20: EtOH 1,5% 10pm Met2	2,56	2,55	3,12	3,81	3,53	3,71	3,34	2,24	2,81
02-20: EtOH 1,5% Met1.1	1,49	2,15	2,83	2,78	2,89	2,74	2,39	2,68	2,63
02-20: EtOH 1,5% Met1.2	1,59	2,11	2,90	3,55	4,19	3,67	3,58	2,76	3,40
02-20: EtOH 1,5% Met2.1	0,69	1,25	1,79	2,03	3,02	3,28	2,45	2,85	2,41
02-20: EtOH 1,5% Met2.2	1,37	1,97	2,90	3,46	4,29	4,83	3,72	1,97	2,85
02-20: EtOH 1,5% Met2.3	1,55	2,52	2,49	2,63	3,06	3,42	3,95	2,88	3,80
02-27: EtOH 1,5% Cr3	1,73	2,43	2,58	2,87	3,16	3,27	2,94	2,44	2,41
02-27: EtOH 1,5% Cr4	1,03	1,88	1,80	1,72	2,46	3,08	3,84	1,57	2,22
02-28: EtOH 1,5%	1,35	1,77	2,58	2,79	3,40	4,63	4,76	4,27	3,29
03-06: EtOH 1,5% #1	1,15	1,88	2,32	3,24	3,31	2,84	2,69	2,27	2,12
03-06: EtOH 1,5% #2	1,63	2,57	2,80	2,85	3,46	3,45	2,49	1,10	0,75
03-06: EtOH 1,5% Cr1	0,51	1,59	1,64	1,70	2,04	2,33	2,11	2,13	1,13
03-06: EtOH 1,5% Cr3	0,71	2,04	2,75	3,52	3,54	4,33	4,22	3,03	3,76
04-18: EtOH 1,5% #1	1,48	2,07	1,90	1,70	2,04	3,00	2,56	3,22	2,58
04-18: EtOH 1,5% #2	1,00	1,64	1,67	1,87	1,78	2,37	3,07	1,85	2,01

05-10: Cr1 EtOH 1,5% mean	1,20	1,79	2,18	2,68	2,91	2,62	4,57	4,43	3,44
05-10: Cr2 EtOH 1,5% mean	1,01	1,44	2,73	2,99	3,69	4,07	3,91	3,60	3,06
07-01: EtOH 1,5% mean	1,39	2,20	2,52	2,56	2,68	2,48	2,06	1,97	1,37
07-09: EtOH 1,5% mean	1,50	2,64	3,39	3,35	3,45	2,87	2,52	1,28	1,58
07-16: EtOH 1,5% mean	1,60	2,22	3,33	3,94	5,41	3,46	2,39	1,21	1,90
07-24: EtOH 1,5% #2 mean	1,35	1,09	1,44	1,94	2,59	2,87	1,85	3,25	3,00
07-24: EtOH 1,5% #1 mean	0,84	1,15	1,97	2,44	2,96	2,62	1,68	1,48	0,97
08-09: EtOH 1,5%	1,29	2,17	2,38	3,05	2,75	2,55	2,57	2,14	2,19
Average EtOH 1.5% N=24X10	1,35	1,99	2,47	2,78	3,19	3,25	3,04	2,48	2,38
Std dev	0,47	0,47	0,57	0,68	0,80	0,70	0,87	0,89	0,87
Std err	0,10	0,10	0,12	0,14	0,16	0,14	0,18	0,18	0,18
02-20: EtOH 2.0% N=1x10	1,41	2,59	3,27	4,17	3,33	1,57	1,35	1,56	2,02
Std dev	1,11	2,24	1,94	3,37	1,67	1,19	1,27	1,87	1,17
Std err	0,35	0,71	0,61	1,07	0,53	0,38	0,40	0,59	0,37
02-20: EtOH 3,0%	1,84	2,86	1,61	0,58	0,43	0,42	0,51	0,32	0,53
07-09: EtOH 3,0% mean	1,94	2,75	1,49	0,69	0,61	1,15	1,06	0,67	1,07
07-24: EtOH 3,0% #2 mean	1,09	2,06	1,72	0,61	0,41	0,30	0,25	0,35	0,30
07-24: EtOH 3,0% #1 mean	0,92	0,75	0,70	0,56	0,29	0,32	0,40	0,80	0,50
08-09: EtOH 3,0%	2,05	2,46	0,95	0,60	0,88	0,96	0,99	0,52	0,53
Average EtOH 3.0% N=5x10	1,57	2,17	1,30	0,61	0,52	0,63	0,64	0,53	0,59
Std dev	0,52	0,86	0,44	0,05	0,23	0,40	0,36	0,21	0,29
Std err	0,23	0,38	0,20	0,02	0,10	0,18	0,16	0,09	0,13
07-01: EtOH 4.0% mean N=1x10	0,86	0,61	0,27	0,65	0,54	0,46	0,61	0,42	1,05
Std dev	0,66	0,70	0,13	0,77	0,36	0,42	0,83	0,29	1,35
Std err	0,21	0,22	0,04	0,24	0,11	0,13	0,26	0,09	0,43

5.12. EtOH induced locomotor activity of seven-day old tfu mutant SG018

02-20: EtOH 1.0% N=1x10	2,35	2,14	1,96	1,66	1,78	2,16	2,46	3,01	3,08
12-27: EtOH 1,5% (SG006)	1,63	1,54	1,98	2,39	2,29	3,24	3,77	3,96	3,61
02-04: EtOH 1,5%	1,94	2,48	2,89	3,07	3,37	2,52	2,89	3,14	3,86
02-14: EtOH 1,5% Met 1	2,59	2,40	2,50	2,79	2,94	3,78	4,45	3,70	4,46
02-14: EtOH 1,5% Met2	2,24	2,33	2,47	3,09	3,05	3,73	3,67	3,53	3,04
02-21: EtOH 1,5%	1,77	1,52	1,98	1,73	2,02	2,67	3,34	2,86	2,78
02-28: EtOH 1,5%	1,14	1,96	2,13	2,30	2,70	3,12	3,40	3,41	4,18
05-10: EtOH 1,5% mean	2,02	2,13	2,54	2,76	2,54	2,79	3,10	3,48	2,96
07-01: EtOH 1,5% mean	2,28	2,24	3,04	2,81	2,55	2,69	2,22	2,90	3,01
07-09: EtOH 1,5% mean	2,84	2,76	2,63	2,97	3,09	4,02	5,62	4,23	4,14
07-31: EtOH 1,5% mean	1,23	1,55	1,80	2,28	1,99	2,43	2,56	2,48	2,02
08-10: EtOH 1,5%	1,89	2,32	2,62	2,83	3,66	3,86	4,27	4,62	5,09
Average EtOH 1.5% N=11x10	1,96	2,11	2,42	2,64	2,75	3,17	3,57	3,48	3,56
Std dev	0,52	0,42	0,40	0,42	0,53	0,59	0,96	0,63	0,89
Std err	0,16	0,13	0,12	0,13	0,16	0,18	0,29	0,19	0,27

02-04: EtOH 2,0%	1,86	2,92	2,87	2,38	2,42	3,43	2,69	2,47	3,47
02-20: EtOH 2,0%	2,33	2,35	1,72	1,72	1,54	2,30	2,56	3,12	4,25
Average EtOH 2.0% N=2x10	2,09	2,64	2,30	2,05	1,98	2,87	2,62	2,79	3,86
Std dev	0,33	0,41	0,81	0,47	0,62	0,80	0,09	0,45	0,55
Std err	0,23	0,29	0,58	0,33	0,44	0,57	0,06	0,32	0,39
02-21: EtOH 3,0%	2,58	2,27	1,58	1,10	1,04	1,88	1,18	1,77	1,24
07-09: EtOH 3,0% mean	1,24	2,33	2,11	1,31	0,88	1,14	1,58	1,75	1,59
07-31: EtOH 3,0% mean	2,84	3,23	1,75	0,92	0,65	0,99	1,25	1,42	2,03
08-10: EtOH 3,0%	2,86	3,09	1,35	0,56	0,58	2,06	1,40	1,16	0,62
Average EtOH 3.0% N=4x10	2,38	2,73	1,70	0,97	0,79	1,52	1,35	1,53	1,37
Std dev	0,77	0,50	0,32	0,32	0,21	0,53	0,18	0,29	0,60
Std err	0,38	0,25	0,16	0,16	0,11	0,27	0,09	0,15	0,30
07-01: EtOH 4.0% mean N=1x10	2,04	2,28	0,57	0,40	0,51	0,39	0,35	0,33	0,60

5.13. EtOH induced locomotor activity of seven-day old wild type SG023

Time (min)	0	1	3	5	7	10	13	16	20
03-22: EtOH 1,5% Cr 3 hf mean	2,05	2,44	3,50	3,79	4,87	6,08	6,12	5,78	6,80
03-22: EtOH 1,5% Cr 2 #1 hf mean	1,26	2,24	3,23	3,73	4,05	4,45	3,94	5,04	4,10
03-22: EtOH 1,5% Cr 2 #2 hf mean	1,59	2,65	2,68	3,06	3,32	3,48	3,25	4,24	3,63
07-01: EtOH 1,5% mean	2,41	2,36	3,77	5,84	7,98	5,81	4,63	3,86	4,23
08-09: EtOH 1,5%	2,92	3,00	4,24	6,19	7,86	7,84	5,39	6,69	4,84
Average SG023 EtOH 1,5% N=50	2,05	2,54	3,48	4,52	5,62	5,53	4,66	5,12	4,72
Std dev	0,66	0,30	0,59	1,40	2,17	1,66	1,14	1,15	1,24
Std err	0,29	0,13	0,26	0,62	0,97	0,74	0,51	0,51	0,55

5.14. EtOH induced locomotor activity of seven-day old WIK SG003

Time (min)	0	1	3	5	7	10	13	16	20	25	30
05-21: EtOH 1,5% #1 mean	1,52	2,11	2,03	2,03	1,87	3,18	5,08	5,38	6,13	4,43	1,51
05-21: EtOH 1,5% #2 mean	1,73	1,78	2,01	1,88	2,46	2,96	4,48	4,09	3,15		
05-21: EtOH 1,5% #3 mean	1,49	1,89	2,22	1,86	1,97	2,98	4,58	6,77	7,94		
05-28: EtOH 1,5% #1 mean	0,84	0,94	1,38	1,91	2,19	3,05	3,08	2,28	2,19	2,16	2,64
05-28: EtOH 1,5% #2 mean	0,47	1,36	1,50	1,51	1,75	2,04	2,51	1,73	2,74	1,70	1,57
05-28: EtOH 1,5% #3 mean	1,06	1,99	3,12	3,91	4,74	5,77	3,51	3,34	1,84	2,75	1,26
05-28: EtOH 1,5% #4 mean	1,90	2,27	2,71	2,84	3,37	4,80	5,23	4,37	4,16	2,24	3,30
06-05: EtOH 1,5% #1 mean	1,64	1,94	2,02	2,77	3,10	3,76	3,45	4,42	3,87	2,60	4,52
06-05: EtOH 1,5% #2 mean	1,33	1,46	2,37	2,04	2,59	4,01	5,25	4,94	4,31	6,28	3,37
Average Wik SG003 EtOH 1,5% N=90	1,33	1,75	2,15	2,31	2,67	3,62	4,13	4,14	4,03	3,16	2,60
Std dev	0,46	0,42	0,54	0,74	0,95	1,12	1,02	1,55	1,95	1,62	1,21
Std err	0,15	0,14	0,18	0,25	0,32	0,37	0,34	0,52	0,65	0,54	0,40

5.15. AMPH induced locomotor activity of seven-day old ABC SG013

Time (min)	0	1	3	5	7	10	13	16	20
05-16: AMPH 0,5mg mean	1,38	1,61	2,17	2,48	2,50	2,42	2,09	1,99	1,77
05-16: AMPH 0,5mg #2 mean	1,04	1,52	2,16	2,17	2,09	2,23	1,93	1,88	1,54
07-23: AMPH 0,5mg/L #1 mean	0,86	1,87	2,43	2,51	2,62	2,71	2,94	2,27	1,99
07-25: AMPH 0,5mg/L #1 mean	1,47	1,90	2,18	2,44	2,45	2,28	2,51	2,35	2,00
07-25: AMPH 0,5mg/L #2 mean	0,64	1,43	1,79	1,77	1,99	2,44	2,26	2,62	2,18
Average AMPH 0.5mg/L N=5x10	1,08	1,67	2,14	2,28	2,33	2,42	2,35	2,22	1,90
Std dev	0,35	0,21	0,23	0,31	0,27	0,19	0,40	0,30	0,25
Std err	0,16	0,09	0,10	0,14	0,12	0,08	0,18	0,13	0,11
05-16: AMPH 2mg mean	1,23	1,78	2,22	2,14	1,93	2,13	1,97	1,77	1,51
05-16: AMPH 2mg #2 mean	0,83	1,87	2,42	2,19	2,01	2,33	2,06	2,00	1,61
07-23: AMPH 2mg/L #1 mean	1,66	2,25	2,29	2,44	2,47	1,99	1,80	1,59	1,47
07-25: AMPH 2mg/L #1 mean	0,89	1,29	1,75	2,20	2,14	2,23	2,09	2,04	1,98
07-25: AMPH 2mg/L #2 mean	0,34	1,76	2,02	1,97	2,30	2,12	2,20	2,08	1,54
Average AMPH 2mg/L N=5x10	0,99	1,79	2,14	2,19	2,17	2,16	2,03	1,90	1,62
Std dev	0,28	0,06	0,14	0,04	0,05	0,14	0,07	0,16	0,07
Std err	0,13	0,03	0,06	0,02	0,02	0,06	0,03	0,07	0,03
05-16: AMPH 5mg mean	1,29	1,78	1,94	2,24	2,36	2,15	1,84	1,51	1,57
05-16: AMPH 5mg #2 mean	1,26	2,04	1,89	2,15	1,86	1,71	1,17	1,24	0,92
Average AMPH 5mg/L N=2x10	1,27	1,91	1,91	2,19	2,11	1,93	1,50	1,37	1,24
Std dev	0,02	0,19	0,03	0,06	0,35	0,31	0,47	0,19	0,46
Std err	0,01	0,13	0,02	0,04	0,25	0,22	0,33	0,14	0,33
05-16: AMPH 10mg mean	0,74	1,58	2,27	2,07	2,46	1,91	1,56	1,31	1,07
05-16: AMPH 10mg #2 mean	1,47	2,20	2,39	2,14	1,82	1,35	1,00	0,96	0,53
07-23: AMPH 10mg/L #1 mean	1,25	1,84	2,59	2,57	2,45	1,88	1,38	0,70	0,50
07-25: AMPH 10mg/L #2 mean	1,47	2,12	2,22	1,95	1,71	1,30	0,79	0,65	0,45
07-25: AMPH 10mg/L #3 mean	1,41	1,68	2,27	2,25	2,39	1,79	1,40	1,14	0,79
Average AMPH 10mg/L N=5x10	1,27	1,88	2,35	2,20	2,17	1,65	1,23	0,95	0,67
Std dev	0,31	0,27	0,15	0,24	0,37	0,30	0,32	0,28	0,26
Std err	0,14	0,12	0,07	0,11	0,16	0,13	0,14	0,13	0,12

5.16. AMPH induced locomotor activity of seven-day old wild type SG019

Time (min)	0	1	3	5	7	10	13	16	20
04-18: AMPH 0,5mg/L	0,49	1,20	1,17	1,19	1,43	1,47	1,51	1,65	1,18
07-01: AMPH 0,5mg/L mean	0,83	1,54	2,52	2,87	2,95	2,40	2,46	2,38	1,82
07-09: AMPH 0,5mg/L mean	1,02	1,71	2,07	2,05	2,43	2,22	2,48	2,02	1,99
07-16: AMPH 0,5mg/L mean	1,00	1,10	1,42	2,03	2,25	2,21	2,33	2,31	1,94
08-09: AMPH 0,5mg/L	1,29	2,10	2,60	2,85	2,37	2,51	2,13	2,45	2,47
Average SG019 AMPH 0.5mg/L N=5x10	0,77	1,44	2,13	2,66	3,07	3,47	3,98	4,47	4,90
Std dev	0,46	0,42	0,72	1,30	1,99	3,22	4,43	5,66	7,41
Std err	0,19	0,17	0,29	0,53	0,81	1,31	1,81	2,31	3,02

02-20: AMPH 2mg/L	0,91	1,73	2,00	1,95	1,84	1,40	0,85	0,81	0,67
02-28: AMPH 2mg/L	1,08	2,16	2,63	2,85	2,88	3,10	3,48	3,47	3,06
04-18: AMPH 2mg/L #1	0,90	1,32	1,33	1,27	1,30	1,77	1,05	1,19	1,16
04-18: AMPH 2mg/L #2	0,74	1,29	2,00	1,95	1,87	1,68	1,93	1,52	1,48
07-01: AMPH 2mg/L mean	1,19	1,67	2,09	2,03	2,14	1,74	1,78	1,79	1,41
07-09: AMPH 2mg/L mean	0,82	1,90	2,31	3,02	2,84	2,78	2,74	2,45	2,24
07-16: AMPH 2mg/L mean	0,89	1,26	1,69	2,24	2,14	2,56	1,57	1,60	1,13
08-09: AMPH 2mg/L	1,82	2,44	2,58	2,51	2,57	2,62	2,36	2,30	2,09
Average SG019 AMPH 2mg/L N=8x10	1,04	1,72	2,08	2,23	2,20	2,21	1,97	1,89	1,65
Std dev	0,35	0,43	0,44	0,56	0,54	0,63	0,87	0,83	0,76
Std err	0,12	0,15	0,15	0,20	0,19	0,22	0,31	0,29	0,27

02-20: AMPH 10mg/L	0,77	1,04	1,45	1,24	0,97	0,65	0,61	0,33	0,44
04-18: AMPH 10mg/L hf mean	1,35	1,51	1,65	1,71	1,33	1,68	0,95	0,64	0,80
07-01: AMPH 10mg/L mean	1,32	1,96	2,16	2,11	1,49	1,03	0,91	0,67	0,60
07-09: AMPH 10mg/L mean	1,83	2,10	1,91	2,65	2,22	2,54	2,41	1,78	1,93
07-16: AMPH 10mg/L mean	1,92	2,00	2,37	2,71	2,78	2,26	2,11	1,82	1,53
08-09: AMPH 10mg/L	1,15	1,16	2,03	2,18	2,23	1,06	1,19	0,64	0,83
Average SG019 AMPH 10mg/L N=6x10	1,39	1,63	1,93	2,10	1,84	1,54	1,37	0,98	1,02
Std dev	0,43	0,46	0,34	0,56	0,68	0,75	0,73	0,65	0,58
Std err	0,17	0,19	0,14	0,23	0,28	0,31	0,30	0,26	0,24

5.17. AMPH induced locomotor activity of seven-day old tfu mutant SG018

Time (min)	0	1	3	5	7	10	13	16	20
07-01: AMPH 0,5mg/L mean	1,53	2,18	2,01	1,76	2,16	1,93	2,13	1,86	1,79
07-09: AMPH 0,5mg/L mean	1,36	2,56	1,77	2,16	2,33	2,27	2,61	2,58	2,44
07-16: AMPH 0,5mg/L mean	1,46	1,94	2,43	2,43	2,66	2,70	2,57	2,28	2,38
07-31: AMPH 0,5mg/L mean	1,76	1,69	2,27	1,86	1,44	1,42	1,02	1,34	1,11
08-10: AMPH 0,5mg/L	2,54	2,68	2,45	2,14	2,29	2,30	1,56	1,85	1,83
Average SG018 AMPH 0.5mg/L N=5x10	1,73	2,21	2,19	2,07	2,18	2,12	1,98	1,98	1,91
Std dev	0,47	0,42	0,29	0,26	0,45	0,48	0,68	0,47	0,54
Std err	0,21	0,19	0,13	0,12	0,20	0,21	0,31	0,21	0,24

02-21: AMPH 2mg/L	1,45	2,04	2,20	2,06	2,03	1,87	1,54	1,39	1,15
02-28: AMPH 2mg/L	1,19	2,25	1,82	1,76	2,38	2,10	1,65	2,05	1,78
07-01: AMPH 2mg/L mean	1,34	1,08	1,55	1,68	1,40	1,55	1,50	1,54	1,40
07-09: AMPH 2mg/L mean	1,92	2,14	2,86	2,55	2,33	2,42	2,47	2,46	2,10
07-16: AMPH 2mg/L mean	1,89	1,68	2,00	1,88	1,81	1,44	1,07	1,15	0,77
07-31: AMPH 2mg/L mean	2,19	1,80	2,64	2,07	1,89	1,66	1,61	1,54	1,59
08-10: AMPH 2mg/L	1,90	1,95	2,23	2,38	2,13	1,77	1,70	1,35	1,28
Average SG018 AMPH 2mg/L N=7x10	1,70	1,85	2,18	2,05	1,99	1,83	1,65	1,64	1,44
Std dev	0,37	0,39	0,45	0,32	0,34	0,34	0,42	0,46	0,44
Std err	0,14	0,15	0,17	0,12	0,13	0,13	0,16	0,17	0,16

02-21: AMPH 5mg/L	1,53	2,25	2,93	2,34	1,97	1,74	1,01	1,51	1,45
02-28: AMPH 5mg/L	2,82	2,84	2,34	2,47	2,03	1,56	2,00	1,65	1,29
Average SG018 AMPH 5mg/L N=2x10	2,17	2,54	2,64	2,40	2,00	1,65	1,51	1,58	1,37
Std dev	0,91	0,42	0,42	0,09	0,04	0,13	0,71	0,10	0,12
Std err	0,64	0,30	0,30	0,06	0,03	0,09	0,50	0,07	0,08

02-21: AMPH 10mg/L	1,44	1,84	1,91	2,03	1,66	2,01	1,30	1,13	1,25
07-01: AMPH 10mg/L mean	1,55	1,83	1,89	2,37	1,43	1,32	1,03	1,06	0,74
07-09: AMPH 10mg/L mean	2,57	2,78	2,24	1,73	2,01	1,53	1,66	1,40	1,26
07-16: AMPH 10mg/L mean	1,16	1,50	1,76	1,42	0,79	0,74	0,96	0,68	0,52
07-31: AMPH 10mg/L mean	2,05	2,07	2,07	1,57	1,24	1,05	0,66	0,78	0,58
08-10: AMPH 10mg/L	1,67	2,08	2,37	1,63	1,30	0,92	0,53	0,60	0,52
Average SG018 AMPH 10mg/L N=6x10	1,74	2,02	2,04	1,79	1,41	1,26	1,02	0,94	0,81
Std dev	0,50	0,43	0,23	0,35	0,41	0,46	0,42	0,31	0,35
Std err	0,20	0,18	0,09	0,14	0,17	0,19	0,17	0,13	0,14

5.18. Locomotor activity of 14-day old ABC SG013 treated with cocaine

Time (min)	0	1	3	5	7	10	13	16	20
Cocaine 0.5mg/L 1x10	0,98	1,56	1,90	2,01	2,08	1,85	1,86	1,86	1,79
std dev	2,33	2,78	2,93	3,03	3,09	2,87	2,88	2,89	2,89
Cocaine 2mg/L 1x10	1,11	1,78	1,74	1,71	1,83	1,73	1,73	1,50	1,53
std dev	2,14	2,95	2,90	2,94	3,19	3,05	3,28	2,86	3,06
Cocaine 5mg/L 1x10	0,64	1,34	1,64	1,76	1,61	1,75	1,79	2,05	1,74
std dev	1,15	2,81	2,76	3,08	3,37	3,14	3,18	3,52	3,26
Cocaine 10mg/L 1x10	1,22	2,09	2,26	1,90	1,90	1,69	1,67	1,59	1,41
std dev	2,46	3,34	3,57	3,19	3,19	2,96	2,97	2,96	2,84

5.19. Locomotor activity of 14-day old ABC SG013 treated with morphine

Placebo #1 14d ABC N=1x10	1,72	2,13	2,60	2,31	2,63	2,60	2,57	2,54	2,61
rd std dev	2,59	3,21	3,38	3,22	3,49	3,46	3,37	3,46	3,57
std dev other macro	1,07	1,06	0,80	0,98	0,67	0,89	0,88	0,99	1,30
std err other macro	0,34	0,34	0,25	0,31	0,24	0,28	0,28	0,31	0,46
Placebo #2 N=1x10	1,50	2,09	2,73	2,87	3,09	2,75	3,02	2,85	3,03
rd std dev	2,09	2,91	3,51	3,62	3,67	3,30	3,63	3,74	3,90
Morphine 0.25mg/L N=1x10	1,88	2,29	2,52	2,73	2,39	2,40	2,70	2,52	2,56
rd std dev	2,92	3,20	3,11	3,40	3,13	2,95	3,46	3,03	3,15
Morphine 0.50mg/L N=1x10	1,49	1,88	2,52	2,35	2,36	2,55	2,65	2,67	2,51
rd std dev	2,70	3,01	3,71	3,49	3,50	3,42	3,36	3,24	3,19
Morphine 1.0mg/L N=1x10	1,41	2,25	2,47	2,59	2,63	2,60	2,72	2,50	2,62
rd std dev	2,45	3,16	3,60	3,46	3,62	3,38	3,38	3,54	3,75
Std err	0,78	1,00	1,14	1,09	1,15	1,07	1,07	1,12	1,19
Morphine 2.0mg/L N=1x10	1,55	2,64	2,81	2,51	2,77	2,73	2,90	2,71	2,82
rd std dev	2,64	3,51	3,51	3,28	3,47	3,38	3,55	3,32	3,67

5.20. Locomotor activity of seven-day old ABC SG013 treated with MPTP 1-5dpf

7-day old, 2 frames/sec:	Control	MPTP 43micM	MPTP 215micM
Mean of mean speed	1,79	0,82	0,29
Std dev	0,98	0,84	0,62
Std err	0,31	0,28	0,20

5.21. EtOH induced hyperactivity after fluphenazine treatment of seven-day old ABC

Time (min)	0	1	3	5	7	10	13	16	20
Control EtOH #1 07-25 mean	1,73	2,21	2,46	3,03	3,15	4,98	5,69	5,84	4,99
Control EtOH #2 07-25 mean	1,51	2,19	2,40	2,81	4,14	4,20	4,20	4,96	3,93
Control EtOH 1,5% #1 07-30 mean	1,53	1,81	2,82	3,50	3,81	5,23	5,28	4,53	4,37
Control EtOH 1,5% #2 07-30 mean	1,52	1,66	2,46	2,77	4,08	4,86	5,10	5,45	3,19
0mg flu - EtOh 1,5% 08-02 mean	2,75	2,98	3,79	5,01	7,35	6,74	4,54	3,91	4,19
Flu 0mg EtOh 1,5% mean	0,98	1,23	2,05	2,72	3,71	3,83	3,43	2,63	1,90
0mg, EtOH 1,5% 08-28 mean	2,04	2,32	2,89	3,81	5,22	4,18	3,10	2,33	2,79
0mg, EtOH 1,5% mean	1,30	1,60	2,30	2,60	3,14	2,85	2,39	4,33	3,77
Average control EtOH 1.5% N=7x10	1,67	2,00	2,65	3,28	4,33	4,61	4,21	4,25	3,64
Std dev	0,53	0,54	0,53	0,81	1,39	1,15	1,16	1,25	0,98
Std err	0,20	0,20	0,20	0,31	0,52	0,43	0,44	0,47	0,37

Pre-treated 2hrs with fluphen:									
Fluphen. 0.1mg/L EtOH 1.5% N=1x10	1,45	2,05	2,67	3,91	4,50	4,37	4,77	4,82	3,91
Flu 0,5mg/L 07-25 #1(+EtOH 1,5%) mean	1,46	1,83	2,76	4,04	5,20	6,06	5,16	3,53	3,69
Flu 0,5mg/L 07-25 #2(+EtOH 1,5%) mean	0,73	1,51	3,50	3,35	4,83	5,59	5,08	3,11	1,95
Avg. flu 0.5mg/L pretr. 2hrs EtOH 1.5% N=2x10	1,09	1,67	3,13	3,70	5,01	5,83	5,12	3,32	2,82
Std dev	0,51	0,23	0,53	0,48	0,26	0,33	0,05	0,30	1,23
Std err	0,36	0,16	0,37	0,34	0,18	0,23	0,04	0,21	0,87

Fluphenazine 2mg/L #2 07-25 (+EtOH 1,5%)	0,94	1,76	3,04	3,82	3,17	2,72	2,52	2,07	1,31
Fluphenazine 2mg/L 2hrs 07-30 + EtOH 1,5%	1,29	1,84	2,58	1,99	1,71	0,84	1,84	2,26	1,61
Flu 2mg, EtOH 1,5% 8-28 mean	1,34	1,65	2,41	2,37	1,47	0,50	0,59	0,41	0,42
Avg. flu. 2.0mg/L pretr. 2hrs EtOH 1.5% N=3x10	1,19	1,75	2,67	2,73	2,12	1,35	1,65	1,58	1,11
Std dev	0,22	0,10	0,33	0,97	0,92	1,19	0,98	1,02	0,62
Std err	0,13	0,06	0,19	0,56	0,53	0,69	0,56	0,59	0,36

Fluphenazine 8mg/L #2 07-25 (+EtOH 1,5%)	1,47	1,86	3,52	2,54	2,60	2,07	2,68	3,56	3,11
Fluphenazine 8mg/L 2hrs 07-30 + EtOH 1,5%	3,25	3,26	1,61	2,61	2,45	1,42	1,00	2,21	2,89
Flu 8mg, EtOH 1,5% mean	4,37	4,19	3,78	2,12	0,60	0,24	0,75	1,30	0,71
Avg. flu. 8.0mg/L pretr. 2hrs EtOH 1.5% N=3x10	3,03	3,10	2,97	2,42	1,88	1,25	1,48	2,36	2,24
Std dev	1,46	1,17	1,19	0,27	1,11	0,93	1,05	1,13	1,32
Std err	0,84	0,68	0,69	0,15	0,64	0,54	0,60	0,66	0,76

Acutely (5min before EtOH 1.5%)									
Fluphenazine 2mg/L Acutely+EtOH 1,5% 07-30	2,89	2,87	4,10	5,17	5,85	6,30	3,18	4,40	4,77
Flu 2mg EtOh 1,5% 08-06 mean	1,37	1,48	2,42	3,70	3,95	1,77	0,72	0,46	0,45
Flu 2mg, EtOH 1,5% 08-28 mean	1,56	1,86	3,51	5,14	5,16	3,36	2,75	2,40	3,80
Avg. flu. 2.0mg/L acutely EtOH 1.5% N=3x10	1,94	2,07	3,34	4,67	4,98	3,81	2,22	2,42	3,01
Std dev	0,83	0,72	0,85	0,84	0,96	2,30	1,31	1,97	2,27
Std err	0,48	0,41	0,49	0,48	0,56	1,33	0,76	1,14	1,31

Fluphenazine 8mg/L Acutely 07-30+EtOH 1,5%	1,81	2,49	4,57	4,95	2,26	0,39	0,33	0,53	0,34
Flu 8mg EtOh 1,5% 08-06 mean	1,07	2,20	4,69	3,57	1,36	0,54	0,41	0,37	0,99
Flu 8mg, EtOH 1,5% 08-28 mean	2,28	2,41	4,10	4,11	3,33	1,11	0,64	0,90	0,61
Avg. flu. 8.0mg/L acutely EtOH 1.5% N=3x10	1,72	2,37	4,45	4,21	2,32	0,68	0,46	0,60	0,65
Std dev	0,61	0,15	0,31	0,69	0,98	0,38	0,16	0,27	0,33
Std err	0,35	0,09	0,18	0,40	0,57	0,22	0,09	0,16	0,19

5.22. SG018 and SG019 locomotor activities on day 14 treated with L-DOPA 1-13dpf

Time (min)	0	1	3	5	7	10	13	16	20
SG019 wt aa treated control N=1x10	0,88	1,92	1,92	2,05	2,52	2,14	2,15	2,22	2,17
SG019 L-DOPA 5.0mg/L + aa treated N=1x10	0,86	1,68	1,99	1,79	2,12	1,85	1,90	2,15	2,38
SG019 L-DOPA 20mg/L + aa treated N=1x10	0,90	1,41	1,93	1,83	1,85	1,96	2,16	1,92	1,93
SG018 tfu aa treated control N=1x10	0,88	1,06	1,33	1,07	0,99	0,95	1,07	1,08	1,06
SG018 L-DOPA 5.0mg/L + aa treated N=1x10	0,81	1,01	1,15	1,44	1,22	1,17	1,14	1,02	0,81
SG018 L-DOPA 20mg/L + aa treated N=1x10	0,70	1,11	0,85	0,68	1,02	0,80	0,80	0,85	0,87

5.23. Rapid EtOH tolerance of seven day-old ABC SG013

Time (min)	0	1	3	5	7	10	13	16	20
07-16: Control #1 mean	1,83	2,19	2,59	4,47	6,39	8,40	5,64	5,29	5,00
07-16: Control #2 mean	2,34	2,41	3,24	3,92	6,92	6,87	5,69	7,02	4,56
07-26: Control #1 mean	2,08	1,86	2,76	4,05	5,93	6,66	5,26	5,18	5,98
07-26: Control #2 3hr mean	2,24	2,32	2,88	4,63	6,62	6,23	4,44	4,77	4,39
07-30: Control #1 mean	1,53	1,81	2,82	3,50	3,81	5,23	5,28	4,53	4,37
07-30: Control #2 mean	1,52	1,66	2,46	2,77	4,08	4,86	5,10	5,45	3,19
07-30: Control #3 mean	1,33	1,68	2,27	3,36	4,30	5,76	7,81	6,76	5,18
08-02: Control #1 mean	1,83	1,67	1,88	3,98	4,65	4,50	4,09	5,03	5,20
08-02: Control #2 mean	1,92	2,07	2,97	3,94	5,01	6,85	4,15	3,82	3,86
Average control EtOH 1.5% N=9x10	1,84	1,96	2,65	3,85	5,30	6,15	5,27	5,32	4,64
Std dev	0,34	0,29	0,41	0,57	1,18	1,21	1,13	1,02	0,82
Std err	0,11	0,10	0,14	0,19	0,39	0,40	0,38	0,34	0,27
07-16: EtOH #1 mean	1,48	1,76	2,72	5,61	6,30	6,47	5,37	6,08	4,60
07-26: EtOH #1 mean	1,94	1,66	2,47	3,33	4,22	5,57	5,34	6,07	4,21
07-26: EtOH #2 mean	1,78	1,58	3,06	4,30	5,11	3,44	4,37	4,72	4,51
07-26: EtOH #3 3hr mean	2,92	2,16	2,76	4,22	4,46	5,18	3,61	4,19	3,32
07-26: EtOH #4 3hr mean	2,60	2,75	2,84	4,34	8,23	8,99	6,78	5,39	7,02
07-30: EtOH #1 mean	1,83	2,29	3,27	4,12	4,16	4,07	5,59	6,05	5,45
07-30: EtOH #2 mean	1,74	1,97	2,73	2,74	3,32	6,04	6,38	4,37	3,54
07-30: EtOH #3 mean	2,08	2,32	2,24	2,97	4,25	5,49	6,23	5,81	3,42
08-02: EtOH #1 mean	2,15	2,47	3,89	5,61	6,72	6,00	5,07	4,88	3,46
08-02: EtOH #2 mean	3,29	2,57	3,43	4,80	6,85	7,11	3,93	4,97	3,01
Avg. pre.exp 1hr d6 EtOH 1.5% N=10x10	2,18	2,15	2,94	4,20	5,36	5,84	5,27	5,25	4,25
Std dev	0,58	0,40	0,49	0,99	1,57	1,55	1,05	0,73	1,23
Std err	0,18	0,13	0,15	0,31	0,50	0,49	0,33	0,23	0,39

5.24. Chronic EtOH tolerance of seven-day old ABC SG013

Time (min)	0	1	3	5	7	10	13	16	20	25
06-19: Control 1,5% EtOH mean	1,77	2,23	2,96	3,62	5,53	5,57	6,75	6,01	4,34	
07-19: Control #1 EtOH 1,5% mean	2,05	2,16	2,65	3,43	4,01	4,80	5,84	4,05	3,27	2,67
07-19: Control #2 EtOH 1,5% mean	2,30	2,35	2,58	3,32	4,01	5,17	6,50	5,41	5,84	4,88
08-06: Control #1 mean	1,36	1,49	2,04	2,75	3,53	5,29	4,95	5,22	4,53	
08-06: Control #2 mean	1,23	1,36	2,22	2,94	2,79	3,55	4,67	4,29	5,06	
Average control EtOH 1.5% N=5x10	1,74	1,92	2,49	3,21	3,98	4,88	5,74	5,00	4,61	3,77
Std dev	0,45	0,46	0,36	0,36	1,00	0,79	0,92	0,81	0,95	1,57
Std err	0,20	0,21	0,16	0,16	0,45	0,35	0,41	0,36	0,42	1,11
06-19: 0,3%-EtOH 1,5% mean	1,51	2,09	2,63	3,13	4,19	4,93	5,86	6,32	4,94	
07-19: 0,3% #1 EtOH 1,5% mean	2,60	2,58	2,40	2,36	2,55	4,02	5,66	4,39	5,97	3,44
07-19: 0,3% #2 EtOH 1,5% mean	1,47	1,53	2,13	2,94	3,08	4,86	7,02	6,76	5,12	6,07
08-06: EtOH 0,3% #1 mean	1,60	1,72	1,95	2,94	3,47	4,11	4,06	3,97	3,48	
08-06: EtOH 0,3% #2 mean	1,23	1,15	1,56	1,88	2,35	2,94	3,54	4,57	5,83	
Average EtOH 0.3% - 1.5% N=5x10	1,68	1,81	2,14	2,65	3,13	4,17	5,23	5,20	5,07	4,76
Std dev	0,53	0,55	0,41	0,52	0,74	0,81	1,41	1,25	0,99	1,86
Std err	0,24	0,24	0,18	0,23	0,33	0,36	0,63	0,56	0,44	1,31
06-19: 0,6%-EtOH 1,5% mean	1,63	2,37	3,04	3,42	4,20	5,03	5,90	4,78	5,47	
07-19: 0,6% #1 EtOH 1,5% mean	2,73	3,05	3,09	3,38	4,84	5,80	6,45	3,76	4,11	2,31
07-19: 0,6% #2 EtOH 1,5% mean	1,70	2,25	2,17	2,53	2,88	4,03	5,29	4,68	4,07	1,34
08-06: EtOH 0,6% #1 mean	1,16	1,34	1,81	2,56	4,50	4,28	3,30	3,18	2,18	
08-06: EtOH 0,6% #2 mean	1,42	1,30	1,81	2,72	3,13	3,05	4,76	3,28	2,43	
Average EtOH 0.6% - 1.5% N=5x10	1,73	2,06	2,38	2,92	3,91	4,44	5,14	3,94	3,65	1,83
Std dev	0,60	0,74	0,64	0,44	0,86	1,04	1,21	0,76	1,36	0,68
Std err	0,27	0,33	0,29	0,20	0,39	0,46	0,54	0,34	0,61	0,48

5.25.Seven-day old ABC SG013 thigmotaxis given 1.5% EtOH

Time (min)	0	1	3	5	7	10	13	16	20
05-23: No drug 2 % obs at wall	61,6	54,3	43,8	45,2	39,9	55,1	67,8	53,1	25,1
05-23: Incub No drug 1 % obs at wall	77,6	57,9	33,5	55,4	55,9	29,7	50,4	49,8	45,3
05-23: Incub No drug 2 % obs at wall	53,4	48,4	50,3	44,4	56,3	62,8	55,6	48,5	50,3
05-30: No drug cross2	56,6	68,0	79,2	40,6	67,5	73,1	39,0	77,6	37,5
05-30: No drug cross 3	65,5	78,0	70,6	58,6	54,1	50,9	48,2	48,3	49,8
06-21: No drug New curve % obs at wall	87,1	64,3	43,2	46,7	47,4	41,9	35,9	37,3	44,6
06-21: 0% Control % obs at wall	31,9	23,6	50,8	55,4	58,9	52,4	39,4	62,2	48,6
07-08: No drug #1, % obs at wall	46,6	51,7	39,9	10,2	32,8	27,3	41,2	27,0	24,1
07-08: No drug #2, % obs at wall	37,5	23,2	21,7	15,7	27,0	23,7	21,3	30,8	38,2
07-23: No drug 1	54,1	35,3	37,3	41,5	37,0	47,8	55,7	58,8	40,5
07-25: No drug 1	41,0	37,2	44,6	45,4	28,8	29,6	32,1	16,8	37,0
07-25: No drug 2	49,8	48,5	42,5	32,4	17,4	39,8	29,0	47,7	27,0
07-30: No drug 1	55,9	45,1	35,0	26,3	32,4	38,3	33,4	21,2	22,6
07-30: No drug 2	35,9	41,7	37,2	59,9	54,9	19,9	24,7	37,1	35,7
08-02: No drug	51,8	35,0	39,8	27,8	26,5	30,3	36,4	29,9	33,4
Average ABC placebo N=15x10	53,7	47,5	44,6	40,4	42,5	41,5	40,7	43,1	37,3
Std dev	15,1	15,6	14,3	15,1	14,9	15,3	12,7	16,6	9,4
Std err	3,9	4,0	3,7	3,9	3,9	4,0	3,3	4,3	2,4

02-28: ABC EtOH 1,5%, % obs at wall	34,9	25,4	43,2	33,8	31,5	33,8	53,7	77,0	80,5
05-23: Incub EtOH 1,5% #1 % obs at wall	27,2	14,5	21,9	24,0	22,3	39,8	52,2	52,8	70,1
05-23: Incub EtOH 1,5% #2 % obs at wall	41,3	41,3	17,6	28,2	39,0	53,1	63,3	74,5	74,7
05-23: EtOH 1,5% #1 % obs at wall	49,8	45,1	28,2	34,3	34,9	55,4	60,7	56,3	70,6
05-23: EtOH 1,5% #2 % obs at wall	50,0	21,9	26,1	34,0	33,8	70,2	73,9	75,2	80,3
05-23: EtOH 1,5% #4 % obs at wall	53,3	54,8	30,8	19,7	28,6	36,9	50,1	58,2	67,2
05-23: EtOH 1,5% #5 % obs at wall	50,9	33,0	32,6	28,5	36,2	38,4	51,0	59,8	69,2
05-30: EtOH 1,5% Cross 2	51,4	33,5	28,1	44,8	52,3	61,1	72,5	81,2	81,9
05-30: EtOH 1,5% Cross 3	52,9	80,1	29,1	37,8	43,5	60,2	78,6	60,8	70,2
05-30: Tol EtOH 1,5% Cr 3	30,5	24,6	28,7	40,6	47,5	64,2	89,1	73,8	57,4
05-30: Tol EtOH 1,5% Cr 2	61,3	45,8	43,4	52,9	60,4	70,8	79,5	80,1	77,5
05-30: Tol No drug Cr3 (EtOH 1,5%)	54,7	35,4	23,8	33,9	32,9	25,2	38,8	45,5	43,9
05-30: Tol No drug Cr2 (EtOH 1,5%)	64,5	45,5	32,7	36,1	44,9	59,0	75,5	78,6	70,2
06-19: Control 1,5% EtOH, % obs at wall	51,6	36,8	38,8	45,4	49,1	52,3	47,7	74,5	54,7
06-21: EtOH 1,5% New curve % obs at wall	18,6	26,4	23,7	32,6	22,2	29,8	41,5	43,7	64,2
06-21: EtOH 1,5% prev untr. % obs at wall	29,0	33,8	35,3	42,6	46,6	36,2	48,3	57,0	51,2
06-21: EtOH 1,5% treated d6 also % obs at	87,8	71,5	43,0	50,8	43,9	75,2	61,2	83,7	90,0
07-08: EtOH 1,5% #1, % obs at wall	31,1	26,9	28,1	43,5	55,6	58,6	60,4	74,8	67,4
07-08: EtOH 1,5% #2, % obs at wall	43,0	27,9	20,6	32,0	25,7	50,2	67,9	75,3	68,3
07-16: Control #1	88,5	47,9	36,6	38,5	57,9	67,6	60,0	74,7	63,3
07-16: Control #2	83,3	57,1	56,1	50,3	54,6	69,6	77,6	71,7	51,6
07-19: Control #1	27,0	25,5	16,8	36,4	29,8	51,1	62,1	56,3	83,1
07-19: Control #2	57,0	36,5	41,8	30,9	32,1	46,1	51,4	57,3	56,5
07-26: Control #1	80,0	60,2	34,5	29,6	35,1	60,6	50,8	56,9	60,4
07-26: Control #2	48,1	43,2	42,2	44,1	61,1	72,1	80,7	65,6	77,4
07-30: Control #1	36,4	38,2	45,1	54,3	52,8	58,6	66,0	70,1	57,6
07-30: Control #2	53,3	46,2	43,8	46,5	47,0	55,3	80,7	66,2	47,6
07-30: Control #3	48,9	41,8	42,8	43,3	62,2	63,4	61,9	74,1	79,7
08-02: Control #1	68,6	42,6	63,9	37,6	50,6	61,7	59,0	70,6	56,7
08-02: Control #2	41,3	43,8	36,2	41,6	42,7	63,2	77,9	57,9	58,2
Average ABC EtOH 1.5% N=30x10	50,6	40,2	34,5	38,3	42,6	54,7	63,1	66,8	66,7
Std dev	18,1	14,4	10,9	8,5	11,8	13,5	13,1	10,8	11,7
Std err	3,3	2,6	2,0	1,5	2,1	2,5	2,4	2,0	2,1

5.26. Seven-day old WIK SG003 thigmotaxis given 1.5% EtOH

Time (min)	0	1	3	5	7	10	13	16	20
05-21: No drug #1 % obs at wall	35,0	13,3	7,8	6,2	8,2	18,5	33,2	34,5	36,2
05-21: No drug #2 % obs at wall	26,5	23,9	19,9	18,3	17,4	13,6	33,4	31,2	29,0
05-28: No drug #1 % obs at wall	44,0	36,9	21,8	16,7	18,5	21,3	24,7	24,0	32,4
05-28: No drug #2 % obs at wall	12,5	16,4	15,9	15,9	14,8	14,1	10,8	23,5	24,4
05-28: No drug #3 % obs at wall	50,1	39,9	21,8	29,2	33,4	36,6	35,4	31,9	46,1
05-28: No drug #4 % obs at wall	59,9	30,5	23,7	31,9	21,6	35,5	49,9	41,4	41,0
06-05: No drug #1 % obs at wall	34,8	34,7	21,3	15,3	21,6	12,0	13,4	15,3	26,1
06-05: No drug #2 % obs at wall	68,9	42,2	22,7	21,6	19,6	21,9	23,5	12,8	12,7
Average WIK placebo N=8x10	41,5	29,7	19,3	19,4	19,4	21,7	28,0	26,8	31,0
Std dev	18,2	10,8	5,2	8,2	7,1	9,6	12,7	9,7	10,4
Std err	6,4	3,8	1,8	2,9	2,5	3,4	4,5	3,4	3,7

Time (min)	0	1	3	5	7	10	13	16	20
05-21: EtOH 1,5% #1 % obs at wall	32,1	12,8	8,7	8,7	10,6	35,7	59,3	36,7	74,5
05-21: EtOH 1,5% #2 % obs at wall	22,5	13,3	9,9	1,7	7,6	25,7	35,3	53,0	70,6
05-21: EtOH 1,5% #3 % obs at wall	39,8	21,3	12,6	11,9	12,4	32,9	40,8	57,4	78,1
05-28: EtOH 1,5% #1 % obs at wall	28,8	35,8	25,5	17,6	27,6	38,8	24,0	24,5	36,5
05-28: EtOH 1,5% #2 % obs at wall	42,6	34,2	7,9	13,2	23,3	24,8	39,4	43,8	52,1
05-28: EtOH 1,5% #3 % obs at wall	29,3	15,9	10,3	17,6	54,4	62,2	57,3	51,3	52,0
05-28: EtOH 1,5% #4 % obs at wall	38,7	20,3	15,8	27,9	32,0	37,6	51,2	46,2	57,1
06-05: EtOH 1,5% #1 % obs at wall	27,5	23,5	17,9	9,1	30,2	45,9	48,8	64,8	52,5
06-05: EtOH 1,5% #2 % obs at wall	49,2	38,5	16,2	4,2	20,3	22,0	45,2	54,2	55,3
Average WIK EtOH 1.5% N=8x10	34,5	26,6	14,9	19,1	34,3	40,8	43,0	41,5	49,4
Std dev	8,6	9,9	5,6	7,9	14,3	12,4	11,1	12,0	13,2
Std err	3,0	3,5	2,0	2,8	5,1	4,4	3,9	4,2	4,7

5.27. Seven-day old SG018 and SG019 thigmotaxis given 1.5% EtOH

SG018:

Time (min)	0	1	3	5	7	10	13	16	20
02-21: No drug, % obs at wall	39,6	19,6	21,5	12,5	10,1	11,1	16,8	12,5	10,4
02-28: No drug, % obs at wall	29,9	27,1	36,5	40,6	14,4	25,8	28,0	27,0	25,1
05-10: No drug, % obs at wall	63,1	90,7	45,3	21,5	32,6	33,6	9,1	34,0	25,1
07-01: No drug 1, % obs at wall	41,2	47,4	58,6	54,8	36,8	58,8	41,4	53,2	48,0
07-01: No drug 2, % obs at wall	38,2	25,3	11,5	10,2	17,8	13,8	14,4	16,1	17,8
07-09: No drug, % obs at wall	47,0	62,3	59,5	65,2	52,0	74,5	51,2	56,8	49,1
07-31: No drug 2	41,5	48,3	40,3	42,7	29,2	30,0	19,6	18,5	33,8
07-31: No drug 3	44,9	37,8	22,1	8,3	13,8	12,2	37,3	22,3	27,9
08-10: No drug 1	67,4	67,5	53,6	37,6	35,1	30,8	26,6	34,6	22,3
08-10: No drug 2	42,3	52,0	42,8	50,4	29,1	33,1	24,1	23,4	27,4
Average SG018 placebo N=10x10	45,5	47,8	39,2	34,4	27,1	32,4	26,9	29,8	28,7
Std dev	11,4	21,8	16,4	20,2	13,0	20,4	13,1	15,0	12,2
Std err	3,6	6,9	5,2	6,4	4,1	6,4	4,1	4,8	3,9

02-21: EtOH 1,5%, % obs at wall	35,9	25,2	22,4	12,6	19,3	13,2	30,0	36,3	58,4
02-28: EtOH 1,5%, % obs at wall	46,9	41,0	27,6	17,0	35,6	41,5	42,4	36,9	37,9
05-10: EtOH 1,5%, % obs at wall	56,9	38,9	35,4	17,2	34,6	44,2	33,0	37,6	37,4
07-01: EtOH 1,5%, % obs at wall	53,2	31,9	25,9	21,7	57,9	47,5	62,3	35,8	41,7
07-09: EtOH 1,5%, % obs at wall	46,8	46,1	24,7	19,2	45,4	56,6	60,5	61,1	67,9
07-31: EtOH 1,5%	59,3	70,4	46,3	24,7	36,8	56,1	63,1	44,1	33,7
08-10: EtOh 1,5%	66,1	36,4	20,8	18,9	33,7	60,1	56,1	70,1	73,5
Average SG018 EtOH 1.5% N=7x10	52,2	41,4	29,0	18,8	37,6	45,6	49,6	46,0	50,1
Std dev	9,9	14,4	9,0	3,8	11,8	15,9	14,2	13,9	16,3
Std err	3,7	5,5	3,4	1,4	4,5	6,0	5,4	5,3	6,1

SG019:

02-27: No drug cross 3, % obs at wall	28,0	29,4	30,6	32,2	32,0	38,0	43,0	23,9	36,1
02-27: No drug cross 4, % obs at wall	33,1	18,1	17,5	16,2	8,5	32,4	29,5	43,2	38,8
02-28: No drug 1, % obs at wall	41,1	21,7	23,7	29,9	22,4	41,6	53,6	44,1	50,7
02-28: No drug 2, % obs at wall	60,0	55,2	29,9	34,8	32,3	43,2	22,7	41,2	30,1
No drug 03-06, % obs at wall	34,5	18,5	11,5	20,5	30,0	29,6	36,4	19,4	28,0
SG019 No drug #1 05-10, % obs at w	21,2	23,9	28,7	29,6	20,6	27,7	35,2	34,9	36,5
SG019 No drug #2 05-10 % obs at wa	48,9	42,3	18,2	18,7	23,9	20,4	42,8	25,9	44,0
No drug 1 04-18, % obs at wall	34,6	32,4	44,8	42,3	37,0	33,6	34,5	33,5	43,1

No drug 2 04-18, % obs at wall	28,9	38,1	31,0	47,2	30,9	16,4	40,0	36,8	22,0
07-01: No drug, % obs at wall	33,5	45,6	19,4	25,1	37,6	22,6	27,3	22,1	15,4
07-09: No drug, % obs at wall	44,5	62,6	41,2	47,7	26,4	40,2	58,7	43,8	38,8
07-16: No drug 1 mean	65,1	58,5	19,9	44,1	39,2	29,0	57,1	41,8	30,2
07-24: No drug 2 mean	41,7	45,3	35,0	21,2	29,1	47,1	37,5	37,9	43,0
07-24: No drug 1 mean	29,6	30,0	26,3	40,0	13,5	20,0	47,3	31,5	27,4
08-09: No drug	74,1	74,5	72,2	44,6	48,9	33,0	47,0	35,8	43,2
Average SG019 placebo N=15x10	41,3	39,7	30,0	32,9	28,8	31,6	40,8	34,4	35,2
Std dev	15,0	17,2	14,8	10,9	10,2	9,2	10,6	8,2	9,5
Std err	3,9	4,4	3,8	2,8	2,6	2,4	2,7	2,1	2,5

02-27: EtOH 1,5% Cross 3, % obs w	22,5	34,2	36,8	18,3	17,7	24,0	25,9	51,6	53,6
02-27: EtOH 1,5% Cross 4, % obs w	33,0	24,9	15,8	17,7	0,8	23,4	29,6	62,2	60,9
03-06: EtOH 1,5% Cross 2 #1, % obs	41,4	39,9	28,0	31,2	37,6	54,1	55,2	70,5	81,2
03-06: EtOH 1,5% Cross 2 #2, % obs	29,2	21,5	29,0	26,3	22,8	27,0	67,9	65,2	79,8
03-06: EtOH 1,5% Cross 1, % obs w	12,5	27,0	42,1	40,1	45,8	44,2	56,4	56,4	60,4
03-06: EtOH 1,5% Cross 3, % obs w	47,3	49,6	36,7	31,6	25,7	37,2	65,0	67,8	76,7
SG019 EtOH 1,5% #1, 05-10 % obs	38,4	34,1	20,4	40,1	44,3	40,4	40,7	60,1	68,9
SG019 EtOH 1,5% #2 05-10 % obs w	25,2	23,3	33,6	35,4	22,1	18,7	32,3	32,3	35,7
EtOH 1,5% #1 04-18 % obs at wall	26,5	32,2	34,5	34,7	24,1	31,0	39,0	52,5	59,3
EtOH 1,5% #2 04-18, % obs at wall	39,5	10,0	28,7	25,9	15,9	33,8	18,9	41,6	43,5
07-01: EtOH 1,5%, % obs at wall	41,5	27,8	30,3	11,5	17,4	46,9	45,2	56,8	52,1
07-09: EtOH 1,5%, % obs at wall	36,2	22,9	14,4	12,8	18,1	38,9	35,4	62,0	60,9
07-16: EtOH 1,5% mean	27,7	18,2	35,6	21,7	40,9	74,2	89,8	95,4	87,0
07-24: EtOH 1,5% #2 mean	76,9	57,0	22,4	12,7	14,3	24,7	32,9	45,7	62,0
07-24: EtOH 1,5% #1 mean	33,2	23,7	19,6	32,1	47,3	54,6	55,8	66,7	48,0
08-09: EtOH 1,5%	44,8	32,4	12,3	11,7	12,7	9,5	18,6	29,9	47,5
Average SG019 EtOH 1.5% N=16x10	36,0	29,9	27,5	25,2	25,5	36,4	44,3	57,3	61,1
Std dev	14,2	11,7	9,0	10,2	13,7	16,1	19,5	15,8	14,6
Std err	3,5	2,9	2,3	2,6	3,4	4,0	4,9	4,0	3,6

5.28. Thigmotaxis of seven-day old ABC SG013 given AMPH

Time (min)	0	1	3	5	7	10	13	16	20
05-16: No drug #1 % obs at wall	34,2	14,1	17,1	27,0	27,5	24,3	20,6	48,7	32,6
05-16: No drug #2 % obs at wall	30,9	34,2	40,8	40,6	23,6	45,5	42,7	43,7	46,7
07-23: No drug 1% obs at wall	54,1	35,3	37,3	41,5	37,0	47,8	55,7	58,8	40,5
07-23: No drug 2% obs at wall	60,2	61,4	83,6	77,0	69,0	73,0	67,8	66,5	45,6
07-25: No drug #1 % obs at wall	41,0	37,2	44,6	45,4	28,8	29,6	32,1	16,8	37,0
07-25: No drug #2% obs at wall	49,8	48,5	42,5	32,4	17,4	39,8	29,0	47,7	27,0
07-30: No drug % obs at wall	35,9	41,7	37,2	59,9	54,9	19,9	24,7	37,1	35,7
Average ABC placebo N=7x10	43,7	38,9	43,3	46,2	36,9	40,0	39,0	45,6	37,9
Std dev	11,1	14,5	20,0	17,1	18,5	18,0	17,4	16,0	7,0
Std err	4,2	5,5	7,5	6,4	7,0	6,8	6,6	6,0	2,7

05-16: AMPH 0,5mg/L #1 % obs at wall	35,3	36,7	53,8	42,3	46,8	45,0	41,8	36,4	63,3
05-16: AMPH 0,5mg/L #2 % obs at wall	46,1	35,4	44,3	28,3	38,4	53,6	51,7	55,5	55,5
07-23: AMPH 0,5mg/L % obs at wall	26,3	30,3	25,8	37,5	23,2	44,5	34,7	64,2	63,7
07-23: AMPH 0,5mg/L #2% obs at wall	63,2	41,7	47,4	38,2	62,2	34,4	34,1	33,7	45,7
07-25: AMPH 0,5mg/L % obs at wall	37,0	50,3	50,2	46,1	45,5	43,8	28,4	54,7	41,2
07-25: AMPH 0,5mg/L % obs at wall	40,1	38,5	31,1	27,2	35,0	45,1	48,2	58,9	41,0
07-30: AMPH 0,5mg/L % obs at wall	25,6	39,0	42,4	31,7	23,8	21,9	25,2	34,0	30,9
Avg. ABC AMPH 0.5mg/L N=7x10	39,1	38,8	42,1	35,9	39,3	41,2	37,7	48,2	48,8
Std dev	12,9	6,2	10,2	7,1	13,8	10,2	9,9	13,0	12,4
Std err	4,9	2,3	3,8	2,7	5,2	3,8	3,7	4,9	4,7

05-16: AMPH 2mg/L #1 % obs at wall	34,2	29,6	20,2	44,0	37,3	34,2	36,7	34,5	36,5
05-16: AMPH 2mg/L #2 % obs at wall	43,9	34,4	37,8	35,1	36,8	52,2	35,8	25,4	23,0
07-23: AMPH 2mg/L #1% obs at wall	57,0	46,6	44,6	45,7	51,7	32,0	28,0	54,9	41,3
07-23: AMPH 2mg/L #2% obs at wall	63,0	39,6	31,4	37,3	24,7	32,8	43,8	59,7	26,9
07-25: AMPH 2mg/L % obs at wall	16,1	23,9	25,8	29,3	36,5	39,0	31,9	35,6	54,5
07-25: AMPH 2mg/L #2 % obs at wall	16,1	24,2	25,8	29,3	36,5	40,4	33,0	35,6	54,5
07-30: AMPH 2mg/L% obs at wall	45,7	59,7	39,1	38,7	31,2	37,8	20,8	26,7	18,6
Avg. ABC AMPH 2mg/L N=7x10	39,4	36,8	32,1	37,0	36,4	38,3	32,9	38,9	36,5
Std dev	18,4	13,0	8,7	6,5	8,2	6,9	7,2	13,3	14,5
Std err	7,0	4,9	3,3	2,4	3,1	2,6	2,7	5,0	5,5

05-16: AMPH 5mg/L #1 % obs at wall	45,2	20,2	24,0	42,3	24,3	40,7	28,1	31,4	30,9
05-16: AMPH 5mg/L #2 % obs at wall	28,8	25,9	35,8	25,5	37,2	57,4	50,5	55,8	25,2
Avg. AMPH 5mg/L, % at w N=2x10	37,0	23,1	29,9	33,9	30,8	49,1	39,3	43,6	28,0
Std dev	11,7	4,0	8,3	11,9	9,1	11,8	15,8	17,3	4,1
Std err	8,2	2,8	5,9	8,4	6,5	8,3	11,2	12,2	2,9

05-16: AMPH 10mg/L #1 % obs at wall	46,5	37,2	23,1	28,7	25,0	26,9	30,7	33,0	31,1
05-16: AMPH 10mg/L #2 % obs at wall	58,5	32,0	45,1	41,8	43,7	46,2	59,0	39,6	35,5
07-23: AMPH 10mg/L #1 % obs at wall	52,4	67,5	42,3	33,8	52,4	28,7	35,0	17,4	28,5
07-23: AMPH 10mg/L #2 % obs at wall	69,2	53,2	44,1	55,7	32,0	54,9	32,9	50,1	46,2
07-25: AMPH 10mg/L #2 % obs at wall	25,4	30,3	36,5	30,6	26,0	42,9	32,0	31,4	46,2
07-25: AMPH 10mg/L #3 % obs at wall	50,5	45,9	46,0	36,9	35,9	39,3	40,8	41,8	49,7
07-30: AMPH 10mg/L % obs at wall	19,6	35,3	33,4	20,4	36,7	23,1	28,1	41,4	31,2
Avg. ABC AMPH 10mg/L N=7x10	46,0	43,1	38,6	35,4	36,0	37,4	36,9	36,4	38,3
Std dev	17,7	13,5	8,3	11,2	9,7	11,6	10,5	10,4	8,8
Std err	6,7	5,1	3,1	4,2	3,7	4,4	4,0	3,9	3,3

5.29. Thigmotaxis of seven-day old wild type SG019 given AMPH

04-18: AMPH 0,5mg/L, % obs at wall	21,6	28,9	21,4	17,3	30,9	35,2	26,1	28,8	31,1
07-01: AMPH 0,5mg/L, % obs at wall	50,1	36,6	22,3	25,2	31,7	36,9	42,4	33,3	35,5
07-09: AMPH 0,5mg/L, % obs at wall	46,0	49,6	33,0	43,6	50,7	41,2	32,7	28,7	42,1
07-16: AMPH 0,5mg/L mean	47,7	49,9	33,5	31,5	37,5	30,5	31,5	37,6	49,3
08-09: AMPH 0,5mg/L	43,3	26,2	32,0	30,0	25,0	41,2	30,3	37,7	44,5
Avg. SG019 AMPH 0,5mg/L N=5x10	41,7	38,2	28,4	29,5	35,2	37,0	32,6	33,2	40,5
Std dev	11,5	11,2	6,1	9,6	9,7	4,5	6,0	4,4	7,2
Std err	5,1	5,0	2,7	4,3	4,4	2,0	2,7	2,0	3,2

04-18: AMPH 2mg/L #1, % obs at wall	35,9	29,5	16,4	8,9	27,7	35,4	28,1	34,8	13,7
04-18: AMPH 2mg/L #2, % obs at wall	18,3	12,3	19,6	26,9	18,9	23,0	31,1	32,8	31,7
07-01: AMPH 2mg/L, % obs at wall	55,7	58,9	33,2	46,4	52,3	41,0	52,0	40,3	59,0
07-09: AMPH 2mg/L, % obs at wall	85,9	68,4	32,6	26,7	31,1	42,6	39,9	28,7	26,7
07-16: AMPH 2mg/L mean	64,5	27,3	52,0	40,0	29,0	25,3	40,4	28,1	43,7
08-09: AMPH 2mg/L	50,4	45,5	36,5	40,9	50,1	47,3	39,3	42,2	41,4
Avg. SG019 AMPH 2mg/L N=6x10	51,8	40,3	31,7	31,6	34,8	35,8	38,5	34,5	36,0
Std dev	23,3	21,1	12,8	13,7	13,3	9,8	8,4	5,8	15,6
Std err	9,5	8,6	5,2	5,6	5,4	4,0	3,4	2,4	6,4

04-18: AMPH 10mg/L, % obs at wall	47,1	34,9	41,7	30,3	27,9	30,9	31,7	41,2	36,0
07-01: AMPH 10mg/L, % obs at wall	41,4	35,9	40,5	32,0	43,7	25,3	34,7	35,4	44,7
07-09: AMPH 10mg/L, % obs at wall	49,2	52,5	12,2	20,5	40,5	24,0	27,0	39,3	59,4
07-16: AMPH 10mg/L mean	61,0	24,7	36,5	36,3	38,0	52,1	23,4	44,0	44,9
08-09: AMPH 10mg/L	49,4	50,3	22,9	29,3	24,3	35,5	38,8	39,6	36,4
Avg. SG019 AMPH 10mg/L N=5x10	49,6	39,7	30,8	29,7	34,9	33,5	31,1	39,9	44,3
Std dev	7,1	11,6	12,8	5,8	8,4	11,3	6,1	3,1	9,5
Std err	3,2	5,2	5,7	2,6	3,8	5,1	2,7	1,4	4,2

5.30. Thigmotaxis of seven-day old tfu mutant SG018 given AMPH

07-01: AMPH 0,5mg/L, % obs at wall	44,1	28,0	36,1	27,7	26,4	19,6	25,8	23,9	21,8
07-09: AMPH 0,5mg/L, % obs at wall	58,4	43,5	17,9	33,0	35,5	28,5	21,1	16,8	22,0
07-16: AMPH 0,5mg/L	46,8	35,8	33,3	39,8	40,8	27,8	39,0	28,2	44,7
07-31: AMPH 0,5mg/L	69,9	63,2	57,8	22,0	33,2	38,5	17,6	38,4	22,6
08-10: AMPH 0,5mg/L	49,3	45,7	32,5	41,1	49,1	35,0	24,6	30,2	23,0
Average SG018 AMPH 0.5mg/L N=5x10	53,7	43,3	35,5	32,7	37,0	29,9	25,6	27,5	26,8
Std dev	10,5	13,2	14,3	8,1	8,5	7,3	8,1	8,0	10,0
Std err	4,7	5,9	6,4	3,6	3,8	3,3	3,6	3,6	4,5

02-21: AMPH 2mg/L, % obs at wall	27,6	35,0	30,8	31,0	29,8	23,7	33,1	20,5	34,2
02-28: AMPH 2mg/L, % obs at wall	44,9	38,9	32,3	31,2	39,2	32,4	16,2	36,3	36,0
07-01: AMPH 2mg/L, % obs at wall	48,5	51,9	32,7	31,8	30,5	33,4	28,2	40,8	37,4
07-09: AMPH 2mg/L, % obs at wall	57,7	61,0	21,0	30,4	24,7	15,6	8,0	16,7	23,1
07-16: AMPH 2mg/L	59,3	41,2	52,5	8,0	22,2	22,6	18,6	33,5	40,6
07-31: AMPH 2mg/L	58,5	60,6	78,8	44,3	40,0	60,0	43,6	40,1	22,7
08-10: AMPH 2mg/L	74,8	60,7	48,8	35,8	31,8	15,8	31,4	29,4	34,1
Average SG018 AMPH 2mg/L N=7x10	53,1	49,9	42,4	30,4	31,2	29,1	25,6	31,0	32,6
Std dev	14,7	11,4	19,4	11,0	6,7	15,4	12,0	9,4	7,0
Std err	5,6	4,3	7,3	4,2	2,5	5,8	4,5	3,6	2,6

02-21: AMPH 10mg/L, % obs at wall	45,0	32,3	15,3	11,3	19,6	29,8	32,0	26,5	0,9
07-01: AMPH 10mg/L, % obs at wall	35,4	48,2	50,6	30,2	25,2	27,3	46,6	37,1	64,5
07-09: AMPH 10mg/L, % obs at wall	54,0	62,9	53,2	19,2	38,9	32,8	25,1	22,2	22,6
07-09: AMPH 10mg/L mean	42,9	61,0	31,1	39,9	4,8	23,6	15,3	15,1	23,0
07-31: AMPH 10mg/L mean	64,3	84,9	75,0	68,9	50,9	55,1	52,4	58,8	47,4
08-10: AMPH 10mg/L	45,9	46,1	49,7	27,9	26,9	24,0	16,4	15,5	28,8
Average SG018 AMPH 10mg/L N=6x10	47,9	55,9	45,8	32,9	27,7	32,1	31,3	29,2	31,2
Std dev	10,0	18,0	20,5	20,2	15,9	11,8	15,5	16,6	22,1
Std err	4,1	7,4	8,4	8,2	6,5	4,8	6,3	6,8	9,0

5.31. Light/dark preference results of ABC SG013, SG018 and SG019

ABC SG013

Min	Placebo #1	Placebo #2	EtOH 1,5% #1	EtOH 1,5% #2
0	4	7	6	4
1	8	6	7	4
2	6	6	8	4
3	6	6	6	6
4	5	5	5	4
5	7	6	3	3
6	8	6	3	3
7	6	6	3	3
8	5	5	1	2
9	4	6	2	4
10	4	6	3	2
11	5	6	0	1
12	5	5	2	1
13	4	5	2	0
14	6	6	0	0
15	6	6	1	1
16	6	6	3	0
17	5	7	2	1
18	4	6	2	0
19	4	5	4	2
20	5	5	3	1
Average	5,25	5,75	2,13	1,5
Std dev	1,18	0,58	1,15	1,26
Std err	0,30	0,14	0,29	0,32

Avg. placebo	5,5	Avg. EtOH	1,81
Std dev	0,35	Std dev	0,44
Std err	0,25	Std err	0,31

SG019

Min	Placebo #1	Placebo #2	EtOH 1,5% #1	EtOH 1,5% #2
0	6	4	5	7
1	8	4	4	6
2	7	5	4	6
3	7	5	5	5
4	7	5	3	4
5	6	4	4	1
6	8	7	1	1
7	6	6	0	2
8	6	5	1	3
9	7	6	1	1
10	7	4	1	0
11	7	5	0	1
12	5	5	1	1
13	7	5	1	0
14	6	5	1	0
15	7	6	1	0
16	7	5	2	0
17	6	6	4	0
18	7	5	4	0

19	6	6	3	1
20	7	6	3	1
Average	6,56	5,38	1,75	0,75
Std dev	0,73	0,81	1,39	0,86
Std err	0,18	0,20	0,35	0,21

Avg. placebo	5,97	Avg. EtOH	1,25
Std dev	0,84	Std dev	0,71
Std err	0,59	Std err	0,50

SG018

Min	Placebo #1	Placebo #2	Placebo #3	EtOH 1,5% #1	EtOH 1,5% #2	EtOH 1,5% #3
0	6	7	3	7	7	5
1	5	7	5	3	4	6
2	3	5	5	2	4	3
3	0	5	6	2	5	3
4	1	6	5	1	4	4
5	0	7	5	2	3	2
6	1	6	5	1	4	2
7	0	6	6	1	3	1
8	2	7	5	1	3	1
9	0	6	5	1	2	3
10	0	7	5	0	3	0
11	1	7	4	1	4	2
12	2	9	6	1	4	4
13	1	8	5	1	4	2
14	1	8	5	1	5	2
15	2	5	4	0	5	0
16	3	6	4	1	6	2
17	1	7	5	2	3	1
18	1	6	3	1	2	3
19	1	5	3	3	4	3
20	1	5	3	4	2	4
Average	1,06	6,56	4,56	1,31	3,56	2,00
Std dev	0,85	1,15	0,96	1,01	1,15	1,21
Std err	0,21	0,29	0,24	0,25	0,29	0,30

Avg. placebo	4,06	Avg. EtOH	2,29
Std dev	2,78	Std dev	1,15
Std err	1,61	Std err	0,67