**Project**

**Relevance of zebrafish** Several aspects make zebrafish suitable as a model organism, and especially for evaluation of new drugs. Zebrafish belongs to the vertebrates and there is a close homology between zebrafish and human, on average approximately 70% sequence homology and gene knock-down can easily be performed using morpholinos.*5* Zebrafish are small and the housing and maintenance costs are therefore low compared to mice or rats. They have a high reproductive rate; a female can produce hundreds of eggs every week, and the embryos develop fast. Although less complex than mammals, the zebrafish larvae brain still contains approximately 100000 neurons at 10 days past fertilization and it can already perform prey and feeding.*6* In addition, zebrafish larvae respond to drugs in a similar way as more advance animals. The structure and molecular pathways of the CNS are highly conserved between mammals and zebrafish, and a functional nervous system is established after only 4–5 days of embryonic development and all major neurotransmitter systems (i.e. dopamine, serotonin, GABA) have been identified.

**Zebrafish behavioral platform** During the last years, we have been successful in developing new techniques that enable us to track very detailed movement patterns, even in compound movements performed in a fraction of a second. Zebrafish larvae have specific and distinct movement patterns (swim bouts) that can be used to model behavioral and cognitive aspects of neurological diseases. We developed an infrastructure for behavioral analysis that records and analyzes the behavior of up to 192 zebrafish larvae in parallel in microtiter plates, using our in-house developed tracking software. The development of this platform has been. Our improved mathematical algorithms have significantly increased the amount of kinematic data that can be handled (300 pictures/second over 3–4h). Importantly, these methods enable unprecedented detection of detailed behaviors. We have implemented over 40 parameters that in detail describe the behavior of zebrafish larvae, gross locomotor activity such as overall swimming distance number of bouts (swim event) and standstill; bout characteristics such as distance, duration and speed per bout and place preference with wall or center preference. In addition, characterizing how zebrafish larvae perform turns would be beneficial but this is a challenging problem since a larvae typically complete fast turns within 10-20 ms. Therefore recording with high speed camera is essential to capture these movements which exclude commercially available systems. Zebrafish display a small repertoire of turns which they use depending on situation and stress level. For example under normal conditions most movements are scouts i.e. short distance movement forward with low speed and angle change. However, other turns comes into use when the larvae prey (J-bends) or escape from a predator (c- and o-bends).*7-9* These turns are characterized by increased speed and larger angle change from the initial facing direction. Furthermore, we have also added a parameter called startle to pin point and count really fast movements with large angle changes useful for quantification of for example convulsions. The possibility to in detail classify and quantify turns during several hours which is beneficial for drug profiling is a unique feature of our system. Analysis of how a drug changes the frequency of different turns will give additional information about drug effects especially in models where the larvae is challenged with an external stimuli. Furthermore, the datasets generated in typical experiments are very large, often containing more than 30000 data points and therefore we have implemented multivariate analysis (Simca) for clustering and classification of evaluated drugs.

In this study we are trying to identify compounds with antipsychotic properties with procognitiv effects. Action sequencing has been found to be deficient in people with schizophrenia or young people at high risk of developing psychosis. We have previously analyzed the behavior of zebrafish larvae using our in house developed platform. However this method bins together results over time slots (30-300s). In this project we will instead perform analysis of sequences of movements e.g. turns in order to identify changes when exposed to drugs that effects major neurotransmitter system in the brain.

**Objectives are:**

Setup a platform for analysis of action sequencing behavior in zebrafish larvae using bioinformatics tools

Perform analysis on a drug effects on action sequencing in zebrafish larvae.

**Codon**

The fish moves forward by discrete swim event so called bouts. In the codon bouts are separated by a b. Each bout could contain one or more turns. Each turn is defined by direction L left or R right, turn classification (see below) and the not yet implemented length (short, medium, long) letters not yet decided.

The fishes are recorded and analyzed in 5 min slots with 1 s pause in between.

Each experiment consist of a number of 5 min recordings, typically 13 thus 65 min experiment.

Under constant light each fish perform approximately 400 bouts per 5 min and does 2 turns per bout on average. This translates to approximately 10000 turns per fish and experiment. Each experiment typically contains 48 fishes thus approximately 480000 turns to analyze per experiment

**Definition turns**

Picking out individual turns of the Swim bouts and divides them into different categories. The result is given in the number of turns (per time window). A turn is defined as a large enough angle instantaneous change in psi, but not necessarily to the same direction, which justifies the dual conditions of the C and O Bends. If two "similar" turns takes place with less than 10 frames (33 ms) intervals, they are joined. For example. two adjacent turns scoots together into a scoot, or a JBend result of a scoot becomes a turn, but a result of a scoot JBend maintained as two separate turns.

The different turns are currently defined like this:

• **S** Scoot: Total angle change <15⁰. Even bouts where no turn is detected is classified as scoots.

• **J** JBend: Total angle change between 15⁰ and 80⁰. The average of the absolute value of the difference between φ and the direction of motion, multiplied by the rate at any time must be less than 4.25 \* 10-5, i.e. a large part of the movement is lateral. The relationship between total angle change and distance swum must be at least 40 degrees / mm. Total swum distance may not be for longer than 0.1486 \* r. The threshold is determined in relation to the well radius.

• **C** CBend: Total angle change> 80⁰. The first continuous turn must be between 80⁰ and 130⁰.

• **O** OBend: Angle Change> 80⁰. First coherent turn must be greater than 130⁰.

• **E** EBend Angle Change> 80⁰. First coherent turn must be less than 80⁰. Covering all the turns that are not captured by CBend and OBend.

• **G** GBend: Total angle change between 15⁰ and 40⁰. Covering all the turns that are not captured by JBend.

• **H** HBend: Total angle change between 40⁰ and 70⁰. Covering all the turns that are not captured by JBend.

• **I** iBend: Total angle change between 70⁰ and 80⁰. Covering all the turns that are not captured by JBend.

* **m** joint turns see above.

Below is an example of output data in FASTA format from an experiment, 1 fish during 5 min

>ZF Recording 2016-04-01; tW: 300s; Start: 122917; End: 123417;

Recording: 1-1; Individual: 9; Drug: Clozapine 1 µM PTZ 2.5 mM

bLsbLibLibLsbLsLsLsmmbLiLsbLgLsbLgLsbLsLsmbLsbLgbLgRsbLibRgbLgbLgbLgLs

bLgbLgbLsLsmbLsbLsLsmbLgbLgbLgbLgbLsLsmbLgbLsRsbLsbLsLsmbLgbLsbLgbLgbL

gbLgbRsbLgbLsbLsLsmbLsbLgbLgRsbLgLsbLgbLsbLsbLebRebLsbLsbLsbLibLsbLsbL

gbLgbLgbLsbLsbLgbLgbLgbRebRiRsmbRiRsRsmmbRgLsbLibRebRhRsmbLibLjbLebReb

LgRsbLgbLgbLsLsRsmbLsbLgLsLsmbLsRsbLgbLgbLobLsLsmbLsbRebLgLsbLgLsbLsLs

mbLibLsLsmbLgLsbLsbLsbLsbLsbLjbLgbLsbLgbLsbLgbLsbLsLgbLgbLgbLjbLiRsbLg

bLgLsbLgbLgbLgLsbLsbLsbLgbLsbLgbRibLsbLgbLgbLgbLjbRcRsLsmbRiRsRsRsmmmb

RebLobLgbLsbLobLgLsRsbRebLgbLsbLsbLiRsbRibLcLsLsmmbRsLgbLgbLhbRjbLcRsb

RgbLobRsbReRsmbRcRsmbRcbRibRsRsmbLobLcbLibRsRsmbLgbLcRsbRsbLgbLibLgLsb

LgbRsbLgbLsbLsLsmbLsbLgbLsbLibLsbLgLsRsbLgbLsLsmbLsRsbLsbLgbLgbLsbLgbL

sLsmbLgbLgLsbRsbLsRsbLsLsmbLiRsbLsbLgLsLsmbLgbLgbLgbLgbLgbRsbLgRsbLgbL

sbLsbLgLsbLgbLgbLsbLgLsbLsLsmbLgbRsbLibLsRsbLsLsmbLgbLsLsmbLgLsbLsbLsL

smbLgbLgbLgLsRsbLgbLgLsbLsbLsbLgbLgRsRsmbLiRsbLibRsbLibLsbLgLgLsbLgbLg

bLgRsbLgbLgLsbLsbLgbLgbRsbRsbLsLsmbLgbLsbLgbLiRsbRibRcbRiRsmbRsbRibRib

LgRsLsbRibLjbRsbRibRebRcRsmbRebRoRsmbLsLsmbLoRsLsLcRsRsmbLibLibLsbLgbL

gbRsLsbRsLsRsbLsRsbLsbLgbLsLsmbLgRsbLgbRgRsLsmbsbsbLgbLgbLsLsmbLgLsbLs

bRsbLgbLsLsmbLsbLgbLgLsbLgLsbRsbLsbLsbRsRsmbLsRsbLgLsLsmbLsbRsbLsbLgbL

sbLsLsmbLsbLgbRsLsLsmbLsbLgRsLsbRsbLgLsRsbLsbLgbLgbLiLsbLgbRsbLiLsbRsL

sbLgbLiLsLsmbLcbLobLcbRsbLgbLsbLobRiRsmbRgbRsbRibLsbRgbRgbRhRsRsmmbRhR

smbsbLsbRobLsbRcRsLsLsRsmmbRobLsbLiLsbLgLsbLibRgbRsbLcRsbRibLoLsmbRibL

obRibRibRoLsRsLsbLgbRhbRobRgbLsRsbLsbLoRsbLgbLsLsmbRsLhbRibLobReRsmbLc

RsbLibsbLgLsLsmbLgbLsbLgbLsbRsbLsbLiRsbLibLgbLsbLgbLgRsbLsLsLsmmbLgbLg

bLiLsRsLsbRsLsLsmbLsRsbLgbLgbLgbRsLgLsbLgLsbLsLgbLgLsbLiLiRsbRsbLgLsRs

bLgLsbLiRsbLgLsbLsLsmbLgbLsLsmbLgbLiRsbLgbLibLgRsbLsRsbsbLgbLgbLibLsLs

LsmmbLsbLsbRsLsbLgLsbLsbRsbLgbLobLibRoRsmbRcbLcbLcLsmbLhbReRsmbLgLsbLj

bRobsbRobLcLsmbLcbLibLibLibLhbRibRibLcbLobLhbLebLiRsbLiLsbLibLiRsbLiRs

bLib