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"Deconstructing the oxygen sensing neuronal network of
Caenorhabditis elegans"

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Abbreviations:

ANOVA ... analysis of variance, AUC ... area under the curve, BL ... base line, DR ... down ramp, DS ... down shift, GCY ... guanylate cyclase, LR ... large reversal, N2 ... wild type *C. elegans* strain, NGM ... nematode growth medium, OT ... omega turn, ROI ... region of interest, SEM ... standard error of the mean, ST ... shallow turn, TWU ... time window used, UR ... up ramp, US ... up shift

1 Introduction

Neurons are simple minded. If they are sufficiently excited, they depolarize. They don't care where the excitation comes from or what happens afterwards. The organisms these simple cells belong to cannot afford to be as simple. They have to deal with an overwhelming flood of signals from their environment (e.g. food, threat, mates) as well as from their internal state (e.g. hungry, frightened, bored).

A network of simple minded cells enables organisms to process all of these signals and convert them into behavior to deal with the challenges the organisms encounter. How activity of neuronal cells can lead to appropriate behavior still leaves us with many questions unanswered.

The most basic of organisms, bacteria, are capable of solving basic problems: what's the best way to get to a food source or what's the best way to escape from a toxic environment. By an uncomplicated behavioral mechanism, the biased random walk, *Escherichia coli* is able to work its way in a gradient to the highest concentration of an attractant or escape from a gradient of a hazardous source (Berg et al, 1972).

Finding the point of highest concentration within a homogeneous, linear gradient is trivial. Finding and reaching a preferred absolute point within a gradient is not. The nematode *Caenorhabditis elegans* has been shown to use a similar strategy to find its way towards the source of an attractant. By a probabilistic occurrence of a set of behaviors (turns and reversals) *C. elegans* is capable to follow a gradient towards its source comparable to the bacterial random walk (Pierce-Shimomura et al, 2005). To achieve this goal from sensory input to behavioral output, a neuronal network has been proposed, where the signal requires a route via 4-5 neurons from sensory to motor neurons (Gray et al, 2005).

C. elegans can do more than follow a gradient to its source. It is also able to find and stay at a preferred optimum within a gradient. Even though it has been shown that *C. elegans* is able to find its way to its preferred temperature in a temperature gradient (Luo et al, 2006), the actual neuronal network and the mechanisms how the environmental signals are integrated into the nervous system and how this leads to a specific behavioral response to find an optimum have not been revealed.

C. elegans has four main classes of oxygen sensory neurons responsible for its oxygen dependent aerotactic behavior. These neurons are able to sense changes in oxygen levels by binding molecular oxygen to different types of soluble guanylate cyclases (GCYs). These cyclases are expressed in various sensory neurons. They produce cyclic

GMP upon activation, which in turn activates TAX-2/TAX-4 ion channels, leading to depolarization of the respective neuron. Two neurons of one class, BAGL and BAGR, are sensing decreases in oxygen by GCY-31 and GCY-33. Four neurons of three classes, URXL, URXR, AQR and PQR sense increases in oxygen by GCY-35 and GCY-36. GCY-31/GCY-33 are thought to be suppressed by binding molecular oxygen, leading to high activity at low oxygen concentrations. The converse effect is thought to be occurring in GCY-35/GCY-36, the higher the oxygen concentration, the more active the cyclases become. (Cheung et al, 2004, Gray et al, 2004, Zimmer et al, 2009)

The sensory openings for AQR, BAG and URX are located in the head, while the sensory opening for PQR is located in its tail region. *C. elegans* should therefore be well equipped not only to sense absolute changes of oxygen concentrations along its path but should also be capable of sensing differences of oxygen concentrations along the length of its body and should therefore be well equipped to identify an optimal oxygen concentration.

C. elegans feed on bacteria. They are maintained by seeding nematode growth medium (NGM) plates with drops of OP50 *E. coli*. Due to the transfer of the bacteria from liquid culture onto the plate, the bacterial solution spreads on the plate forming a center lawn of uniform thickness and a thicker bordering region. Cultured *C. elegans* prefer the border of the bacterial feeding lawn. This has been linked to a specific oxygen concentration at the border and it has been proposed, that *C. elegans* moves to the corresponding range of oxygen concentration because it is a marker for the possible presence of larger quantities of its food source in its real environment (soil, rotting fruits, etc). Oxygen concentrations on the feeding plate have been shown to be around 20% on the agar, ~17-18% on the bacterial lawn and ~13-14% at the thicker border of the lawn. (Gray et al, 2004)

Behavioral experiments exposing animals of the laboratory wild type strain N2 to a constant oxygen gradient of 4-21% had been performed in the laboratory of Manuel Zimmer. These experiments showed, that after 30 minutes animals accumulated in an area corresponding to oxygen concentrations ranging from 13-18%, with most animals found at 16%. Due to these findings this range of oxygen concentrations will be the focus of interest in experiments exposing animals to a constantly changing oxygen environment.

In previously published gradient assays, N2 animals show a preference for oxygen concentrations of 10-12% (Chang et al, 2006, Zimmer et al, 2009) or 7-8% (Gray et al, 2004). The preference at these concentrations in contrast to 16% oxygen has been explained by hyperoxia avoidance to reduce oxidative damage. The published gradients ranged from 0-21% oxygen which could explain different behavior compared to the

unpublished 4-21% gradients performed in the laboratory of Manuel Zimmer. Another explanation for the difference between these two gradient results could be that starved animals are more likely to move towards oxygen concentrations associated with food, even if these pose a possible threat to an animal.

Previous behavior experiments done in the laboratory of Manuel Zimmer using neuropeptide depleted *C. elegans* mutants revealed a severe defect in the behavioral response to changing oxygen concentrations regardless of the strength of the stimulus. These basic findings lead to the assumption, that neuropeptides play a significant role either in oxygen sensing or in the modulation of the corresponding activity in the neuronal circuit to elicit an appropriate behavioral response. Due to these findings, experiments will focus on comparisons of N2 and neuropeptide mutants.

So far over a hundred individual neuropeptides have been identified in *C. elegans* and have been classified in three families (Li et al, 2010). These families are the neuropeptide like proteins (nlp), the insulin like proteins (ins) and finally the FMRF like proteins (flp). After transcription of flp genes and translation into pro-proteins, these proteins are imported into dense core vesicles, where they are further processed into the functional neuropeptide parts by pro-protein convertases. The pro-protein convertase KPC-2/EGL-3 is expressed in most neurons and is therefore a prime candidate for further investigation of neuropeptides in the nervous system. (Thacker et al, 2000, Kass et al, 2001)

Aim of this thesis is to address the following points of interest

- Identify the most likely neuronal circuit of interest using the published connectome from sensory neurons to motoneurons responsible for the most important behavioral responses.
- Define oxygen stimulus protocols to best capture signal integration and behavioral response.
- Record the activity of oxygen sensing neurons using calcium imaging in wild type and mutants of interest and analyse the signal with respect to response to a strong stimulus and gradual oxygen concentration changes. Determine if the sensory signal is directly transformed into behavioral output or if the signal is modified within the network of interest.
- Record and analyse the behavior of *C. elegans* in response to oxygen stimuli in a quantitative manner in wild type and mutants of interest to enable a bottom up statistical analysis how oxygen signals are transformed from the sensory neurons to behavior. The use of cell kill as well as mutant strains and rescues allows a step by

step deconstruction of the network of interest. To achieve this, two major points have to be addressed:

- Identify and describe the main behavioral patterns *C. elegans* uses to navigate an environment with changing oxygen concentrations
- Modify existing informatics tools for the analysis of the identified behavioral patterns from the behavioral experiments in a quantitative manner.

1.1 The behavioral responses of *C. elegans*

The moving behavior of *C. elegans* has been extensively studied and described. *C. elegans* moves forward by propagating sinusoidal movements along its body.

For drastic changes of bearing, two main behaviors were identified and described in detail.

Worms can change their bearing by engaging in "omega turns", where the body initially bends until the nose gets close to the tail upon which forward movement resumes. It has been described, that without stimulus *C. elegans* interrupts its forward runs at least twice a minute with such omega turns (Croll, 1975a,b).

A second means to change the bearing has been described as "reversals", where worms stop their forward movement and move backwards within their tracks for a short period of time before forward movement in a changed direction resumes (Croll, 1975a,b).

It has been shown using comparisons of experimental assays and computer modeling worm behavior, that these two main behavioral tactics suffice to describe unstimulated runs as well as runs when engaging in attractant chemotaxis (Pierce-Shimomura et al, 1999).

More detailed analysis revealed, that the rate of omega turns and reversals is dependent on the feeding state of an animal as well as on the state of its environment. If animals are transferred from food to agar plates without food, worms display a local search behavior manifesting in an increased rate of reversals and omega turns during the first minutes off food. 10-50 minutes after the removal from food the frequency of reversals and omega turns drops drastically from 1-2 to ~0.3 per minutes while the worms move forward in long, uninterrupted runs. (Wakabayashi et al, 2004, Gray et al, 2005) The reversal frequency is not only dependent on feeding state, but has also been shown to be dependent on temperature and humidity as well as developmental stage and adult age (Zhao et al, 2003).

Reversals have been linked to an escape response from predatory fungi which is triggered by a slight touch at the neck. This response relies on engaging in a quick reversal after the touch occurs. Additionally head movements are suppressed while a reversal is taking place. (Maquire et al, 2011)

In recent years it has been discussed, that in addition to the behaviors described, *C. elegans* uses turns with large turning angles and gradual curves to navigate when they are off food to effectively follow sensory cues while staying on the move (Luo et al, 2006, Iino et al, 2009, Kim et al, 2011). These turns have been labeled as "shallow turns" and have been shown to be the most common form of reorientation in traveling behavior to efficiently find new food sources (Kim et al, 2011).

1.1.1 Definition of behavioral responses analyzed in this thesis

- Omega turn (OT)

Omega turns in the literature describe turns where worms bend their bodies until their nose touches their tail forming a shape reminiscent of the greek letter " Ω ".

These turns only constitute a fraction of turns where worms drastically change their body posture and bearing.

Omega turns analyzed in this thesis include all turns, where worms change the shape of the body from an elliptical to a circular outline in addition to drastically change the angular speed of their centroid while doing so.

All of these turns disrupt the sinusoidal wave animals are using to propel themselves forward. Only after the turn is completed, the normal forward movement by sinusoidal wave propagation continues.

- Shallow turn (ST)

STs in this thesis describe all events, when worms slightly change the shape of their bodies from an elliptical outline towards a circular one, without an immediate drastic change of angular speed of the centroid and without disrupting the sinusoidal wave thus continuing to move forward while turning. Observing moving single worms these events seem to be actively controlled, probably integrating sensory input on the move. It can be seen, that animals use these movements to course correct a turn that has already started, e.g. starting a 90 degree turn, and adding another 90 degree turn immediately after in the opposite direction, thus continuing along the original bearing. The feature to analyze STs was developed for this thesis.

The STs discussed here therefore correspond to the "short turn" subset of the shallow turns described in Kim et al, 2011, with the addition of "correcting turns" not leading to an actual change in direction.

- Turn angle

Turn angles describe the smaller angle between the bearing of a worm before and after an OT or ST has taken place. The feature to analyze turning angles of omega and shallow turns was developed for this thesis. For a detailed description see Materials and Methods.

- Large reversals (LR)

A reversal takes place, when a worm stops and moves backwards within its tracks for a certain period of time. Reversals lasting for one or two headswings have been categorized as short, reversals with at least three headswings as long reversals (Gray et al, 2005). In contrast to this a distinction between large and short reversals in this thesis is based on the length of the reversal normalized to the length of the worm. With the behavioral paradigms used in this work, large and short reversals show a very similar pattern of occurrence throughout the individual experiments. Therefore only large reversals will be analysed and discussed in detail.

1.2 Neuronal circuit of interest

The complete wiring diagram of the nervous system of *C. elegans* has been published as early as 1986 (White et al, 1986). To analyse *C. elegans* behavior in the context of the oxygen signal sensed by the oxygen sensory neurons AQR, BAG and URX, a neuronal network of interest was identified from the published connectome.

The main points of interest for this circuit were:

- candidate pre-motorneurons responsible for turns and reversals
- connections from the sensory neurons of interest to the candidate pre-motorneurons within one to two interneurons. This constraint was added due to the strong interconnectivity of the *C. elegans* nervous system by gap junctions and the fact, that information of neuronal activity in *C. elegans* is not conveyed by action potentials but rather by passive signal propagation (Goodman et al, 1998) or plateau potentials (Mellem et al, 2008). This leads to the assumption, that a signal will be modified by each step from one neuron to the next which in turn will lead to a modification of the original signal beyond recognition and would therefore not be likely to lead to a behavioral response according to the sensory input.

This might not be the case for a strong stimulus, where the activation is large enough to surpass any other network activity eliciting a strong behavioral response. Contrary in a gradually changing environment like a chemical gradient, it is reasonable to assume, that only neuronal activity that is not modified too far by being integrated into the activity state of many interneurons can elicit a meaningful response.

Pre-motorneurons identified for reversals are AVA and AVE (Chalfie et al, 1985, Li et al, 2011). For identification of the pre-motorneurons required for turns, evidence based information was gathered for RIM, RMG and SMD. RMD and RIG were included into this list because they constitute the rest of neurons innervating muscles in the neck region, namely the 3rd and 4th layer of muscles from the tip of the nose. (White et al, 1986) Using the muscles in the neck is essential for being able to engage in a turn. Due to this fact, these neurons were included as well even though there is no evidence so far, that

they are required for turns. RMD neurons are included because they are thought to be cross inhibitors of SMD (White et al, 1986). The removal of SMD has been shown to decrease the frequency of omega turns, while killing RIV had the opposite effect thus increasing the frequency of OTs (Gray et al, 2005). RIM has been shown to suppress movements of the head, counteracting activity from SMD or RMD (Pirri et al, 2009)

The synaptic connections for the node-edge network created to depict the identified neuronal connections from sensory to motoneurons are based on Chen et al, 2006.

Nodes colorcode	Description	Edges colorcode	Description
yellow	sensory neurons	blue	gap junction
red	interneuron	black	chemical synapse
blue	pre-motoneurons	green	chemical synapse to motoneuron
light green	motoneurons		
dark green	muscles		

Table 1: Definition of nodes and edges in the neuronal circuit of interest

Abbreviation	Description	Source
flp-x	FMRF like peptides	(Li et al, 2008)
eat-4	glutamate	(Serrano-Saiz et al, 2013)
ser-2	7-transmembrane GPCR	(Tsalik et al, 2003)
ser-4	serotonin receptor	(Tsalik et al, 2003)
glr-1,2	glutamate AMPA ionotropic receptor	(Brockie et al, 2001)
glr-3-6	glutamate kainate ionotropic receptor	(Brockie et al, 2001)
nmr-1,2	NMDA ionotropic receptor	(Brockie et al, 2001)
AVR-15	glutamate gated chloride channel	(Dent et al, 1997)
AVR-14	glutamate gated chloride channel	(Cook et al, 2006)

Table 2: Neuropeptides, neurotransmitters and receptors in the neuronal circuit of interest.

The candidate neuronal circuit of interest (Figure 1) was generated using Cytoscape v2.8.3 (Shannon et al, 2003, Smoot et al, 2011). Numbers shown above the edges correspond to the number of synapses found for this particular connection between two neurons. Abbreviations next to nodes note receptors and neurotransmitters with published expression patterns for the corresponding neurons.

eat4 ... glutamatergic (Serrano Ruiz 2013)
 flp-1 ... wormbook (12.2013) GPCR (Tsailik 2003)
 ser-4 ... serotonin receptor (Tsailik 2003)
 glr1.2 ... glutamate AMPA ionotropic receptor (Brookie 2001)
 glr1.3-6 ... glutamate kainate ionotropic receptor (Brookie 2001)
 flp-18 ... flippase (Chen et al 2006)
 AVR-15 ... glutamate gated chloride channel (Dent 1997)
 AVR-14 ... shows reduction in reversals (Cook 2006)

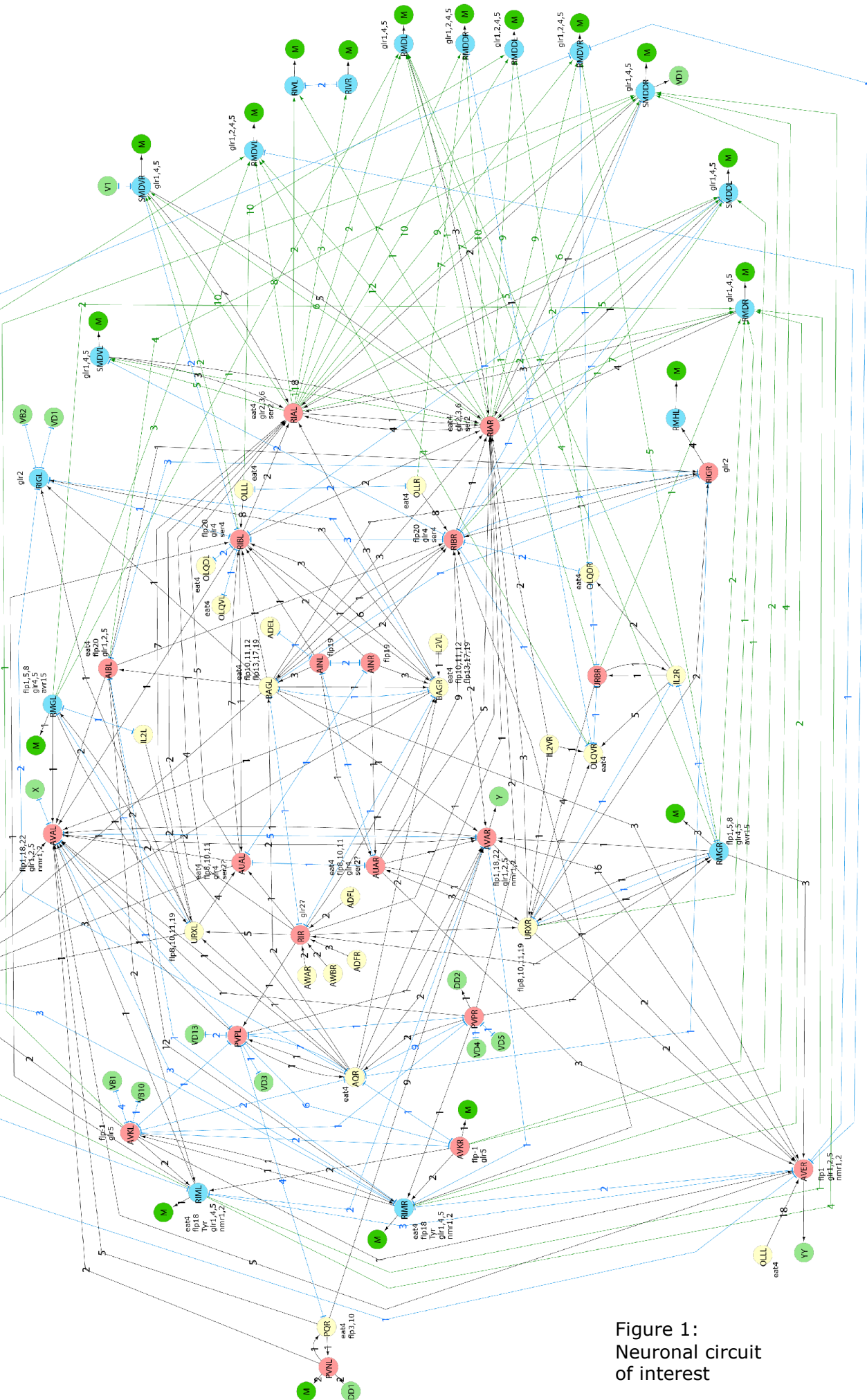


Figure 1:
Neuronal circuit
of interest

2 Results and discussion

2.1 Calcium imaging

The activity of the oxygen de- and increase sensory neurons BAG and URX is tested in an N2 (strain ZIM226) and *egl-3 n150* CX9191 (strain ZIM266) background. Both strains contain the same extra-chromosomal array expressing GCaMP5k under the *flp-8* and the *flp-17* promoter. *Flp-8* is expressed in URX, *flp-17* in BAG. GCaMP5k is a genetically encoded calcium indicator (Akerboom et al, 2012) which links calmodulin to an inactive form of GFP. Upon binding of Ca^{2+} to calmodulin the conformation of GFP changes and becomes fluorescent. Depolarization of a neuron leads to an intracellular release of Ca^{2+} . GCaMP therefore is a useful second messenger in visualizing neuronal activity.

A description of the strains used and the protocol how animals were treated and recorded can be found in the materials and methods section. Plots of all individual recordings can be found in the addendum section 5.1).

The neuropeptide mutant *egl-3 n150* has been shown to be depleted of most neuropeptides (Husson et al, 2006) which make it the first obvious target for investigation. The comparison of the neuronal activity of the oxygen sensors in N2 and *egl-3 n150* strain CX9191 should show, if the sensory activity of the oxygen sensory neurons BAG and URX is dependent on neuropeptides.

Due to variations in the expression level of GCaMP, high concentrations of GCaMP molecules in a sensory neuron can occur. If the concentration is high enough, Ca^{2+} ions which are released from one GCaMP molecule are immediately bound by another leading to a high basal level of fluorescence without actual neuronal activity taking place. This buffering of Ca^{2+} in the GCaMP pool can lead to a loss of sensitivity in case of an actual depolarization of the neuron of interest. The increase of fluorescence from the subsequent release of Ca^{2+} can either be very small or overall missing, if most of the present GCaMP molecules are Ca^{2+} saturated. To avoid experiments biased by this fact, recordings with an F0 higher than 2500 were excluded from analysis.

2.1.1 Neuronal activity in an oxygen shift paradigm

To study the response of the sensory neurons to a harsh stimulus, animals were exposed to subsequent oxygen shifts from 21-4% and back to 21%, oxygen concentrations are always balanced with nitrogen. The oxygen "down shift" (DS) took place 100 seconds, the "up shift" (US) 460 seconds after the start of the experiment.

step	duration [s]	oxygen [%]	nitrogen [%]
1	100	21	79
2	360	10	90
3	150	21	79

Table 3: Gas concentrations and switch times used during the shift paradigm, length of the experiment: 610s

In all subsequent shift plots, the time between DS and US is marked by the dark grey area. In the shown average plots, the black line indicates the average $\Delta F/F_0$ of all recordings, while the light grey area displays the corresponding standard error of the mean (SEM).

The x-axes states the time in seconds from start to end of the experiment, the y-axes the percent increase of fluorescence compared to the F_0 fluorescence level of the respective recorded neuron. Numbers below the plot indicate areas of interest used in statistical analysis.

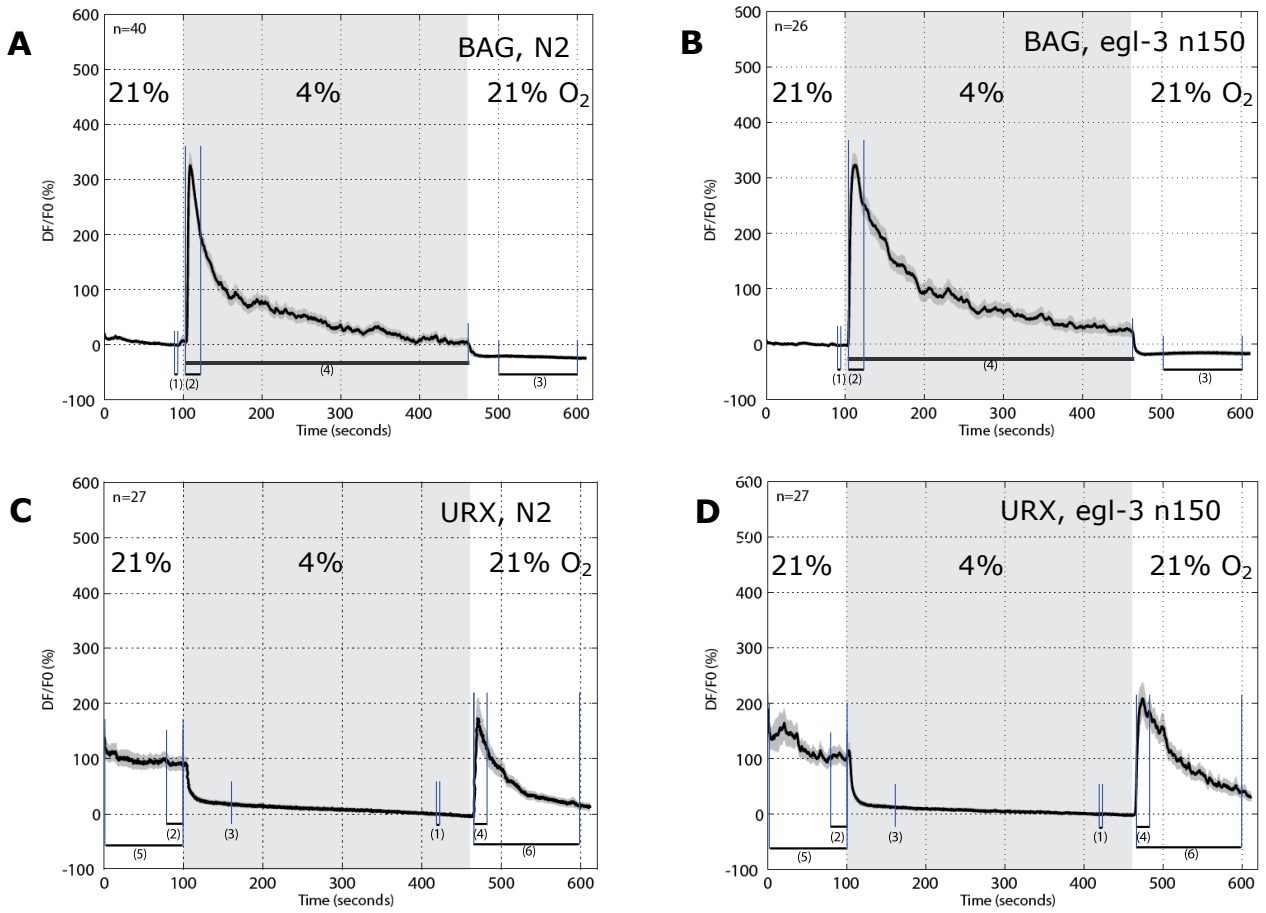


Figure 2: Recordings of the neuronal activity of BAG and URX in an N2 and egl-3 n150 background in a shift paradigm. The beginning of the shaded, grey area at 100s states a shift from 21-4% oxygen. The end of the shaded, grey area at 460s states a shift from 4-21% oxygen. (A) Average plot of 40 single recordings of the activity of the decreasing oxygen sensor BAG in N2. BAG was responsive in all recordings. (B) Average plot of 26 single recordings of the activity of the decreasing oxygen sensor in the egl-3 n150 strain CX9191. BAG was responsive in all recordings. (C) Average plot of 27 single recordings of the activity of the increasing oxygen sensor URX in N2. In four of the recordings, the URX neurons did not show any response. (D) Average plot of 27 single recordings of the activity of the increasing oxygen sensor URX in the egl-3 n150 strain CX9191. URX was responsive in all of the recordings.

BAG response egl-3 n150 compared to N2				URX response egl-3 n150 compared to N2			
		TWU [s]	significant difference			TWU [s]	significant difference
(1)	avg F0	80-84	ns	(1)	avg F0	410-414	ns
(2)	avg dF/F0	105-125	ns	(2)	avg dF/F0	80-100	ns
(3)	avg dF/F0	500-600	ns	(3)	avg dF/F0	130-131	ns
(4)	AUC dF/F0	105-459	+/* (P < 0.02)	(4)	avg dF/F0	465-485	ns
				(5)	AUC dF/F0	1-100	ns
				(6)	AUC dF/F0	465-600	+/** (P < 0.005)

Table 4: Comparison of N2 to the egl-3 n150 mutant recordings of BAG and URX (Figure 2) using unpaired t-test. AUC ... area under the curve. Find all corresponding statistics in the addendum section 3.1).

BAG in the N2 background displays a strong, immediate activation upon oxygen DS with a mean maximum of over 300% of F0 fluorescence. The initial activation recedes over time, but BAG displays recurring activity until the oxygen US takes place. Immediately after the oxygen US the fluorescence drops to a constant level below the initial fluorescence at the start of the experiment.

URX in the N2 background is active during high oxygen concentration levels, the neurons display activity from the start of the experiment accordingly. Immediately after the DS has taken place, the activity of URX is strongly reduced within 10s. URX stays inactive for the complete period of low oxygen. After the US has taken place, URX displays strong activation, with its peak 5-8 seconds after the US has taken place.

2.1.2 Concluding remarks shift paradigm

Neuropeptides absent in the *egl-3 n150* mutant do not influence the baseline activity nor the immediate response to oxygen DS or US in either BAG or URX. What can be observed is a change in the overall activity of the respective neurons after the corresponding shift has taken place. One reason for this could be due to neuropeptides released downstream suppressing the activity of the sensory neurons. Another reason could be that reduced inhibition in the overall downstream network due to the lack of inhibiting neuropeptide leads to an increased network activity leading to a higher rate of activating feedback to the respective sensory neurons.

2.1.3 Neuronal activity in an oxygen ramp paradigm

To study whether the preference of animals to oxygen concentrations between 15-18% is already mediated by the activity of the sensory neurons itself, animals were exposed to an oxygen ramp paradigm. This paradigm challenges animals with a gradual decrease in oxygen from 21% to 4% ("down ramp", DR) over the time of 180 seconds. After a 180 seconds resting period at 4%, an oxygen "up ramp" (UR) takes place, again increasing the oxygen concentration from 4% to 21%. Oxygen is always balanced with nitrogen.

step	duration [s]	oxygen [%]	nitrogen [%]
1	10	21	79
2	180	21-04	79-96
3	180	04	96
4	180	04-21	96-79
5	60	21	79

Table 5: Gas concentrations and switch times used during the ramp paradigm, length of the experiment: 610s

In the following plots DR and UR are indicated by grey shaded areas. The three white areas in the DR area correspond to oxygen concentrations 18-17%, 16-15%, 14-13%

from left to right. The white areas in the UR area correspond to oxygen concentrations 13-14%, 15-16%, 17-18% from left to right.

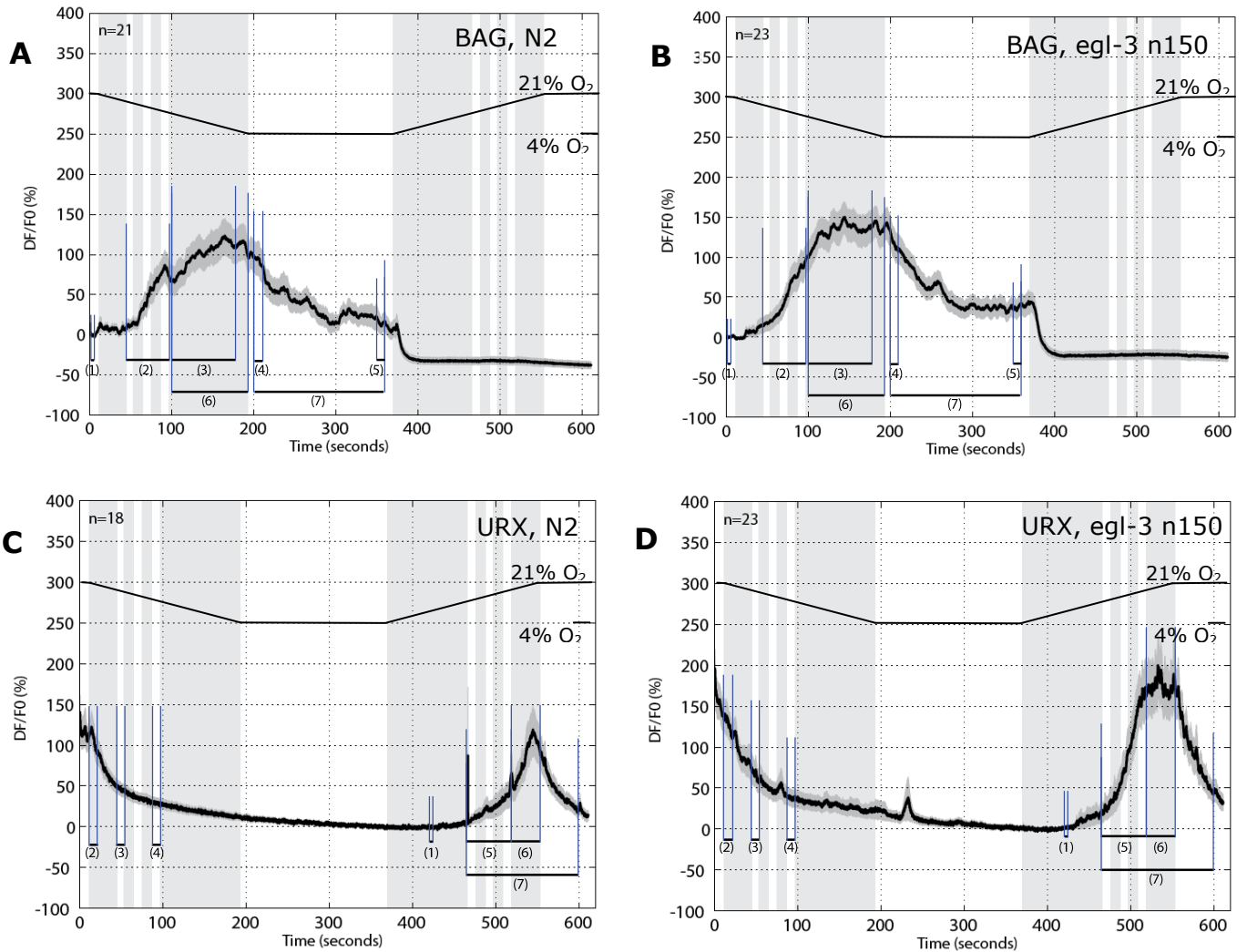


Figure 3: Recordings of the neuronal activity of BAG and URX in an N2 and egl-3 n150 background in a ramp paradigm. The duration of the DR from 21 to 4% oxygen is marked by the first grey area from 10s to 190s. The duration of the UR from 4 to 21% oxygen is marked by the second grey area from 370s to 550s. (A) Average plot of 21 single recordings of the activity of the decreasing oxygen sensor BAG in the N2 strain. BAG was not responding in 4 recordings. (B) Average plot of 23 single recordings of the activity of the decreasing oxygen sensor BAG in the egl-3 n150 strain CX9191. BAG did not display any activity in one of the recordings. (C) Average plot of 18 single recordings of the activity of the increasing oxygen sensor URX in the N2 strain. In 2 of the recordings the URX neurons did not display any activity. The single spike at 13% oxygen in the UR is due to an artifact in a single frame of a single recording. (D) Average plot of 23 single recordings of the activity of the increasing oxygen sensor URX in the egl-3 n150 strain CX9191.

TWU [s] significant difference				TWU [s] significant difference			
BAG response egl-3 n150 compared to N2				URX response egl-3 n150 compared to N2			
(1)	avg F0	1-4	ns	(1)	avg F0	410-414	ns
(2.1)	avg dF/F0	53-64*	ns	(2)	avg dF/F0	10-20	ns
(2.2)	avg dF/F0	64-75*	ns	(3)	avg dF/F0	53-63	ns
(2.3)	avg dF/F0	75-86*	ns	(4)	avg dF/F0	97-108	ns
(2.4)	avg dF/F0	86-97*	ns	(5.1)	avg dF/F0	476-487\$	ns
(2.5)	avg dF/F0	97-108*	ns	(5.2)	avg dF/F0	487-497\$	ns
(3)	avg dF/F0	160-180	ns	(5.3)	avg dF/F0	497-508\$	** (P < 0.01)
(4)	avg dF/F0	200-210	ns	(5.4)	avg dF/F0	508-519\$	** (P < 0.005)
(5)	avg dF/F0	350-360	ns	(6)	avg dF/F0	519-540+	** (P < 0.01)
(6)	AUC dF/F0	100-190	ns	(7)	AUC dF/F0	476-600	** (P < 0.005)
(7)	AUC dF/F0	200-360	ns				

Table 6: Comparison of N2 to the egl-3 n150 mutant recordings of BAG and URX in the shift paradigm (Figure 3) using unpaired t-test. * ... TWUs 53-64s, 64-75s, 75-86s, 86-97s and 97-108s correspond to the oxygen concentration changes of interest from 18-13%. \$... TWUs 476-487s to 508-519s correspond to oxygen concentrations 14-18% respectively. + ... TWU 519-540s captures the maximum response of the UR paradigm. AUC ... area under the curve. Find all corresponding statistics in the addendum section 3.1).

BAG in the N2 background (Figure 3A) shows increasing activity after the decreasing oxygen has passed 18% and keeps increasing, until the ramp has reached 4% oxygen. Only after the oxygen concentration stays constant at 4%, the activity gradually declines, but stays above baseline level, until the UR starts. On average 6s after the start of the UR, the activity of BAG decreases to below baseline level. Compared to the activity traces before the DR has started, the activity trace during the UR is astonishingly smooth. Seeing the activity of BAG at the oxygen concentrations 13-4% and given the fact, that GCY-31/GCY-33 are active at low oxygen concentrations, this points to an active suppression of BAG activity, if oxygen concentrations increase again after a previous decrease. If this suppression only happens if the oxygen level has previously dropped below a specific threshold, concentration remains target of investigation. The absence of neuropeptides does not influence the activity of BAG in the ramp paradigm as can be seen in Table 6. In the shift paradigm a slight increase of the Area Under the Curve (AUC) after the DS was observed (Table 4). It could be that the increase in AUC is only seen after a strong activation of BAG in the neuropeptide depleted background. In the ramp paradigm the maximum increase in fluorescence is at about 120% while the increase in the shift paradigm was found at around 300%.

Due to the oxygen sensor proteins GCY-35/GCY-36 whose activity is positively correlated with the environmental oxygen concentration, URX displays high activity immediately at the beginning of the experiment at 21% oxygen (Figure 3C). The neuronal activity of

URX decreases upon start of the DR ramp. The strong decrease after the start of the DR changes to a less narrow reduction of activity, once the oxygen concentration passes 18%. During the resting period at 4% oxygen, URX does not display any activity. Even after the UR has started, the activity only increases, after 13% oxygen has been passed. After this concentration, the activity steadily increases until the maximum activity at around 20% oxygen has been reached. After the UR has ended the activity of URX decreases back to the level of F0 fluorescence. During the DR URX behaves as expected. During the UR URX displays activity after 13% oxygen has been passed. A comparison of the activity of URX in N2 and *egl-3 n150* mutant background shows, that the absence of neuropeptides leads to an overall increased activity of URX during the UR response (Table 6).

2.1.4 Concluding remarks ramp paradigm

The activity of BAG during the DR shows increasing activity only after 18% oxygen has been passed and keeps increasing its activity until 4% oxygen has been reached. The neuronal activity follows the expected pattern upon a constantly decreasing oxygen concentration. The neuronal activity at 4% follows the pattern already described in the shift paradigm.

The interesting part is the decrease after the UR has started. On average 6 seconds after the start of the UR, where the oxygen concentration has not yet even reached 5%, the activity of BAG drops to below baseline level. At this level of oxygen concentration, GCY-31/GCY-33 should still be active, only gradually decreasing the activity of BAG while the oxygen concentration rises. The immediate drop of activity and the continued smoothness of the curve until the end of the recording suggest, that a suppression of BAG from an upstream neuron takes place.

A reasonable assumption would be that this suppression is mediated by the increasing oxygen sensor URX. This is not the case due to the fact, that URX starts its activity only after increases in oxygen have passed 13%. A suppression of BAG can therefore not be mediated by URX.

An alternative suppressor of BAG would be AQR. AQR is expressing the increasing oxygen soluble guanylate cyclases GCY-35/GCY-36 like URX. Furthermore, AQR is presynaptic to both BAG neurons while the URX neurons are not, which strengthens the theory, that AQR is the neuron suppressing the activity of BAG upon start of the UR. This would also suggest, that in a ramp paradigm AQR activates sooner than URX does which is puzzling due to the fact, that they use the same oxygen sensors, GCY-35/GCY-36. In oxygen shift paradigms the activity of AQR in a 1h starved shift or ramp paradigm in an N2 background has not yet been shown. In an *npr-1* mutant background and an on food oxygen US paradigm, the AQR US response has been shown to be overall

comparable to the URX response, even though the strength of the response is increased compared to URX (Busch et al, 2012).

Another possible neuron suppressing the activity of BAG could be IL2VL, which is presynaptic to BAGR and has been shown to convey oxygen sensation as well.

Neuropeptides do not influence the activity of the decreasing oxygen sensory neuron BAG in the ramp paradigm. The absence of neuropeptides does lead to a stronger UR response mediated by URX. An explanation for this behavior could be increased activity in network downstream of URX. Since URX is gap junctioned to RMG, an increased downstream activity would lead to a feed back and an increase in URX in turn.

2.1.5 Comparison of neuronal activity and behavior in the oxygen paradigms

Shift paradigm

The average sum of the activity traces from BAG and URX recordings in an N2 background provide an approximation of the input that the neuronal network is exposed to during the DS US paradigm. This summed activity of the neurons is compared to the omega turn and reversal behavior average of 23 individual behavior recordings of an oxygen DS US paradigm to check for a correlation between the neuronal activity and the . As in the calcium imaging recordings, the grey shaded area indicates the time between DS and US, the mean fraction of events taking place is shown as a black line, the SEM in dark grey.

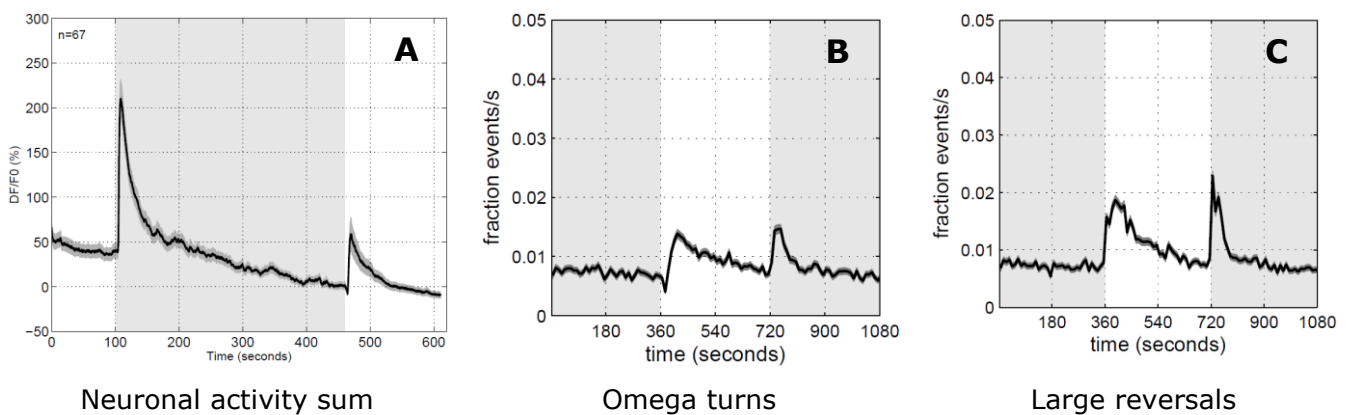


Figure 4: Comparison of the neuronal activity sum of BAG and URX (A) with omega turns (B) and large reversals (C) in an oxygen shift paradigm in N2.

The time scales of calcium imaging and behavior assay experiments are not identical, but the period between DS and US is 360s seconds in both. For both DS and US the neuronal activity resembles to some degree the behavioral responses. Both reversals and omega turns show a significant increase after DS and US have taken place, both show sustained increased activity after the initial DS response until the US takes place.

However, omega turns and reversals display a modified response upon DS. The omega turns show a delay of about 60s before the response takes place, the reversals show the maximum also about 60s after the shift has taken place. This indicates that the signal from the sensory neurons is not directly translated into immediate behavior, a modification of the signal downstream of the sensory neuron takes place.

It is unclear, if the decrease in omega turns after the DS correlates with the time until URX is completely inactive and thus explains this behavior.

Ramp paradigm

Again the neuronal activity in all recordings of BAG and URX in the ramp paradigm were used to create an average plot. The activity sum of the neurons is compared to the omega turn and reversal behavior average of 23 individual behavior recordings of an oxygen DR-UR 21%-4%-21% paradigm.

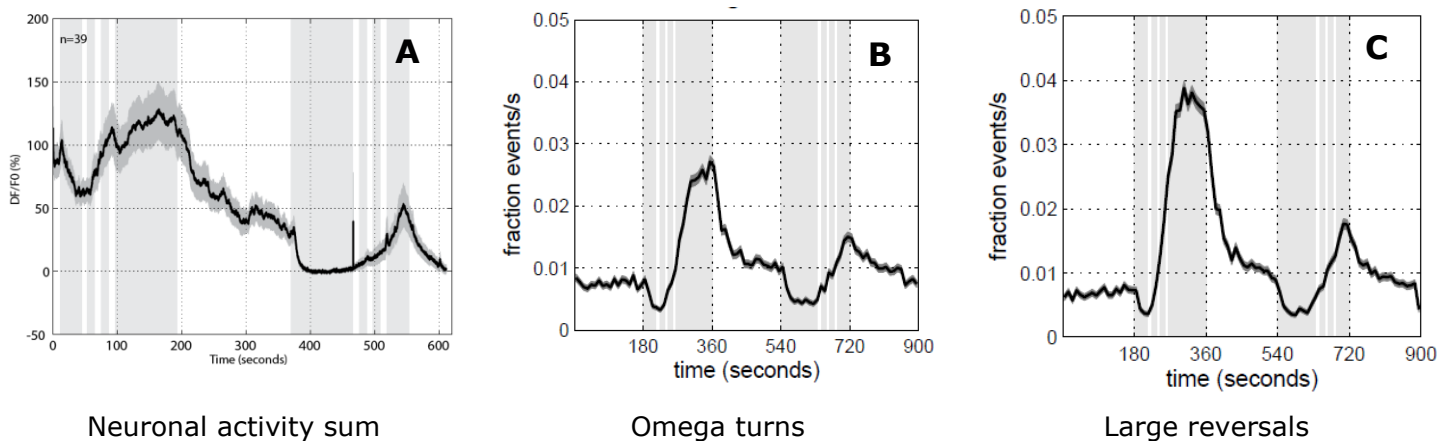


Figure 5: Comparison of the neuronal activity sum of BAG and URX (A) with omega turns (B) and large reversals (C) in an oxygen ramp paradigm in N2.

The activity sum of BAG and URX (Figure 5) resembles the behavioral responses in DR and UR for both omega turns and large reversals. The combined activity of BAG and URX correlates with the responses of these two behavior types in the ramp paradigm. During the DR the combined activity of URX and BAG leads to a minimum when the oxygen concentration passes from 18 to 17% and increases again, until the maximum activity has been reached at the end of the ramp. Activity stays high at low oxygen concentrations, until an UR takes place.

Immediately after the UR starts, the activity is suppressed to an overall minimum. This minimum is maintained, until the oxygen concentration of 13% has been surpassed and the activity reaches its maximum at the end of the UR upon which activity decreases again.

Omega turns and reversals are behaviors that can be triggered in response to aversive stimuli. They enable animals to move away from an undesirable environment. In light of

this, it is favorable to increase this type of behavior, if an animal moves in a direction where the conditions worsen and it is favorable to decrease these types of behavior, if an animal moves toward a direction, where the conditions improve. In the light of an animal finding preferred condition in the middle of a gradient, it would be optimal, if the probabilities of suppressing and increasing aversive behavior balance each other. A further strategy would be to suppress turning and reversing behavior until an animal has reached a region of preferred oxygen, while within this area, the turn and reversal activity is increased again to search the vicinity for the absolute optimum.

If the activity of the oxygen sensory neurons is directly converted into aversive behavior like omega turns and reversals, it would be advantageous to have low activity if an animal moves into the direction of its preferred oxygen concentrations and has high activity if animals move away from it.

The activity sum of BAG and URX in Figure 5A represents this behavior. During the DR, activity decreases until 17% oxygen has been passed, activity decreases until the animal has reached the upper bound of its preferred concentration. After 17% oxygen has been reached, activity rises again due to an increase in BAG. Once an animal has reached a region where the oxygen concentration approaches the optimum of 16%, turning and reversals could improve finding the absolute optimum within this region.

A further explanation, why the rise in activity is narrow after 17% oxygen has been passed towards a lower concentration could be that decreases in oxygen could lead to a hypoxic environment which is a threat to an animal. Finding an optimum could therefore be better and finer tuned while searching in the increasing oxygen concentration direction than in the decreasing.

Once 13% oxygen has been passed, the activity increases until its maximum has been reached. Once the oxygen concentration is unfavorable but constant at 4% oxygen, the activity gradually declines. If an animal has reached an area where the concentration is unfavorable but has been at a favorable area before, searching activity only gradually declines, only after a sufficient search of the area, searching diminishes and forward movement to find another area takes place.

Once the oxygen starts increasing from an unfavorable concentration, activity is suppressed. Turning and reversals are not required, as long as oxygen increases towards preferred concentrations. Activity increases again only after the lower bound of preferred oxygen concentrations, 13%, has been passed. For as long as an animal is within lower and upper bounds of the preferred oxygen concentrations, the activity increases only slowly as the oxygen concentrations increase. A strong increase only takes place, after the upper bound has been surpassed.

2.1.6 Concluding remarks

As concluding remarks to the calcium imaging section, BAG and URX are sufficient to explain omega turn and large reversal behavior in a changing oxygen environment. The combined activity of BAG and URX is sufficient to detect the upper and the lower bound of the preferred oxygen concentrations 14-17%. It remains unclear, if the activity of the sensory neurons themselves in the ramp paradigm can be explained solely by the activity of the GCY oxygen sensory proteins, or if the neuronal activity is controlled by upstream neurons integrating further information of oxygen changes to reach the critical inactivation and activation pattern at the upper and lower bound of the preferred oxygen concentrations.

As was seen in the shift paradigm the activity sum of the two sensory neuron types BAG and URX can directly explain a most of the actual behavior, but important parts of the DS behavior still differ from the neuronal activity. The signal has to be modified along the way to enable the actual behavior.

Neuropeptides have only limited influence on the overall activity of these neurons and do not change the function of reacting to increasing or decreasing oxygen concentration or working specifically at oxygen concentrations of interest. It is unclear, if neuropeptides work on the sensory neurons themselves, modifying their activity or if the absence of neuropeptides causes affects mainly the downstream network activity which then leads to a feedback to the sensory neurons themselves.

2.2 Behavior analysis

An extensive analysis of the different types of behavior of N2 compared to mutant and cell kill strains has been done to identify significant differences. Due to the extensive nature of this analysis, most of the results, behavior plots and the corresponding statistical analysis results have been moved to additional results sections 5.2.x) in the addendum. The following results focus on the findings most relevant for the discussion of the topic of this thesis.

During all of the following experiments in the shift paradigm, the gas mixture stays constant for the duration described. After the duration given, the oxygen/nitrogen levels are shifted to the next gas concentrations described. As stated in the Calcium imaging section, a change from a higher to lower oxygen concentration is called a "down shift" (DS), a change from a lower to a higher one an "up shift" (US) respectively. The oxygen/nitrogen gas concentrations take 12s to reach the final concentrations throughout the whole assay plates.

step	duration [s]	oxygen [%]	nitrogen [%]
1	360	21	79
2	360	10	90
3	360	21	79

Table 7: Gas concentrations and switch times used during the shift paradigm, length of the experiment: 1080s

This paradigm confronts animals with two intense stimuli, the first a DS from 21 to 10%, the second an US from 10-21% of oxygen. This paradigm has been shown to elicit a strong and robust behavioral response and should therefore be well suited to identify the role of neurons and neuropeptides within the oxygen sensing network.

	strain	n_{experiments}	n_{animals}
N2	N2	23	3152
N2 BAG cell kill	CX11697	11	1288
N2 AQR/URX cell kill	CX7102	13	1584
egl-3 n150	CX9191	15	1166
egl-3 n150 BAG cell kill	ZIM300	11	1593
egl-3 n150 AQR/URX cell kill	ZIM301	13	1525
egl-3 n729	MT1541	13	1065
sbt-1 ok901	RB987	9	1015
flp-1 ok2811*	ZIM144	10	1065
flp-8 pk360	flp-8 (PK360)	10	1263
flp-10 n4543	MT14676	7	1091
flp-17 n4894	MT15933	7	935
flp-10 n4543 x flp-17 n4894	MT15973	11	1375
egl-3 n150 P _{flp-17} ::egl-3 (09)	ZIM284	4	223

Table 8: Strains used in shift assays, the number of individual experiments and overall number of moving animals in all respective experiments. * All recordings of this strain were done by Ingrid Hums. All corresponding movies were reanalyzed using a developed software for this thesis. For details of the software see the Materials and Methods section.

In previous gradient assays it has been shown, that the highest fraction of N2 animals is found at an oxygen concentration of about 13%. On a feeding plate, the oxygen concentration in the center of an OP50 bacterial cell lawn was 17%, in the thicker border of the lawn 13%. It has been discussed that lower oxygen concentrations therefore signal the presence of food to *C. elegans*. (Gray et al, 2004)

In all of the following behavior ramp paradigms used, the oxygen concentration during the "down ramp" (DR) period is gradually decrease by 0.00094% each second, until a final concentration of 4% oxygen has been reached. The nitrogen concentration is increased respectively. The same gradual increase/decrease is applied during the "up ramp" (UR) period in reverse order for oxygen/nitrogen respectively.

Changes of the oxygen/nitrogen concentration happen nearly instantaneously, the concentrations are also changing nearly instantaneously throughout the assay plates due to the small changes over time.

step	duration [s]	oxygen [%]	nitrogen [%]
1	180	21	79
2	180	21-04	79-96
3	180	04	96
4	180	04-21	96-79
5	180	21	79

Table 9: Gas concentrations and switch times used during the ramp paradigm, length of the experiment: 900s

This paradigm confronts the animals with gradually changing oxygen concentrations without providing spatial information about the change. This paradigm elicits a robust behavioral response and is well suited to study the animals in respect to their behavior when the oxygen concentration passes the preferred conditions.

	strain	n _{experiments}	n _{animals}
N2	N2	23	3302
N2 BAG cell kill	CX11697	10	1191
N2 AQR/URX cell kill	CX7102	13	1664
egl-3 n150	CX9191	9	881
egl-3 n150 BAG cell kill	ZIM300	10	1400
egl-3 n150 AQR/URX cell kill	ZIM301	9	1050
egl-3 n729	MT1541	10	1206
sbt-1 ok901	RB987	9	1158
flp-8 pk360	flp-8 (PK360)	10	1368
flp-10 n4543	MT14676	7	1069
flp-17 n4894	MT15933	7	1038
flp-10 n4543 x flp-17 n4894	MT15973	11	1469

Table 10: Strains used in ramp assays, the number of individual experiments and the overall number of moving animals in all respective experiments.

The movies of all behavior experiments were analysed using the wormTracker software that was partially developed for this thesis. Please find a detailed description of the software in the Materials and Methods section.

All behavior plots are averages from individual recordings using 30s binning. Regarding the plots in the ramp paradigm: Thin white lines during the DR period state oxygen concentrations of 18-17 (212-224s), 16-15 (235-245s), 14-13% (256-267s) and in the UR period of 13-14 (635-646s), 15-16 (657-667s), 17-18% (687-689s) respectively.

2.2.1 Omega turns and turn angles

2.2.1.1 Behavior in the shift paradigm

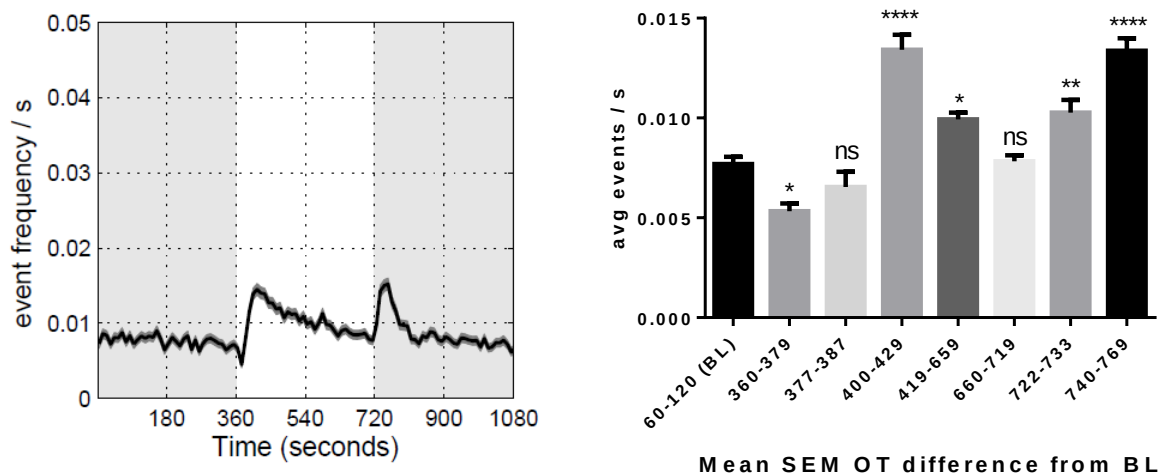


Figure 6: Average plot of OTs of 23 recordings of N2 animals in the shift paradigm. Statistics: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

- (1) Baseline (BL) data describes the average turning rate within a 60s period where the animals are not subject to an external stimulus and before any stimulus has taken place. The time window used (TWU) to acquire data averages ranges from 60-120s in the experiment.
- (2) The OT rate is initially significantly reduced after the DS takes place, the minimum is found between 10 to 30 seconds after the DS.
- (3) After the initial reduction, a significant increase in OTs takes place. The maximum of this increase is found between 419-429s (TWU 360-430s), 60-70s after the DS has taken place.
- (4) The OT rate stays significantly increased after the DS has taken place but gradually decreases until it returns to BL level after 180 seconds.
- (5) The US elicits an increase in OTs without previous reduction. The maximum is found between 749-769s, 30-50s after the US. The average events/s in the TWU 740-769s showing the maxima and is significantly above BL level.

As can be seen in Figure 6, a sudden drop in the environmental oxygen concentration leads to a reduction of OTs followed by a significant increase. The reduction of OTs coincides with a significant reduction in speed, which can also be observed at the same TWU. This provides an animal with time to sample the environment before movement continues. Observing individual animals after a DS has taken place, slowing and

sampling of the environment can be seen. Only after has been sufficient sampling, movement is resumed, an increase in OTs after this sampling provides a search strategy to return to a favored oxygen environment.

As discussed above, the BAG neuron pair senses decreases in the surrounding oxygen concentration. After an oxygen DS has occurred, BAG stays active, until a subsequent US takes place (Figure 2A). The neuronal activity of BAG after a DS is reflected in the corresponding OT behavior with the exception of an initial reduction of OTs immediately after the DS has taken place. This reduction in OTs is also not reflected in the activity sum of BAG and URX (Figure 4A). This leads to the assumption, that a time dependent inhibitory action in the circuit downstream of BAG and URX takes place.

An interpretation of the network activity would be that inactivation of the URX pair and AQR upon a DS leads to a corresponding decrease of OTs which is then overruled by BAG activity leading to an increase in OTs. The BAG neuron shows increased but decaying activity until an US takes place. This also means that the activity of AQR/URX is required to maintain OT BL level. If AQR and URX are removed from the network, BL level should be lower, but this is not the case, the level is actually increased. Another theory would be that the activation of BAG induces a short lived suppression, but leads to an increase for as long as it is active. In succession, the OT rate during the first 10-30 seconds is suppressed while favouring slowing and reversal responses. Probably slowing by a reduction of input to or increased inhibition of AVB and engaging in reversals.

After the drastic increase of OTs, the enhanced event frequency gradually decreases until it reaches BL level after about 6 minutes. This correlates with the sustained neuronal activity of BAG after a DS which stays above BL until an US occurs. This points towards a direct involvement of BAG with sustained OTs, leading to an overall increased rate of OTs, while the animals are again susceptible to other stimuli.

The overall shape of the DS response follows the neuronal activity of BAG. The activity in BAG does not show a later onset but an immediate response. The DS behavior is basically mediated by the BAG activity, but the initial OT inhibition happens somewhere downstream of BAG.

Maximum and amplitude of DS and US response are comparable, 0.0139/0.0149 maximum and 0.0081/0.0099 amplitude event frequency/s respectively. The time until the event frequency is back to baseline after the US is shorter than after the DS, it takes only around 90s to return to BL level which corresponds with the neuronal activity of URX. Unlike the DS response, the US response seems to act like a direct forward network from the sensory neuron to the OT response. US and DS response seem to affect different parts of the network.

The following identified phases of DS and US will be used as reference for comparisons with mutant, cell kill and rescue lines.

- OT BL comparison at TWU 60-120s.
- OT DS suppression at TWU 360-379s.
- OT DS maximum response at TWU 400-429s.
- Secondary DS response at TWUs 419-659s and 660-719s.
- OT US response at TWUs 722-733s and 740-769s.

TWU [s]	N2 BAG cell kill (CX 11697)	N2 AQR/URX cell kill (CX 7102)	egl-3 n150 (CX 9191)	egl-3 n150 BAG cell kill (ZIM300)	egl-3 n150 AQR/URX cell kill (ZIM301)	egl-3 n729 (MT1541)	sbt-1 ok901 (RB987)
BL (60-120)	ns	+/*	ns	ns	+/*	+/*	ns
360-379	ns	ns	+/*	ns	+/*	+/*	+/*
400-429	-/*	+/*	ns	-/*	ns	ns	-/*
419-659	ns	+/*	ns	ns	+/*	+/*	-/*
660-719	ns	+/*	+/*	ns	+/*	+/*	ns
722-733	ns	ns	+/*	+/*	ns	ns	ns
740-769	ns	-/*	ns	ns	ns	ns	-/*

Table 11: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.1), the statistics in section 5.3.2.1).

As was shown in the calcium imaging section, the absence of neuropeptides lead to minor increases in the overall responses to changes in oxygen of either BAG or URX. Analysis of behavior recordings of neuropeptide depleted egl-3 mutants show, that the propagation of the sensory neuron activity to the N2 response relies heavily on neuropeptides. Center of discussion are three different neuropeptide mutants, egl-3 n150, egl-3 n729 and sbt-1. For discussion of the egl-3 mutation, the strains CX9191 egl-3 n150 and MT1541 egl-3 n729 were selected, both of which carry a point mutation disrupting wt function. Mass spectrometry analysis revealed that out of 75 identified neuropeptides only 2 are present in the egl-3 n150 allele and showed depletion of all 75 neuropeptides in the egl-3 n729 allele (Husson et al, 2006).

7B2 is a chaperone protein expressed by gene sbt-1 and is required for the proteolytic activation of EGL-3 (Lindberg et al, 1998). According to mass spectrometry analysis, that 50 of 91 neuropeptides are absent in the sbt-1 mutant. (Husson et al, 2007)

The different depletion pattern of neuropeptides in these mutants provide a baseline for the identification of neuropeptide candidates required for oxygen sensing behavior. To identify candidates, neuropeptides expressed in neurons of the proposed neuronal circuit of interest (Figure 1) were taken into consideration.

Neuropeptide	Expression pattern	egl-3 n150	egl-3 n729	sbt-1 ok901
FLP-1	AVA, AVE, AVK, RIG, RMG	+ (1 of 6)	-	+ (5 of 5)
FLP-3	PQR	-	-	-
FLP-5	RMG	not detected	not detected	+ (1 of 3)
FLP-7	RMDV/SMDV	-	-	-
FLP-8	AUA, URX (RMG/ADA)	not detected	not detected	-
FLP-10	AUA, BAG, PQR, URX	not detected	not detected	not detected
FLP-11	AUA, BAG, URX	-	-	+ (3 of 3)
FLP-12	BAG	not detected	not detected	not detected
FLP-13	BAG	-	-	+ (5 of 6)
FLP-17	BAG	not detected	not detected	not detected
FLP-18	AVA, RIG, RIM	-	-	+ (2 of 6)
FLP-19	BAG, URX, AIN	-	-	-
FLP-20	RIB/AIB	not detected	not detected	not detected
FLP-22	AVA, SMD	-	-	+ (1 of 1)

Table 12: Expression pattern of neuropeptides in the neuronal circuit of interest. + ... neuropeptide present, - ... neuropeptide absent Neuropeptides and the respective expression patterns according to Kim et al 2004 and Li et al 2008. Presence and absence of neuropeptides in corresponding mutants according to Husson et al, 2006 and Husson et al, 2007.

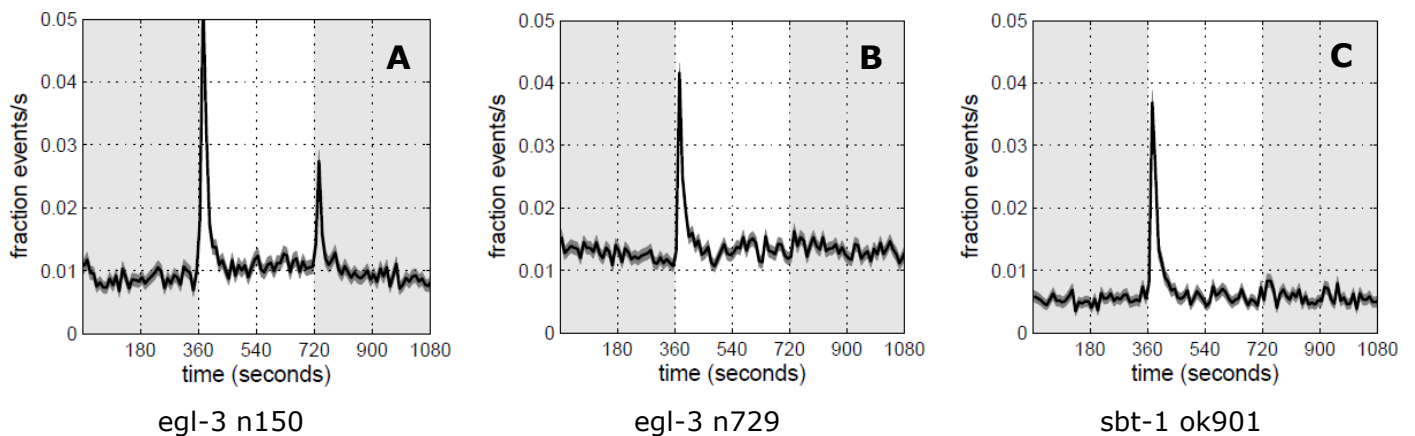


Figure 7: Average plots of OTs in the shift paradigm of the neuropeptide depleted mutants egl-3 n150, egl-3 n729 and sbt-1 ok901 using 15, 13 and 9 individual recordings respectively

The OT BL levels of egl-3 n150 and sbt-1 ok901 are not significantly different from N2 while the BL level of egl-3 n729 is significantly increased (Figure 7, Table 11).

Furthermore, the OT BL of both N2 AQR/URX and egl-3 n150 AQR/URX cell kill lines are significantly increased compared to the N2 BL.

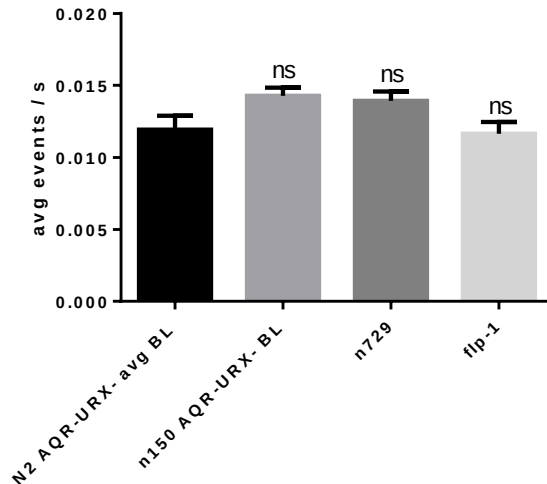


Figure 8: Results from one way ANOVA and Dunnett's multiple comparison test of the **N2 AQR/URX cell kill OT BL vs. mutants**. Error bars indicate SEM.

The OT BL of N2 AQR/URX, egl-3 n150 AQR/URX cell kill lines and egl-3 n729 all display a BL level that is not significantly different from one another (Figure 8).

Considering OT BL differences, a neuropeptide present in egl-3 n150 and sbt-1 but absent in egl-3 n729 is considered a candidate for inhibition of the basal OT rate.

The OT BL of the N2 AQR/URX cell kill line is significantly increased compared to the N2 OT BL, but not significantly different from egl-3 n729. Furthermore, the egl-3 n150 AQR/URX cell kill line shows a significant increase compared to both N2 and egl-3 n150 and is not significantly different from either egl-3 n729 or the N2 AQR/URX cell kill line (Table 11, Figure 8). All of these findings point towards a neuropeptide that is present in egl-3 n150 and sbt-1, absent in egl-3 n729 and expressed in either AQR or URX or an immediate downstream neuron.

FLP-1 is a candidate neuropeptide for the OT BL, which is expressed in egl-3 n150 and sbt-1 but not in egl-3 n729. flp-1 is expressed in RMG, which gap junctions to URX and in RIG which receives synaptic input from URX and gap junctions to AQR.

Other candidate neuropeptides are FLP-5 and FLP-10, which are expressed in URX and RMG respectively, but for which neuropeptide depletion has not been tested in the aforementioned literature. To test this theory, cell kill lines of RMG and RIG would be required.

All of the discussed neuropeptide depletion mutants show a significant increase of OTs at TWU 360-379s, immediately after the DS occurs. The initial decrease in OTs after DS at this TWU in the N2 behavior is replaced by this increase. This increase ends sooner than the wt response, at TWU 400-429s the response in egl-3 n729 and sbt-1 ok091 is significantly decreased compared to wt. This also means that the continued activity of

BAG after the initial DS response is not propagated through the neuronal circuit. The propagation of this signal therefore relies solely on neuropeptides.

In the N2 AQR/URX cell kill line, the OT decrease upon DS is present, and the following OT response is not significantly different from the N2 response. In the *egl-3 n150* AQR/URX cell kill line, the OT increase upon DS is significantly increased compared to N2, but significantly lower than the *egl-3 n150* response (Table 11). A rescue of neuropeptides in the *egl-3 n150* background under the *flp-17* promoter, which is exclusively expressed in BAG, shows a significant decrease of the DS response compared to the *egl-3 n150* DS response (Table 13).

Two different theories could explain this behavior. There could be a balance of opposing neuropeptides modifying the DS response. The *egl-3 n150* DS response is significantly higher than the one of *egl-3 n729* or *sbt-1*. Since the *egl-3 n729* mutant is more heavily neuropeptide depleted than the *egl-3 n150* mutant, this leads to the assumption, that there are at least two different neuropeptides mediating inhibition and activation of the OT DS response.

In the *egl-3 n150* mutant inhibiting neuropeptides for the DS response are missing, but neuropeptides mediating an increase in the response are still present, while both inhibitors as well as activators are missing in the *egl-3 n729* and *sbt-1* mutant. The activating neuropeptide could be expressed in AQR/URX or in an immediate downstream neuron, since the DS response in the *egl-3 n150* AQR/URX cell kill line is significantly lower than the *egl-3 n150* DS response.

In this theory, there is one neuropeptide present in *egl-3 n150* and absent in *egl-3 n729* and *sbt-1*, which increases the OT response upon DS, which is expressed in AQR/URX or a downstream neuron and a second, absent in all three mutants, which inhibits the DS response.

No analysed neuropeptide shows the depletion pattern required for the activating neuropeptide. Candidates are the neuropeptides FLP-5, FLP-8, FLP-10, FLP-18 and FLP-20, which show expression in URX or a downstream neuron but which have not been analysed for depletion. Neuropeptides eligible for inhibiting the DS response would be ones that are absent in all mutants or that show expression in BAG and depletion in *egl-3 n729* and *sbt-1* but not in *egl-3 n150*. These would be FLP-7, FLP-10, FLP-12, FLP-17, FLP-19 and FLP-20.

There still is an initial suppression of OTs after the DS in the N2 AQR/URX cell kill strain, which suggests, that the neuropeptides suppressing the BL and the initial response upon DS are two different kinds.

Another theory to explain the DS behavior in the neuropeptide depleted mutants would be that there is an underlying DS response that does not rely on neuropeptides. The

explanation for the increased DS OT in egl-3 n150 would still be the same with FLP-10 and FLP-20 as candidates. Since this underlying response leads to an immediate, substantial increase in OTs, it is likely to assume, that this is a basic escape response that is modified by neuropeptides. This response enables even neuropeptide depleted animals to escape from a possible hazardous stimulus. In N2 animals, this escape response is modified by a delicate balance of neuropeptides, enabling animals to react in a more fine tuned way to changes in their environment.

The US response at TWU 740-769s is missing in the egl-3 n729 and sbt-1 ok901 mutants (Figure 7B and C). The egl-3 n150 response at the same TWU is significantly increased compared to the wt response (Table 11). This response requires AQR/URX with no involvement of BAG and is neuropeptide dependent. Since there is no response in egl-3 n729 and sbt-1 while there still is a response in egl-3 n150, neuropeptides absent in the first two but present in the later and which show expression in URX or a downstream neuron but not in BAG are required and responsible for the US response. Candidate neuropeptides are FLP-5, FLP-8, FLP-10, FLP-18 and FLP-20 which show expression in URX or URX downstream neurons and correspond to the depletion pattern described or which have not been analysed.

TWU [s]	flp-1 ok2811 (ZIM144)	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	egl-3 n150 Pflp- 17:egl-3(09) (ZIM284)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	+ /****	ns	ns	ns	ns
360-379	ns	ns	ns	+ /****	ns
400-429	ns	ns	ns	ns	ns
419-659	+ /****	ns	ns	ns	ns
660-719	+ /****	ns	ns	+ /**	ns
722-733	+ /****	+ /*	+ /****	+ /****	ns
740-769	+ /****	ns	ns	ns	ns

Table 13: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.1), the statistics in section 5.3.2.1).

To address questions regarding the OT BL, flp-1 and flp-10 neuropeptide mutants were analysed (Table 13). The flp-1 mutant indeed shows an OT BL that is significantly increased compared to N2 and not significantly different from the BL of egl-3 n729, N2 AQR/URX cell kill and egl-3 n150 AQR/URX cell kill (Table 13, Figure 8). A significant increase of the US response at TWUs 722-733s and 740-769s shows, that FLP-1 has an OT inhibiting effect downstream of AQR or URX. The neuropeptide responsible for the US response is a different one. The flp-10 mutant does not show an increased OT BL, FLP-10 is not responsible for inhibiting the OT BL.

To address the question of inhibiting and activating neuropeptides in the DS response, flp-8, flp-10 and flp-17 mutants were analyzed, but none of the mutants show a DS response that is different from the N2 response.

To address which neuropeptides are responsible for the US response, flp-8 and flp-10 mutants were analyzed. In both cases the US response was still present; these two neuropeptides are not responsible for mediating the response. Interestingly the US response in the flp-10 mutant was significantly increased compared to the N2 response at TWU 722-733s. This would mean that the amplitude of the US response is controlled by both inhibiting and activating neuropeptides as well.

The difference between the underlying response in DS and US means, that activity leading to responses to oxygen in- and decreases merge only late within the network to produce the response. As a proposed model AQR/URX are active while the oxygen concentration is high and lowers the OT turning rate via RMG/RIG and FLP-1. When a DS occurs, AQR and URX are inactive, the release of FLP-1 is lowered if not completely stopped and the inhibition of OTs stops with it, leading to a strong increase in OTs mediated by the activity of BAG. Neuropeptides other than FLP-1 are responsible for an initial decrease in OTs to suppress the first, substantial increase.

2.2.1.2 Concluding remarks

As a concluding remark to omega turns in the shift paradigm, the US response is mediated solely by neuropeptides. There is an underlying DS response that is not dependent on neuropeptides, but is completely masked by multiple neuropeptides. Both DS and US response are dependent on a balance of different inhibiting and activating neuropeptides. This leads to the assumption, that as long as there is no strong aversive stimulus requiring animals to immediately escape, responses are fine tuned under a tightly controlled balance of different neuropeptides to enable optimal responses in a gradually changing environment.

2.2.1.3 OT angles in the shift paradigm

When an animal engages in an OT, the bearing before and after the turn differs. The OT turn angle describes this angle in degrees and describes, if an animal heads back to where it came from (turn angle lower than 30 degrees) or if it merely adjusts the bearing. Narrow angles enable an animal to move away from unfavorable environments, while flat angles enable an animal to explore the environment to search for the most favorable conditions.

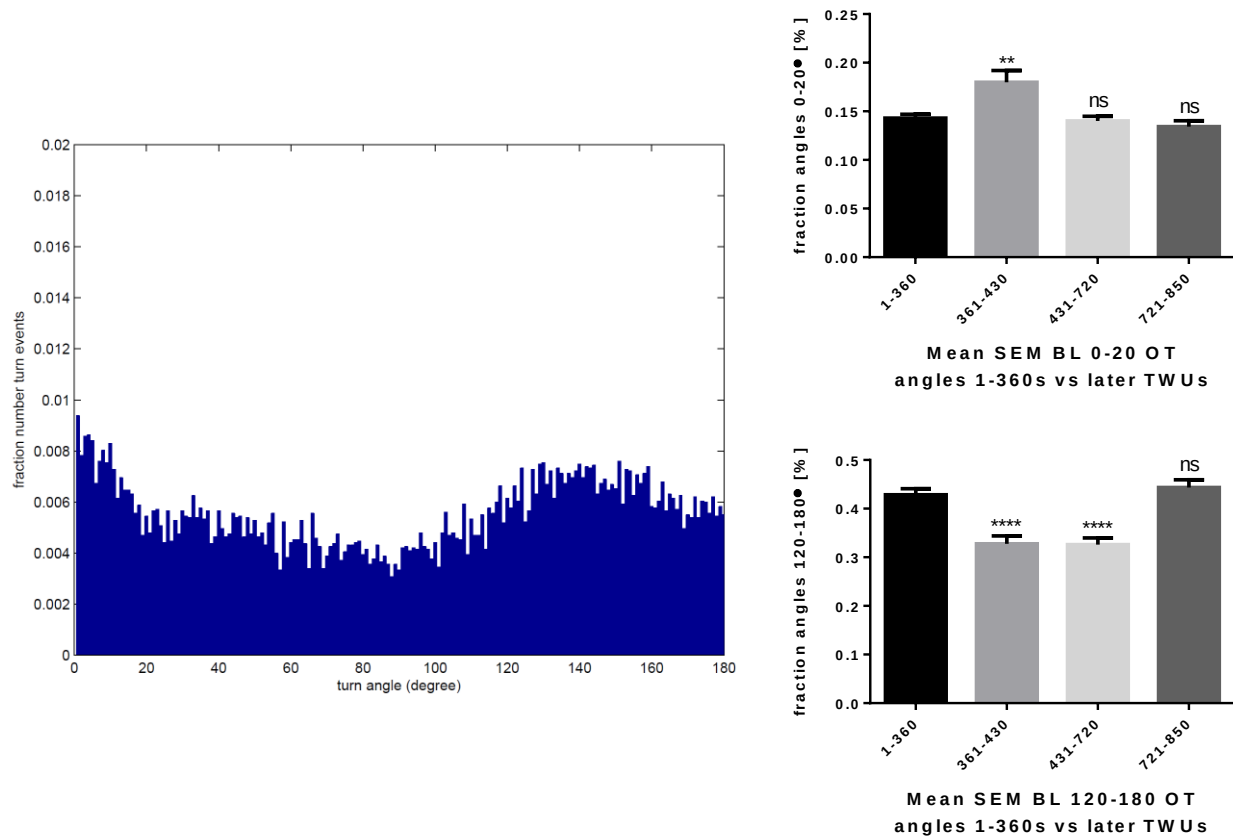


Figure 9: Normalized OT angles of 18556 OTs in 23 recordings. Statistics: BL OT angles at TWU 1-360s in degrees compared to later TWUs in N2, results from one way ANOVA and Dunnett's multiple comparison test, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

When plotting the OT turn angles normalized to the overall 29183 OT events of 23 individual recordings at TWU 1-1080s, the plot shows a bimodal distribution, with one maximum below 20 degrees and another at around 140 degrees. These results can be interpreted, that turns that have been previously described as omega turns can be categorized in two different types and can be set apart by their turning angle.

If taking turning events from the whole recording into consideration, then 12.0% of all turns are found around the first maximum (turns of 0-20 degrees), 43.8% around the second maximum (turns of 120-180 degrees).

An analysis of different TWUs covering time frames of interest within the shift paradigm shows the following statistics:

	1-360s	361-430s	361-720s	431-720s	721-850s	1-1080s
% at 0-20	11.5	17.2	13.1	12.2	11.4	12.0
% at 120-180	49.3	33.0	35.2	35.7	46.8	43.8

Table 14: Distribution of OT angles at different TWUs

When the 0-20 degree angles from OT angle BL at TWU 1-360s are compared to the angles of other TWUs, the only one that is significantly different is TWU 361-430s, immediately after the DS (Figure 9). Here turns with a turn angle between 0-20 degrees are significantly increased compared to BL level. In the following discussion, angles of 0-20 degrees will be termed "**narrow angles**".

When analyzing the turn angles of 120-180 degrees, all of the TWUs between DS and US are significantly lower than the BL turn angles. Just the TWU after the US is not significantly different from the angles at BL TWU (Figure 9). In the following discussion, angles of 120-180 degrees will be termed "**flat angles**".

There is a significant change from flat to more narrow OT angles after the DS has taken place. A DS can be interpreted as a more severe stimulus thus favoring escape responses over exploring. The turn angles are not different from BL during or after the US has occurred. Here flat OT angles are favored over narrow angles favoring explorative behavior.

TWU [s]	N2 BAG cell kill (CX11697)	N2 AQR/URX cell kill (CX7102)	egl-3 n150 (Cx9191)	egl-3 n150 BAG cell kill (ZIM300)	egl-3 n150 AQR/URX cell kill (ZIM301)	egl-3 n729 (MT1541)	sbt-1 ok901 (RB987)
0-20	+/**	+/**	+/**	ns	+/**	+/**	+/**
120-180	-/**	-/**	-/**	-/**	-/**	-/**	-/**

Table 15: OT angles at TWU 1-360s in degrees, Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding statistics in the addendum section 5.3.2.3.

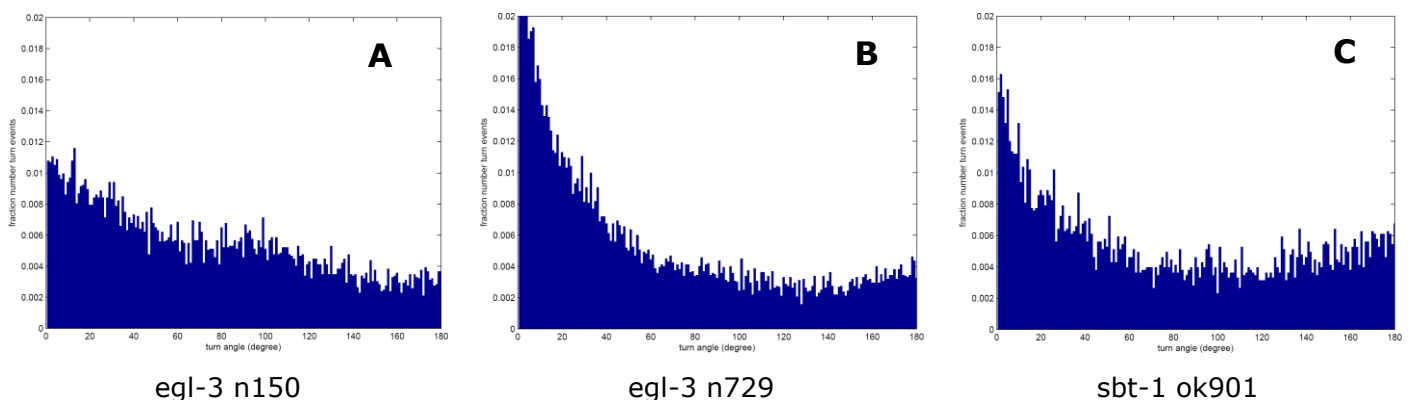


Figure 10: Normalized OT angles of (A) 10951 OTs in egl-3 n150, (B) 16037 OTs in egl-3 n729 and (C) 6077 OTs in sbt-1 ok901.

The normalized plots of turn angles in the three neuropeptide depleted mutants show a strong deviation from the N2 distribution (Figure 10). Neuropeptides absent in the egl-3

n150 mutant inhibit narrow and promote flat turn angles (Figure 10A). In the egl-3 n729 mutant, the increase in narrow turning angles and the decrease in flat angles is even more pronounced in the egl-3 n150 mutant strain (Figure 10B). Neuropeptides seem to promote flat angles while inhibiting narrow angles. In the sbt-1 mutant, turn angles below 21 degrees are significantly in- and angles above 120 degrees significantly decreased, even though the differences are weaker compared to the other egl-3 mutants (Figure 10C, Table 15).

TWU [s]	egl-3 n150 BAG cell kill (ZIM300)	egl-3 n150 AQR/URX cell kill (ZIM301)	egl-3 n729 (MT1541)	sbt-1 ok901 (RB987)
0-20	ns	+/**	+/**	ns
120-180	+/**	ns	ns	+/**

Table 16: OT angles at TWU 1-360s in degrees, Results from one way ANOVA and Dunnett's multiple comparison test of **egl-3 n150 vs. mutant strains**, + ... significant increase compared to egl-3 n150, - ... significant decrease compared to egl-3 n150, ns ... no significant difference. Find all corresponding statistics in the addendum section 5.3.2.4).

When the neuropeptide depleted mutants are compared to the egl-3 n150 mutant, the strongest inhibition of flat angles and increase in narrow angles can be found in the egl-3 n729 mutant, which shows the strongest neuropeptides depletion. Neuropeptides are promoting flat turn angles and inhibit narrower angles.

According to Table 16 there is no significant difference between the egl-3 mutants n150 and n729 in angles above 120-180 degrees. Neuropeptides promoting these turns are absent in both mutants. This inhibition is weaker in the sbt-1 mutant where the angles are still significantly reduced compared to N2. This suggests that there are more than one neuropeptides promoting suppression of flat turn angles. One of these neuropeptides is still present in the sbt-1 mutant while both inhibiting neuropeptides are absent in the egl-3 n729 and egl-3 n150 mutants.

FLP-5 is present in sbt-1 but absent in egl-3 n150 and egl-3 n729. This neuropeptide is expressed in RMG, a motoneuron which could synaptically modify the response of the RMD head motoneurons leading to a modification of turning angles.

FLP-11, FLP-18 and FLP-22 have the same pattern of presence/absence as FLP-5 and would be candidates as well for promoting more flat angles.

The increase of turns below 21 degrees is strongest in the egl-3 n729 mutant (Figure 10). There seem to be at least two neuropeptides involved in inhibiting these angles, because the number of egl-3 n729 angles is significantly higher compared to egl-3 n150 or sbt-1 which in turn are significantly higher compared to N2 (Table 15, Table 16). Neuropeptides absent in egl-3 n729 but present in egl-3 n150 and sbt-1 are responsible

for inhibiting these angles. Neuropeptides absent in all mutants are responsible for a further inhibition of these angles.

FLP-1 has limited expression in egl-3 n150 and full expression in sbt-1 while being absent in egl-3 n729 (Table 12) which makes it a good candidate for suppression of narrow turn angles. Further candidates are FLP-10, FLP-12, FLP-17 and FLP-20 which all were not included in the mass spectrometry analysis described above.

TWU [s]	flp-1 ok2811 (ZIM144)	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	egl-3 n150 P _{flp-17} :egl-3(09) (ZIM284)	flp-8 pk360 strain flp-8 pk360
0-20	+ /****	ns	ns	ns	ns
120-180	- /****	ns	ns	- /****	ns

Table 17: OT angles at TWU 1-360s in degrees, Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutant strains**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding statistics in the addendum section 5.3.2.3).

To investigate the involvement of neuropeptides in turn angles, neuropeptides FLP-10 and FLP-17 were investigated, but the turn angle distribution was not different from wt, excluding these two neuropeptide mutants from turn angle analysis.

The neuropeptide FLP-1 is involved in both increasing narrow turning angles and reducing flat angles compared to N2. But both of the turning angles are significantly different from the egl-3 n729 behavior, where the strongest difference has been observed. FLP-1 is involved in mediating turn angles, but is not the only neuropeptide required to explain wt behavior.

2.2.1.4 Concluding remarks

The different preference for narrow turning angles and reduction in flat angles after the DS and in neuropeptide depleted mutants add to the theory, that there is a robust, underlying escape response in the oxygen sensing network and that this response is independent of neuropeptides. Neuropeptides fine tune not only the probability of omega turns in case of changes in the environmental oxygen concentration, but also fine tune the immediate behavior after a turn to enable exploration of the environment to find optimal conditions, if escape responses are not required.

2.2.1.5 Behavior in the ramp paradigm

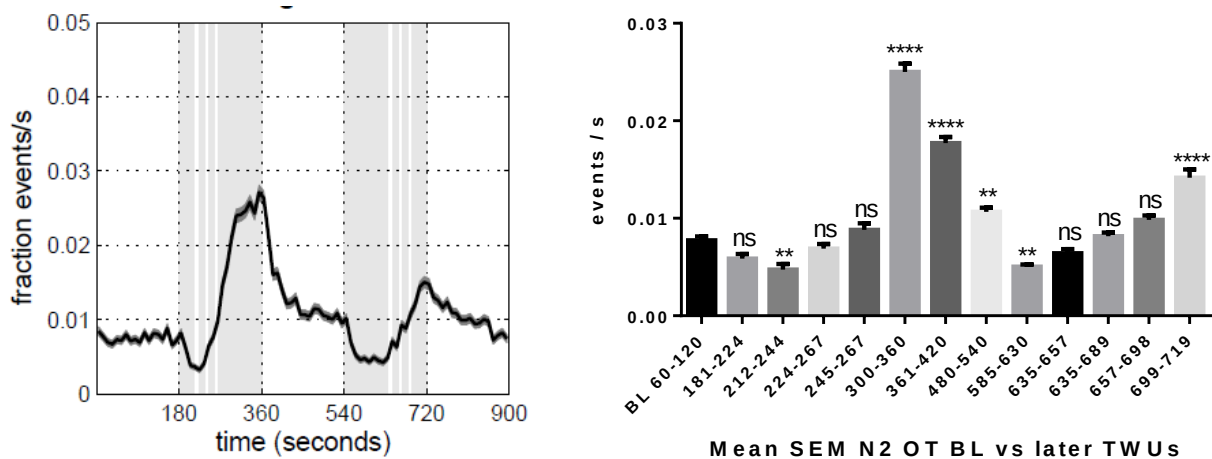


Figure 11: Average of OTs of 23 recordings of N2 animals in the ramp paradigm. Statistics: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

- (1) After the DR starts, OTs are inhibited, until 17% oxygen has been reached. OTs at TWU 212-244s are significantly decreased compared to BL level.
- (2) The OT rate starts to rise again and increases back to BL level when 13% oxygen has been reached (TWU 245-267s).
- (3) After 13% has been passed, the OT rate increases further until it plateaus even before the ramp has reached 4% oxygen. The increase is significant and with 0.0249 events/ s three times as high as BL level (TWU 300-360s).
- (4) Immediately after the UR has reached 4%, and the oxygen concentration stays constant, the OT rate drops over the next 60s (TWU 361-420s) until it plateaus again at a level that is still significantly higher than BL level (TWU 480-540s).
- (5) Immediately after the oxygen concentration starts increasing from 4% upwards, OTs are significantly suppressed until an oxygen concentration of 13% has been reached (TWU 585-630s).
- (6) After 13% O₂ has been passed, the OT rate gradually increases again and this gradual increase continues, until the oxygen concentration has reached 21% and stays constant.
- (7) The OT rate is only significantly different from BL level, after 18% oxygen has been passed (TWU 600-719s). After 21% has been reached, the OT rate gradually drops back to BL level.

During the first part of the UR BAG is inactivated and URX is still not active (Figure 3, Figure 5). The activity sum of these neurons could enable the drop in the rate of OTs compared to the OT rate at constant 4% oxygen. Only after 13% oxygen has been

reached and URX is activated, the OT rate increases again. When the oxygen concentration rises above 13%, the OT rate increases accordingly. The farther from the preferred oxygen concentration, the higher the rate of OTs becomes. The OT rate rises above BL level after the oxygen concentration passes 18%.

TWU [s]	N2 BAG cell kill (CX11697)	N2 AQR/URX cell kill (CX7102)	egl-3 n150 (Cx9191)	egl-3 n150 BAG cell kill (ZIM300)	egl-3 n150 AQR/URX cell kill (ZIM301)	egl-3 n729 (MT1541)	sbt-1 ok901 (RB987)
BL (60-120)	ns	+/**	ns	ns	+/**	+/**	ns
181-224	ns	+/**	+/**	ns	+/**	+/**	ns
212-244	ns	+/**	+/**	ns	+/**	+/**	+/**
224-267	ns	+/**	+/**	ns	+/**	+/**	+/**
245-267	ns	ns	+/**	ns	+/**	+/**	+/**
300-360	-/**	ns	-/**	-/**	ns	ns	-/**
361-420	-/**	ns	-/**	-/**	ns	ns	-/**
480-540	ns	+/**	ns	ns	+/**	ns	-/**
585-630	+/**	+/**	+/**	+/**	+/**	+/**	ns
635-657	+/**	+/**	ns	+/**	+/**	+/**	ns
635-689	ns	+/**	ns	ns	+/**	+/**	ns
657-698	ns	ns	ns	ns	+/**	+/**	-/**
699-719	ns	ns	ns	ns	ns	+/**	-/**

Table 18: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.2), the statistics in section 5.3.2.8).

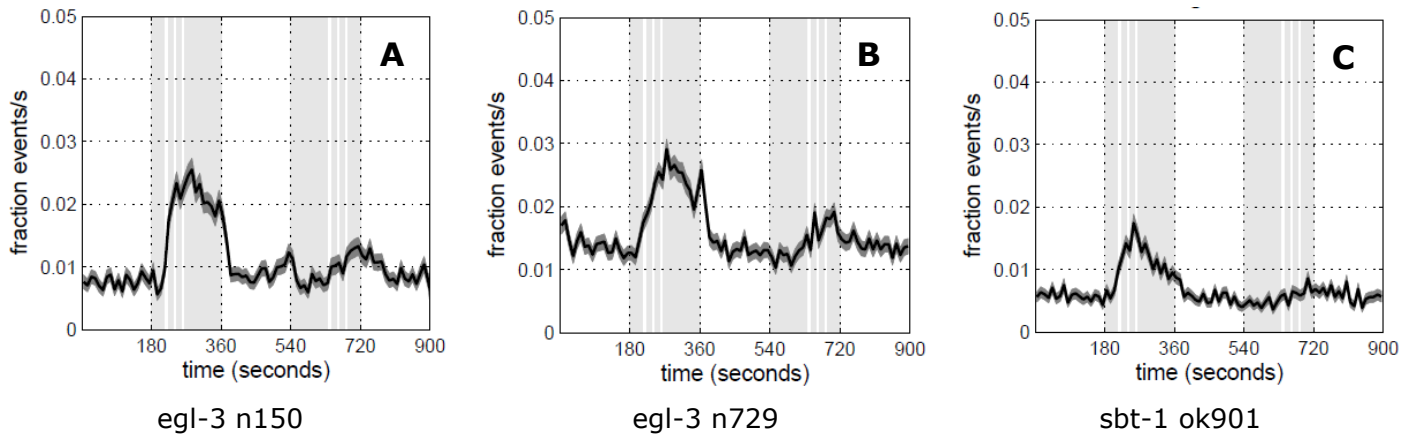


Figure 12: Average plots of OTs in the ramp paradigm of the neuropeptide depleted mutants egl-3 n150, egl-3 n729 and sbt-1 ok901 using 9, 10 and 9 individual recordings respectively

In the egl-3 n150 mutant, the inhibition of OTs at the start of the DR is missing as has been seen in the shift assays. This is not due to missing activity in BAG or URX, the neuronal activity in the egl-3 n150 mutant is not significantly different from N2. (Figure 12A, Table 18) As in the shift assays, the response starts sooner, the rise starts at the time an oxygen concentration of 18% is passed which coincides with an increase of activity in BAG (Figure 3A). The amplitude of the DR response of egl-3 n150 is not significantly different from the N2 response, but the onset occurs earlier as does the increase in OTs. The secondary increase of OTs which is mediated by the slow decay of

BAG activity over time is missing in the OT response like in the shift paradigm and is probably mediated by neuropeptides as well. The decrease of OTs upon the onset of the UR is missing in *egl-3 n150* as well, only after 13% oxygen have been passed, the UR behavior is not different from N2 any longer.

As discussed before, the OT BL in the *egl-3 n729* mutant is significantly increased compared to both N2 and *egl-3 n150*. It has already been discussed, that FLP-1 is a prime candidate for inhibiting the OT BL. Once the later TWUs are corrected for the increased BL level, *egl-3 n729* behaves exactly like *egl-3 n150* which is interesting given the fact, that the US response in the shift paradigms is missing in *egl-3 n729* compared to *egl-3 n150* (addendum 5.3.2.9).

In the *sbt-1* mutant, the DR response compared to N2 occurs sooner and the amplitude is significantly reduced (Figure 12C). The onset of the DR response is the same as in *egl-3 n150*, but the amplitude is also significantly reduced. The response during constant 4% oxygen is significantly lower compared to N2. At the start of the UR, the OT rate is not significantly different from N2, but that is not because the drop in OTs is present, but because the OT rate was already at this rate during the 4% period. The UR response after 13% oxygen is significantly reduced compared to N2.

In all neuropeptide depleted mutants the initial inhibition of OTs upon the start of the DR as well as the UR is missing, neuropeptides required to facilitate this inhibition should be depleted in all neuropeptide mutants. Taking the neuropeptide depletion pattern in the different strains into account, candidates for the initial OT inhibition in the DR are FLP-3, FLP-7, FLP-8 and FLP-19 which have been shown to be depleted in the respective mutants and FLP-10, FLP-12, FLP-17 and FLP-22, which were not included in the respective mass spectrometry analysis (Table 12).

The DR OT response is not different from N2 in the *egl-3* mutants, but is significantly lower than the response of N2, *egl-3 n150* and *egl-3 n729* in the *sbt-1* mutant (Table 18). This could be due to a turn promoting neuropeptide, that is present in *egl-3 n150* and *egl-3 n729* and absent in *sbt-1 ok901*. This presence/absence pattern does not occur in the neuropeptides that have been analysed by mass spectrometry (Table 12). Another reason for the behavior of *sbt-1* in the DR could be, that neuropeptides present in *sbt-1* but absent in the other two *egl-3* mutants are inhibiting OTs after a DR occurs. Neuropeptide candidates for this would be FLP-13, FLP-18 or FLP-22, which show the described expression pattern. For both cases the neuropeptides FLP-8, FLP-10, FLP-12, FLP-17 and FLP-20 are valid candidates, because they were not included in the mass spectrometry analysis (Table 12).

The prolonged OTs after the end of the DR are absent in all neuropeptide depleted mutants. Candidates are neuropeptides that are absent in all depleted mutants and are either expressed in BAG or in downstream neurons, since the same behavior can be seen in the BAG cell kill lines. Candidates are FLP-5, FLP-7, FLP-8, FLP-10, FLP-12, FLP-17, FLP-18, FLP-19 and FLP-20 according to Table 12.

The DR response after the initial inhibition is not significantly different from the N2 response in the two *egl-3* mutants, but the response is significantly decreased in *sbt-1* compared to N2 (Table 18).

In the shift paradigm, *egl-3* n150 still displayed an US response, while it was missing in the *egl-3* n729 and *sbt-1* mutant. Taken into account, that an UR response takes place in the *egl-3* n729 mutant showing depletion in all neuropeptides tested, it seems likely, that neuropeptides present in *sbt-1* but absent in the *egl-3* mutants are responsible for inhibiting OTs. To gain a functional UR response, a balance between promoting and inhibiting OTs seem likely. Candidates for promotion of OTs in the UR are therefore neuropeptides which are absent in all neuropeptide depleted mutants, which include FLP-5, FLP-7, FLP-8, FLP-10, FLP-12, FLP-17, FLP-18, FLP-19 and FLP-20 according to Table 12. Candidates for inhibition of OTs in the UR are neuropeptides which are present in *sbt-1* but absent in the *egl-3* mutants including FLP-5, FLP-10, FLP-11, FLP-12, FLP-13, FLP-17, FLP-18, FLP-20 and FLP-22 (Table 12).

TWU [s]	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	ns	ns	ns
181-224	ns	-/*	ns
212-244	ns	ns	ns
224-267	ns	ns	ns
245-267	ns	ns	ns
300-360	ns	-/***	-/****
361-420	ns	ns	ns
480-540	ns	ns	ns
585-630	+/**	+/**	+/**
635-657	+/***	+/***	+/*
635-689	+/***	+/***	ns
657-698	+/***	+/***	ns
699-719	ns	ns	ns

Table 19: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.2), the statistics in section 5.3.2.8).

All of the neuropeptide mutants *flp-8*, *flp-10* and *flp-17* show, that the initial inhibition of OTs at the start of the DR is still present, none of these neuropeptides are involved, which removes them from the list of candidates (Table 19).

Both neuropeptide mutants flp-8 (expressed in URX, AUA and RMG) and flp-17 (expressed in BAG) display a significantly reduced maximum of the DR response at TWU 300-360s (Table 19). This not only means, that they are involved in promoting OTs during a DR, but also that the N2 response in a gradually decreasing oxygen concentration also relies on a balance between inhibiting and promoting neuropeptides. flp-8 is expressed in URX, AUA and RMG. Since URX is inactive during the time of the DR, it can be assumed, that FLP-8 released from AUA or RMG strengthens the OT response after a DR has started. BAG gap junctions to RIR which is synaptically connected to AUA. Downstream targets of AUA are RIA and RIB which both synapse onto SMD and RMD neurons associated with turns.

FLP-10 is not involved in the DR behavior.

All of the neuropeptide mutants flp-8, flp-10 and flp-17 show a significant increase at the start of the UR at TWU 585-630s (Table 19). All of these neuropeptides could be responsible for mediating the initial UR inhibition of OTs.

flp-10 and flp-17 mutants show a strong increase in the actual UR response after the oxygen concentration has passed 13%. FLP-10 which is expressed in BAG, AQR, URX and PQR could simply inhibit the UR response. The result for FLP-17 is puzzling, since it is expressed only in BAG which is already inactive during the UR response. FLP-17 could have a long lasting effect on the OT circuit.

2.2.1.6 Concluding remarks

As seen in Figure 11, animals moving from a high oxygen environment towards a preferred oxygen concentration inhibit their OT turning rate enabling them to move uninterrupted towards preferred concentrations. While within an area of preferred oxygen, the turning rate rises again, enabling animals to explore the area to find a local optimum, but the turning rate never rises above BL level as long as they are within their preferred oxygen concentrations. Only after the upper bound of the preferred concentration has been passed, the OT rate rises again drastically, when animals sense that they are moving towards a low oxygen environment.

Neuropeptides absent in all neuropeptide depleted mutants are required for mediating the initial inhibition of OTs at the start of the DR. Instead, the OT rate starts rising at the begin of the DR. This in leads to an OT response that has reached its maximum while the oxygen concentration is actually well within the preferred oxygen concentration. This shift of OT activity to an earlier point in time suggests that neuropeptides are essential to keep OTs at BL level while animals navigate within their preferred oxygen concentration. Without neuropeptides, any increases in oxygen lead to an immediate

strong response of OTs making it harder for animals to reach and stay at a preferred oxygen concentration.

The strong increase that can be seen in the DS paradigm does not appear in the DR. A gradual activation of BAG does not seem to elicit a strong non-neuropeptide dependent response while an immediate activation does. OT behavior can only be observed for as long as the oxygen concentration is still decreasing. After the decrease has stopped, the OT activity returns to BL without staying increased compared to the N2 response. As has been already discussed at the shift paradigm, the secondary DS response relies on neuropeptides, in neuropeptide depleted mutants, this response is completely missing. The same can be seen in the ramp behavior.

2.2.1.7 OT angles in the ramp paradigm

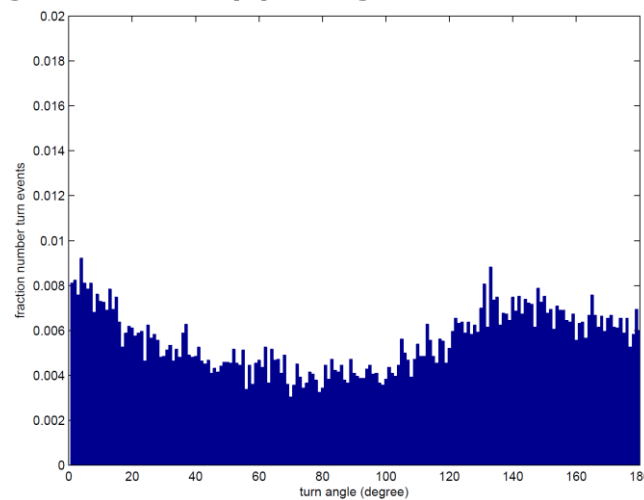


Figure 13: Normalized N2 OT angles of 22474 events, TWU 1-900s

The turning angles observed in the ramp paradigm are not different from the shift paradigm turning angles and show two maxima, one below 20 degrees and one around 140 degrees. If the different time frames of the ramp paradigm are compared to the BL turning angle level (unstimulated period from 1-180s), the following distribution can be observed.

	1-180s	181-267s	268-360s	361-450s	451-540s	541-656s	657-720s	721-900s
% at 0-20	11.5	14.8	17.1	12.0	10.4	10.5	8.6	9.3
% at 120-180	50.2	42.7	33.1	39.9	43.7	46.5	53.4	55.6

Table 20: N2 OT angle distribution in different TWUs

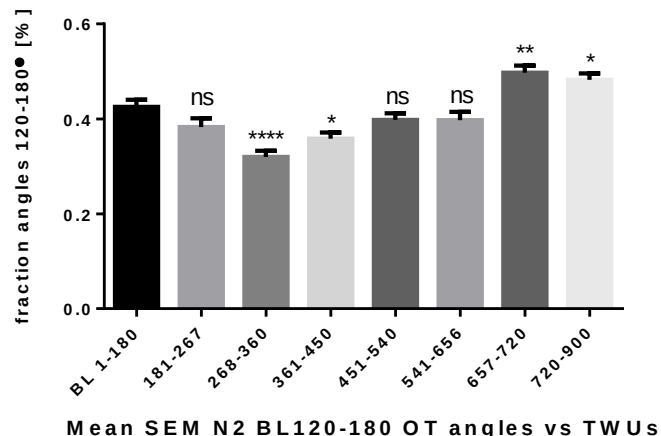
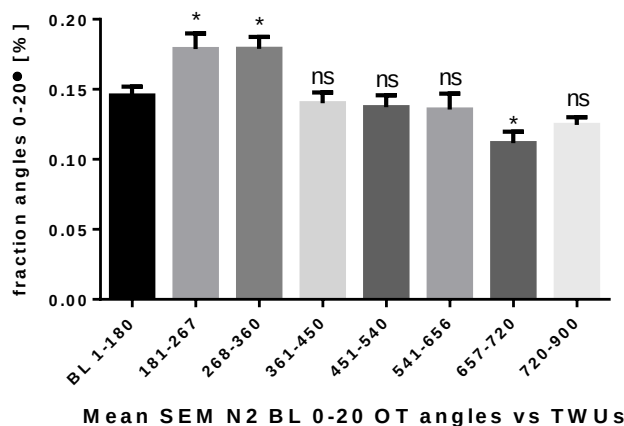


Figure 14: Results from one way ANOVA and Dunnett's multiple comparison test of **BL OT angles vs. later TWU in N2**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

In Figure 14 the 0-20 degree turning angles at TWUs 181-267s (21-13% oxygen) and 268-360s (13-4% oxygen) covering the whole DR period show a significant increase compared to BL level. TWU 657-720s (13-21% oxygen) shows a significant decrease of narrow angles. Flat turning angles show an inverse distribution, inhibiting flat turns during the DR period and increasing flat turns at the end of the UR and after the UR has ended. This is comparable to the results of the shift paradigm, where narrow angles are in- and flat angles decreased after the DS has occurred.

The differences in turning angles between N2, mutant and cell kill lines are the same as in the shift paradigms and will only be discussed briefly.

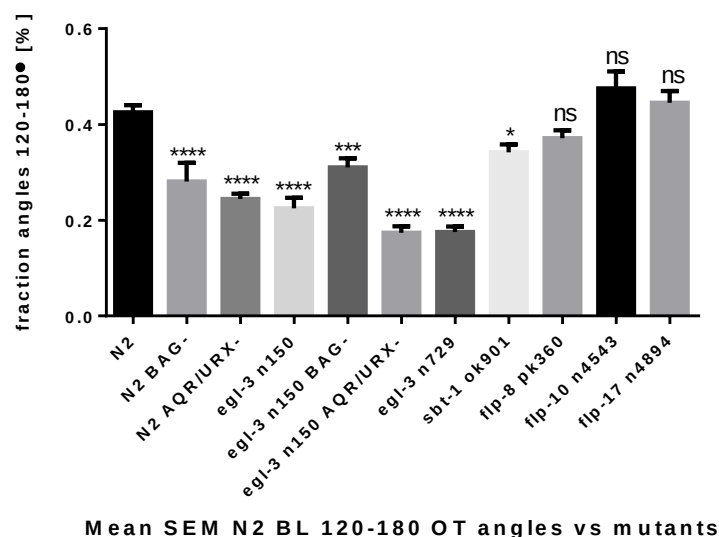
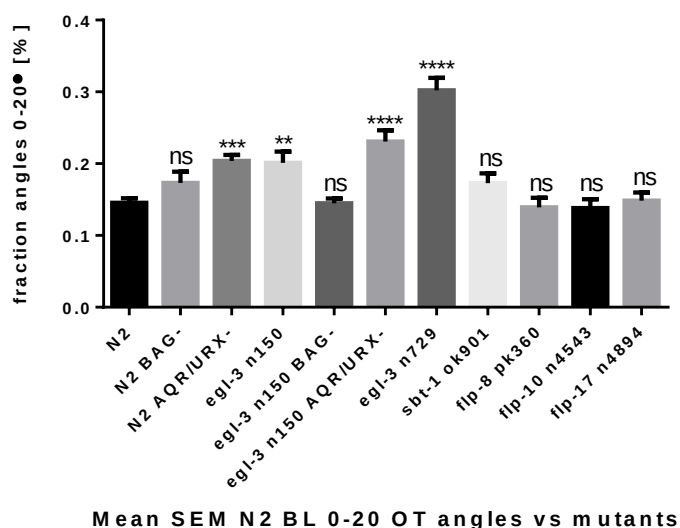


Figure 15: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL OT angles vs. mutant strains**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

As seen in Figure 15 narrow OT angles are significantly in- and flat OT angles are significantly decreased in all of the neuropeptide depleted mutants *egl-3 n150*, *egl-3 n729* and *sbt-1* compared to N2. This reduction/increase pattern is more severe in the more heavily neuropeptide depleted mutant *egl-3 n729* compared to the least depleted mutant *sbt-1*.

All of the analysed neuropeptide mutants *flp-8*, *flp-10* and *flp-17* show a turn angle distribution very similar to the N2 distribution for both angles of 0-20 and 120-180 degrees and can therefore be excluded from further analysis.

2.2.1.8 Concluding remarks

The favoring of narrow over flat angles during the DR points towards an escape response, while the inhibition of narrow angles during the UR points towards modified local search behavior.

These results show, that the behavior in a gradually changing oxygen environment is not only dependent on harsh responses like OTs and reversals, but that it is also dependent on fine tuning of the angles after turns to enable proficient search behavior.

There seems to be an underlying preference for narrow turning angles that does not require neuropeptides. Neuropeptides mask this preference, inhibit narrow angles and promote flat angles. Due to the varying narrow/flat ratio in the different neuropeptide depleted mutants it can be assumed, that this ratio depends on a balance of multiple inhibiting and promoting neuropeptides.

The removal of either BAG or AQR/URX from the network in an N2 background drastically change the narrow/flat angle ratio as well towards an increase in narrow angles over flat ones (Figure 15). This leads to the assumption, that neuropeptides from both BAG and AQR/URX modify the ratio. Since this ratio is different from the corresponding ratio in an *egl-3 n150* BAG or AQR/URX cell kill line background, it can be assumed, that neuropeptides from other neurons are involved in adjusting the narrow/flat angle ratio as well (Figure 15).

2.2.2 Shallow turns

2.2.2.1 Behavior in the shift paradigm

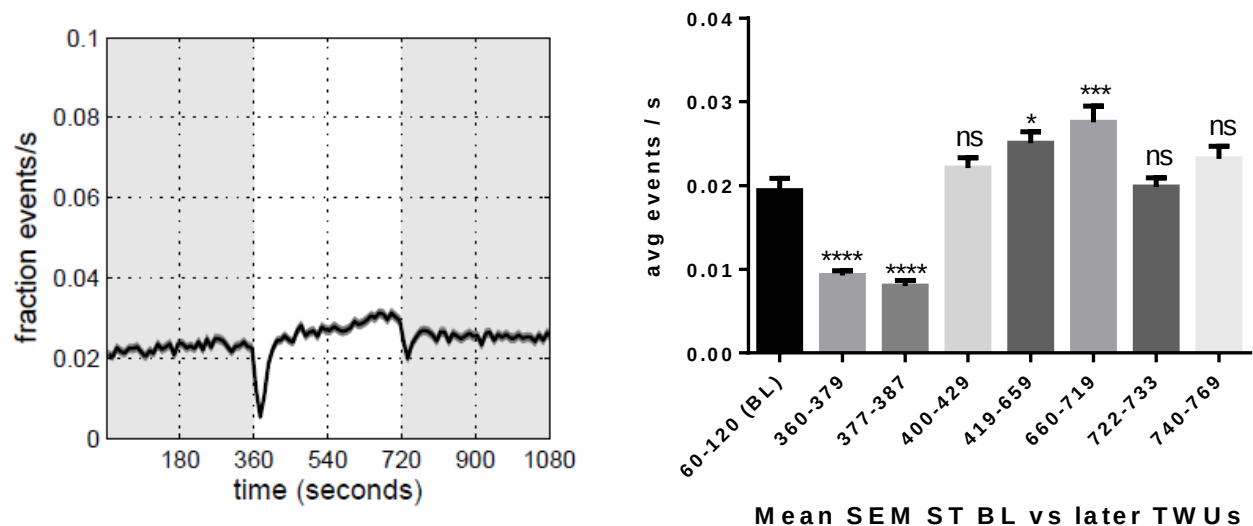


Figure 16: Average plot of STs of 23 recordings of N2 animals in the shift paradigm. Results from one way ANOVA and Dunnett's multiple comparison test of **BL ST angles vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference.

- (1) The average ST BL rate within TWU 60-120s is nearly three times as high as the BL OT rate.
- (2) Immediately after the DS occurs, STs are inhibited until a minimum at 389s is reached. The average ST events/s at TWU 377-389s are significantly decreased compared to BL.
- (3) 40s after the DS the event rate has returned to BL level.
- (4) The rate of STs increases constantly over the next 320 seconds. The average events/s at TWU 660-719s are significantly increased compared to BL level.
- (5) Immediately after the US occurs, the increased BL is suppressed back to BL level, the minimum is found between 739 and 749s. The average ST turning rate at TWU 722-733 is no significantly different from BL. The suppression amplitude is 0.0090 events/s, this is a significant drop from before US to after US.

As can be seen in Figure 16, STs are strongly inhibited in the first 30s after the DS followed by a steady increase until an US occurs. Upon US the ST rate returns to BL level. The decrease in STs corresponds with a general slowing of animals. Since ST occur out of movement, it is not surprising, that the ST rate is reduced, when the overall speed drops.

TWU [s]	N2 BAG cell kill (CX11697)	N2 AQR/URX cell kill (CX7102)	egl-3 n150 (Cx9191)	egl-3 n150 BAG cell kill (ZIM300)	egl-3 n150 AQR/URX cell kill (ZIM301)	egl-3 n729 (MT1541)	sbt-1 ok901 (RB987)
BL (60-120)	-/*	ns	-/****	ns	ns	-/***	-/****
360-379	ns	ns	+/****	+/**	+/****	+/****	+/****
377-387	+/*	+/*	+/****	+/**	+/****	+/**	+/****
400-429	ns	ns	-/****	ns	ns	-/***	-/****
419-659	ns	ns	-/****	ns	ns	-/****	-/****
660-719	-/*	ns	-/****	ns	ns	-/****	-/****
722-733	ns	+/**	ns	ns	+/*	-/**	-/****
740-769	-/**	ns	-/****	ns	-/*	-/****	-/****

Table 21: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutant strains**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.3), the statistics in section 5.3.2.5).

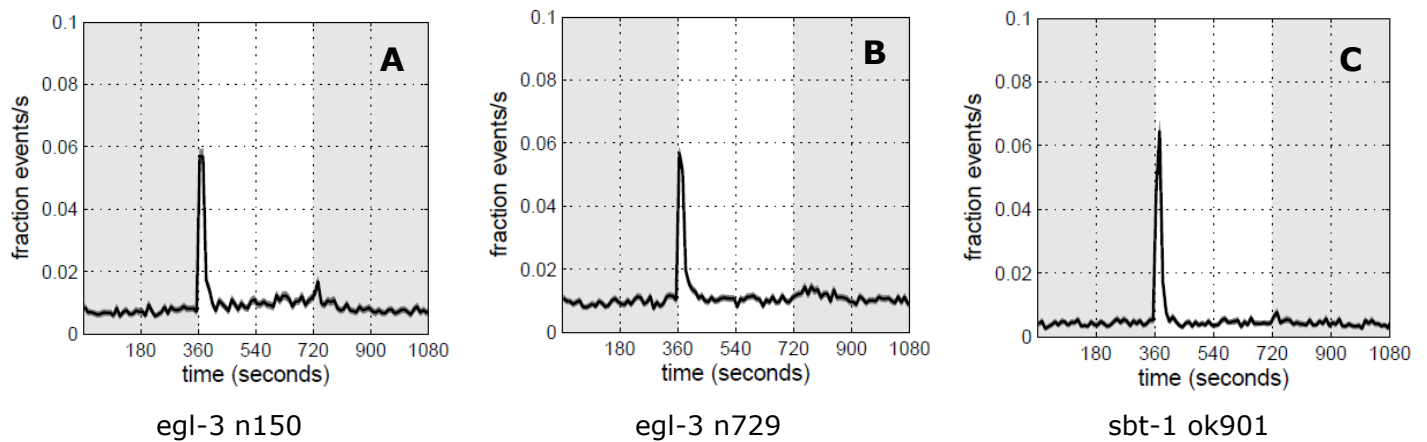


Figure 17: Average plots of STs in the shift paradigm of the neuropeptide depleted mutants egl-3 n150, egl-3 n729 and sbt-1 ok901 using 15, 13 and 9 individual recordings respectively

The ST BL at TWU 60-120s is significantly reduced in all of the neuropeptide depleted mutants (Figure 17, Table 21). In all of these mutants a strong increase in STs immediately after the DS replaces the N2 inhibition of turns. The absence of neuropeptides leads to a drastic and significant increase of STs at TWUs 360-379 and 377-387s. This occurs at the same time as the increase of OTs. It seems that a drop in oxygen elicits an immediate response leading to an increased rate in turns, omega turns as well as shallow turns. Neuropeptides seem to suppress the already discussed underlying response for a certain period of time to enable animals to engage in a less drastic response.

Since there are no significant differences between the neuropeptide depleted mutants, the main candidate neuropeptides are those which are absent in all three mutant strains. According to Table 12 these are FLP-3, FLP-5, FLP-7, FLP-8, FLP-10, FLP-12, FLP-13, FLP-17, FLP-19 and FLP-20.

TWU [s]	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	ns	ns	ns
360-379	ns	ns	ns
377-387	ns	ns	ns
400-429	ns	ns	ns
419-659	ns	ns	ns
660-719	ns	ns	ns
722-733	ns	ns	ns
740-769	ns	ns	ns

Table 22: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutant strains**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.3), the statistics in section 5.3.2.5).

Test of the neuropeptide mutants flp-8, flp-10 and flp-17 show, that these neuropeptides are not involved in modifying the ST BL or oxygen shift behavior.

2.2.2.2 Behavior in the ramp paradigm

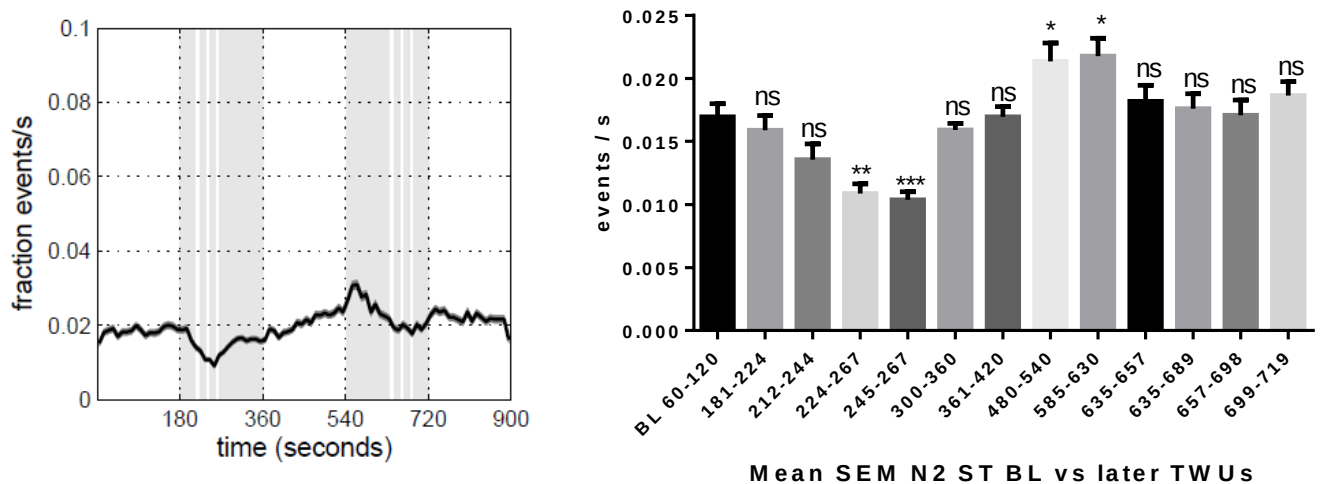


Figure 18: Average plot of STs of 23 recordings of N2 animals in the ramp paradigm. Statistics: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference.

- (1) The rate of STs drops after the DR has passed 20% oxygen. This drop continues, until 14% oxygen has been reached. At TWU 245-267s (oxygen concentration 15-13%) the average ST rate is significantly decreased compared to the ST BL level.
- (2) After 13% oxygen has been passed, the ST rate gradually increases again. The increase of the average ST rate becomes significantly higher than BL level in TWU 480-540s where the oxygen concentration stays constant at 4%.
- (3) After the UR starts, the ST rate is still significantly increased in TWU 585-630s.
- (4) Only after the oxygen concentration has increased above 13% oxygen, the ST rate returns to BL at TWU 635-657s and stays at this level.

TWU [s]	N2 BAG cell kill (CX 11697)	N2 AQR/URX cell kill (CX 7102)	egl-3 n150 (Cx 9191)	egl-3 n150 BAG cell kill (ZIM 300)	egl-3 n150 AQR/URX cell kill (ZIM 301)	egl-3 n729 (MT 1541)	sbt-1 ok901 (RB987)
BL (60-120)	ns	ns	-/****	ns	ns	-/***	-/****
181-224	ns	ns	-/***	ns	+/*	ns	-/****
212-244	ns	ns	ns	+/**	+/****	ns	-/*
224-267	ns	ns	+/*	+/****	+/****	+/**	ns
245-267	+/*	ns	+/**	+/****	+/****	+/****	ns
300-360	ns	ns	ns	+/****	+/****	+/*	-/****
361-420	ns	ns	-/**	+/****	+/****	ns	-/****
480-540	ns	ns	-/****	ns	ns	-/****	-/****
585-630	-/*	ns	-/****	ns	ns	-/****	-/****
635-657	ns	ns	-/**	ns	ns	-/*	-/****
635-689	ns	ns	-/*	ns	ns	ns	-/****
657-698	ns	ns	-/**	ns	ns	ns	-/****
699-719	-/**	ns	-/****	ns	ns	-/*	-/****

Table 23: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.4), the statistics in section 5.3.2.10).

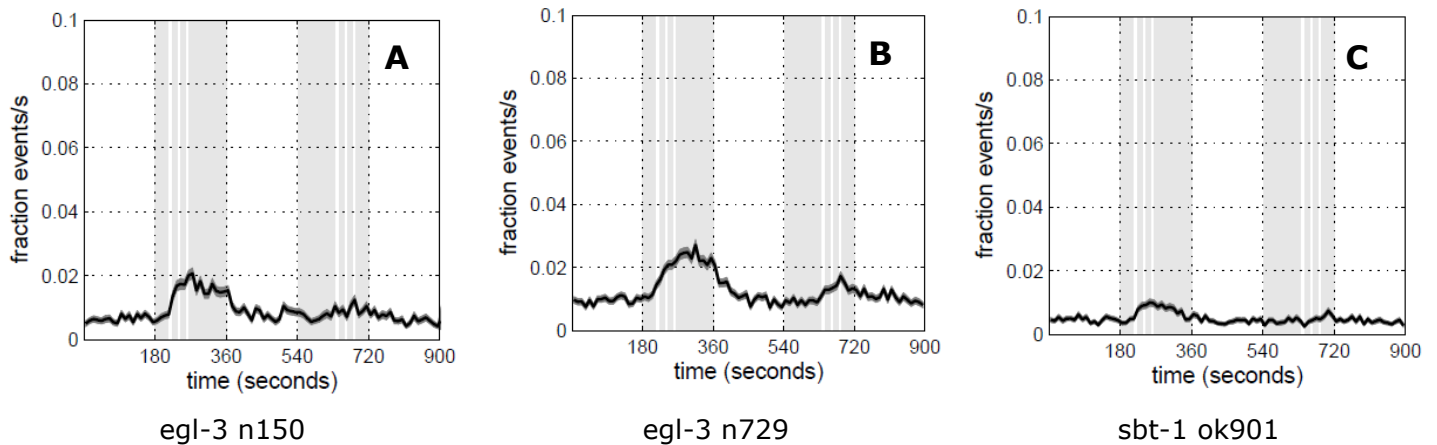


Figure 19: Average plots of STs in the ramp paradigm of the neuropeptide depleted mutants *egl-3 n150*, *egl-3 n729* and *sbt-1 ok901* using 9, 10 and 9 individual recordings respectively

The reduced ST BL has already been discussed in the shift paradigm. As seen in Figure 19A-C, in all of the neuropeptide depleted mutants the drop in STs after the onset of the DR is replaced by an increase in STs after 18% oxygen has been passed. Without neuropeptides the ST response in a DR and the following resting period at 4% oxygen basically follows the activity of BAG. Neuropeptides mask the underlying response as has been discussed in the shift paradigm. Neuropeptides facilitate an inhibition of STs once a DR occurs until the preferred oxygen concentration of 13% has been passed, in the DR they are therefore required to increase the chance for animals to reach a preferred oxygen concentration by modifying the traveling behavior. Due to the similar responses of the neuropeptide depleted mutants, a detailed analysis of differently acting neuropeptides is not feasible. Candidate neuropeptides absent in all neuropeptide

depleted mutants are FLP-3, FLP-5, FLP-7, FLP-8, FLP-10, FLP-12, FLP-13, FLP-17, FLP-19 and FLP-20 (Table 12).

TWU [s]	flp-10 n4543 (MT 14676)	flp-17 n4894 (MT 15933)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	ns	-/*	ns
181-224	ns	-/**	ns
212-244	ns	-/**	ns
224-267	ns	-/*	ns
245-267	ns	-/*	ns
300-360	ns	ns	ns
361-420	ns	ns	ns
480-540	ns	ns	ns
585-630	ns	ns	ns
635-657	ns	ns	ns
635-689	ns	ns	ns
657-698	ns	ns	ns
699-719	ns	ns	ns

Table 24: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.4), the statistics in section 5.3.2.10).

Tests of the neuropeptide mutants flp-8 and flp-10 show, that they are not involved in modifying the ST BL or ramp behavior. The neuropeptide mutant flp-17 displays a reduced rate of STs during the DR leaving it as a prime candidate for DR ST inhibition.

2.2.2.3 Concluding remarks

During the DR the rate of STs drops, until the lower bound of the preferred oxygen concentration of 13% has been passed. The probability of starting an ST decreases, the more the oxygen decreases to the preferred oxygen concentration. After 13% oxygen has been passed, the ST rate gradually increases again. The ST probability increases, the further the concentration decreases from the preferred oxygen concentration. The same behavior can be observed in the UR until 13% oxygen has been reached from which point onwards the ST rate remains at BL level. STs are most likely part of the traveling behavior to find new food sources while moving. STs are always inhibited when animals move towards a preferred oxygen concentration.

As discussed in the OT section, there appears to be an underlying response not dependent on neuropeptides facilitating an escape response. This response is masked by a balance of multiple neuropeptides to provide more fine tuned behavior dependent on the environmental situation.

2.2.2.4 Shallow turn angles

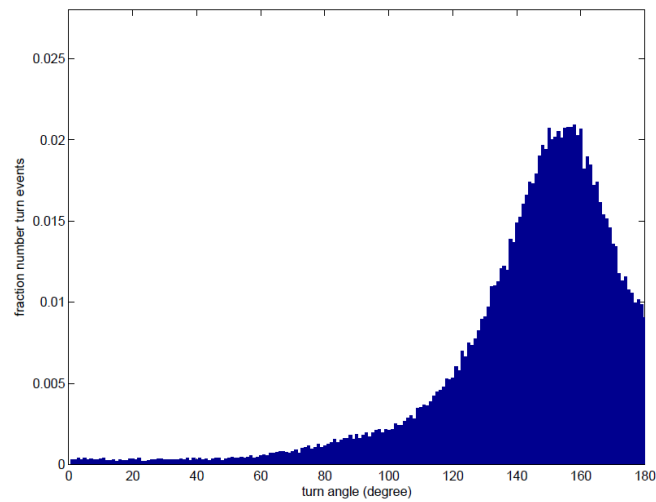


Figure 20: Normalized N2 ST angles of 75317 ST events at TWU 1-1080s in the shift paradigm.

The majority of turns (85.9%) show a change of bearing larger than 120 degrees, with its maximum at 150 degrees. This finding correlates with the turning angles of shallow turns described in Kim et al (2011). Turns with an angle close to 180 degrees result mainly from turns displaying two subsequent steering events. The first steering event is a strong head swing that would change the bearing by 90 degrees. This first head swing is immediately followed by a second head swing in the opposite direction bringing the worm back to its original bearing.

Since this distribution does not change significantly in any of the TWUs nor in any of the cell kill or mutant strains or the ramp paradigm, ST angles will not be further discussed in detail.

2.2.3 Large reversals

2.2.3.1 Behavior in the shift paradigm

Reversals have been distinguished by duration and length into long and short reversals. Short reversals (SR) have been defined as reversals not longer than 0.25 length of the worm. Large reversals are all reversals longer than short reversals. Since LRs and SR show a very similar distribution in all of the behavior assays, therefore only LRs will be discussed in detail.

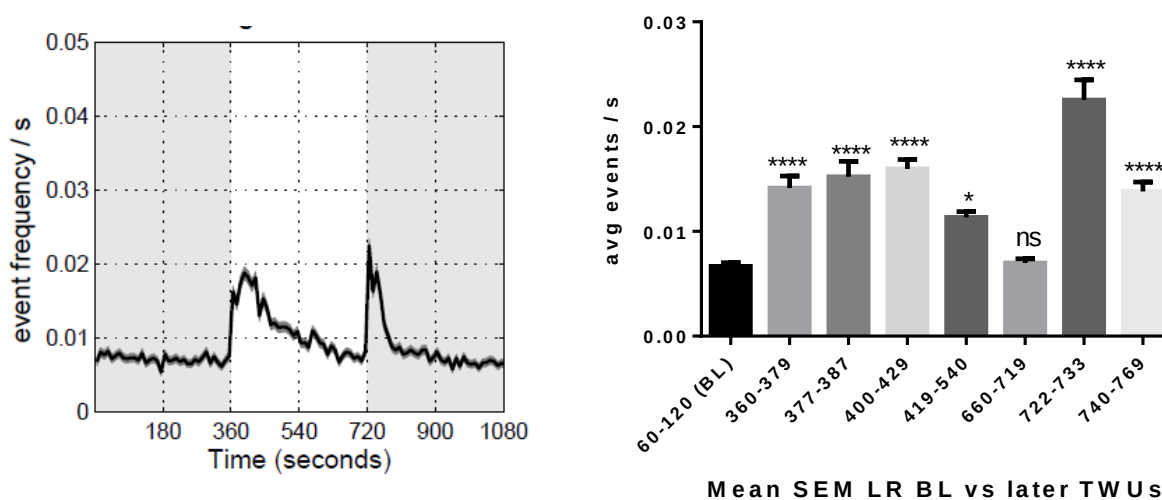


Figure 21: Average plot of LRs of 23 recordings of N2 animals in the shift paradigm. Statistics: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

- (1) BL data describes the average reversal rate within TWU 60-120s.
- (2) Immediately after the DS the rate of reversal rises significantly compared to BL level, there is no inhibition of LR compared to OTs.
- (3) The rate of LRs stays significantly increased at TWU 419-540s.
- (4) Three minutes after the DS the LR rate has returned to BL level (TWU 660-719s).
- (5) Immediately after the US, LR rise a significantly compared to BL level, this increase stays significant for 60s until the LR rate returns to BL level.

The following identified time frames of interest will be used as reference for comparison with mutant, cell kill and rescue lines:

- LR BL at TWU 60-120s
- LR DS response at TWU 377-387s and 400-429s
- Increased LR event rate at TWU 419-540s.
- LR rate returned to BL level at TWU 660-719s.
- LR US response at TWUs 722-733s and 740-769s

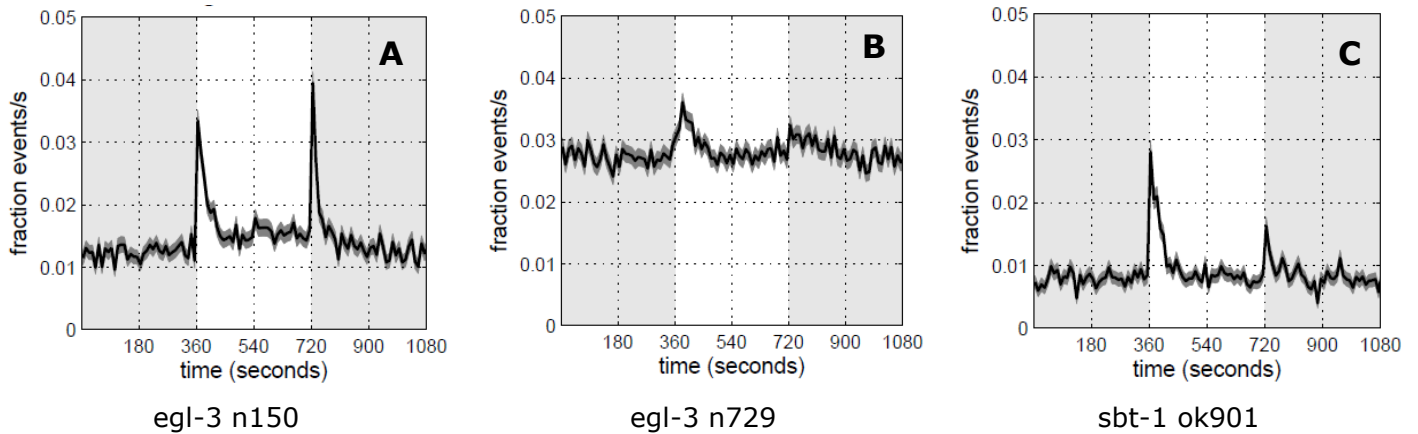


Figure 22: Average plots of LR in the shift paradigm of the neuropeptide depleted mutants *egl-3 n150*, *egl-3 n729* and *sbt-1 ok901* using 15, 13 and 9 individual recordings respectively

TWU [s]	N2 BAG cell kill (CX11697)	N2 AQR/URX cell kill (CX7102)	<i>egl-3</i> <i>n150</i> (Cx9191)	<i>egl-3 n150</i> BAG cell kill (ZIM300)	<i>egl-3</i> <i>n150</i> AQR/URX cell kill (ZIM301)	<i>egl-3</i> <i>n729</i> (MT1541)	<i>sbt-1</i> <i>ok901</i> (RB987)
BL (60-120)	ns	ns	+/**	+/*	+/*	+/*	ns
360-379	-/*	ns	+/*	-/*	+/*	+/*	+/*
377-387	ns	ns	+/*	-/*	+/*	+/*	ns
400-429	-/*	ns	ns	-/*	+/*	+/*	-/*
419-659	ns	ns	+/*	ns	+/*	+/*	ns
660-719	ns	+/*	+/*	+/*	+/*	+/*	ns
722-733	ns	-/*	+/*	+/*	-/*	ns	ns
740-769	ns	-/*	ns	ns	ns	+/*	-/*

Table 25: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.5), the statistics in section 5.3.2.6).

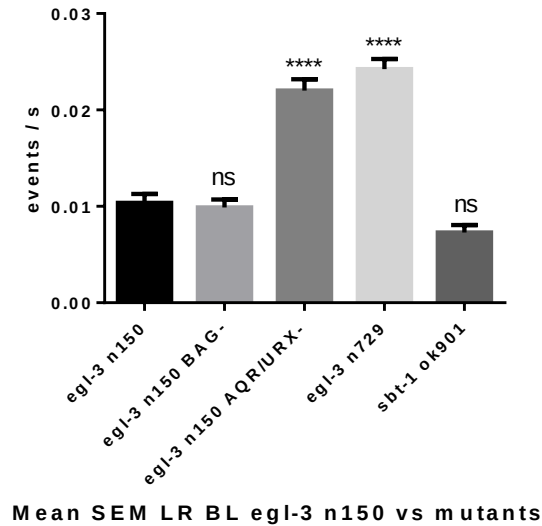


Figure 23: Results from one way ANOVA and Dunnett's multiple comparison test of egl-3 n150 vs. mutant strains, + ... significant increase compared to egl-3 n150, - ... significant decrease compared to egl-3 n150, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

Both egl-3 n150 and egl-3 n729 mutants show a significantly increased LR BL compared to N2 while the LR BL of sbt-1 does not (Table 25). Since the LR BL of the egl-3 n150 AQR/URX cell kill is significantly increased, but the N2 AQR/URX cell kill is not (Table 25), a neuropeptide downstream of AQR/URX is required for inhibition of the LR BL. Due to the fact, that the LR BL of the egl-3 n150 AQR/URX cell kill line is increased compared to the egl-3 n150 mutant but lower than the corresponding BL of the egl-3 n729 BL (Figure 23), it seems likely that more than one neuropeptide is involved in inhibiting the BL. Neuropeptides present in sbt-1 but absent in egl-3 n150 and egl-3 n729 are inhibiting the LR BL. According to Table 12, candidates for LR BL inhibition are FLP-1, FLP-11, FLP-13, FLP-18 and FLP-22 which show this absence pattern and FLP-10, FLP-12, FLP-17 and FLP-20 which were not detected in the mass spectrometry analysis.

The LR DS response at TWU 360-379s is significantly increased in all neuropeptide depleted mutants (Table 25). Due to the increased LR BL level at the TWUs after the DS the LR rates are significantly increased or not significantly different from N2 in the egl-3 mutants (Table 25). The sbt-1 mutant shows a significant reduction of LR in TWU 400-429s comparable to the reduction in the N2 BAG or egl-3 n150 BAG cell kill lines at the same TWU (Table 25). Neuropeptides from or downstream of BAG absent in the sbt-1 mutant could be required for the prolonged increased LR rate after a DS has occurred. Candidates for these findings are FLP-5, FLP-7, FLP-8, FLP-10, FLP-12, FLP-13, FLP-17, FLP-18, FLP-19 and FLP-20 (Table 12).

The US response in the *egl-3* n729 mutant cannot be distinguished from the increased LR BL level and can therefore not be included in the analysis. The LR US response is significantly increased in the *egl-3* n150 mutant compared to N2, while it is significantly decreased in *sbt-1* (Table 25). Transmission of the US response either depends on a balance between excitatory and inhibitory neuropeptides or on at least two inhibitory neuropeptides. For the second case both neuropeptides would be absent in the *egl-3* n150 mutant while the *sbt-1* mutant shows one absent, one present. Neuropeptide candidates present in *sbt-1* and absent in *egl-3* n150 are FLP-1, FLP-5, FLP-10, FLP-11, FLP-12, FLP-13, FLP-17, FLP-18 FLP-20 and FLP-22 (Table 12). Candidates for neuropeptides absent in all mutants are FLP-3, FLP-7, FLP-8, FLP-10, FLP-12, FLP-17, FLP-19 and FLP-20 (Table 12).

TWU [s]	flp-1 ok2811 (ZIM144)	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	+ /****	ns	ns	ns
360-379	ns	ns	+ /****	ns
377-387	+ /**	+ /*	+ /*	ns
400-429	+ /****	+ /*	ns	ns
419-659	+ /****	ns	ns	ns
660-719	+ /****	ns	ns	ns
722-733	+ /****	ns	+ /***	ns
740-769	+ /****	ns	ns	ns

Table 26: Results from one way one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.5), the statistics in section 5.3.2.6).

Regarding the inhibition of the LR BL, FLP-1 is the only one of the tested neuropeptide mutants that shows a significantly increased BL compared to N2 (Table 26). The increase is still significantly lower than the LR BL of *egl-3* n729, indicating that FLP-1 is only one of multiple neuropeptides inhibiting the LR BL.

Both *flp-10* and *flp-17* mutants show a significantly increased DS response compared to N2 (Table 26), indicating they are both negative regulators of the LR DS response.

The *flp-17* mutant shows a response at TWUs 360-379s and 377-387s that is not significantly different from the *egl-3* n150 response (addendum 5.3.2.7), while the response at TWU 400-429s is not significantly different from the N2 response (Table 26). FLP-17 is a prime candidate for inhibiting the initial, underlying DS response while the prolonged LR response of increased reversals is mediated by another neuropeptide.

FLP-8 and FLP-10 are not involved in the US response. The *flp-17* mutant shows a significant increase compared to the N2 response (Table 26), while it is not significantly different from the *egl-3* n150 response (addendum 5.3.2.7). Since FLP-17 is expressed solely in BAG and BAG is not active during the US response, release of FLP-17 could

cause long lasting inhibitory action in the LR network downstream of both BAG and AQR/URX.

2.2.3.2 Concluding remarks

The immediate increase in LRs upon DS and US suggests a direct transmission of the signal from the sensory neurons to the motorneurons controlling reversals. The proposed oxygen sensing network shows, that both BAG neurons synapse onto the AVE and AVA neuron pairs, which have been shown to elicit reversals (Chalfie et al, 1985, Li et al, 2011). Additionally BAGL synapses onto AIBL, which has been shown to inhibit the forward movement neuron AVB (Wakabayashi et al, 2004). Activation of BAG could increase the probability of reversals at the expense of forward movement by activating AVA and AVE and inhibiting AVB. Both URX neurons synapse onto AVE, AQR synapses to AVA. URX furthermore synapses to AUA which in turn shows synaptic connections to both AVA and AVE (Figure 1). All of these findings strengthen the theory, that activity of the oxygen sensory neurons BAG and URX is directly transmitted to reversal motorneurons.

Resembling the discussed OT behavior, the LR DS response consists of two parts. The first part, being an underlying response and taking place even without the presence of neuropeptides provides a robust escape response. Amplitude and duration of this underlying response is modulated by a balance of multiple different neuropeptides. The second part is solely dependent on neuropeptides enabling a refined search behavior.

2.2.3.3 Behavior in the ramp paradigm

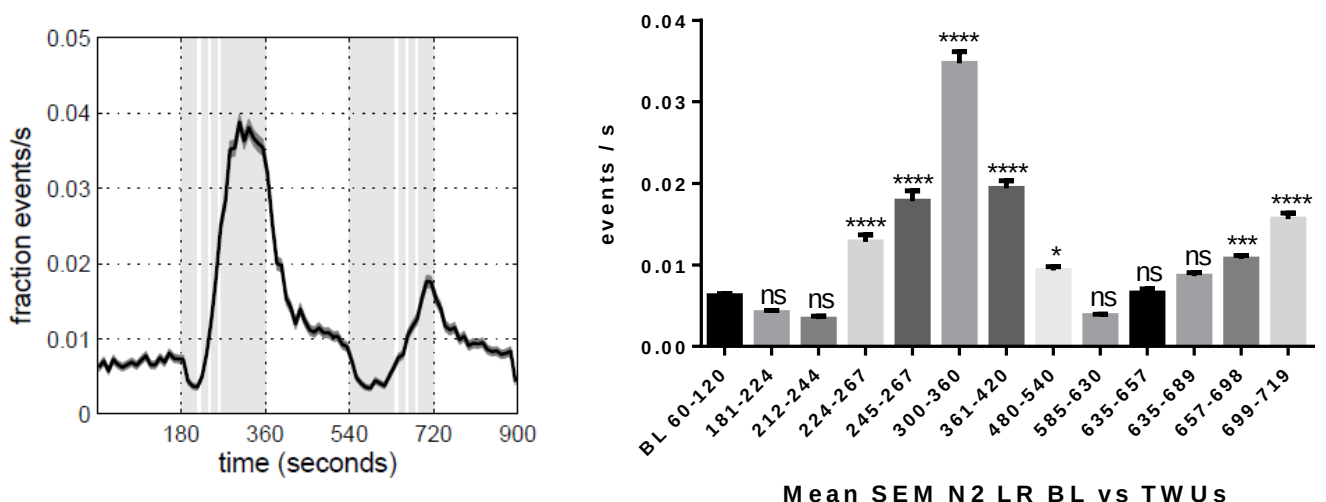


Figure 24: Average plot of LRs of 23 recordings of N2 animals in the ramp paradigm., Statistics: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 BL vs. later TWUs**, + ... significant increase compared to BL, - ... significant decrease compared to BL, ns ... no significant difference. Error bars indicate standard error of the mean (SEM).

- (1) After the DR starts, the LR rate drops, until 17% oxygen has been reached, the average LR rate at TWU 212-244s is significantly decreased compared to BL level.
- (2) The LR rate starts to rise again and increases back to BL level when 13% oxygen has been reached at TWU 245-267s.
- (3) After 13% oxygen has been passed, the LR rate increases further until it plateaus even before the oxygen concentration has reached 4% oxygen. The increase is significant and nearly three times as high as BL level (TWU 300-360s).
- (4) Immediately after the UR has reached 4% and the oxygen concentration stays constant, the LR rate decreases over the next 60s (TWU 361-420s) until it plateaus again at a level that is still significantly higher than BL level (TWU 480-540s).
- (5) Immediately after the oxygen concentration starts increasing from 4% upwards, the LR drops again and stays significantly decreased until an oxygen concentration of 13% has been reached at TWU 585-630s. In comparison to the OT rate, this decrease is not significantly below LR BL level.
- (6) After 13% oxygen has been passed, the LR rate gradually increases again until the oxygen concentration has reached 21%.
- (7) The LR rate is only significantly different from BL level after 18% oxygen has been passed at TWU 600-719s. After 21% oxygen has been reached, the LR rate gradually drops back to BL level.

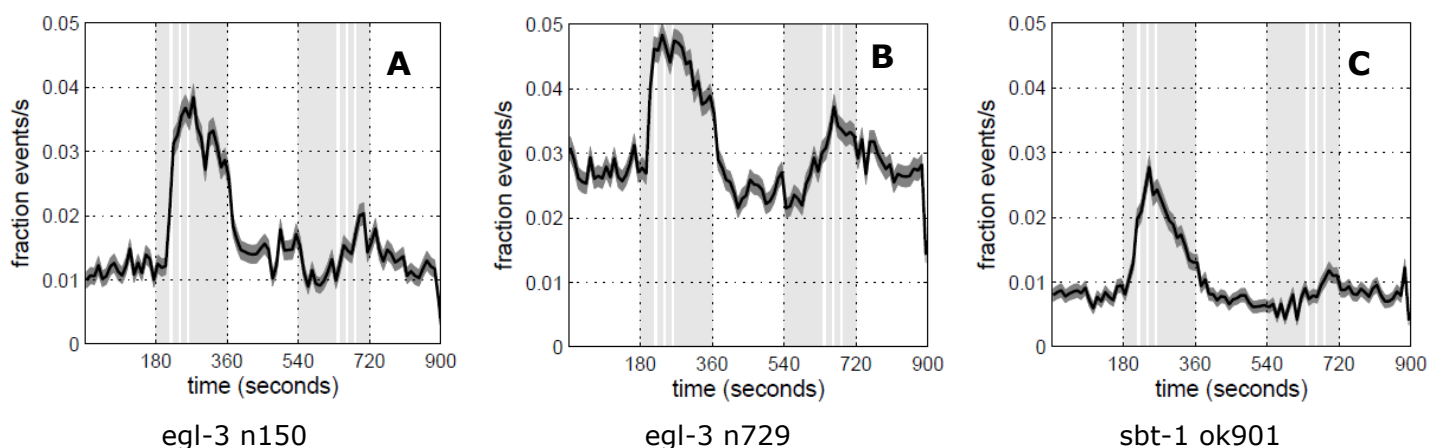


Figure 25: Average plots of LR rates in the ramp paradigm of the neuropeptide depleted mutants *egl-3 n150*, *egl-3 n729* and *sbt-1 ok901* using 9, 10 and 9 individual recordings respectively

TWU [s]	N2 BAG cell kill (CX 11697)	N2 AQR/URX cell kill (CX 7102)	egl-3 n150 (CX 9191)	egl-3 n150 BAG cell kill (ZIM 300)	egl-3 n150 AQR/URX cell kill (ZIM 301)	egl-3 n729 (MT 1541)	sbt-1 ok901 (RB987)
BL (60-120)	+/**	ns	+/**	ns	+/**	+/**	ns
181-224	+/**	ns	+/**	+/**	+/**	+/**	+/**
212-244	+/**	ns	+/**	+/**	+/**	+/**	+/**
224-267	ns	ns	+/**	ns	+/**	+/**	+/**
245-267	-/**	ns	+/**	-/**	+/**	+/**	ns
300-360	-/**	-/**	-/**	-/**	ns	ns	-/**
361-420	-/**	ns	ns	-/**	+/**	+/**	-/**
480-540	ns	ns	+/**	ns	+/**	+/**	ns
585-630	+/**	ns	+/**	+/**	+/**	+/**	ns
635-657	+/**	ns	+/**	+/**	+/**	+/**	ns
635-689	ns	ns	+/**	+/**	+/**	+/**	ns
657-698	ns	ns	ns	ns	+/**	+/**	ns
699-719	ns	-/**	ns	ns	+/**	+/**	-/**

Table 27: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.6), the statistics in section 5.3.2.12).

Differences in the LR BL have already been discussed in the shift paradigm section. All of the neuropeptide depleted mutants in Figure 25 show a missing drop of the LR rate at the start of the DR which is replaced by a premature increase of LRs instead. Also in all of these mutants the maximum DR response is shifted compared to N2 leading to a premature decline in the absolute LR rate before the DR has ended. This decrease leads to a significantly reduce LR rate in the case of the egl-3 n150 and sbt-1 mutant at TWU 300-360s compared to N2 (Table 27). The prolonged increased rate of LRs after the end of the DR at TWU 361-420s is missing in the sbt-1 mutant (Table 27). Due to the increased LR BL rate this behavior cannot be clearly stated for the two egl-3 mutants. The UR behavior is not significantly different from N2 in the sbt- mutant 1 (all TWUs between 585-719s in Table 27) leading to the assumption, that neuropeptides required for the UR behavior are still present in the sbt-1 mutant. If the average LR rates of the egl-3 mutants are normalized to N2 LR BL levels, their response is not significantly different from the N2 behavior (addendum 5.3.2.12).

The amplitude of the LR UR response seems to be dependent on LR promoting and inhibiting neuropeptides. Neuropeptides absent in the egl-3 mutants but present in sbt-1 could be required to inhibit the UR response. Candidates for this depletion pattern would be FLP-5, FLP-8, FLP-10, FLP-11, FLP-12, FLP-13, FLP-17, FLP-18, FLP-20 and FLP-22 (Table 12).

TWU [s]	flp-10 n4543 (MT14676)	flp-17 n4894 (MT15933)	flp-8 pk360 strain flp-8 pk360
BL (60-120)	ns	ns	ns
181-224	ns	ns	ns
212-244	ns	ns	ns
224-267	+/**	ns	ns
245-267	+/**	ns	ns
300-360	ns	ns	-/**
361-420	ns	ns	ns
480-540	ns	ns	ns
585-630	+/*	+/**	ns
635-657	+/*	+/**	ns
635-689	+/*	+/**	ns
657-698	ns	+/**	ns
699-719	ns	ns	ns

Table 28: Results from one way ANOVA and Dunnett's multiple comparison test of **N2 vs. mutants**, + ... significant increase compared to N2, - ... significant decrease compared to N2, ns ... no significant difference. Find all corresponding panels in the addendum section 5.2.6), the statistics in section 5.3.2.12).

FLP-8 influences the maximum LR response during the DR and has been shown to be missing in the sbt-1 ok901 mutant which also has a reduced maximum LR response at TWU 300-360s (Table 28). flp-8 is expressed in URX and AUA (Table 12). Since BAG mediates the DR response, FLP-8 released from AUA would be a prime candidate. BAG gap junctions to RIR which in turn is synaptically connected to AUA (Figure 1). Activity in BAG can lead to the release of FLP-8 in AUA which in turn additionally increases the LR response. FLP-8 has no influence on any other part of DR or UR behavior. FLP-8 is not involved in mediating the initial DR inhibition.

FLP-10 is not involved in mediating the initial DR inhibition at TWU 212-244s, but inhibits the DR rising response at TWU 245-267s (Table 28). flp-10 is expressed in BAG, URX and AUA (Table 12) which all connect to reversal interneurons AVA and AVE (Figure 1). Together with FLP-8 FLP-10 could be involved in balancing the maximum LR response during the DR. The absence of FLP-10 leads to a significant increase during the start of the UR response at TWU 585-630s. At this time neither BAG nor URX are active leaving AUA as a prime target for mediating the initial LR DR inhibition.

FLP-17 is not involved in the initial inhibition of the DR response. The absence of FLP-17 removes the LR inhibition upon UR. All LR rates during the UR are significantly higher compared to N2, the LR rates increase even before 13% oxygen has been reached, the maximum response is sooner and higher than in N2. This is consistent with the findings of the shift paradigm.

2.2.3.4 Concluding remarks

As can be seen in Figure 24, during the DR the LR rate drops corresponding to the decreasing activity of URX, while BAG is still inactive until 18% oxygen has been

reached. As long as animals move from a high oxygen concentration towards the upper bound of their preferred concentration, reversals are inhibited. After animals pass a concentration of 14% oxygen indicating that the preferred concentration has been passed, moving towards unpreferred or even dangerous oxygen concentrations, the rate of LRs is drastically increased. Once a stable oxygen environment has been reached, the increased LR rate drops again to a slightly elevated LR rate.

The reversal rate during the UR again follows the activity pattern of both BAG and URX (Figure 3, Figure 5). Once the UR starts and BAG is inactivated, the LR rate drops below BL level until 13% oxygen has been reached and URX becomes active. The LR rate gradually increases and rises above BL level, after the preferred oxygen concentration of 16% has been passed. After the oxygen concentration has reached a constant 21%, the elevated LR rate gradually drops back to BL level.

The LR behavior in DR, intermediate period and UR mirrors the OT ramp behavior. The only difference between LRs and OTs is the amplitude of the respective response, after the preferred concentrations in DR and UR have been passed where LRs show a higher maximum response compared to OTs.

As in the shift paradigm, the N2 response relies on a balance of multiple neuropeptides.

2.3 Concluding remarks behavior assays

Results of the calcium imaging of both shift and ramp paradigm show, that the wild type behavior in response to changes in the oxygen concentration corresponds to the activity sum of the oxygen sensory neurons BAG and URX. In a ramp paradigm BAG starts its activity after the upper bound of the preferred oxygen concentration 18%, URX after the lower bound of the preferred oxygen concentration of 13% has been passed. Within an oxygen gradient the change of activity in these neurons could provide sufficient information to find and stay within the bounds of its preferred oxygen concentrations.

C. elegans reacts to changes in oxygen concentrations by multiple different behavioral strategies. All of these behavioral strategies require the oxygen sensors BAG and AQR/URX to sense changes in the oxygen environment. The activity sum of BAG and URX are sufficient to explain LR and partially explain OT behavior by direct transmission of the signal to motoneurons. ST behavior is inversely coupled to the activity sum of BAG and URX.

The transmission of sensory information in a changing oxygen environment from oxygen sensory neurons to motoneurons to elicit specific behavioral responses through the network relies on multiple neuropeptides. Analysis of different neuropeptide depleted mutants lead to neuropeptide candidates involved in behaviors in response to changes in the oxygen environment stated in the tables below.

Shift paradigm	Neuropeptide candidates	Multiple neuropeptides involved
Omega turns		
Baseline	flp-1 , flp-5	
DS response	flp-5, flp-7, flp-12, flp-17, flp-19, flp-18, flp-20	+
US response	flp-5, flp-10 , flp-18 and flp-20	+
Shallow turns		
Baseline	flp-3, flp-5, flp-7, flp-12, flp-13, flp-19, flp-20	+
DS response	flp-3, flp-5, flp-7, flp-12, flp-13, flp-19, flp-20	
US response	flp-3, flp-5, flp-7, flp-12, flp-13, flp-19, flp-20	
Large reversals		
Baseline	flp-1 , flp-11, flp-12, flp-13, flp-18, flp-20, flp-22	
DS prolonged response	flp-5, flp-7, flp-12, flp-13, flp-18, flp-19, flp-20	
US response	flp-1, flp-3, flp-5, flp-11, flp-12, flp-13, flp-17 , flp-18, flp-19, flp-20, flp-22	+

Table 29: Neuropeptide candidates for different behaviors in the shift paradigm. Neuropeptide candidates in bold have been shown to affect the respective behavior. + ... Analysis did show, that multiple neuropeptides are required for the respective behavior.

Ramp paradigm	Neuropeptide candidates	Multiple neuropeptides involved
Omega turns		
DR inhibition	flp-3, flp-7, flp-12, flp-19, flp-22	
DR response	flp-8 , flp-12, flp-13, flp-17 , flp-18, flp-20, flp-22	
Resting period	flp-5, flp-7, flp-12, flp-18, flp-19, flp-20	
UR inhibition	flp-5, flp-7, flp-8 , flp-10 , flp-12, flp-17 , flp-18, flp-19, flp-20	
UR response	flp-5, flp-10 , flp-11, flp-12, flp-13, flp-17 , flp-18, flp-20, flp-22	+
Shallow turns		
Candidates	flp-3, flp-5, flp-7, flp-12, flp-13, flp-19, flp-20	
Large reversals		
DR response	increase in all NP mutants (flp-8 , flp-10 , flp-17)	+
UR response	increase in all NP mutants, (flp-10 , flp-17)	+
UR inhibition	flp-5, flp-10 , flp-11, flp-12, flp-13, flp-17 , flp-18, flp-20, flp-22	+

Table 30: Neuropeptide candidates involved in different behaviors in the ramp paradigm. Neuropeptide candidates in bold have been shown to affect the respective behavior. + ... Analysis did show, that multiple neuropeptides are required for the respective behavior.

OT angles	Neuropeptide candidates	Multiple neuropeptides involved
Flat turn promotion	flp-5, flp-11, flp-18, flp-22	+
Narrow turn inhibition	flp-1 , flp-20	+

Table 31: Neuropeptide candidates involved flat turn promotion and narrow turn inhibition. Neuropeptide candidates in bold have been shown to affect the respective behavior. + ... Analysis did show, that multiple neuropeptides are required for the respective behavior.

Furthermore the study of the signal transmission from sensory neurons to motoneurons resulting in behavioral responses shows, that upon changes in the oxygen concentration, two different responses take place. The first is a strong response independent from neuropeptides. This response leads to a very strong but short increase in OTs, STs and LRs after a DS stimulus and leads to a strong increase of narrow turning angles. This can be interpreted as a robust escape response to a harsh stimulus.

Neuropeptides efficiently mask this stereotypical escape response if changes in the environmental oxygen concentration are not associated with an immediate threat to the animal and mediate highly fine tuned responses to gradual oxygen changes. This fine tuned behavior enables *C. elegans* to efficiently sample the environment in response to gradual changes and is probably required to efficiently reach and stay at preferred oxygen concentrations.

As an outlook for further elucidation of the neuropeptides essential for the transmission of the oxygen sensory information to the proper behavior to reach and stay at a preferred oxygen concentration oxygen gradient assays in wild type and neuropeptide mutant strains would be the best approach.

3 Materials and methods

3.1 Calcium imaging

For all calcium imaging recordings a custom PDMS microfluidic device previously described (Zimmer et al, 2009) was used. The channel containing the worm was connected to a reservoir containing a 5mM Tetramisol S-Basal buffer solution. Tetramisol (Thienpont et al, 1966) is an anthelmintic drug paralyzing various nematodes. The gas channel was connected to a mass flow controller (Voegtlin Instruments) mixing the required oxygen-nitrogen concentrations from respective bottles of the pressurized gases and introducing the mixture at a constant flow rate of 50ml/min. All parts were connected by Tygon tubing (0.02 in ID, 0.06 in OD; Norton) or polyethylene tubing (0.066 in ID, 0.095 in OD; Intramedic) using 23G Luerstub adapters (Intramedic). All recordings were done using an epifluorescence microscope equipped with a Cooled pE-100 excitation system. Images were acquired with an Andor iXon 397 EMCCD camera and MetaMorph software (Universal Imaging), streaming images at a rate of 10 frames per second. Exposure time to elicit fluorescence from expressed GCaMP5k was 100ms.

For all experiments, well fed adult worms displaying a single row of eggs were expressing the marker gene were transferred without food onto a food free NGM plate and starved for an hour. After this hour, the tubing between chip and reservoir was disconnected on the chip end. By using a syringe on the reservoir end of the tubing, a drop of buffer with Tetramisol was placed on top of an animal on the NGM plate. Inverting the flow using the syringe, the drop of buffer including the animal was sucked up again. The tubing was reconnected to the chip, using the syringe, the animal was gently transferred into the chip and positioned in the recording channel. Animals were left resting for 5 minutes before the start of the actual experimental recordings.

A custom MetaMorph tracking journal was used to acquire the fluorescence values of the neurons of interest. A tracking algorithm provided by MetaMorph is used to follow a largely defined tracking region containing the neuron. Further a region close to the neuron but without scattering light from neighboring light sources is chosen to acquire background fluorescence. A third region of interest (ROI) is manually drawn tightly around the neuron of interest. This ROI is defined at the frame containing the maximum response from this neuron to include the complete size of the responding neuron. At each frame the built-in tracking algorithm moves the tracking region to best fit the tracking region of the previous frame. The differences of the tracking region in x and y compared to the last frame are used to update the positions of the background region and ROI accordingly.

Fluorescent values from pixels below the calculated threshold are excluded from further analysis. Neurons containing GCaMP display a baseline fluorescence even in an inactive state. Due to the fact, that expression levels of GCaMP differ greatly between individual animals, a static threshold to exclude pixels within the ROI not based on fluorescence from the neuron does not yield good results. To exclude these signals, a dynamic thresholding algorithm is used. At each frame, a dynamic threshold is calculated using minimum and maximum intensity values from pixels within the ROI.

$$F_{thresh} = ROI_{min F} + \frac{(ROI_{max F} - ROI_{min F})}{100} * 30$$

Equation 1: F_{thresh} ... fluorescence threshold, $ROI_{min F}$... minimum fluorescence at current ROI, $ROI_{max F}$... maximum fluorescence at current ROI

Pixels displaying a fluorescence value below the calculated fluorescence threshold are excluded. At each frame the average fluorescence intensity from pixels above threshold is recorded.

ROI fluorescence values and background fluorescence values are written to a text file. A custom Matlab (The Mathworks) script is used to calculate $\Delta F/F_0$ from background subtracted fluorescence values of individual recordings and plot trial average graphs.

3.2 Behavior assays

3.2.1 Behavior experiment settings

For all behavior assay experiments a setup was used as described in Zimmer et al, 2009. Briefly discussed the setup consisted of a mass flow controller (Voegtlin Instruments) mixing the required oxygen-nitrogen concentrations from respective bottles of the pressurized gases. A custom LabView 10 (National Instruments) software was used to control gas mixture and flow rate of the flow meter. Tubing connected the flow meter to a custom plexiglass device. If placed on an NGM plate and sealed with a metallic ring to avoid gas exchange with the outside, the area of gas flow within the device was 60x60x0.7mm. All flow rates used were 100mL/min. Recordings were done using StreamPix 5 Software (Norpix) controlling a digital 4 megapixel CCD camera (Jai genicam GigE) with a resolution of 2048x2048, 8bit, at a rate one frame per 333ms. Illumination was provided by a 24V LED Light Unit and a PD2-5024 (CCS) power supply. Light intensity was kept within a range of 150-220.

For the actual assay ~180-200 adult, gravid animals displaying one row of eggs per assay were manually transferred from a feeding plate to the center of a 14cm NGM plate without bacteria to avoid providing food for the animals on the assay plate in the process. The center of a 9cm Whatman filter paper was cut away, the paper placed on the NGM plate and thoroughly soaked with 1mL of a 20mM $CuCl_2$ solution to prevent

animals from leaving the area of interest. The animals were left for 1h in the darkened behavior room to disperse on the plate and switch from local search to traveling behavior. Temperature was kept at $\sim 20^{\circ}\text{C}$, humidity at $\sim 30\text{-}50\%$. After the hour, the plate was transferred to the recording area, placed under the plexiglas device and left for 5 minutes at a controlled gas concentration mixture of 21% oxygen, 79% nitrogen. After the initial 5 minutes the following gas flow protocol was used to challenge the animals. All recordings were analysed by a custom Matlab (The Mathworks) script described in the next section.

3.2.2 wormTracker Software

The wormTracker is a Matlab based software analyzing movies to identify trajectories and behavior of objects moving over time. Basis for this was a GUI software previously described (Ramot et al, 2008). The previous wormTracker derived from the published software used in the laboratory of Manuel Zimmer was a series of loosely connected Matlab scripts performing individual steps of tracking, analysis and quantification of *C. elegans* behavior movies without GUI. The main structure of this software was rewritten to increase usability and enable automated large scale analysis.

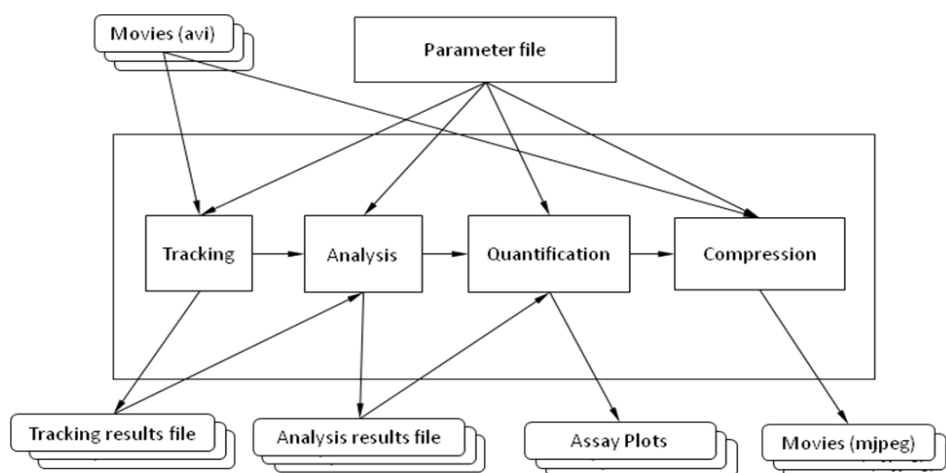


Figure 26: Workflow of the developed wormTracker

The software design is modular, the individual parts of the workflow are able to work independently from one another. This way a user is able to access and stop at any step of the workflow, given the required input files are provided.

Minor changes were done at the tracking step to improve the subsequent quality of OT analysis and providing quality reports about the movies analysed. Major changes were done at the analysis step, replacing the previous method of identifying and analyzing reversals and adding methods to identify and analyse STs and turning angles. The

quantification step was rewritten to enable the analysis of both single recordings and trial averaging.

3.2.2.1 Tracking step

At the tracking step a provided movie is analysed frame by frame. At each frame the image is converted into a black and white image using an intensity threshold. Each pixel with intensity above the defined threshold is converted to black, below to white. Using the Matlab regionprops package, the image is analysed for regions of connected white pixel between a lower and an upper threshold corresponding to the size of the animals. The range of the connected regions used to identify objects in this thesis was 40-120 pixels. For each of these identified objects specific attributes are calculated, the most important of these attributes being trajectory of the object's centroid (time series of x-y coordinates), speed, size of an object in pixels, direction in degrees, eccentricity and angular speed. Angular speed is calculated from the derivative of direction within three frames. Eccentricity is based on the overall outline of the current object ranging from ellipsoid to circular. The eccentricity of a circle is 0, the eccentricity of an ellipse is 1. At each frame the x-y coordinates of identified objects are matched to the x-y coordinates of objects in the last frame to follow them over time leading to the track of an animal for as long as the object can be followed. If the size of a connected region is below or above the specified range, an object is discarded. This inevitably leads to the situation, that an object at frame-1 has no successor at the current frame. In this case, the corresponding track is ended and the object is no longer followed. This in turn leads to tracks of different length. Since most behaviors require a specific amount of time to be initiated and completed, tracks with a length below 20 frames are discarded.

In the previous tracker there was an issue, if two animals ran into each other. In most cases this lead to the false identification of an omega turn event. To avoid this behavior, objects that increase their size from one frame to the next for more than 50 pixel, they are excluded from the analysis effectively ending the tracks of the two animals involved. All tracks are saved as a Matlab structure file for further analysis.

3.2.2.2 Analysis step

During the analysis step the attributes of each track found in the tracking step are screened to identify the different types of behavior, which can be defined by the following parameters.

Omega turns:

OTs are identified by a characteristic increase in angular speed and decrease in eccentricity above and below specific thresholds respectively.

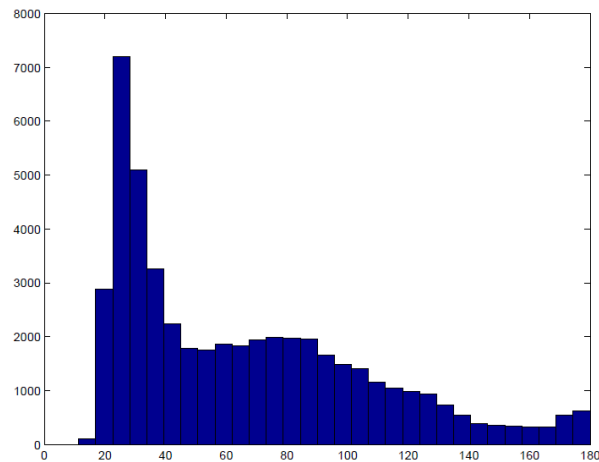


Figure 27: Angular speed distribution of 48701 OTs identified by the previous wormTracker. X axis shows the angular speed, Y axis the number of OTs within a bin.

Plotting the average angular speed of all OTs identified by the previous wormTracker (Figure 27) showed a bimodal distribution with a minimum at 50. Analysis of turns with an average angular speed below 50 showed, that these turns fall into the shallow turn category and are therefore excluded from the omega turn category in the developed version of the wormTracker. Events found within reversals are excluded as well.

Shallow turns:

The identification of STs was not included in the previous version of the wormTracker and was developed for this thesis. STs are identified by a characteristic decrease in the eccentricity of an object while displaying only a minor change in angular. In these cases the centroid of the object does not change its bearing strong enough to elicit changes in angular speed, while the animal itself is changing its body posture sufficiently to execute a turn. All analysis done in this thesis used a threshold of 0.87 for eccentricity and 50 for angular speed. Events found within reversals are excluded.

Turning angles:

Turning angles of omega and shallow turns are calculated using the Pythagorean equation for Euclidian distance followed by the calculation of the alpha angle in a triangle

with known sides. The three points in space used to calculate the sides are the coordinates of (A) the middle point of the turn of interest, (B) 10*frame rate before (A) and (C) 10*frame rate after (A).

$$a = \sqrt{(x_A - x_B)^2 + (y_A - y_B)^2}; b = \sqrt{(x_A - x_C)^2 + (y_A - y_C)^2}; c = \sqrt{(x_B - x_C)^2 + (y_B - y_C)^2}$$

Equation 2: Pythagorean equation for Euclidian distance

$$\alpha = a \cos \left(\cos \left(\frac{a^2 + b^2 - c^2}{2ab} \right) \right)$$

Equation 3: turning angle in radians

$$\alpha' = \frac{\alpha * 180}{\pi}$$

Equation 4: turning angle in degree

Reversals:

The identification of reversals was completely rewritten for this thesis. Reversals are identified by characteristic changes in bearing and angular speed at a starting as well as an ending event. Starting and ending events have to occur within a specified timeframe.

All identified behaviors are added to the Matlab structure of the corresponding tracks. After the analysis is done, the modified structure is saved as a new Matlab structure file.

3.2.2.3 Quantification step

At the quantification step all tracks identified in the tracking step and behavior information from the analysis step are gathered. At this step omega turns, shallow turns, reversals and speed are quantified in the following manner. A matrix with the dimension of the length of the experiment times the number of tracks is created for each type of behavior. At the start time of each behavior of each track a 1 is entered into the corresponding cell of the matrix. If specified, this matrix is reshaped to create bins over time to reduce noise. From the resulting matrix mean and standard deviation are calculated and subsequently plotted. In this thesis all speed data was calculated and plotted without binning, all turns and reversals were calculated plotted with 30s binning.

3.2.2.4 Performance analysis of the developed software

To test the performance of the developed tracker, movies showing individual animals were extracted from behavior assay movies by a custom Matlab script. The path of the animal was plotted into the movie. Behavioral patterns identified by the analysis step were plotted onto the path using color coding to distinguish between different behaviors.

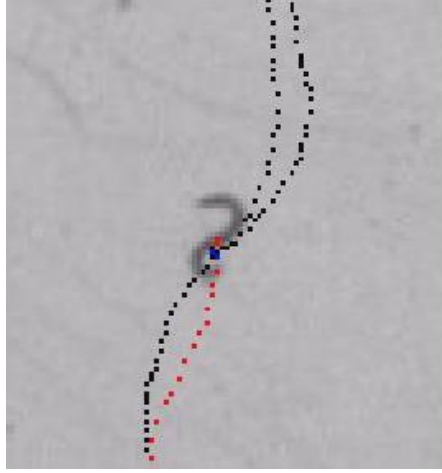


Figure 28: Caption of the movie of a single animal. The image shows the animal in the center, the x-y coordinates of the centroid over time is plotted in black. The animal is engaging in an OT (centroid coordinates plotted in blue) after having performed a reversal (centroid coordinates plotted in red).

Thusly created single movies were manually analysed, correct and incorrectly annotated as well as missed behavioral events were recorded. For comparison of improved performance, the same amount of time of single movies from the previously used tracker and the developed version were analysed. Single tracks were extracted from four different behavior movies of 1h starved N2 animals in a 21-10-21% oxygen shift paradigm experiment. Tracks with a length of at least 4 minutes covering either DS or US were chosen for the analysis.

Sensitivity (true positive rate), precision (positive predictive value) and fraction correct were calculated as follows:

TP ... true positive (correctly annotated event)

FP ... false positive (incorrectly annotated event)

FN ... false negative (missing event)

$$Sensitivity = \frac{\sum TP}{\sum TP + \sum FN}$$

Equation 5

$$Precision = \frac{\sum TP}{\sum TP + \sum FP}$$

Equation 6

$$Fraction \text{ correct} = \frac{\sum TP}{\sum TP + \sum FP + \sum FN}$$

Equation 7

N2	Time analysed	291 min	tracks	31
	OT	ST	LR	SR
Sensitivity	0.80	-	0.84	0.78
Precision	0.54	-	0.70	0.66
Fraction correct	0.47	-	0.62	0.55

Table 32: Performance of the previous tracker

N2	Time analysed	293 min	tracks	44
	OT	ST	LR	SR
Sensitivity	0.76	0.89	0.88	0.91
Precision	0.85	0.89	0.91	0.88
Fraction correct	0.67	0.80	0.81	0.88

Table 33: Performance of the developed wormTracker

Comparison of the performances of previous and developed tracker show that the changes introduced to the tracking of animals increased all categories of the detection of OTs. The detection of STs is well above 75% in all categories. The completely rewritten detection of reversals is also well improved in all categories compared to the previous version.

3.2.3 Statistical analysis

Data gathered from individual behavioral recordings and calcium imaging recordings were quantified using different custom Matlab (The Mathworks) scripts retrieving unbinned data for all TWUs. All statistical analysis was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com

3.2.4 Strains

All animals were kept in an incubator at constant 20C. Strains were maintained by transferring 4 worms at the L4 developmental stage to a new nematode growth medium (NGM) feeding plate seeded with OP50 *E. coli* bacteria. All strains except ZIM284 were provided by the laboratory.

Transgenic animals of strain ZIM284 were generated by injecting a mix of pSM plasmids ZIM281 carrying genomic *egl-3::SL2::GFP* under the *flp-17* promoter (expressed in BAG), pSM plasmids carrying co-injection marker mCherry under the *myo-3* promoter (expressed in body wall muscles) and empty pSM plasmids adjusted to a total concentration of 100ng/uL plasmid DNA to yield heritable extra-chromosomal complex plasmid arrays. Well fed young adult hermaphrodites of the *egl-3* n150 strain displaying none to a maximum of five eggs were injected 3-5 times into the gonads with the standard injection volume using a Zeiss Axiovert S100 microscope and an Eppendorf

FemtoJet with Eppendorf Femtotips microinjection needles. Surviving injected animals were transferred to NGM feeding plates and kept under standard conditions as described above. After 2-4 days F1 progeny displaying mCherry co-injection marker fluorescence under a Zeiss fluorescent stereoscope were singled out onto individual NGM feeding plates. After 2-3 days the progeny of the singled out F1 animals were re-inspected for co-injection marker fluorescence. Strains with F2 animals displaying non-mosaic co-injection fluorescence in more than 50% of the progeny were considered candidate strains. Animals of the candidate strains displaying GFP expression in the BAG neurons were used for behavior assays. Candidate strain number 09 showed the best rescue results in a shift paradigm and was therefore used for further experiments.

Strain	Genotype/Description	Parent strain	Origin
N2	Wild-type <i>C. elegans</i> var Bristol. Generation time is about 3 days. Brood size is about 350. Also CGC reference 257. Isolated from mushroom compost near Bristol, England by L.N. Staniland. Cultured by W.L. Nicholas, identified to genus by Gunther Osche and species by Victor Nigon; subsequently cultured by C.E. Dougherty. Given to Sydney Brenner ca. 1966. Subcultured by Don Riddle in 1973. <i>Caenorhabditis elegans</i> wild isolate.	N2	CGC
CX11697	kyIs536; kyIs538 kyIs538 = An integrated chromosomal array containing a PCR product with the glb-5 promoter driving a cDNA containing the p12 domain of Human Caspase3 and sl2 gfp injected at 5 ng/ul, w/ 1ng/ul of elt-2::mCherry for a co-injection marker, and 100ng/ul of pSM, kyIs536 = An integrated chromosomal array containing a PCR product with the flp-17 promoter driving a cDNA containing the p17 domain of Human Caspase3 and sl2 gfp injected at 4 ng/ul, w/ 10ng/ul of elt-2::gfp or a co-injection marker, and 100ng/ul of pSM. kyex3290 The strain contains an integrated plasmid array containing a two caspase system under the glb-5 and flp-17 promoters, with an expression overlap exclusively in the BAG neuron pair, leading to apoptosis of these cells in early development.		Bargmann lab
CX7102	lin-15(n765) qaIs2241 X URX, AQR, PQR cell kill strain. qaIs2241= gcy-36:: egl-1 + gcy-35::gfp + lin-15(+) The strain carries an integrated plasmid array containing a two caspase system under the gcy-36 and egl-1 promoters, with an expression overlap in the AQR neuron and the URX neuron pair. This leads to apoptosis of these cells in early development.		Bargmann lab
CX9191	egl-3(n150ts)V crossed out integrated transgene to recover egl-3 single mutant The strain carries a point mutation from G to A in the gene of the neuropeptide pre-protein convertase EGL-3. Using mass spectrometry it has been shown that out of 75 identified neuropeptides only 2 are present in the egl-3 mutation allele (Husson, 2006).	CX5075	Bargmann lab: Makoto
ZIM300	kyIs536; kyIs538; egl-3(n150ts) BAG- x egl-3 kyIs538 = An integrated chromosomal array containing a PCR product with the glb-5 promoter driving a cDNA containing the p12 domain of Human Caspase3 and sl2 gfp injected at 5 ng/ul, w/ 1ng/ul of elt-2::mCherry for a co-injection marker,	CX11697, Ingrid Hums CX9191	

	and 100ng/ul of pSM, kyIs536 = An integrated chromosomal array containing a PCR product with the flp-17 promoter driving a cDNA containing the p17 domain of Human Caspase3 and sl2 gfp injected at 4 ng/ul, w/ 1ng/ul of elt-2::mCherry for a co-injection marker, and 100ng/ul of pSM. kyex3290 The strain is based on a cross of the neuropeptide depleted egl-3 n150 strain Cx9191 with the N2 BAG cell kill strain CX11697.	
ZIM301	lin-15(n765) qaIs2241 X; CX7102, Zimmer lab: Ingrid Hums egl-3(n150ts) CX9191 URX ⁻ x egl-3 URX, AQR, PQR cell kill strain. qaIs2241= gcy-36::egl-1 + gcy-35::gfp + lin-15(+) The strain is based on a cross of the neuropeptide depleted egl-3 n150 strain Cx9191 with the N2 AQR/URX cell kill strain CX7102.	
ZIM144	flp-1(ok2811)IV RB2126 Zimmer lab: Ingrid Hums outcrossed 6x The strain carries a 500bp deletion in the flp-1 gene.	
MT1541	egl-3 (n729) V CX12861 Zimmer lab: Ingrid Hums non-transgenic worms derived from CX12861 with lost transgene The strain MT1541 carries a missense mutation from G to E in the gene of the neuropeptide pre-protein convertase EGL-3. Mass spectrometry analysis of this mutation showed a depletion of all 75 neuropeptides tested (Husson et al, 2006).	
RB987	sbt-1(ok901) V. CGC The strain carries a 1382bp deletion mutation in the sbt-1 gene. This gene is translated into the chaperone protein 7B2 which is required for the transport and activation of the pre-protein convertase EGL-3. Neuropeptide depletion in this mutant has been shown before (Husson et al, 2007). This mutant phenocopies the n729 behavior with only half the neuropeptides missing compared to n729 or n150.	
flp-8 (PK360)	flp-8 (PK360) Chris Li (Bargmann lab stock) outcrossed 3x The strain carries a 1400bp deletion in the flp-8 gene.	
MT15973	flp-10(n4543);flp-17(n4894) MT14676, MT15933 This strain is a cross of flp-10 and flp-17 mutant lines which each carry a 1kbp deletion in the respective gene of interest.	
ZIM284	egl-3 (n150) mzmEx190 CX9191 Zimmer Lab: Michael Sonntag mzmEx190 = Ex[Pflp-17::egl-3_genomic::SL2::GFP; Pmyo-3::mCherry] Pflp-17::egl-3_genomic::SL2::GFP [pMS01] injected at 5 ng/ul Pmyo-3::mCherry injected at 5 ng/ul A rescue construct using the flp-17 promoter coupled to wt egl-3 has been used to rescue the neuropeptide expression in BAG. The rescue construct was injected into worms of the egl-3 n150 strain Cx9191.	
MT14676	flp-10(n4543) IV Niels Ringstad (NYU)	
MT15933	flp-17(n4894) IV Niels Ringstad (NYU)	
ZIM226	mzmEx156 N2 Zimmer Lab: Tina Schroedel mzmEx 156 = Ex[Pflp-8::GCaMP5K;Pflp-17::GCaMP5K;Punc-122::gfp] injected: Pflp-8::GCaMP5K 5ng/ul + Pflp-17::GCaMP5K 5ng/ul + Punc-122::gfp 15ng/ul	
ZIM266	egl-3(n150ts)V CX9191, Zimmer Lab: Ingrid Hums mzmEx156 ZIM226 mzmEx 156 = Ex[Pflp-8::GCaMP5K;Pflp-17::GCaMP5K;Punc-122::gfp] injected: Pflp-8::GCaMP5K 5ng/ul + Pflp-17::GCaMP5K 5ng/ul + Punc-122::gfp 15ng/ul	

Table 34: Strains used in behavioral and calcium imaging experiments

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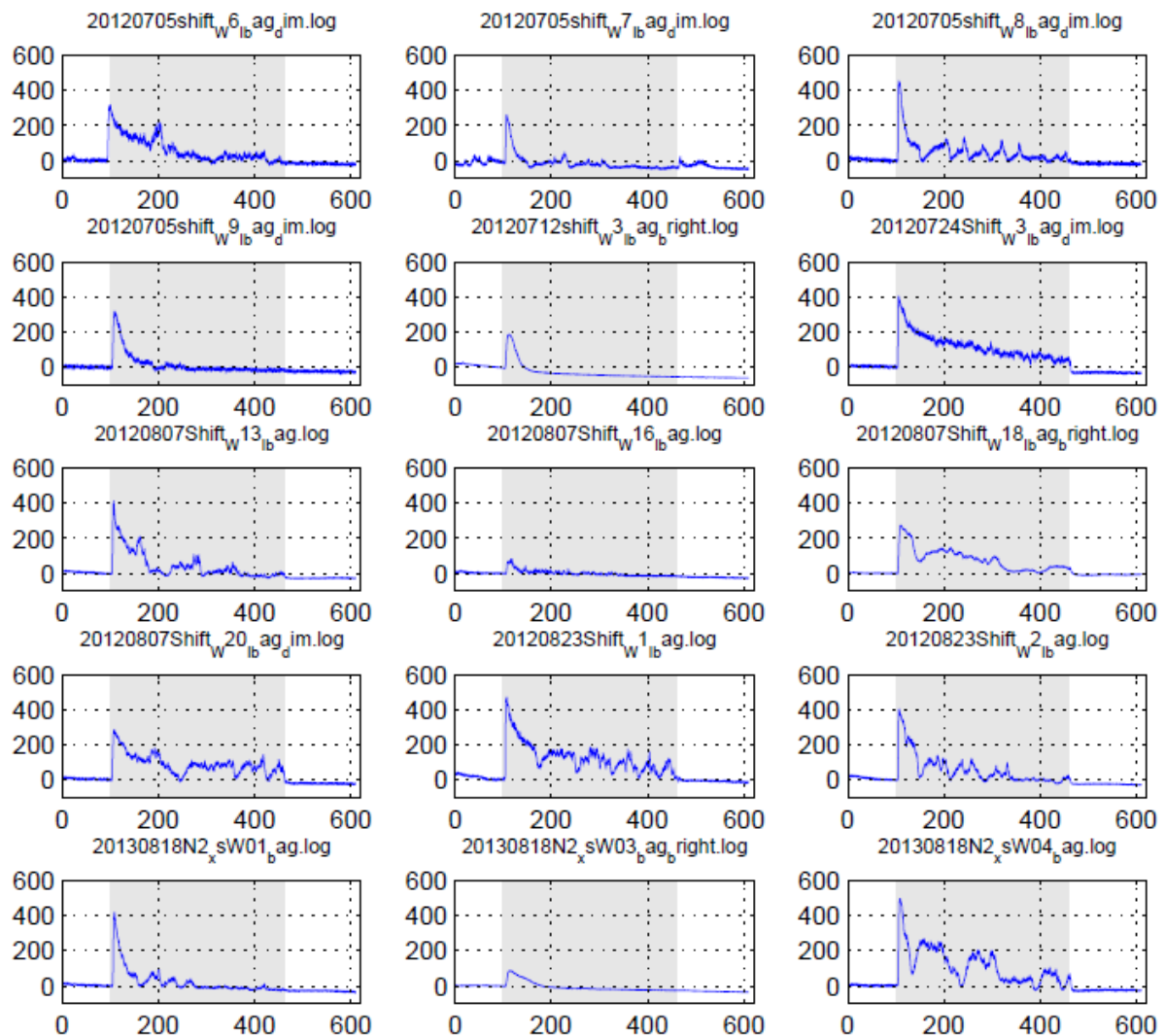
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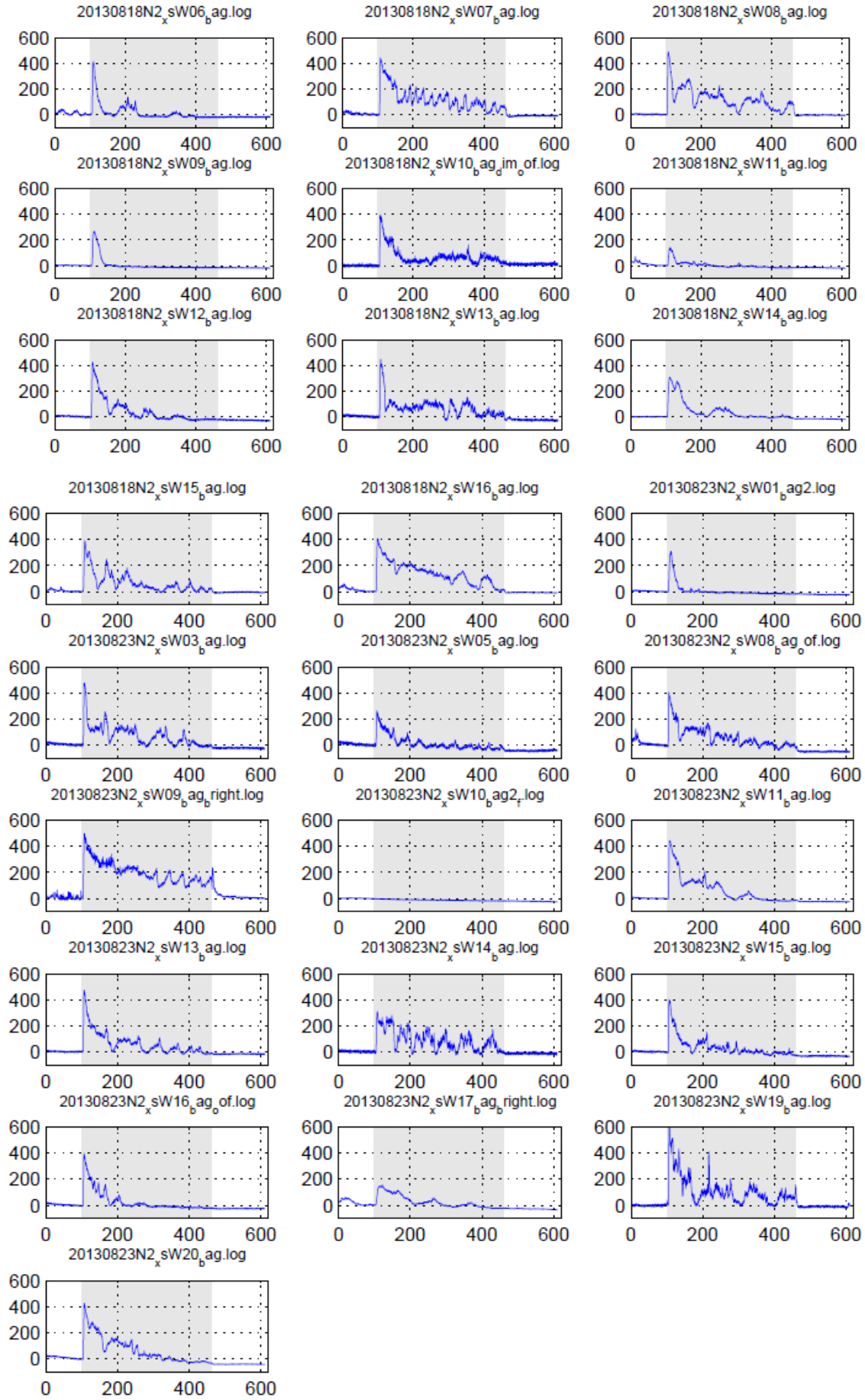
5 Addendum

5.1 Single calcium imaging traces

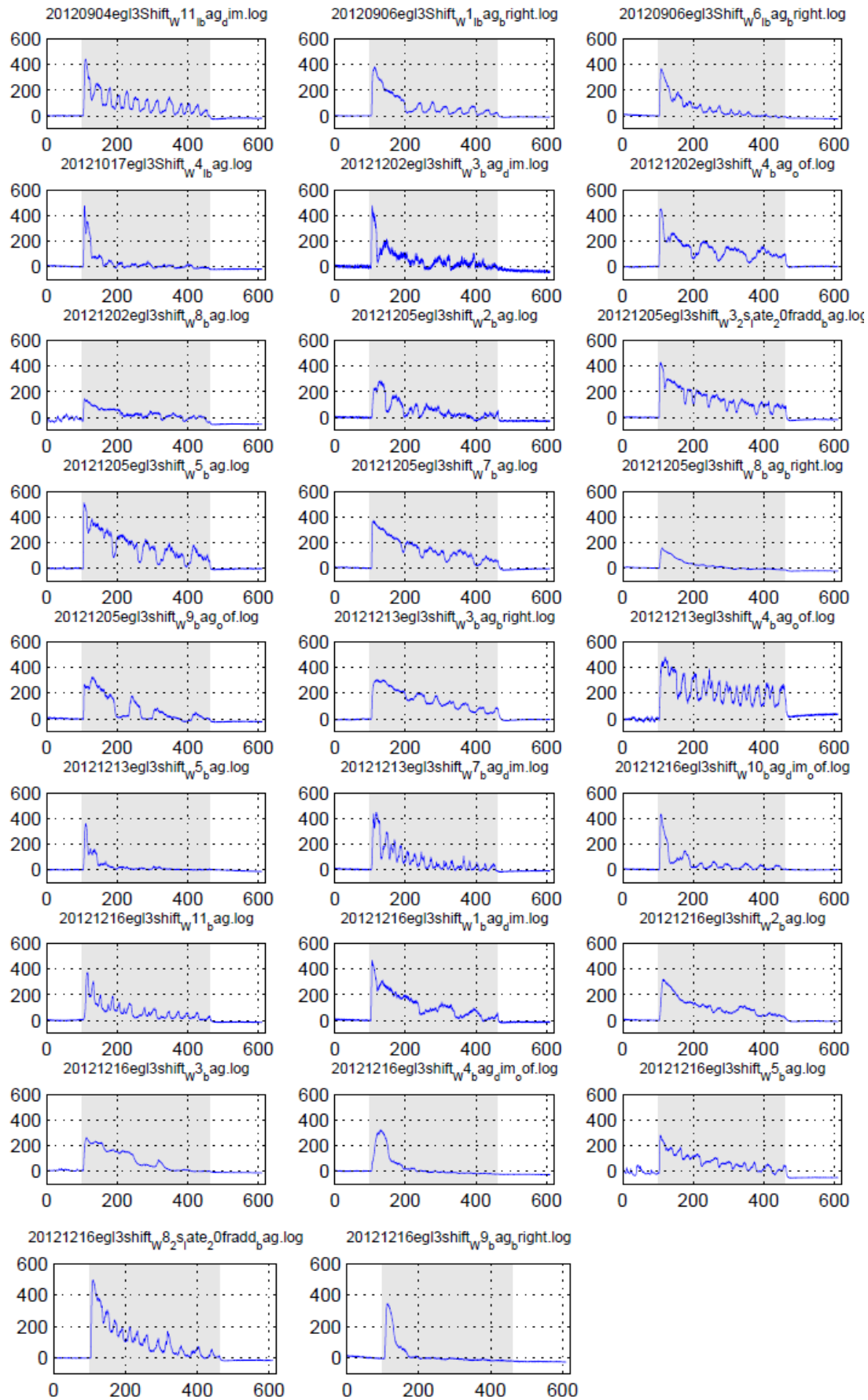
The following section contains all single traces of the recordings used to generate the corresponding average plots discussed in the first section. Recordings where the filename contains the letter "I" between recording number and neuron of interest were done by Ingrid Hums using same strain and same oxygen paradigm protocol as stated. BAG and URX have been reported to be tonic signaling neurons. The constant high concentration of increased intracellular Ca^{2+} is thought to be mediated by L-type voltage gated Ca^{2+} channels (L-VGCC), IP3 and ryanodine receptor Ca^{2+} channels. (Busch et al, 2011)

BAG in an N2 background (strain ZIM226) in the shift paradigm

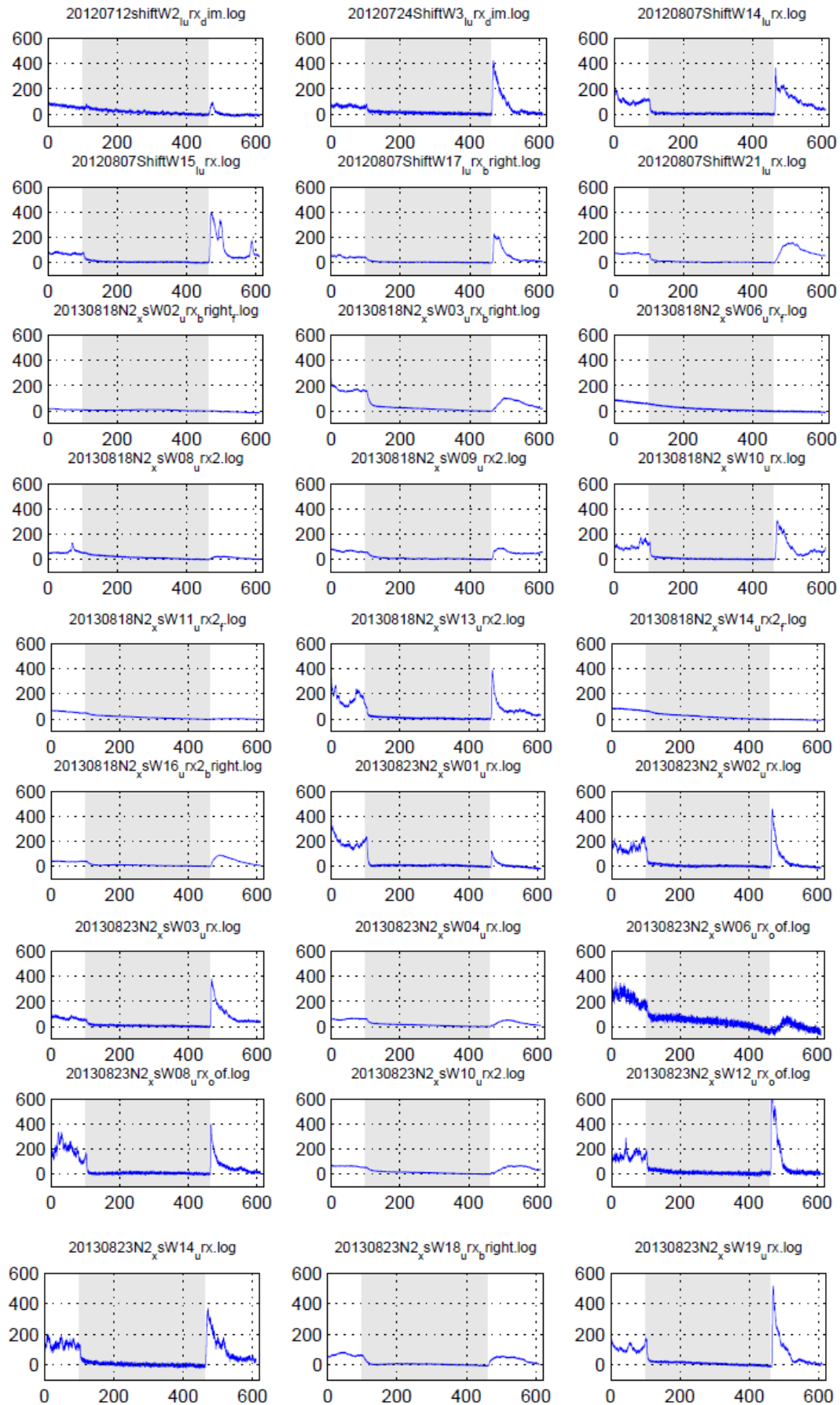




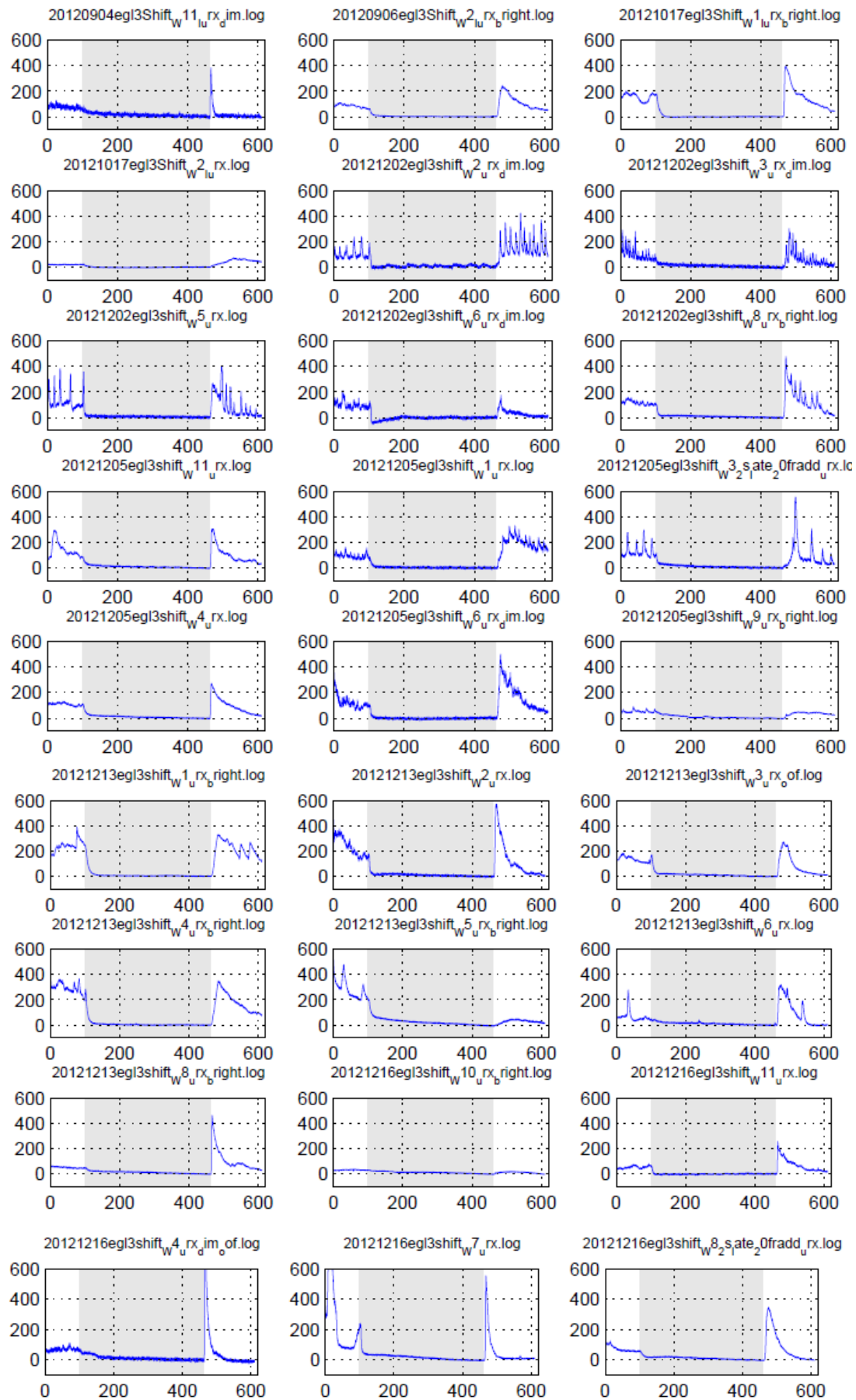
BAG in an egl-3 n150 background (strain ZIM266) in the shift paradigm



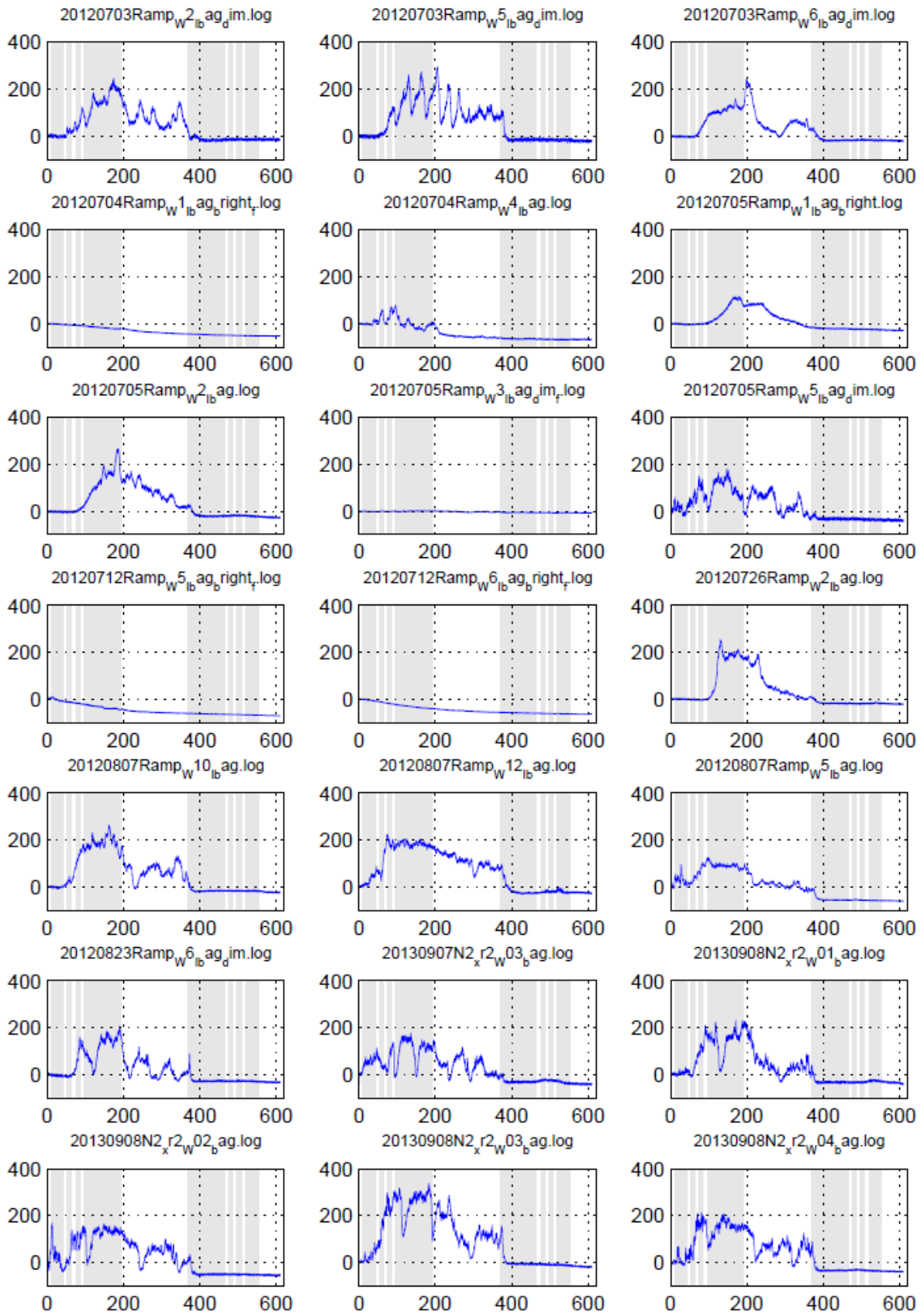
URX in an N2 background (strain ZIM226) in the shift paradigm



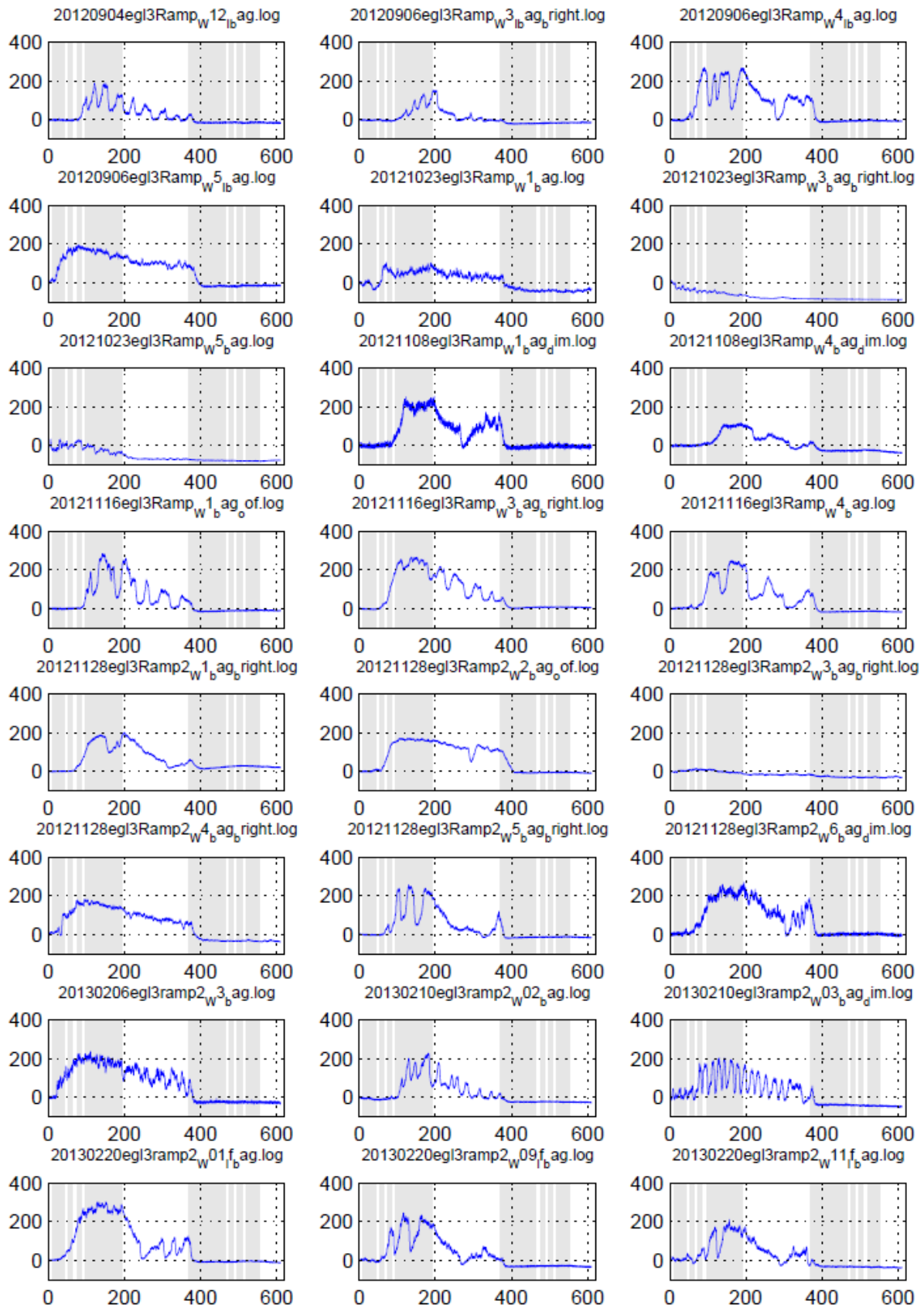
URX in an egl-3 n150 background (strain ZIM266) in the shift paradigm



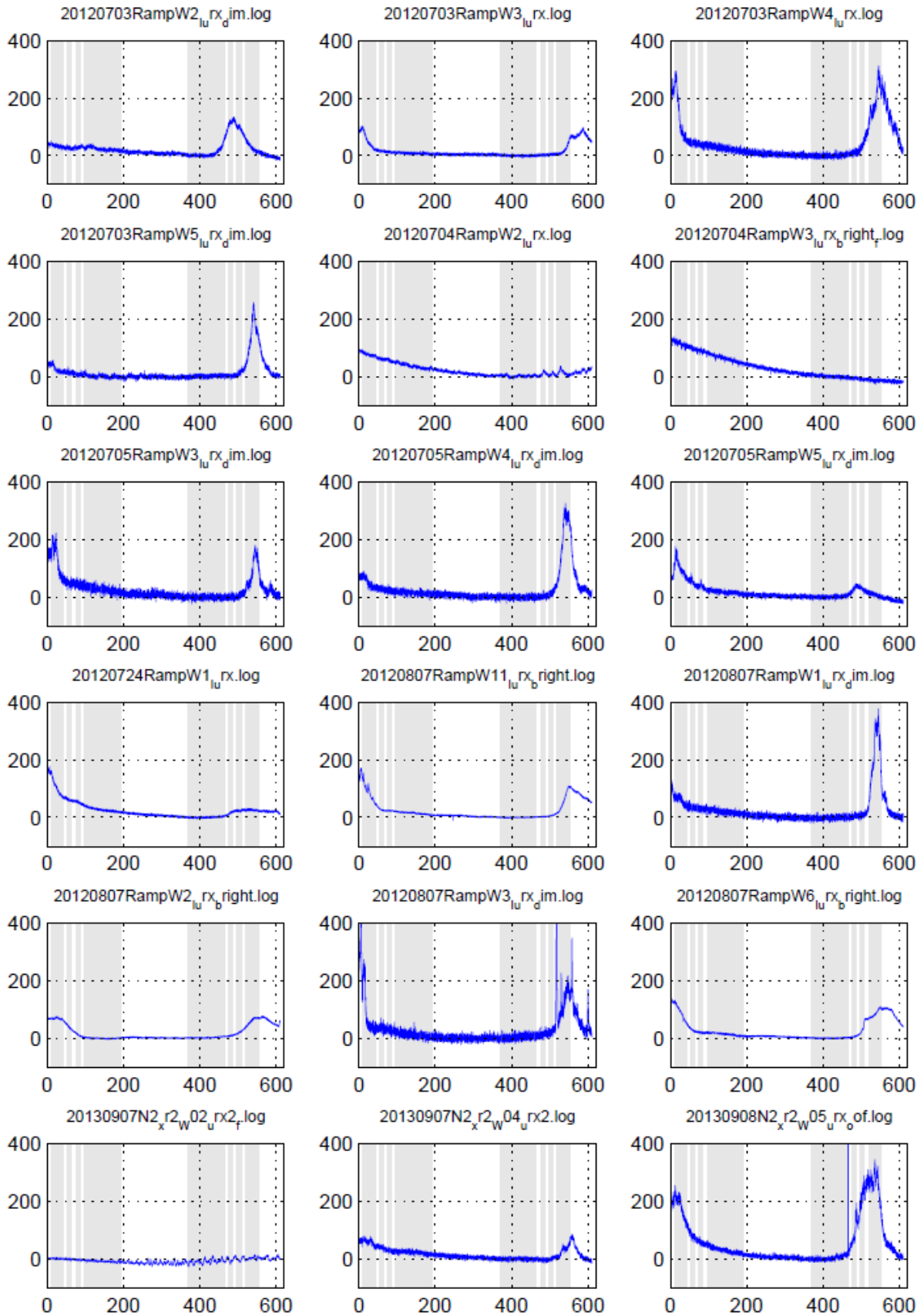
BAG in an N2 background (strain ZIM226) in the ramp paradigm



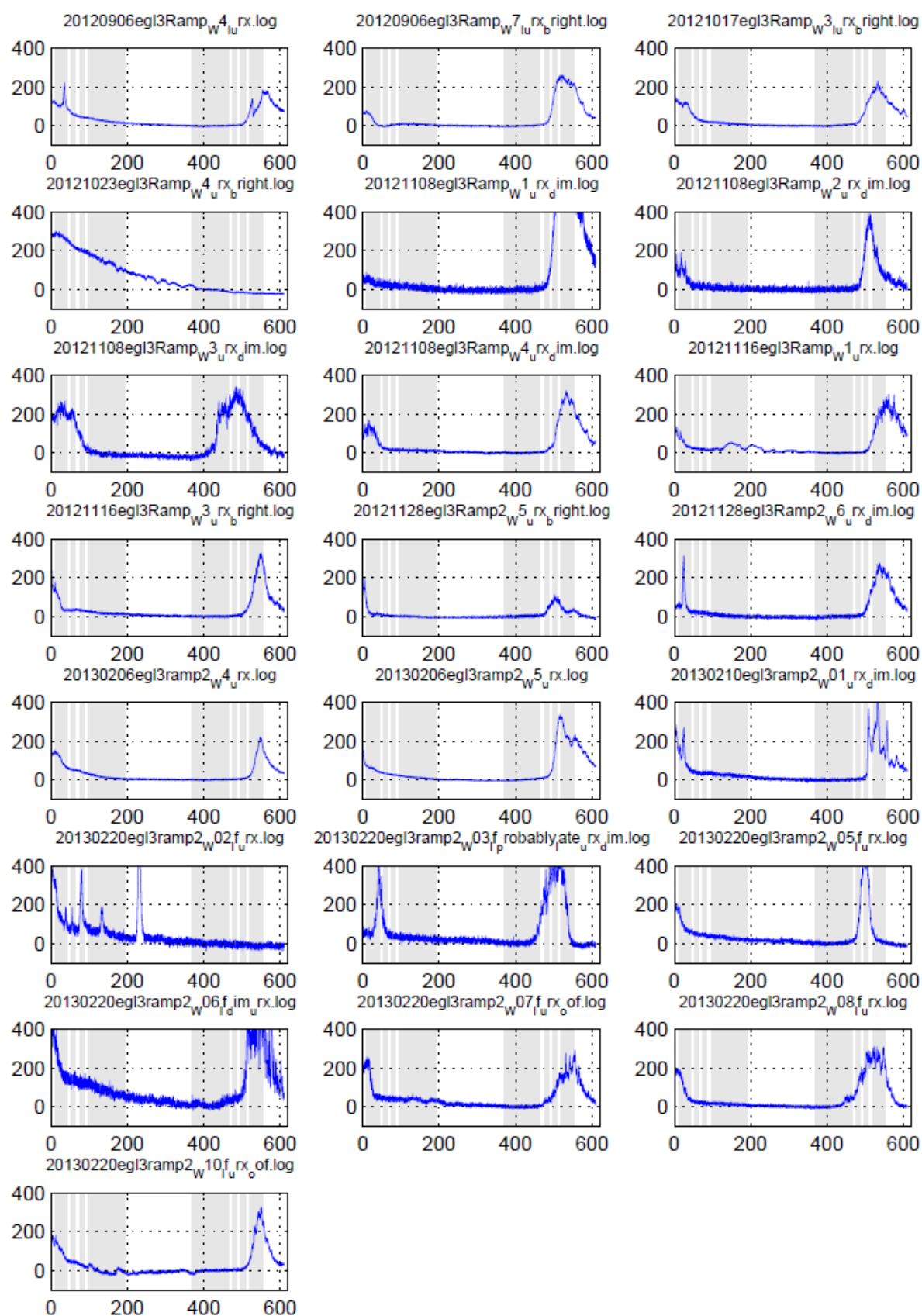
BAG in an egl-3 n150 background (strain ZIM266) in the ramp paradigm



URX in an N2 background (strain ZIM226) in the ramp paradigm



URX in an *egl-3 n150* background (strain ZIM266) in the ramp paradigm

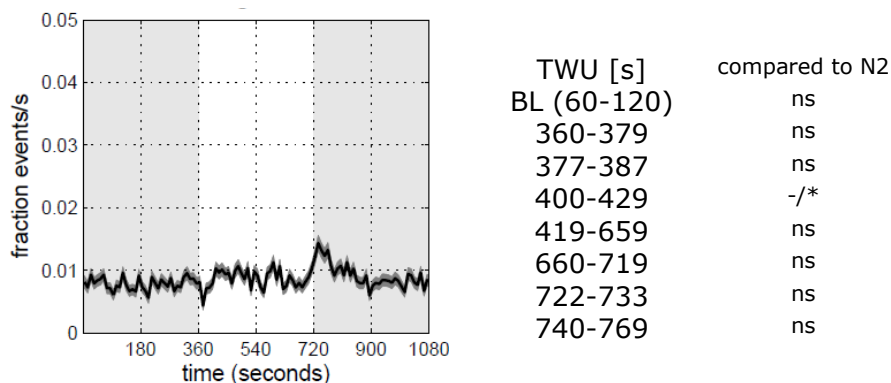


5.2 Behavior results

All statistics in the following section are results from one way ANOVA and Dunnett's multiple comparison test of the discussed strain compared to N2 or *egl-3 n150*. Find the actual comparisons including SEM in the addendum statistics section 5.3.2). + ... significant increase, - ... significant decrease, ns ... no significant difference.

5.2.1 Omega turns in the shift paradigm

N2 BAG cell kill strain CX11697



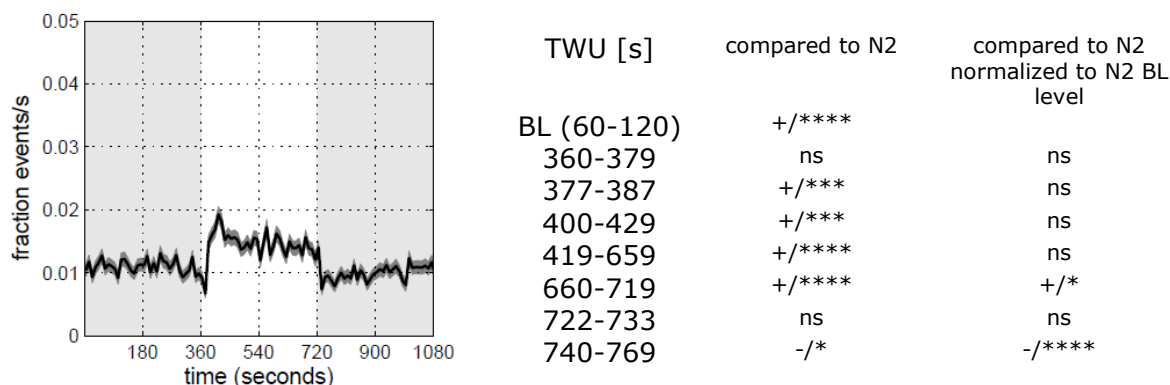
BAG does not affect the OT BL. There is no response to decreasing oxygen concentrations, BAG is required to mediate DS behavior. The absence of BAG does not influence the US behavior.

OT angles

- The OT angles of 0-20 degrees at BL TWU 1-360s are significantly increased (18.6% vs. 11.4%)
- The angles of 120-180 degrees at the same TWU are significantly decreased (27.4% vs. 49.3%).
- This angle distribution is present in all other TWUs as well.

There is a difference in turning angles in the N2 BAG cell kill line even though the TWU is previous to any stimulus. The results suggest that BAG promotes turning angles above 120 degrees and inhibits angles below 20 degrees regardless of stimulus. The second maximum around turn angle 140 degrees is completely missing. Either low activity of BAG at high oxygen still is sufficient to mediate turn angle modification or neuropeptides from BAG have a long lasting effect on turn angle preferences.

N2 AQR/URX cell kill strain CX7102



AQR/URX are required to suppress the BL OT turning rate. The difference between the N2 and N2 AQR/URX⁻ BL OT rate is exactly the same as the difference between the minima of the two strains found between 369-389s. This suggests, that the drop in OTs after the DS is independent from OT BL level, its absolute amplitude is not changed by an increased BL level.

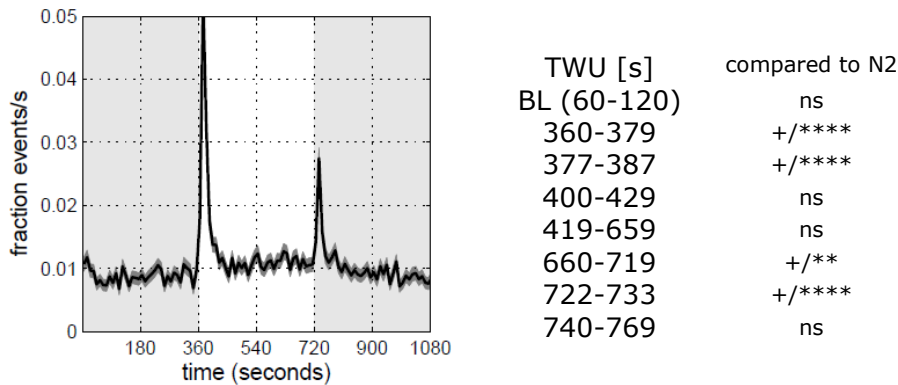
The US response is missing; AQR and URX are responsible for this response. The turning rate returns to BL level within 20-50s, faster than expected. An explanation for this behavior could be that BAG activity raises the OT rate. Once BAG is inactivated by a rise in oxygen concentration, the OT rate drops immediately back to BL level. This also leads to the conclusion, that an increase of OTs mediated by BAG is additive to the overall OT BL level. URX has been shown to partially mediate hyperoxia avoidance (Chang et al, 2006), which could be the reason for the increased OT BL in the absence of URX.

OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (21.5%) compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (27.3%) compared to wt.

AQR/URX decrease turns with an angle lower than 21 degrees and promote angles higher than 120 degrees. Whether this is mediated by normal signal transmission or by neuropeptides is not clear.

egl-3 n150 strain Cx9191



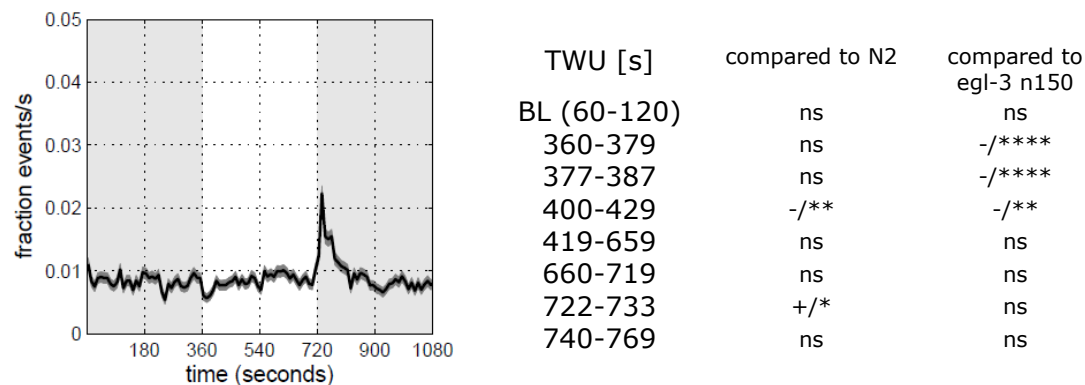
The absence of neuropeptides strongly affects both DS and US response. The decrease of OTs immediately after the DS is missing and replaced by a strong and immediate increase in OTs leading to a maximum over four times the strength of the wt response. The US response occurs earlier and stronger as well. Neuropeptides are essential for the wt response of both de- and increases in oxygen.

OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (21.5%) compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (27.3%) compared to wt.

Neuropeptides absent in the *egl-3 n150* mutant inhibit narrow and promote flat turn angles.

egl-3 n150 BAG cell kill strain ZIM300



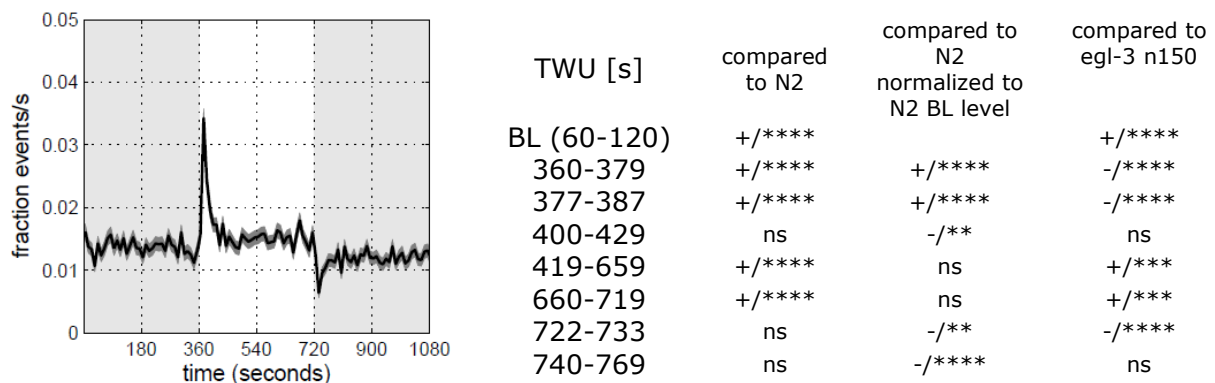
Neuropeptides from BAG do not influence any other part of the OT response of the oxygen sensing network apart from the DS response.

OT angles

- Angles below 21 degrees at TWU 1-360s are increased (14.4%), but not significantly compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (29.3%) compared to wt.

The turn angles below 21 degrees are not significantly higher than wt, but lower than in the *egl-3 n150* mutant. This points towards at least two different neuropeptides in balance required for the proper inhibition of narrow turning angles. The decrease of the turn angles above 120 degrees are comparable to the *egl-3 n150* mutant.

egl-3 n150 AQR/URX cell kill strain ZIM301



The OT baseline difference is not caused by neuropeptides absent in the *egl-3 n150* mutant. There is no difference between N2 and *egl-3 n150* AQR/URX⁻ BL on this behalf. This can be explained by normal transmission of the activity of AQR/URX and inhibition of OTs by a downstream neuron or by neuropeptides that are still present in the *egl-3 n150* neuropeptide mutant.

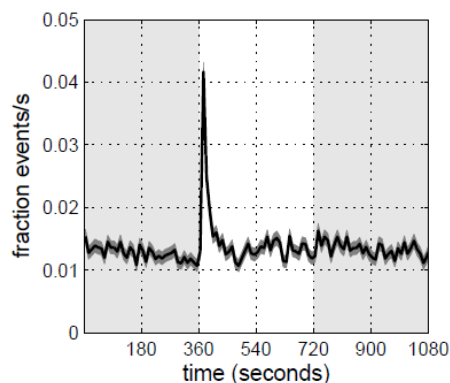
Neuropeptides from AQR/URX or from a downstream neuron are involved in the inhibition of the amplitude of the initial DS OT response.

OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (23.9%) compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (17.5%), compared to wt.

The decrease in flat turn angles is even more severe in the *egl-3 n150* AQR/URX cell kill line than in the *egl-3 n150* mutant. Neuropeptides still present in this mutant and expressed in AQR/URX or a close downstream are responsible for promoting flat turn angles.

egl-3 n729 strain MT1541



TWU [s]	compared to N2	compared to N2 normalized to N2 BL level	compared to egl-3 n150
BL (60-120)	+ /****		+ /****
360-379	+ /****	+ /****	- /***
377-387	+ /****	+ /****	- /****
400-429	ns	- /**	ns
419-659	+ /***	- /**	ns
660-719	+ /****	ns	ns
722-733	ns	ns	- /**
740-769	ns	- /**	ns

The difference to N2 suggests, that neuropeptides absent in egl-3 n729 are suppressing the OT BL. As was shown in the egl-3 n150 mutant, the absence of neuropeptides removes the decrease of OTs upon DS and leads to an immediate increase in OTs without delay. Since this mutant has been shown the most severe neuropeptide depletion it is reasonable to assume, that the US response is dependent solely on neuropeptides.

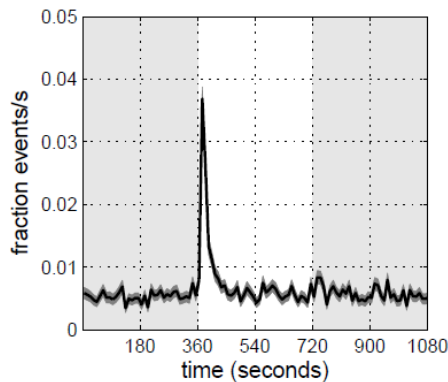
OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (31.8%) compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (16.6%), compared to wt.

The increase in narrow turning angles and the decrease in flat angles is even more pronounced in the egl-3 n150 mutant strain. Neuropeptides seem to promote flat angles while inhibiting narrow angles.

Turning angles leading to an escape after a turn are present and enhanced if neuropeptides are absent. As has been seen in the OT behavior of neuropeptide depleted mutants, there is a robust escape response that is modified by neuropeptides to mediate fine tuned searching behavior.

sbt-1 ok901 strain RB987



TWU [s]	compared to N2	compared to egl-3 n150
BL (60-120)	ns	ns
360-379	+ /****	- /****
377-387	+ /****	- /*
400-429	- /*	- /*
419-659	- /***	- /****
660-719	ns	- /****
722-733	ns	- /****
740-769	- /****	- /****

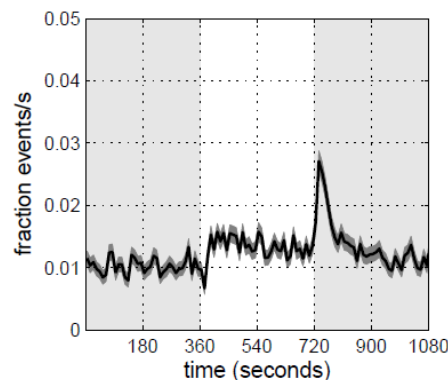
The OT BL turning rate is not significantly different than wt but significantly lower than the n729 mutant. The interpretation would be, that a neuropeptide present in n150 and sbt-1 but absent in n729 is responsible for suppressing the basal OT rate. The higher BL of the N2 AQR/URX⁻ is phenocopied in the behavior of n729 which leads to the assumption, that a neuropeptide from AQR/URX are responsible for the suppression of OT without oxygen stimulus.

OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (19.4%) compared to wt.
- Angles above 120 degrees at the same TWU are significantly decreased (31.0%), compared to wt.

Turn angles below 21 degrees are significantly in- and angles above 120 degrees significantly decreased, even though the differences are weaker compared to the other egl-3 mutants.

flp-1 ok2811 strain ZIM144



TWU [s]	compared to N2	compared to N2 normalized to N2 BL level
BL (60-120)	+ /****	
360-379	ns	ns
377-387	+ /**	ns
400-429	ns	ns
419-659	+ /****	ns
660-719	+ /****	ns
722-733	+ /****	+ /**
740-769	+ /****	+ /****

When N2 AQR/URX⁻, n150 AQR/URX⁻, n729 and flp-1 OT BL are compared, there is no significant difference between them.

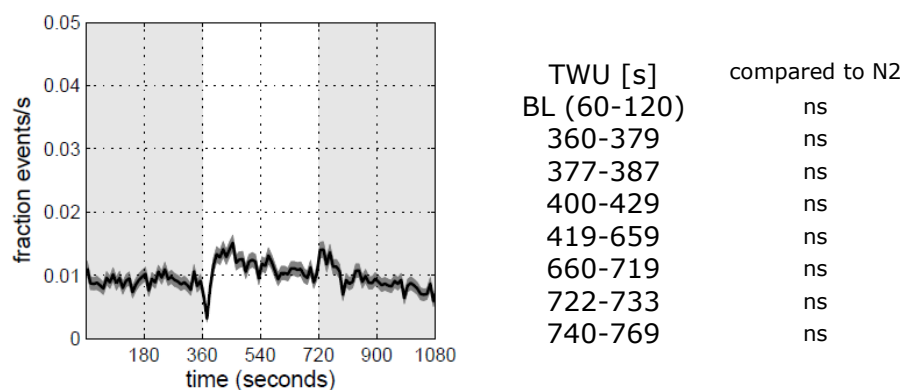
It has been shown, that flp-1 has an effect on body posture. Worms having a flp-1 defect show an increased body wave amplitude. Worms over expressing flp-1 show a very flat body posture (Nelson et al, 1998).

OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (23.1%) compared to wt and are significantly increased to egl-3 n150 but are still significantly lower than egl-3 n729.
- Angles above 120 degrees at the same TWU are significantly decreased (22.2%), compared to wt, are significantly decreased compared to egl-3 n729 but are not significantly different from egl-3 n150.

flp-1 is involved in lowering narrow turns and are involved in increasing flat turn angles, but flp-1 is not the only neuropeptide involved.

flp-8 pk360 strain flp-8 pk360



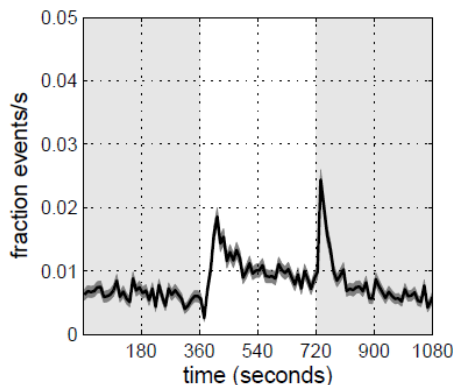
This mutant has been tested to check for involvement in the URX US response. The results suggest, that flp-8 does not have a part in either DS or US response.

OT angles

- Angles below 21 degrees at TWU 1-360s are not significantly different from wt.
- Angles above 120 degrees at the same TWU are not significantly different from wt.

flp-8 is not involved in modifying turning angles.

flp-10 n4543 strain MT14676



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	+/*
740-769	ns

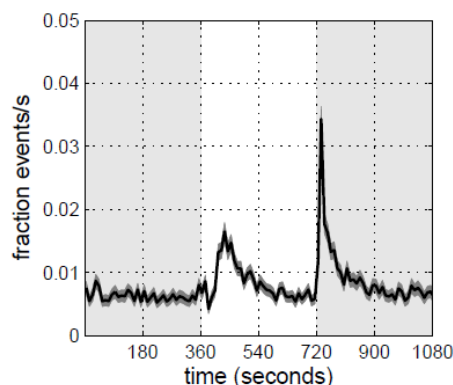
Even though the average wt and flp-10 mutant plots look different, the differences are not significant. flp-10 does not seem to be involved in the OT BL or in the OT DS responses. The US response however is significantly increased, flp-10 could play a role in inhibiting the US response.

OT angles

- Angles below 21 degrees at TWU 1-360s are not significantly different from wt.
- Angles above 120 degrees at the same TWU are not significantly different from wt.

flp-10 is not involved in modifying the turning angles.

flp-17 n4894 strain MT15933



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	+/*****
740-769	ns

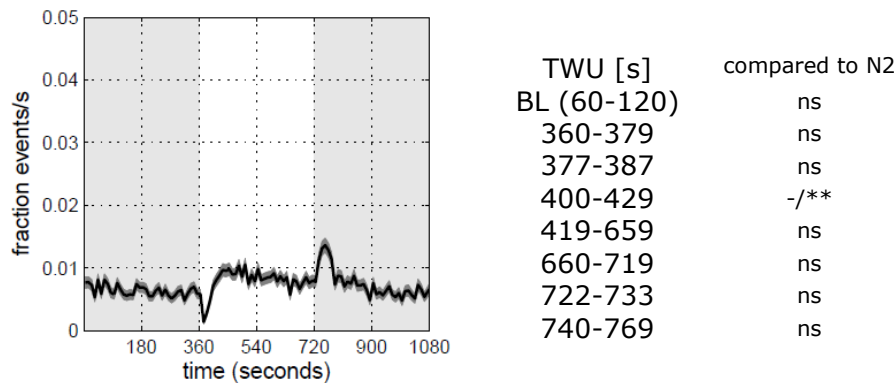
This result is unexpected. It looks like the absence of flp-17 which has been shown to be only in BAG and when BAG is inactivated increases OTs upon US. flp-17 could have a long lasting effect. After a release upon DS, it suppresses OTs when BAG is no longer active and an US occurs, to make sure, that animals do not turn back to where they came from and the DS has taken place.

OT angles

- The turn angles below 21 degrees are not significantly different from wt.
- The turn angles above 120 degrees are not significantly different from wt.

flp-17 is not involved in modifying turn angles.

flp-10 n4543 x flp-17 n4894 strain MT15973



The OT DS response is missing in the flp-10xflp-17 double mutant. What is unexpected is, that the US response is not different from the N2 response, while the individual mutants both show a significant increase.

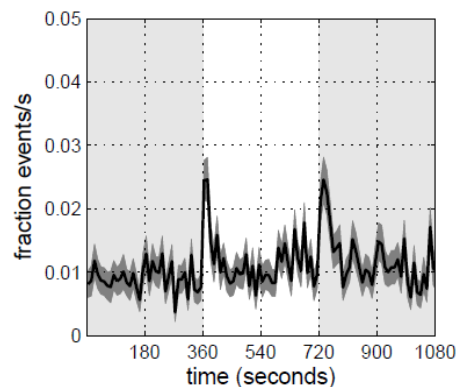
OT angles

- Angles below 21 degrees at TWU 1-360s are significantly increased (20.0%) compared to wt, are not significantly different from egl-3 n150 and are significantly lower than egl-3 n729.
- Angles above 120 degrees at the same TWU are significantly decreased (26.1%), compared to wt, and are significantly increased compared to egl-3 n150 and egl-3 n729.

Either flp-10 or flp-17 are definitely one of the neuropeptides required to suppress tight turning angles and are the ones that are absent in the n150 and sbt-1 mutants. If we take flp-1 into account it still does not add up to the huge increase of narrow turns in the egl-3 n729 mutant. There probably are at least three different neuropeptides responsible for suppressing narrow turns, but flp-10/17 and flp-1 are definitely involved.

Furthermore flp-10/17 are also involved in promoting flat angles instead. But because egl-3 n150 and egl-3 n729 are significantly lower, there have to be other neuropeptides involved as well. The problem is, that flp-10 and flp-17 alone do not display a turning angle pattern different from wt. Only if both of them are missing, the turning angles change.

egl-3 n150 Pflp-17::egl-3 strain ZIM284



TWU [s]	compared to N2	compared to egl-3 n150
BL (60-120)	ns	ns
360-379	+ / ****	- / *
377-387	+ / **	- / ****
400-429	ns	ns
419-659	ns	ns
660-719	+ / **	ns
722-733	+ / ****	ns
740-769	ns	+ / *

Neuropeptides from BAG decrease the amplitude of the DS response.

OT angles

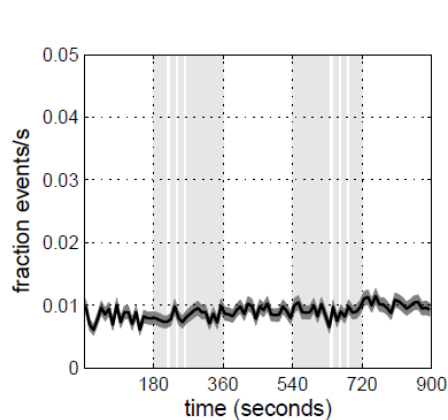
- Angles below 21 degrees are not significantly different from egl-3 n150
- Angles above 120 degrees are not significantly different from egl-3 n150

The turn angles are not significantly different from the egl-3 n150 mutant.

Neuropeptides from BAG are not involved in modifying the turning angles. This supports the turn flp-17 mutant data.

5.2.2 Omega turns in the ramp paradigm

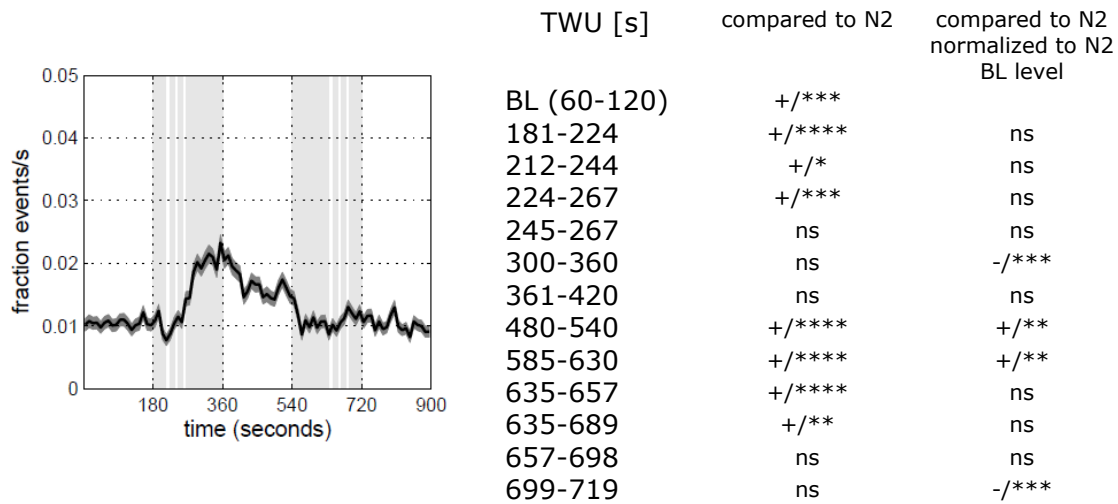
N2 BAG cell kill strain CX11697



TWU [s]	compared to N2
BL (60-120)	ns
181-224	ns
212-244	ns
224-267	ns
245-267	ns
300-360	- / ****
361-420	- / ****
480-540	ns
585-630	+ / ****
635-657	+ / *
635-689	ns
657-698	ns
699-719	ns

In the average plot there is no response to DR nor UR. BAG is required for the DR response. The inactivation of BAG could be required for the suppression of OTs upon start of the DR. This suppression might be required for the subsequent increase in OTs upon rising oxygen concentrations above 17%.

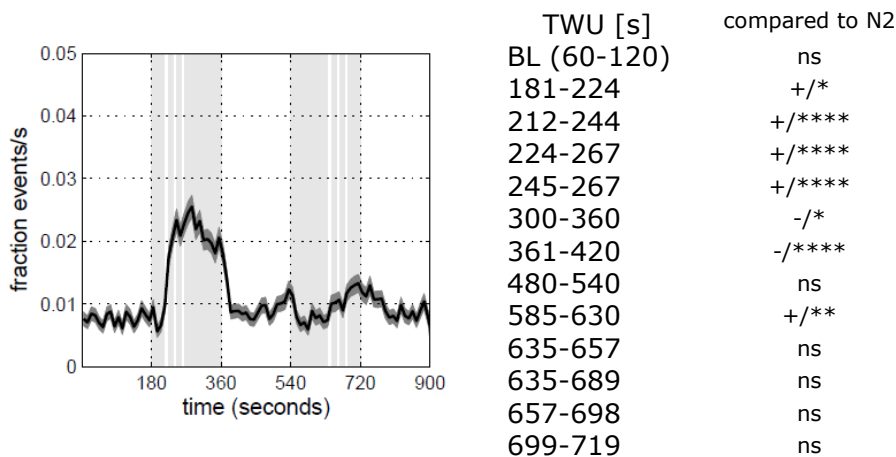
N2 AQR/URX cell kill strain CX7102



The DR response is not significantly different from wt, if it is BL corrected. The OT rate stays increased after the oxygen concentration stays constant at 4%. Even if AQR/URX are inactive, they are required to suppress OTs after a DR has occurred.

The UR response is missing. The inhibition of OTs upon onset of the UR is dependent on AQR/URX, even though URX is not active until a concentration of 13% oxygen has been passed. The UR suppression is also not dependent on BAG alone, but seems to require the presence of both BAG and AQR/URX.

egl-3 n150 strain Cx9191



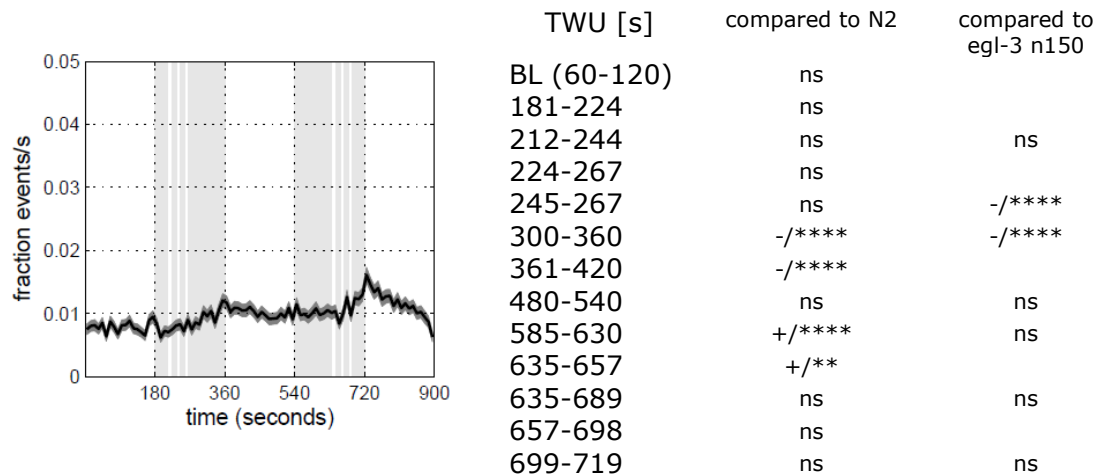
The inhibition of OTs at the start of the DR is missing as has been seen in the shift assays. This is not due to missing activity in BAG or URX, the neuronal activity in the egl-3 n150 mutant is not significantly different from N2.

As in the shift assays, the response starts sooner, the rise starts at the time an oxygen concentration of 18% is passed which coincides with an increase of activity in BAG. The amplitude of the DR response is not significantly different from the wt response, but the onset happens earlier. Also the increase in OTs drops earlier than in wt. The secondary increase of OTs which is mediated by the slow decay of BAG activity over time is missing

in the OT response like in the shift paradigm and is probably mediated by neuropeptides as well.

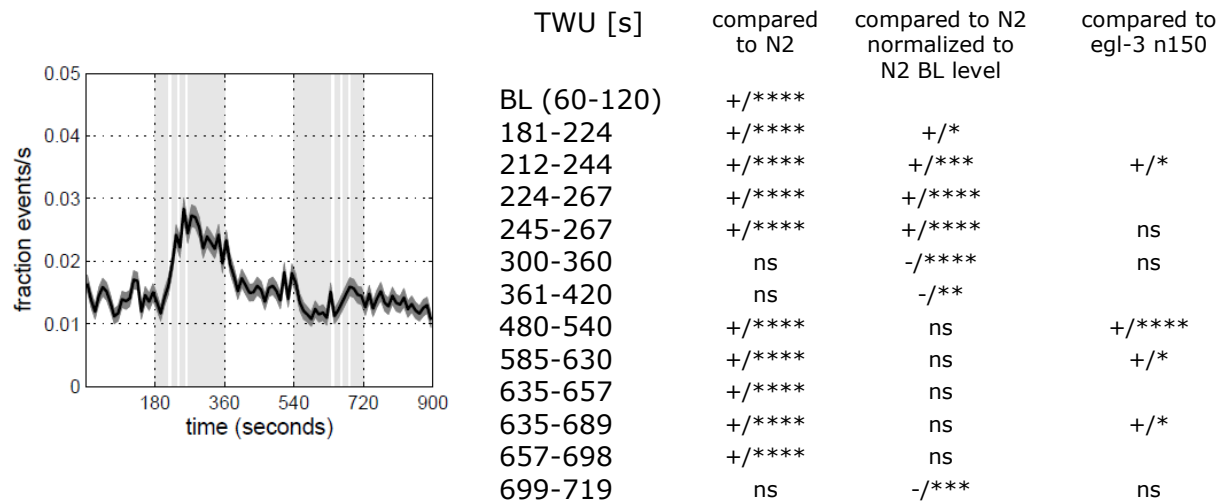
The decrease of OTs upon the onset of the UR is missing as well, only after 13% oxygen have been passed, the UR behavior is not different from wt any longer.

egl-3 n150 BAG cell kill strain ZIM300



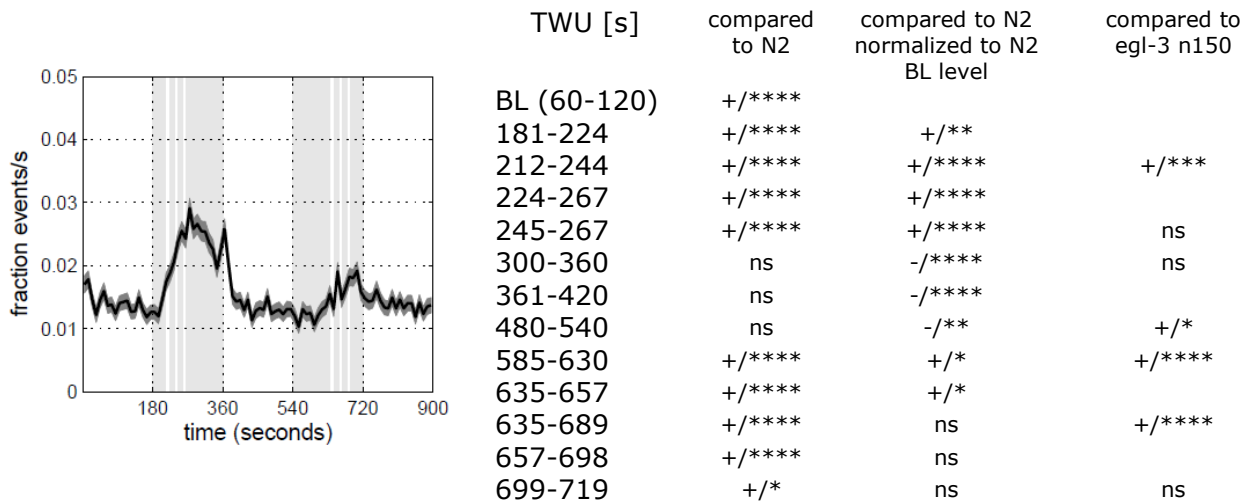
Except for the OT suppression at the UR onset, BAG is not required for the UR response. Furthermore, neuropeptides are not involved in modifying the further response to rising oxygen after 13% oxygen has been passed.

egl-3 n150 AQR/URX cell kill strain ZIM301



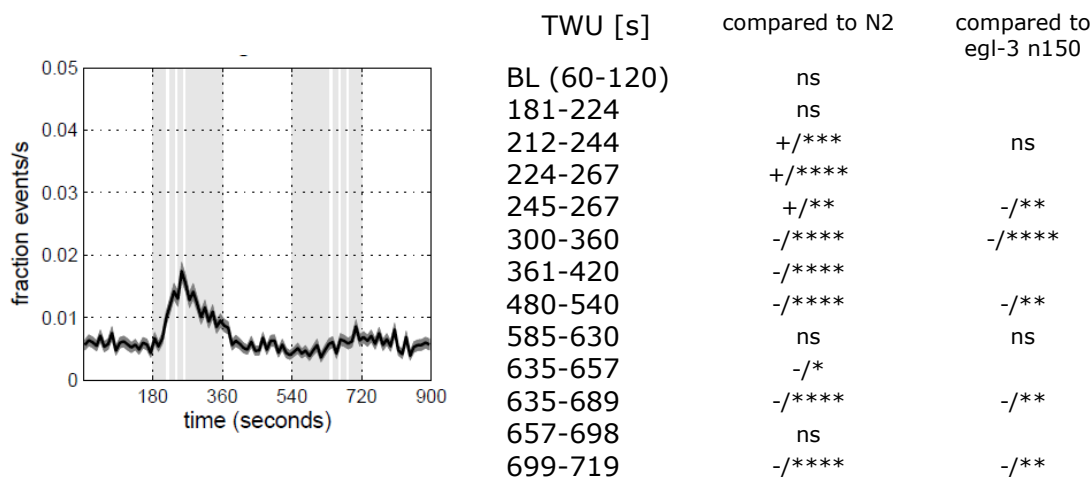
The drop of OTs after the onset of the UR seems to be due to the inactivation of BAG. A further suppression seems to require neuropeptides.

egl-3 n729 strain MT1541



After BL correction the OT rate is not significantly different from egl-3 n150 in any of the TWUs. Except for the increased BL, the egl-3 n729 mutant behaves like the egl-3 n150 mutant.

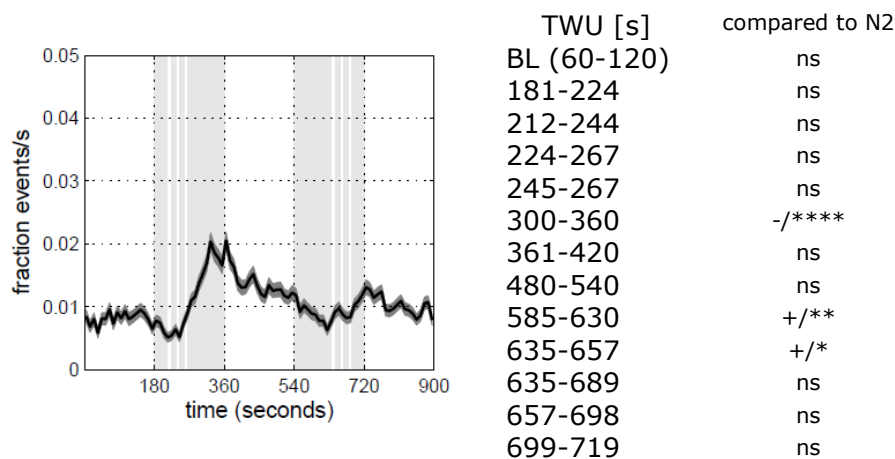
sbt-1 ok901 strain RB987



Compared to N2 the DR response occurs sooner, but the amplitude is significantly reduced. The onset of the DR response is the same as in egl-3 n150, but the amplitude is also significantly reduced. The secondary activity during 4% oxygen is significantly lower compared to N2.

At the start of the UR, the OT rate is not significantly different from N2, but that is not because the drop in OTs is present, but because the OT rate was already at this rate during the 4% period. The UR response after 13% oxygen is significantly reduced compared to N2.

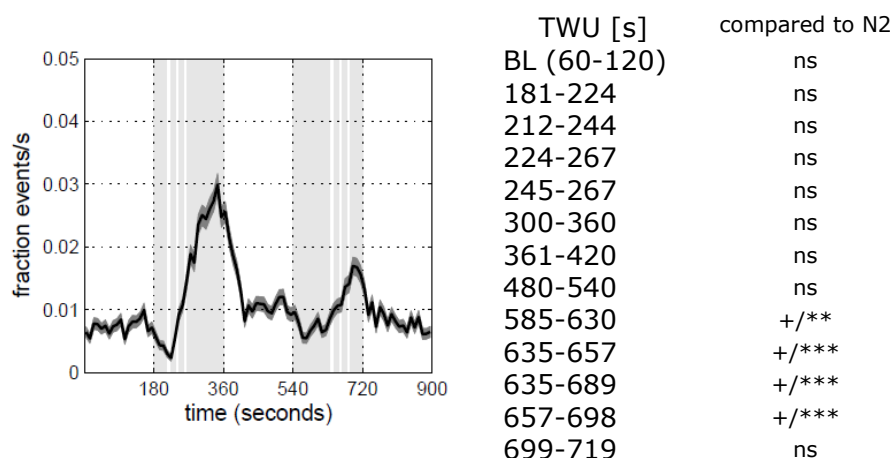
flp-8 pk360 strain flp-8 pk360



The DR inhibition is unchanged compared to N2, as is the increase in OTs after the 18% oxygen threshold has been passed. flp-8 is not involved in modifying search behavior in the DR. But the maximum amplitude at TWU 300-360s of the response is significantly lower than N2. flp-8 seems to play a role in promoting OTs in the network downstream of BAG. flp-8 is expressed in URX, AUA and RMG. Since URX is inactive during the time of the DR, it can be assumed, that flp-8 released from AUA or RMG strengthens the OT response after a DR has started. BAG gap junctions to RIR which is synaptically connected to AUA. Downstream targets of AUA are RIA and RIB which both synapse onto SMD and RMD neurons associated with turns.

flp-8 also shows a defect in the OT inhibition after the UR has started. What is puzzling is, that flp-8 is expressed in URX, which could in theory release flp-8 to inhibit turns at the beginning of the UR, but during this time URX is still inactive. If flp-8 is partially promoting turns via AUA, then flp-8 would no longer be released after BAG inactivates thus leading to a decrease in turns for as long as URX is inactive.

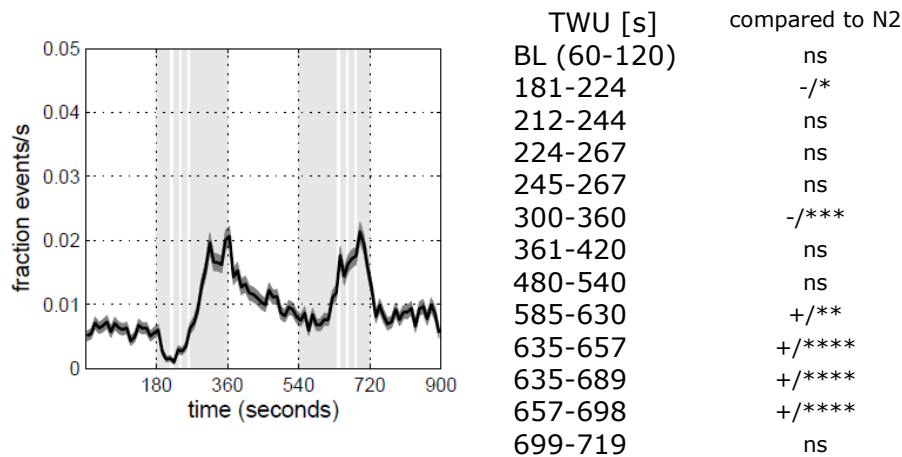
flp-10 n4543 strain MT14676



flp-10 seems to play a part in suppressing OTs upon the onset of the UR. Furthermore, the onset of the increase in OTs after 13% oxygen is sooner than in wt.

The average plots of wt and flp-10 look basically the same. The results of DR suppression and sooner increase after 13% oxygen has been passed are significant, but it could be, that there simply still are too few experiments. In the analysis of the shift paradigm, flp-10 did not seem to have an influence on the behavior and this could be the case here as well.

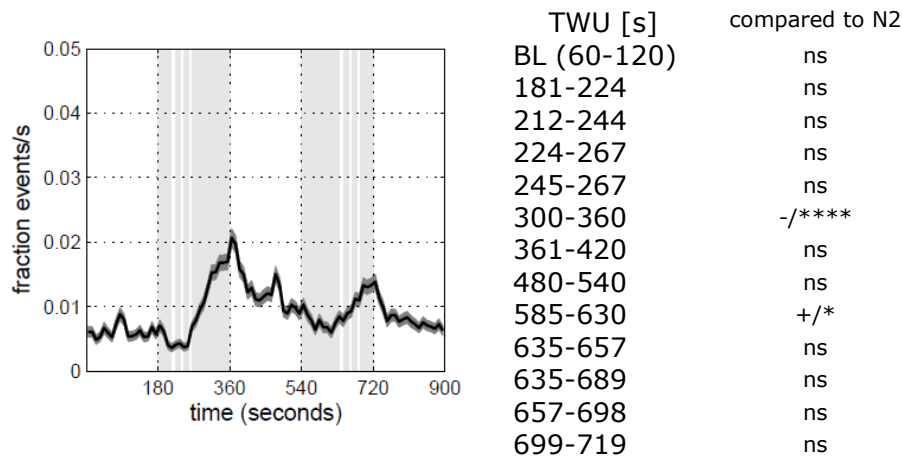
flp-17 n4894 strain MT15933



flp-17 seems to be involved in both DR and UR. In the DR its absence leads to a stronger OT inhibition compared to wt. The maximum DR response is significantly lower than the N2 response.

In the UR response, the inhibition is missing and the response is higher than the wt response. The results can be interpreted, that upon flp-17 release, the OT rate is increased counteracting the initial inhibition and supporting the later response. Once BAG is inactivated and flp-17 is no longer released, the loss of flp-17 leads to an overall decrease in OTs that's seen as an initial inhibition and then further limits the amplitude of the UR response.

flp-10 n4543 x flp-17 n4894 strain MT15973



- The OT rate at TWU 300-360s is not significantly different from the flp-17 mutant.
- The OT rate at TWU 585-630s is not significantly different from the flp-17 mutant.

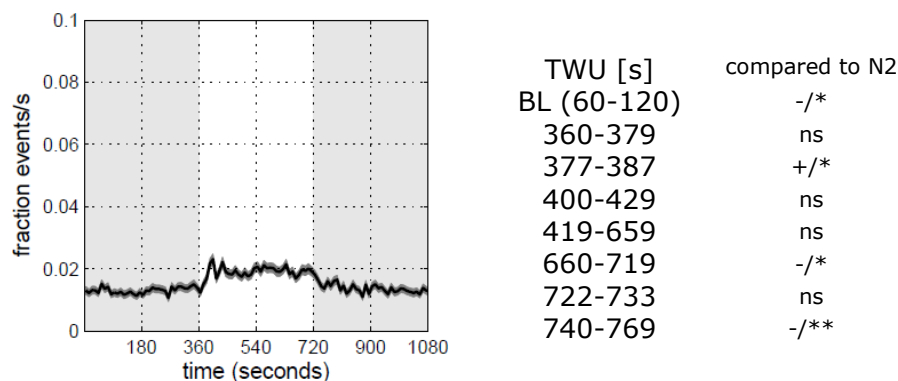
The increased suppression of the flp-17 mutant alone is no longer there.

At the DR OT response the effect of the flp-17 mutant is still there and not changed by the additionally missing flp-10.

The suppression of OTs after the onset of the UR is still higher than wt, but its not significantly different from either flp-10 or flp-17 alone. This effect is not additive.

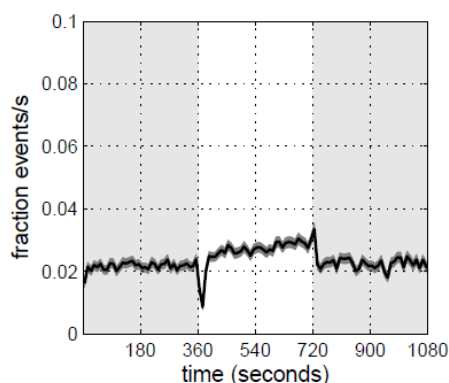
5.2.3 Shallow turns in the shift paradigm

N2 BAG cell kill strain CX11697



BAG is required to mediate the initial DS inhibition. The missing US inhibition can be attributed to the missing inactivation of BAG.

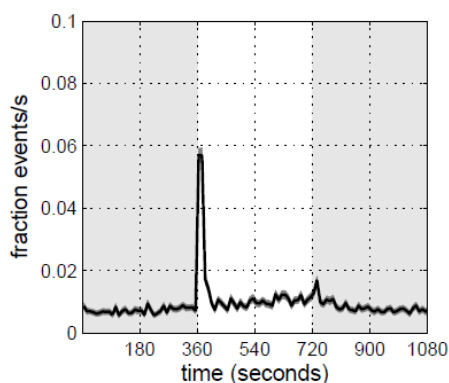
N2 AQR/URX cell kill strain CX7102



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	+/*
400-429	ns
419-659	ns
660-719	ns
722-733	+/**
740-769	ns

AQR/URX are required for the immediate US ST inhibition, but the ST rate returns to BL level without AQR/URX probably due to the inactivation of BAG. The DS inhibition is still present, but not as strong as when AQR/URX are there.

egl-3 n150 strain CX9191

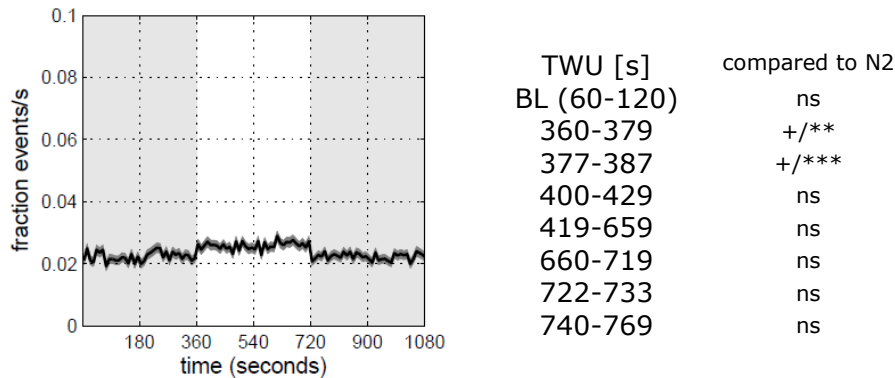


TWU [s]	compared to N2
BL (60-120)	-/**
360-379	+/**
377-387	+/**
400-429	-/**
419-659	-/**
660-719	-/**
722-733	ns
740-769	-/**

egl-3 n150 worms show a reduced rate of STs per animal compared to wt (11 STs per animal compared to 24/animal in wt). Neuropeptides missing in egl-3 n150 are required for STs. The speed of egl-3 n150 animals is significantly reduced compared to wt. The missing speed could be the reason for these animals not engaging in STs. The STs measured here take place during movement.

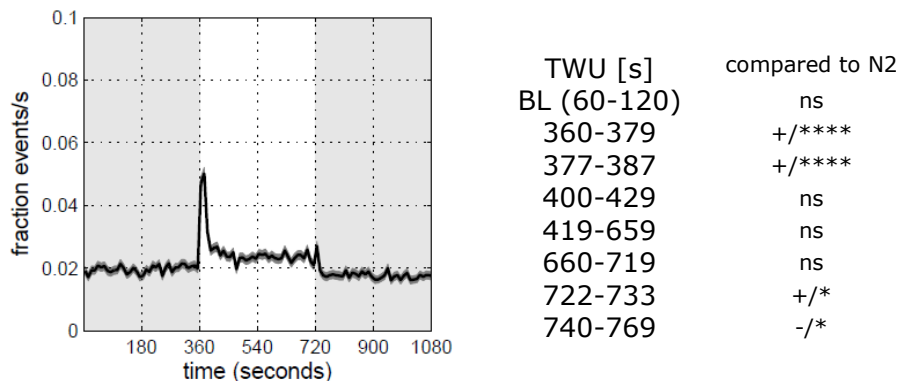
Neuropeptides are required to maintain the ST BL. The absence of neuropeptides leads to a drastic and significant decrease of STs. This happens at the same time as the increase of OTs. It seems, that a drop in oxygen elicits an immediate response leading to an increased rate in turns. At this point nearly all animals are engaged either in reversing, OTs or STs. Neuropeptides seem to suppress the "hardwired response" for a certain period of time to enable animals to engage in a less drastic response.

egl-3 n150 BAG cell kill strain ZIM300



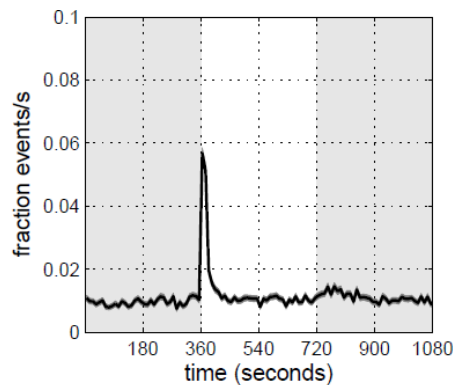
The strain behaves exactly as the N2 BAG cell kill strain. Compared to the egl-3 n150 mutant, the ST BL is at N2 level. The presence of BAG in a neuropeptide depleted background inhibits the ST BL.

egl-3 n150 AQR/URX cell kill strain ZIM301



BAG and AQR/URX are required to be present and active in the network, and only then the ST BL is inhibited probably by a downstream neuron, mediated by neuropeptides. Once one of the sensory neurons is missing, this inhibition is not longer active. This is fairly certain, because the ST BL is also significantly lower in the other neuropeptide depleted strains egl-3 n729 and sbt-1 ok901 where the oxygen sensing neurons are still active. The initial DS response is not different from egl-3 n150. AQR/URX do not seem to influence the DS ST response with or w/o neuropeptides. The US inhibition is missing, in this respect the egl-3 mutant is not significantly different from the N2 AQR/URX cell kill strain.

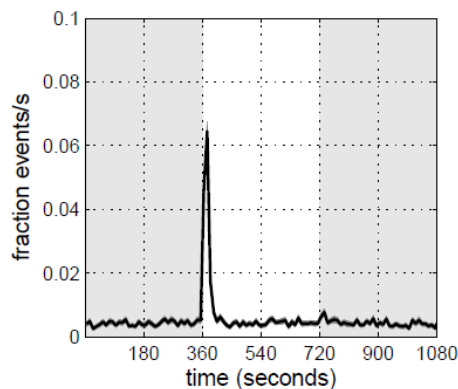
egl-3 n729 strain MT1541



TWU [s]	compared to N2
BL (60-120)	-/**
360-379	+/**
377-387	+/**
400-429	-/**
419-659	-/**
660-719	-/**
722-733	-/**
740-769	-/**

None of the TWUs are significantly different from egl-3 n150. None of the neuropeptides present in n150 but absent in n729 have an influence on the ST behavior.

sbt-1 ok901 strain RB987

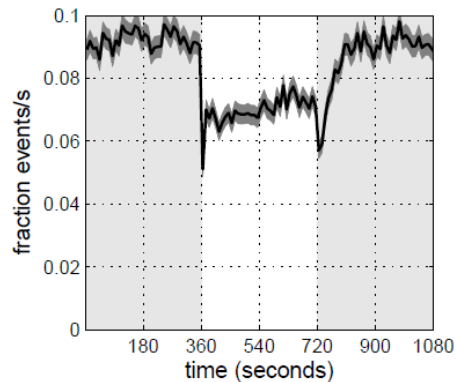


TWU [s]	compared to N2
BL (60-120)	-/**
360-379	+/**
377-387	+/**
400-429	-/**
419-659	-/**
660-719	-/**
722-733	-/**
740-769	-/**

Neuropeptides present in egl-3 n150 and egl-3 n729 but absent in sbt-1 ok901 are either still increasing the ST BL or neuropeptides present in sbt-1 ok901 but absent in egl-3 n150 and egl-3 n729 are further inhibiting the ST BL. There are at least two different neuropeptides involved in the adjustment of the ST BL.

The DS response at TWU 360-379s is not modified by any of the neuropeptides present in any of the neuropeptide mutants. This means, that neuropeptides absent in all of these mutants are responsible for the inhibition of the hardwired DS response.

flp-1 ok2811 strain ZIM144

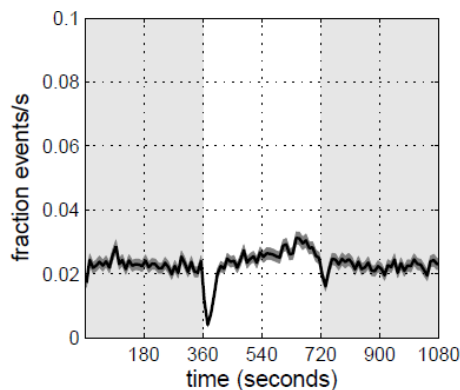


TWU [s]	compared to N2
BL (60-120)	+ /****
360-379	+ /****
377-387	+ /****
400-429	+ /****
419-659	+ /****
660-719	+ /****
722-733	+ /****
740-769	+ /****

The flp-1 mutant has a severe locomotive defect. This defect leads to "loopy" animals which show an abnormally low eccentricity. This in turn leads to the detection of STs where no STs actually take place. Even though a significant suppression of STs can be detected at the time of DS and US where the ST rate is significantly decreased.

flp-1 is therefore not the neuropeptide required to suppress STs at DS or US. The shape of the graph suggests, that activity of AQR/URX are required to increase the ST rate, after the DS where AQR/URX are inactive, the overall ST rate drops significantly and increases again after the US.

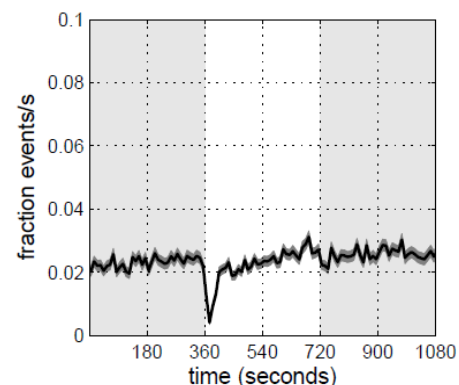
flp-8 pk360 strain flp-8 pk360



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-8 is not involved in suppression of increase of ST BL, DS or US response.

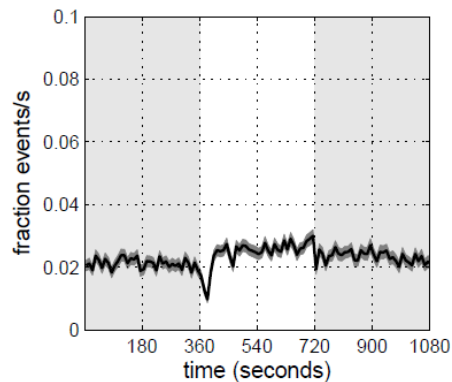
flp-10 n4543 strain MT14676



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-10 is not involved in modifying ST BL or shift responses.

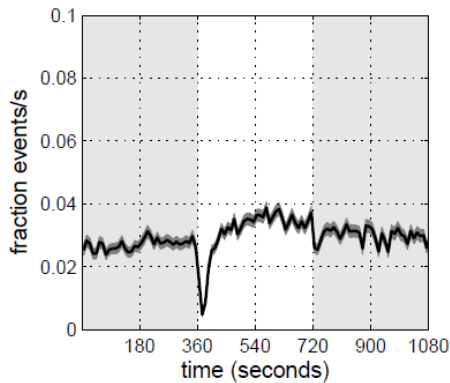
flp-17 n4894 strain MT15933



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-17 is not involved in modifying ST BL or shift responses.

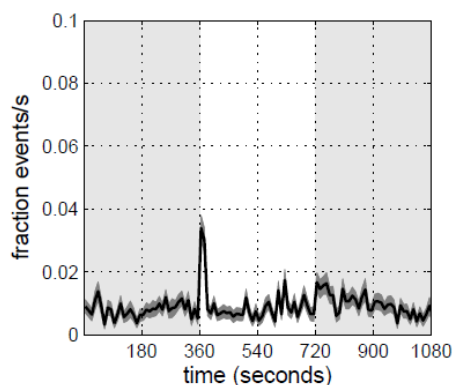
flp-10 n4543 x flp-17 n4894 strain MT15973



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-10 and flp-17 are not involved in any of the ST responses or BL.

egl-3 n150 Pflp-17:egl-3(09) strain ZIM284

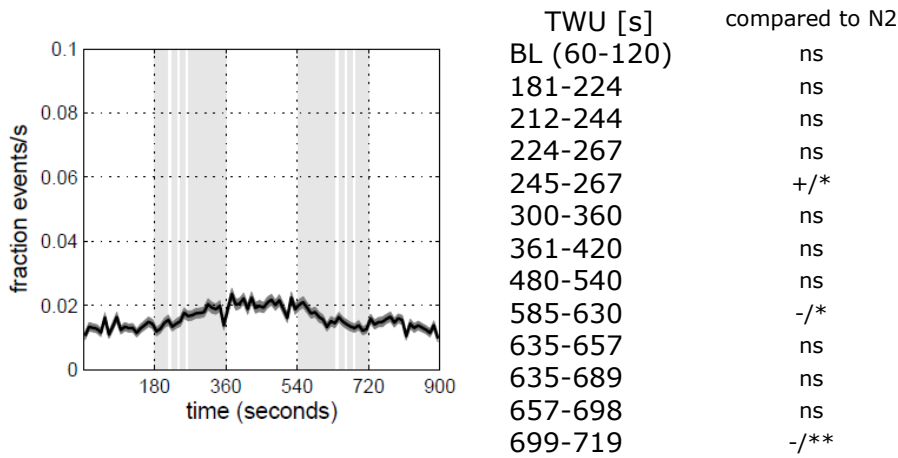


TWU [s]	compared to N2
BL (60-120)	-/**
360-379	+/**
377-387	ns
400-429	-/**
419-659	-/****
660-719	-/**
722-733	ns
740-769	ns

Neuropeptides from BAG have no influence on the ST BL. Neuropeptides from BAG itself do not induce the ST suppression, but lower the initial "hardwired" response.

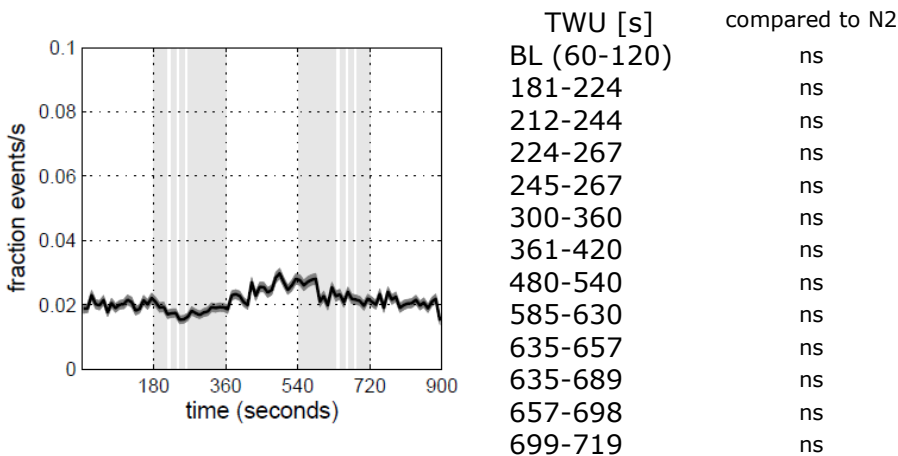
5.2.4 Shallow turns in the ramp paradigm

N2 BAG cell kill strain CX11697



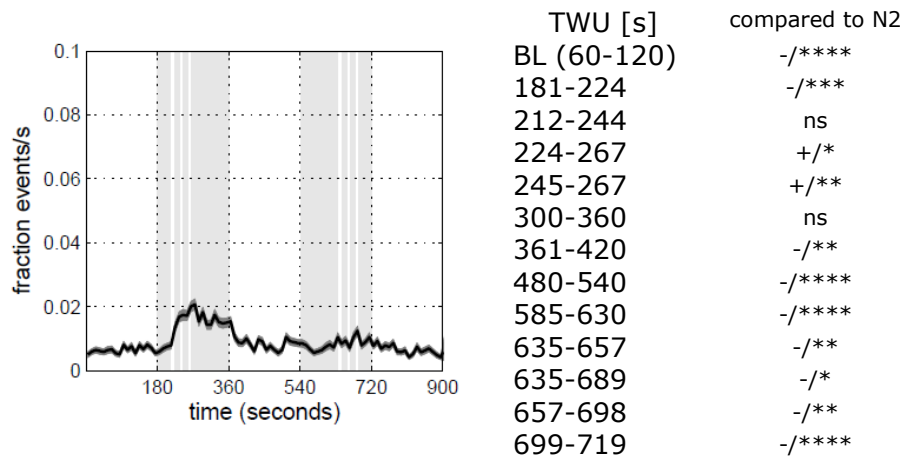
BAG is not involved in modifying the ST BL. BAG is required for sensing decreases in oxygen, the ST response is dependent on BAG activity.

N2 AQR/URX cell kill strain CX7102



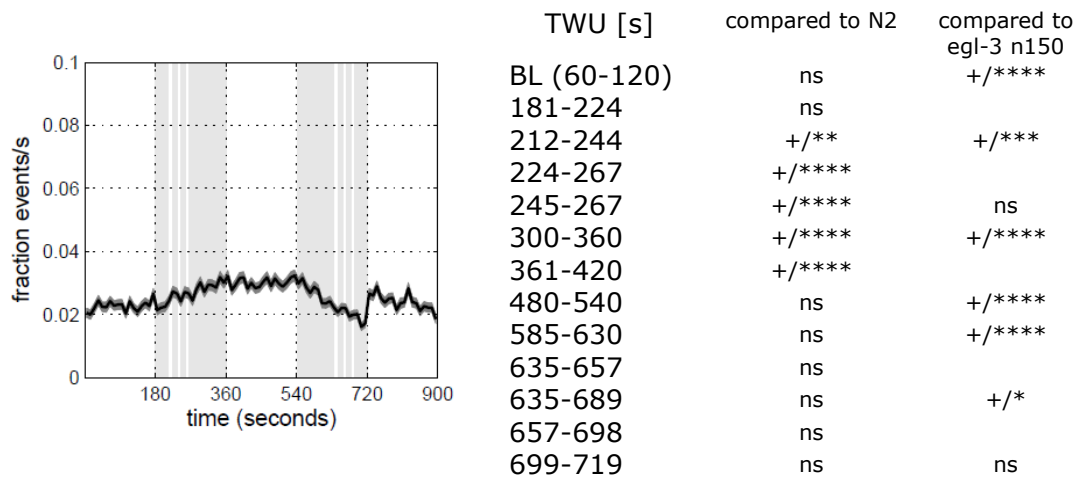
AQR/URX are not involved in modifying the ST BL. They also have no influence on the DR behavior. The UR behavior is unchanged compared to wt. It be, that AQR/URX are not required to facilitate the change of an increased ST rate back to BL after BAG is no longer active.

egl-3 n150 strain Cx9191



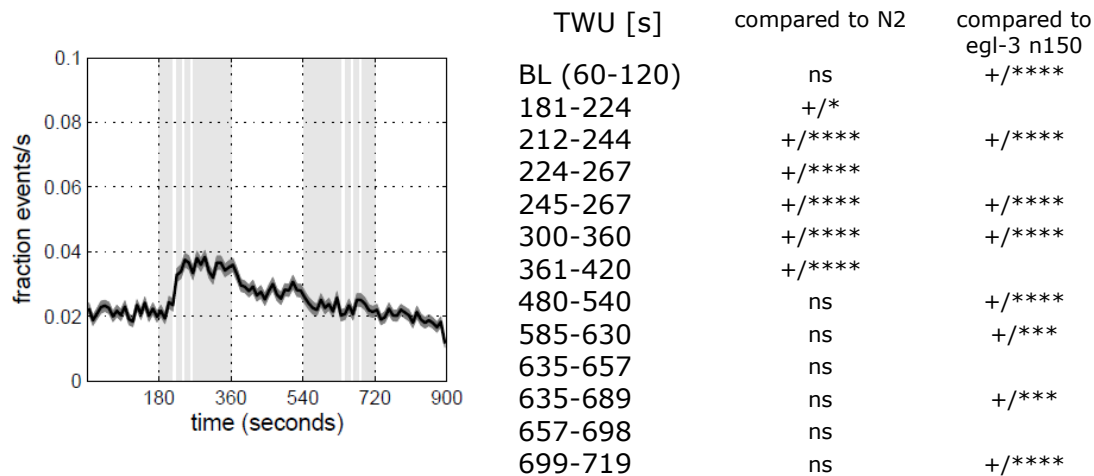
As discussed in the shift paradigm, neuropeptides are essential for maintaining the ST BL. After the oxygen concentration has passed 18%, the ST turning rate increases significantly and stays significantly increased, until the DR has stopped. As seen in the shift paradigm, BAG elicits an underlying ST response once BAG is active. This response is suppressed by neuropeptides. In the neuropeptide depleted mutant, animals are more likely to turn after they approach a preferred oxygen concentration. As in the shift paradigm, the UR does not show any response.

egl-3 n150 BAG cell kill strain ZIM300



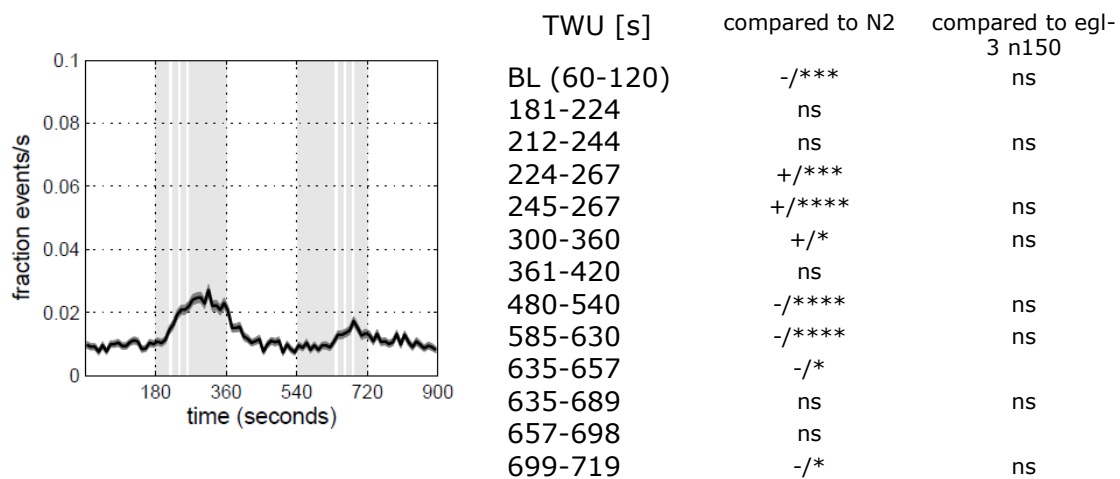
The differences in BL and non responsiveness to changes in oxygen have been discussed in the shift paradigm.

egl-3 n150 AQR/URX cell kill strain ZIM301



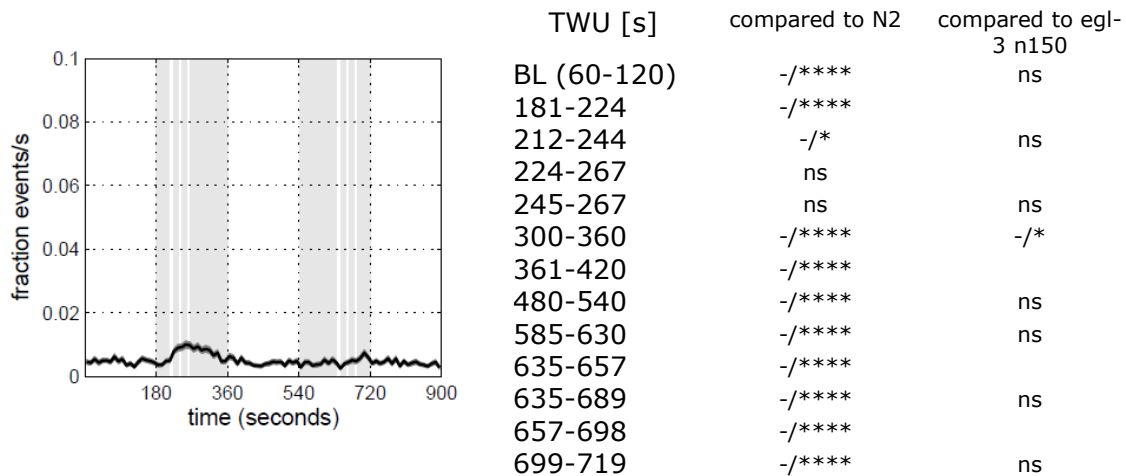
The ST BL rate relies on intact BAG, AQR and URX. If one of them is missing, the turning probability is significantly increased. This increase is additive to the underlying BAG response in response to decreases in oxygen. The DR turning response follows the neuronal activity of BAG in a neuropeptide depleted background.

egl-3 n729 strain MT1541



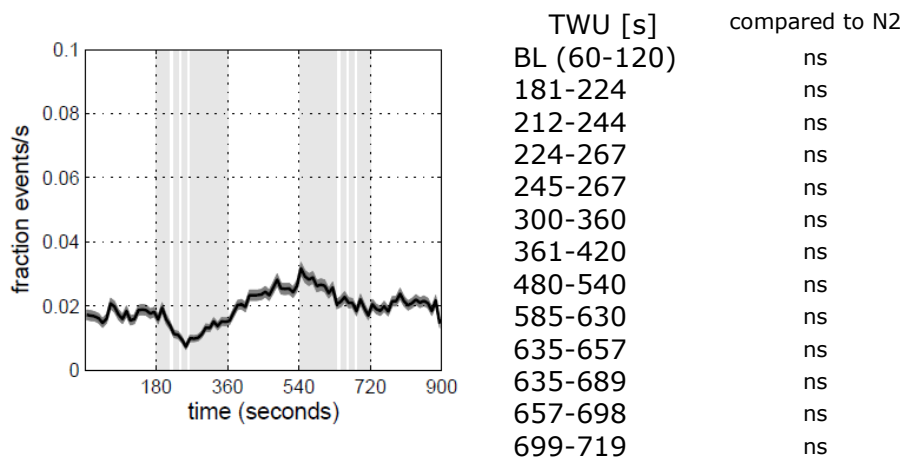
None of the neuropeptides present in egl-3 n150 but absent in egl-3 n729 have an influence on DR or UR behavior.

sbt-1 ok901 strain RB987



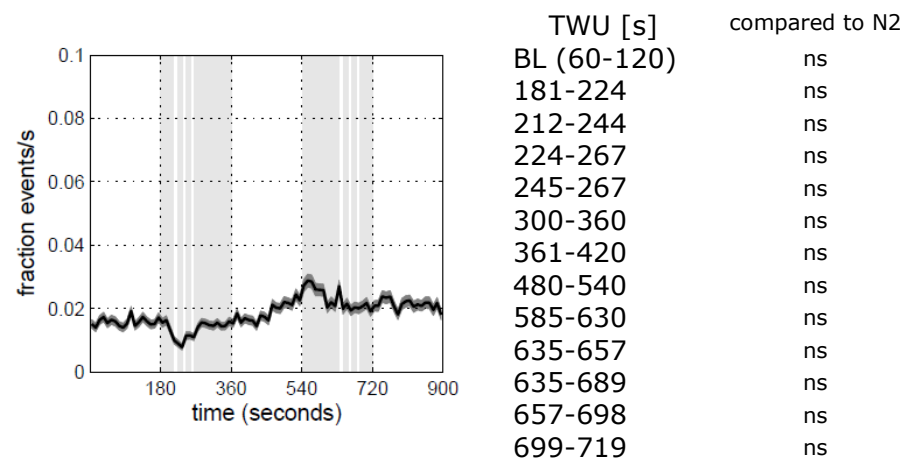
The only difference to the egl-3 n150 mutant is a lower DR response after 18% oxygen has been passed.

flp-8 pk360 strain flp-8 pk360



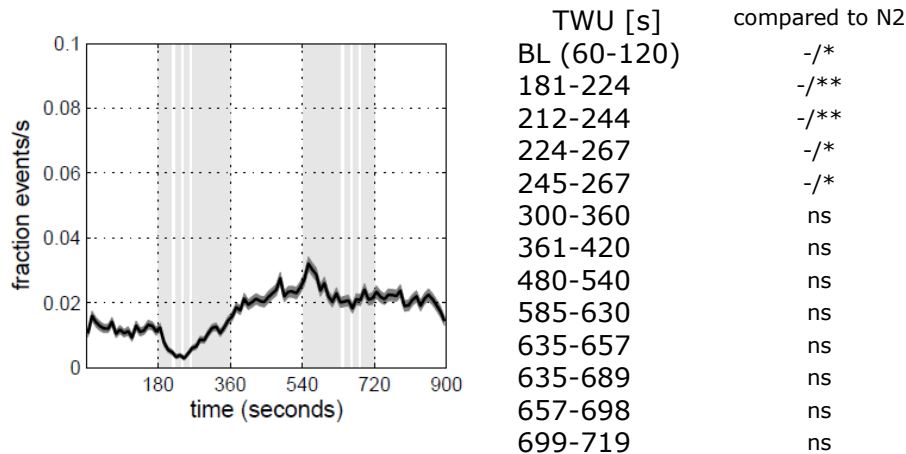
flp-8 is not involved in any ramp ST behavior.

flp-10 n4543 strain MT14676



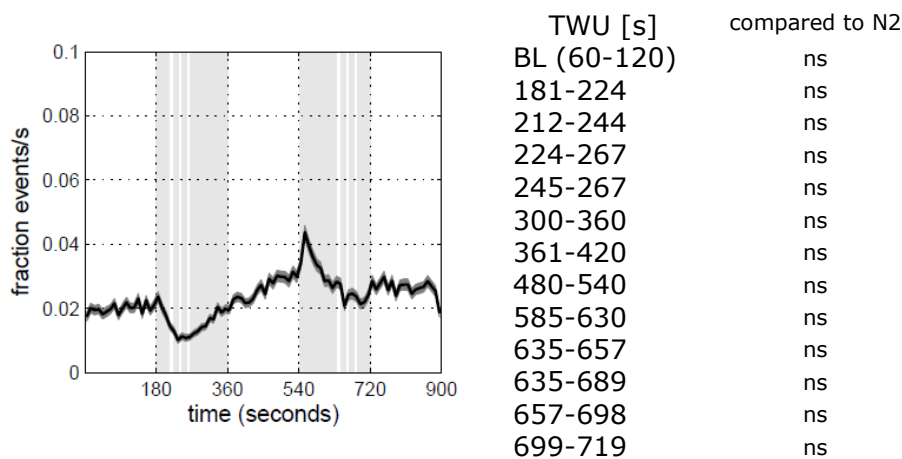
flp-10 is not involved in any ramp ST behavior.

flp-17 n4894 strain MT15933



flp-17 shows an inhibited the ST rate during the DR after 18% oxygen has been passed. This could be due to a decreased ST BL.

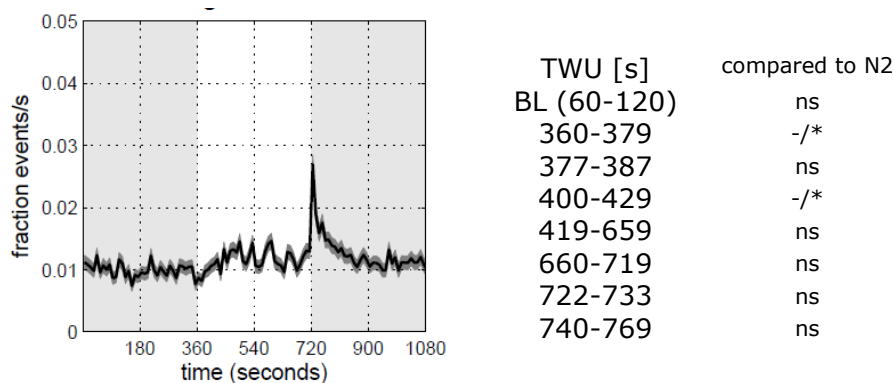
flp-10 n4543 x flp-17 n4894 strain MT15973



The combination of flp-10 and flp-17 is not involved in any ramp ST behavior.

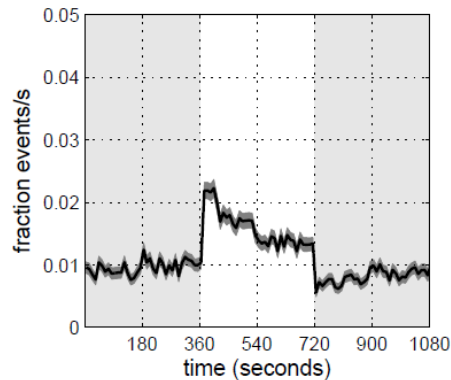
5.2.5 Large reversals in the shift paradigm

N2 BAG cell kill strain CX11697



Absence of BAG removes the DS LR response and does not influence the US response.

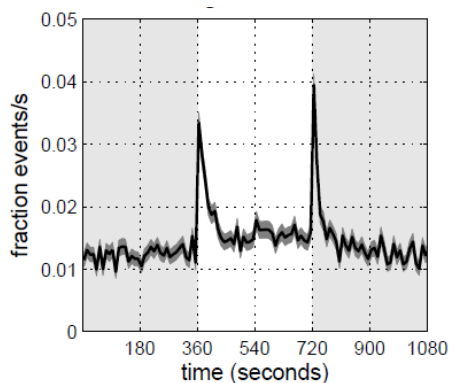
N2 AQR/URX cell kill strain CX7102



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	+/*
722-733	-/*
740-769	-/*

AQR/URX do not influence the BL LR rate. The US sensing neurons do not influence the initial LR DS response. The LR turning rate does not return to BL in the DS aftermath until an US occurs. The US LR response is completely missing. The LR rate returns to BL level immediately after the US occurs. This suggests, that there is either another neuron sensing oxygen USs inhibiting LRs at this point or the inactivation of BAG by the US leads to the drastic drop in LRs.

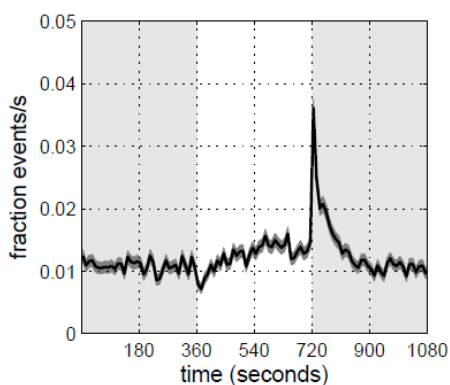
egl-3 n150 strain Cx9191



TWU [s]	compared to N2	compared to N2 normalized to N2 BL level
BL (60-120)	+/**	
360-379	+/*	+/*
377-387	+/*	ns
400-429	ns	
419-659	+/*	
660-719	+/*	ns
722-733	+/*	+/*
740-769	ns	ns

Neuropeptides inhibit the LR BL level, absence leads to a significant increase. Both DS and US response are significantly increased compared to wt level. These neuropeptides are not from BAG dependent, since the N2 BAG cell kill line shows not difference in the US response.

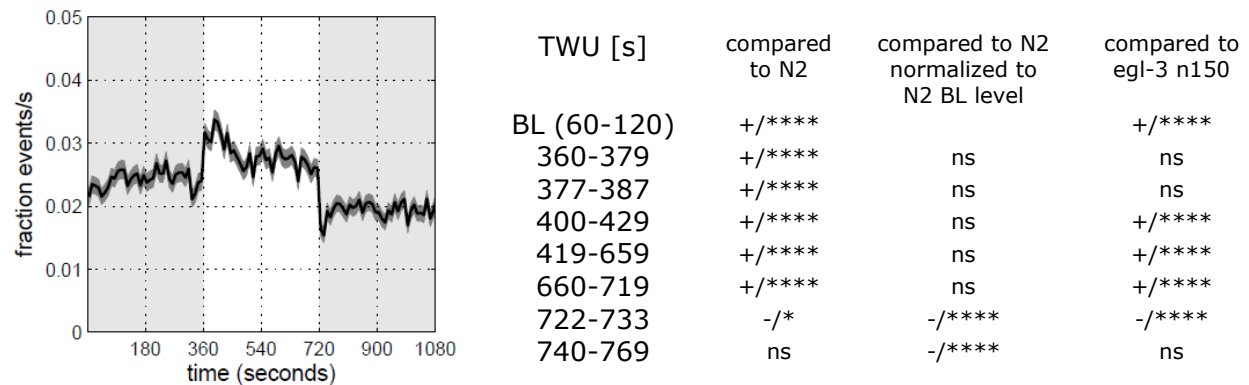
egl-3 n150 BAG cell kill strain ZIM300



TWU [s]	compared to N2	compared to egl-3 n150
BL (60-120)	+/*	ns
360-379	-/*	-/*
377-387	-/*	-/*
400-429	-/*	-/*
419-659	ns	ns
660-719	+/*	ns
722-733	+/*	ns
740-769	ns	ns

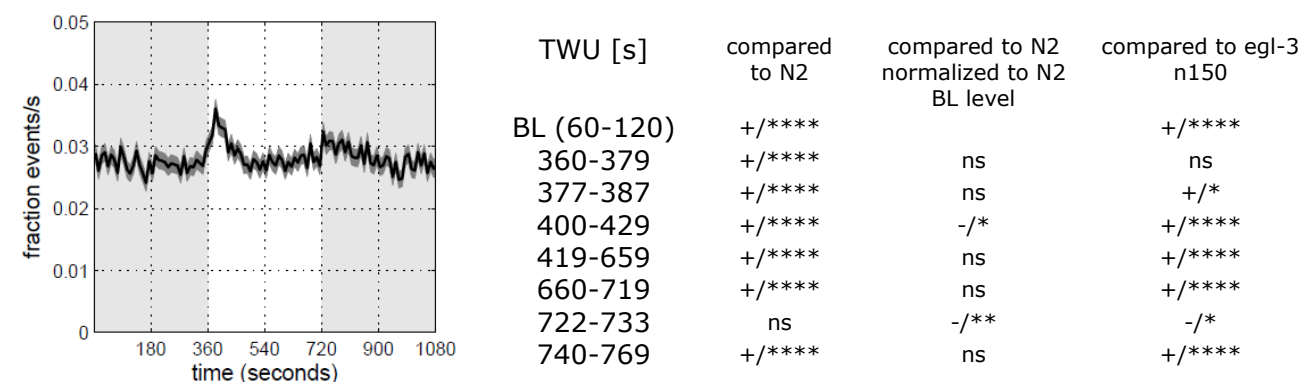
The LR DS response is significantly lower than wt but not significantly different from the N2 BAG cell kill strain.

egl-3 n150 AQR/URX cell kill strain ZIM301



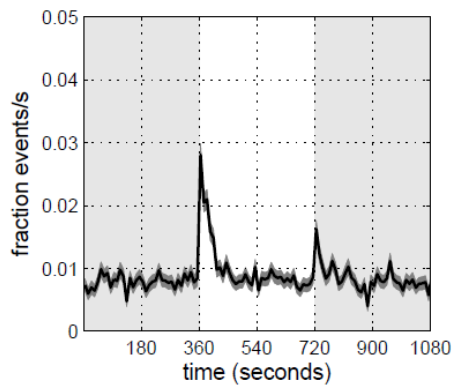
As discussed above, neuropeptides inhibit the LR BL. Additionally the activity of AQR/URX inhibit the LR BL as well. In the absence of both, the LR BL is nearly thrice as high as the wt BL. At the US a reduction of LRs takes place, after BAG is inactive. This reduction does not rely on neuropeptides absent in the egl-3 n150 mutant.

egl-3 n729 strain MT1541



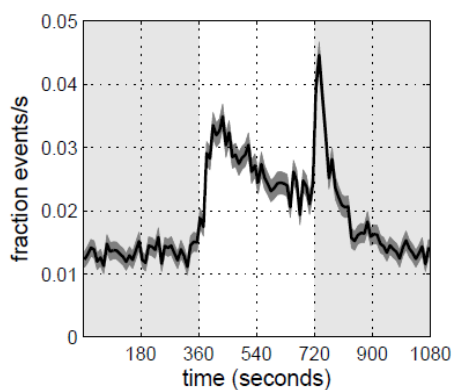
The LR BL is significantly increased compared to both N2 as well as egl-3 n150 LR BL. The LR BL level is dependent on more than one neuropeptide. The DS response of the egl-3 n1729 mutant is significantly higher than the egl-3 n150 response. If this increase is due to the higher BL is unclear. The US response at TWU 722-733s is not different from N2 but significantly lower than egl-3 n150. If an existing response is hidden within the increased BL is unclear.

sbt-1 ok901 strain RB987



TWU [s]	compared to N2	compared to egl-3 n150
BL (60-120)	ns	ns
360-379	+/**	ns
377-387	ns	ns
400-429	-/*	-/**
419-659	ns	-/**
660-719	ns	-/**
722-733	ns	-/**
740-769	-/*	-/**

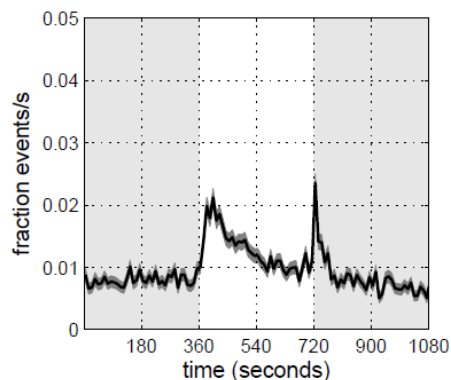
flp-1 ok2811 strain ZIM144



TWU [s]	compared to N2	compared to N2 normalized to N2 BL level
BL (60-120)	+/**	ns
360-379	ns	ns
377-387	+/**	ns
400-429	+/**	+/**
419-659	+/**	+/**
660-719	+/**	+/**
722-733	+/**	+/**
740-769	+/**	+/**

flp-1 inhibits both OT and LR BL level and reduces the LR DS and US responses. The LR BL is significantly lower than egl-3 n729 LR BL indicating, that flp-1 is only one of multiple neuropeptides inhibiting the BL.

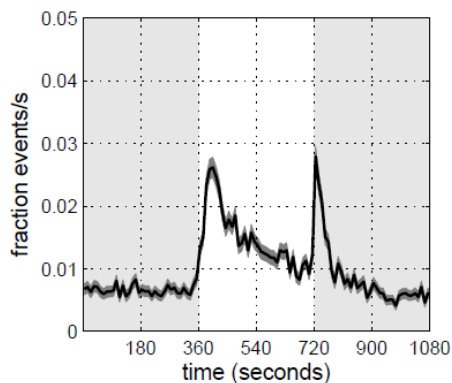
flp-8 pk360 strain flp-8 pk360



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-8 is not involved in mediating LRs in response to oxygen sensation.

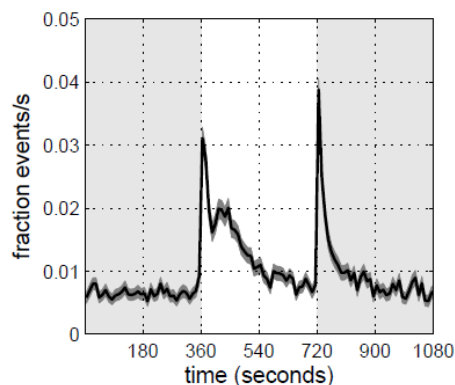
flp-10 n4543 strain MT14676



TWU [s]	compared to N2
BL (60-120)	ns
360-379	ns
377-387	+/*
400-429	+/*
419-659	ns
660-719	ns
722-733	ns
740-769	ns

flp-10 is not involved in modifying the LR baseline. Absence of flp-10 increases the DS response, but does not modify the US response.

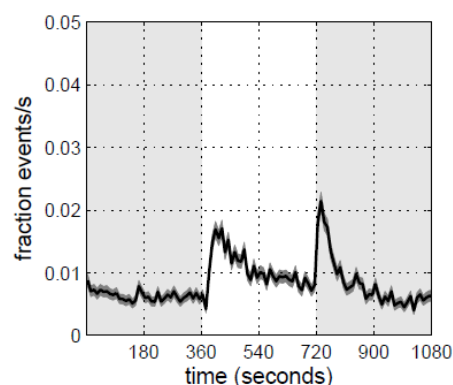
flp-17 n4894 strain MT15933



TWU [s]	compared to N2
BL (60-120)	ns
360-379	+/****
377-387	+/*
400-429	ns
419-659	ns
660-719	ns
722-733	+/***
740-769	ns

flp-17 is not involved in inhibiting the LR baseline. flp-17 seems to be involved in inhibiting the initial DS LR response within the first 10 seconds as well as inhibiting the US response.

flp-10 n4543 x flp-17 n4894 strain MT15973

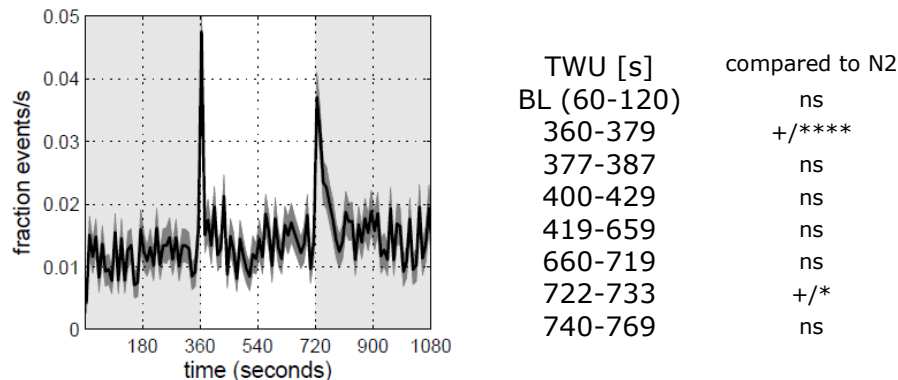


TWU [s]	compared to N2
BL (60-120)	ns
360-379	-/***
377-387	ns
400-429	ns
419-659	ns
660-719	ns
722-733	ns
740-769	ns

The LR response after the DS starts delayed compared to wt. Either flp-10 or flp-17 are required to induce the immediate increase in LR. Since this does not occur in the N2 AQR/URX cell kill line, flp-10 from URX is not responsible. Either flp-10 from another neuron or flp-17 from BAG itself are required to increase the LR response after a DS. The

flp-10 x flp-17 double mutant does not show an involvement in the DS or US LR response.

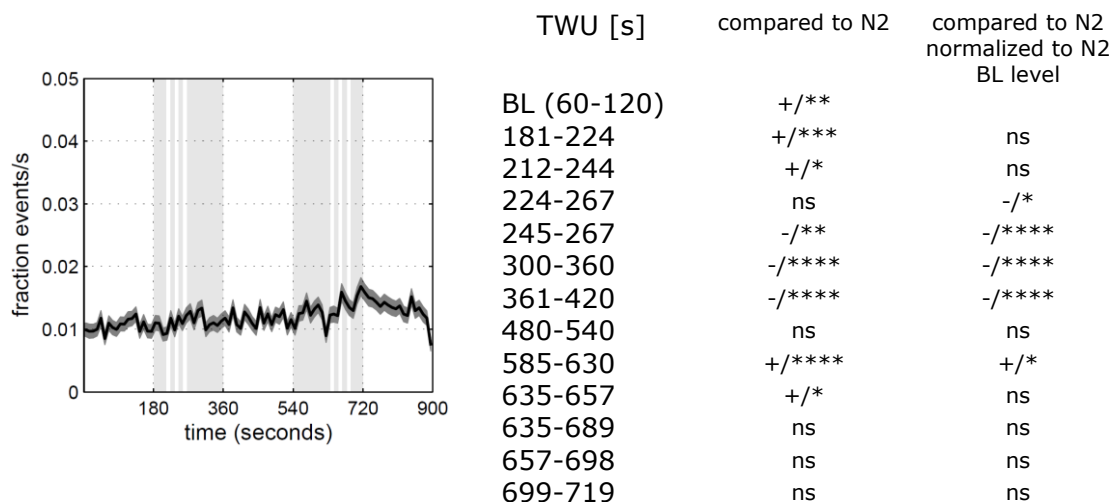
egl-3 n150 Pflp-17:egl-3(09) strain ZIM284



None of the LR responses are significantly different from the egl-3 n150 mutant, neuropeptides from BAG do not affect LR responses in the oxygen sensing network, neuropeptides in the LR response pathway have to be downstream from BAG.

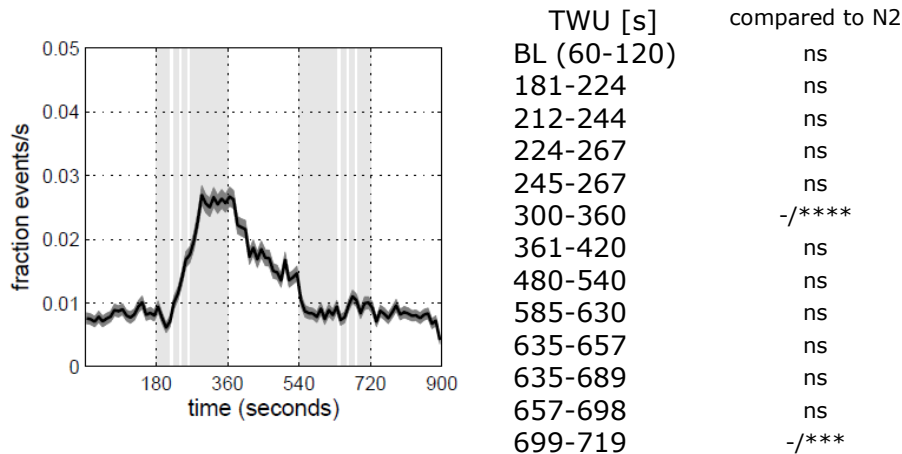
5.2.6 Large reversals in the ramp paradigm

N2 BAG cell kill strain CX11697



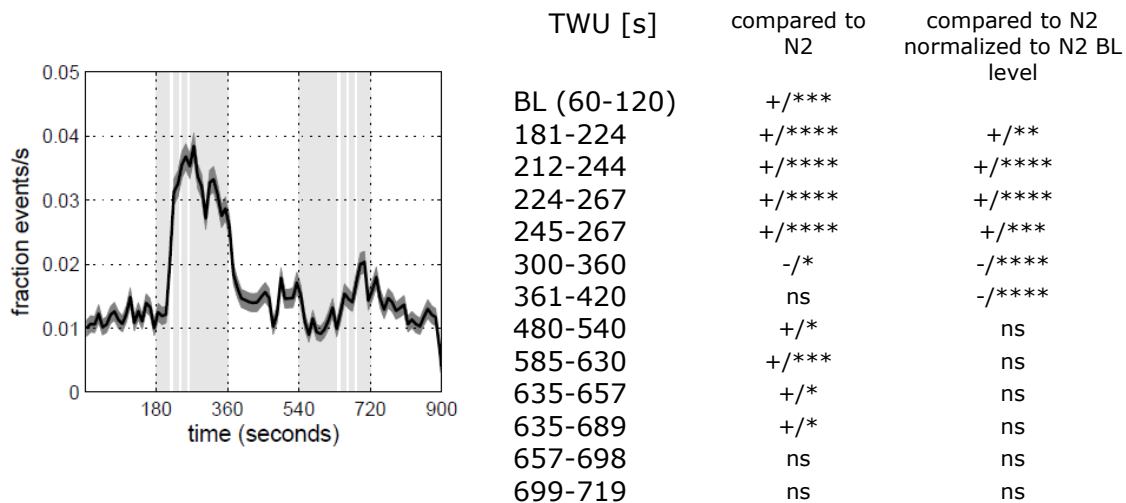
The DR response requires BAG. In addition, the drop in LRs after the start of the UR is missing as well, since the inactivation of BAG while AQR/URX are still not active does not take place. Sensation and reaction to a drop in oxygen is required to for a drop in OTs or reversals upon a following increase in oxygen to enable animals to identify the preferred oxygen concentration.

N2 AQR/URX cell kill strain CX7102



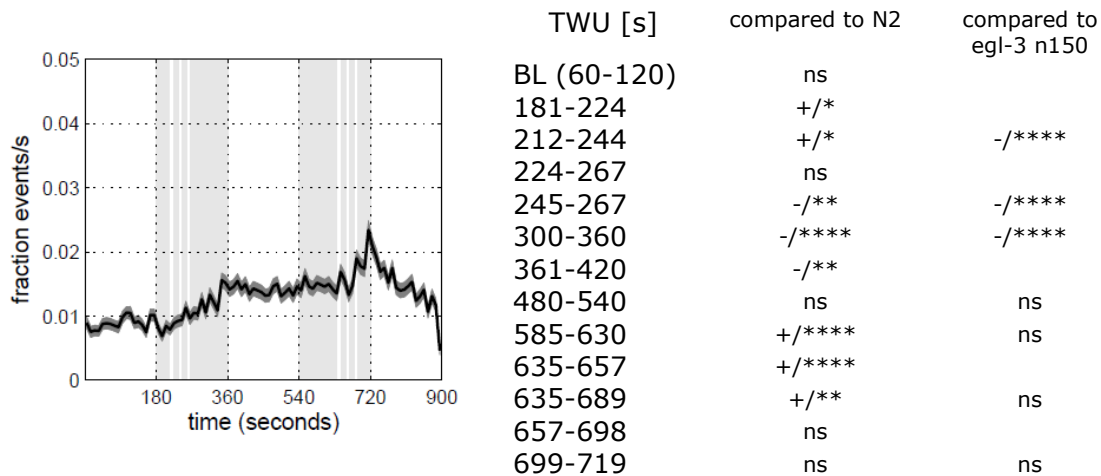
AQR/URX influence the amplitude of the DR LR response after the preferred oxygen concentration has been passed. AQR/URX are required for the UR LR response after the oxygen concentration has passed 13% oxygen.

egl-3 n150 strain Cx9191



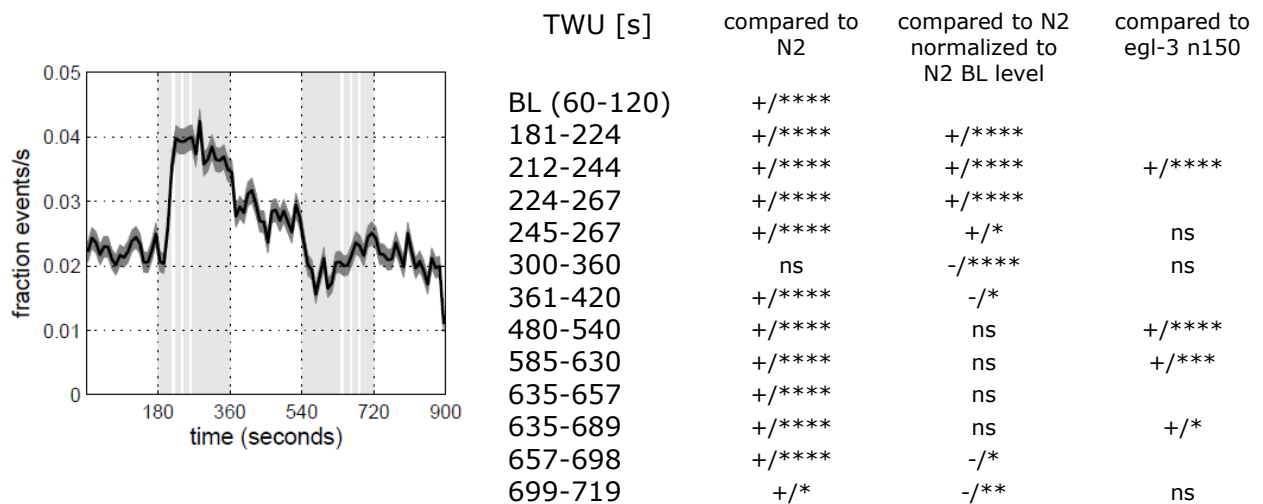
The drop in LRs at the start of the DR is missing replaced by a significant increase in LRs. This increase continues throughout the DR, even though the maximum response is shifted to an earlier time compared to wt. The amplitude of the DR response of N2 and the egl-3 n150 mutant are not significantly different, neuropeptides absent in this mutant are not responsible for the height of the DR response. The modification of the DR response in the N2 AQR/URX cell kill line seems to be not dependent on neuropeptides. If normalized to N2 BL level, the UR response seems to be unaffected by the lack of neuropeptides.

egl-3 n150 BAG cell kill strain ZIM300



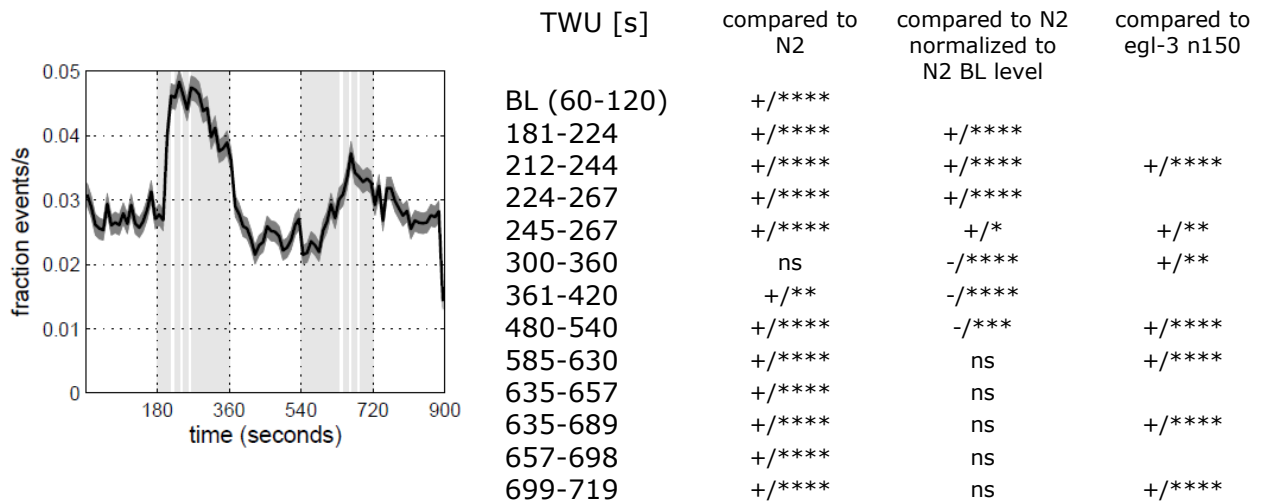
The LR rate is not significantly different from the respective TWUs in the N2 BAG cell kill line.

egl-3 n150 AQR/URX cell kill strain ZIM301



The drop in LRs at the start of the DR is missing and the response starts earlier as compared to N2. All other responses are not different from the N2 AQR/URX cell kill line, if the LR rate is normalized to N2 BL level. Absent AQR/URX in a neuropeptide depleted background have no influence on DR or UR behavior. Neuropeptides possibly still present in the egl-3 n150 mutant and expressed in AQR/URX have no influence on the DR or UR behavior.

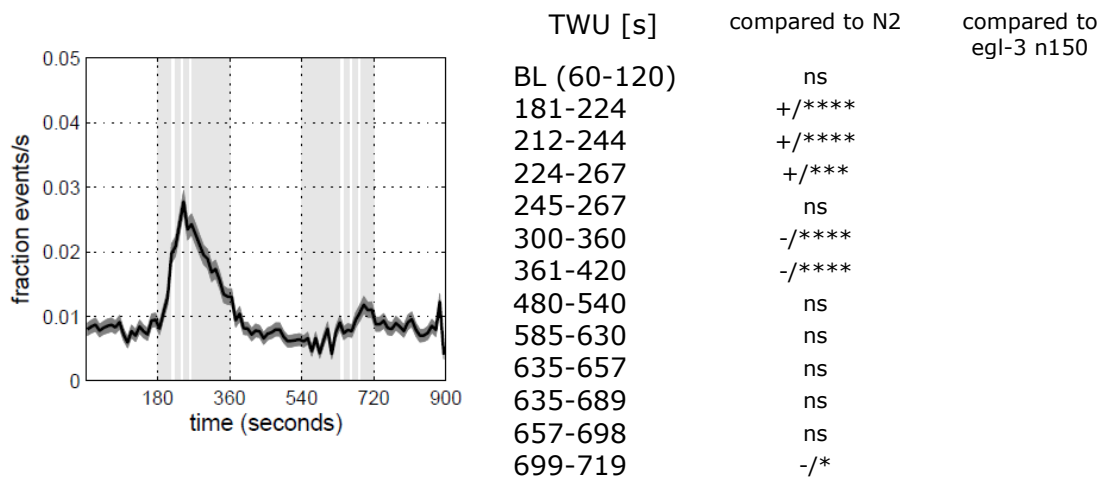
egl-3 n729 strain MT1541



The LR BL depends on at least two different neuropeptides, neuropeptides absent in both egl-3 n150 and egl-3 n729 in addition to neuropeptides only absent in egl-3 n729.

This difference could also influence the initial DR response at TWU 212-244s, which is significantly increased in the egl-3 n729 mutant compared to egl-3 n150. The rest of the DR and UR responses are not significantly different in the both mutants, if they are normalized to N2 BL level.

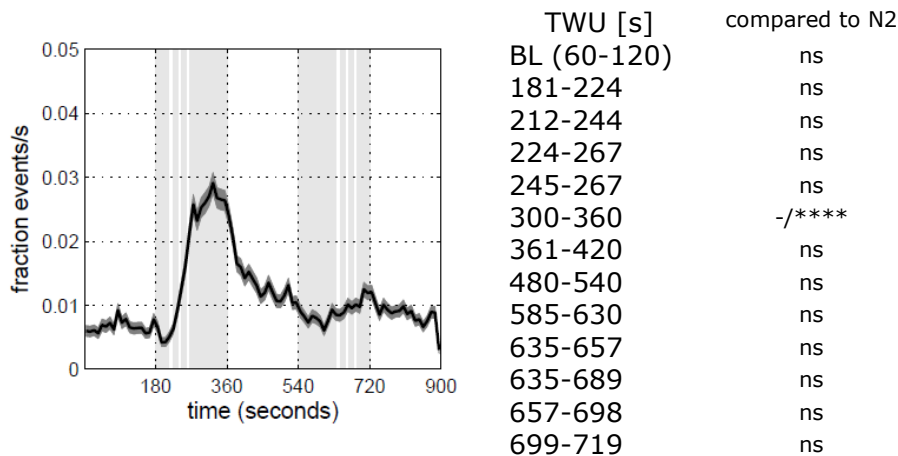
sbt-1 ok901 strain RB987



As discussed above, neuropeptides missing in the egl-3 n150 and n729 mutants but present in sbt-1 ok901 are responsible for suppressing the LR BL.

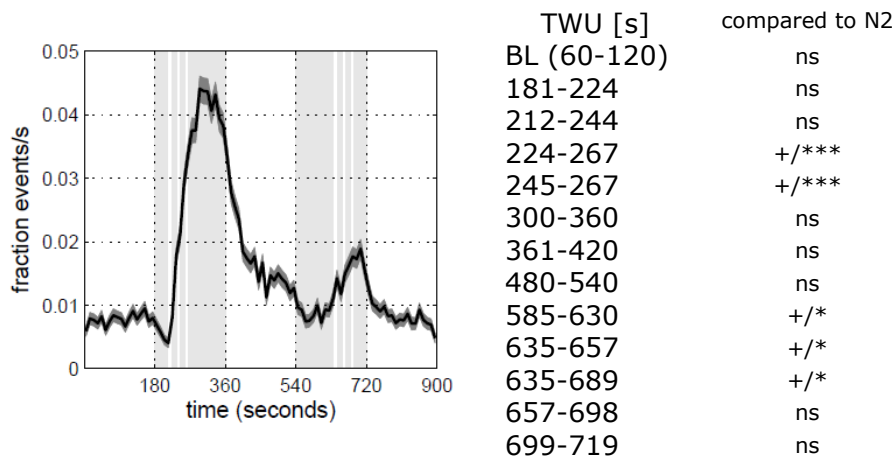
Furthermore, neuropeptides missing in all of the egl-3 and in the sbt-1 mutant are required for the initial DR inhibition of LRs and in the premature maximum of LRs before the optimal oxygen concentration has been reached.

flp-8 pk360 strain flp-8 pk360



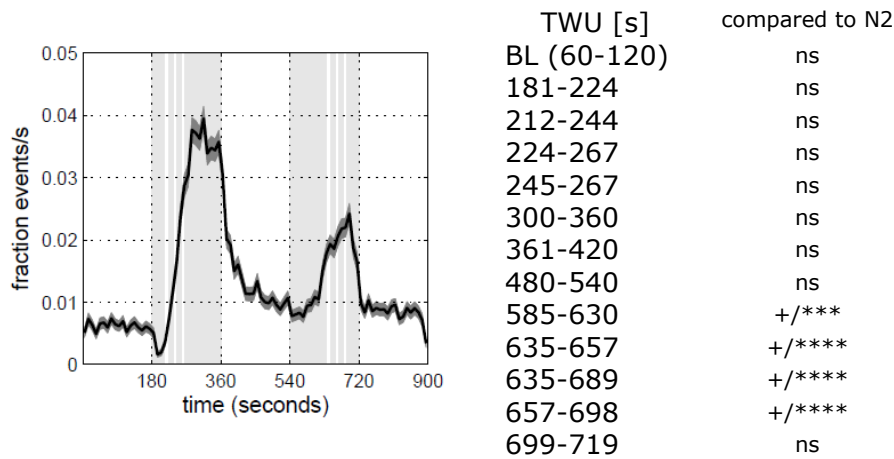
flp-8 influences the maximum LR response during the DR and has been shown to be missing in the sbt-1 ok901 mutant which also has a reduced maximum LR response. flp-8 is expressed in URX and AUA. Since BAG mediates the DR response, flp-8 released from AUA would be a good candidate. BAG gap junctions to RIR which in turn is synaptically connected to AUA. Activity in BAG can lead to the release of flp-8 in AUA which in turn additionally increases the LR response. flp-8 has no influence on any other part of DR or UR behavior. flp-8 is not involved in mediating the initial DR inhibition.

flp-10 n4543 strain MT14676



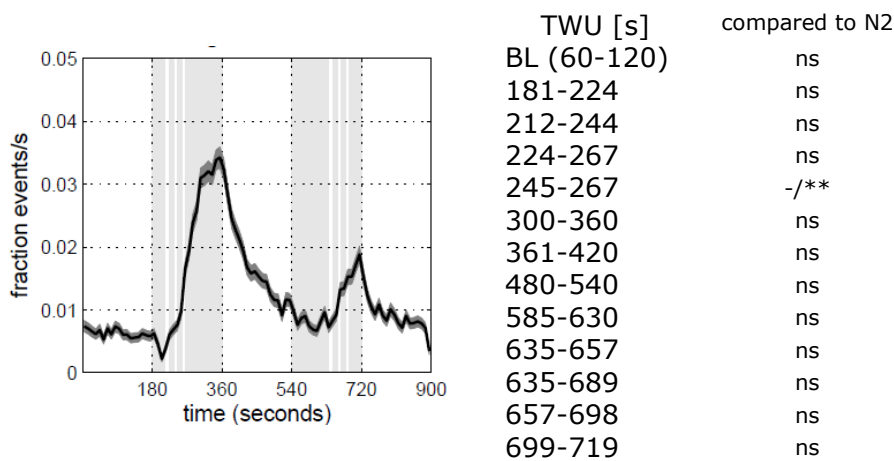
flp-10 is not involved in mediating the initial DR inhibition, but inhibits the DR maximum response. flp-10 is expressed in BAG, URX and AUA which all connect to reversal interneurons AVA and AVE. Together with flp-8 flp-10 could be involved in the balancing the maximum LR response during the DR. The absence of flp-10 leads to a significant increase during the start of the UR. At this time neither BAG nor URX are active leaving AUA as a prime target for mediating the initial LR DR inhibition.

flp-17 n4894 strain MT15933



flp-17 is not involved in the initial inhibition of the DR response. The absence of flp-17 removes the LR inhibition upon UR. All LR rates during the UR are significantly higher compared to N2, the LR rates increase even before 13% oxygen has been reached, the maximum response is sooner and higher than in N2.

flp-10 n4543 x flp-17 n4894 strain MT15973



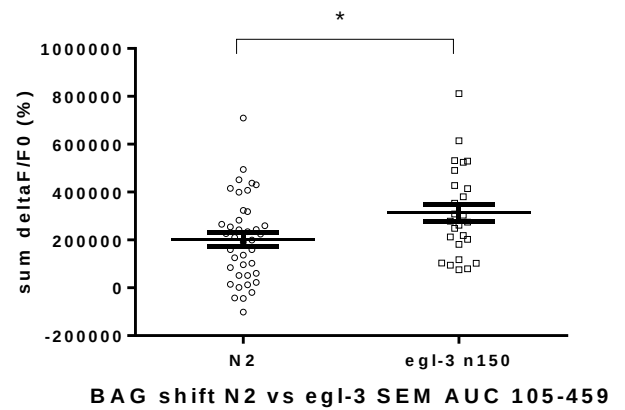
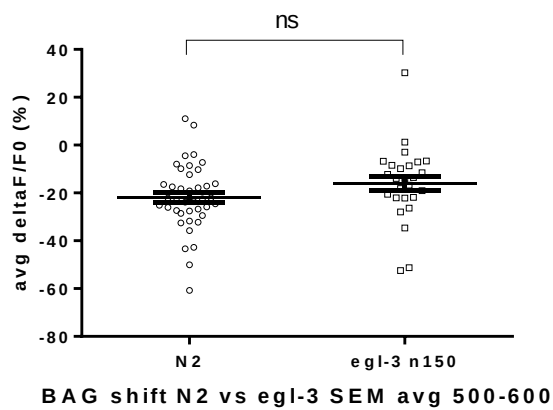
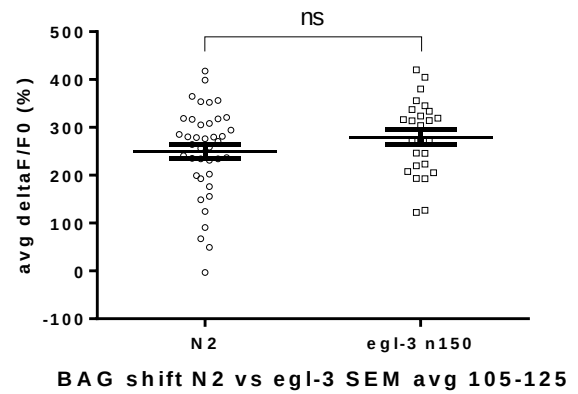
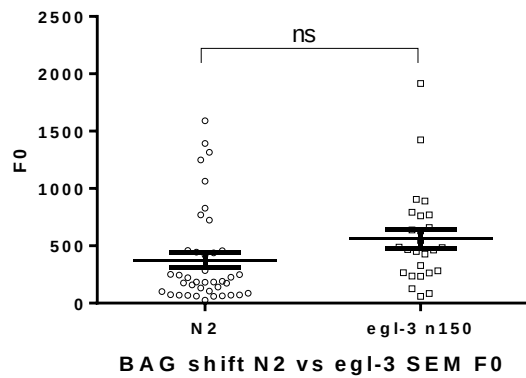
flp-10 and flp-17 seem to keep each other's effect in balance.

5.3 Statistics

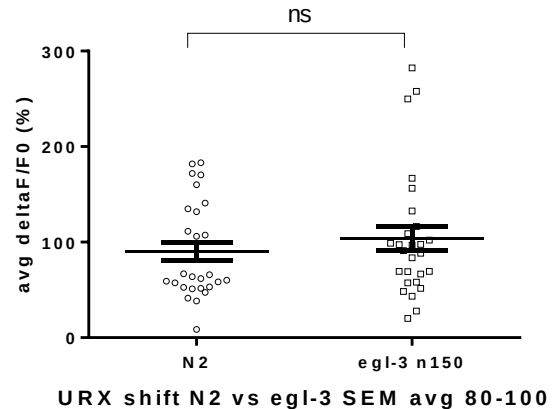
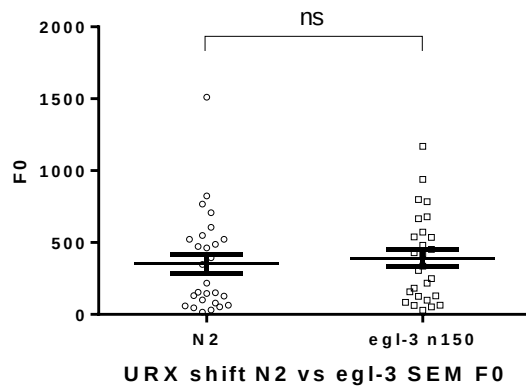
5.3.1 Calcium imaging

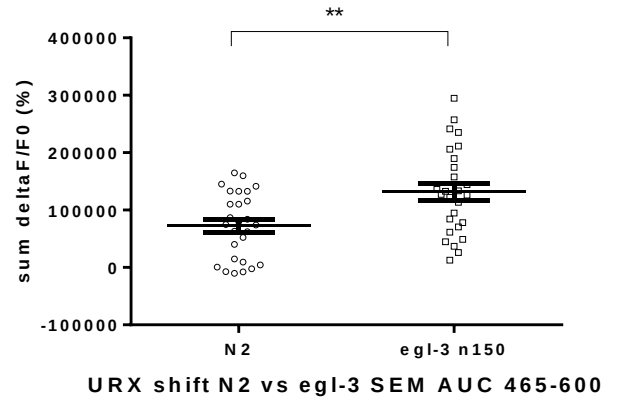
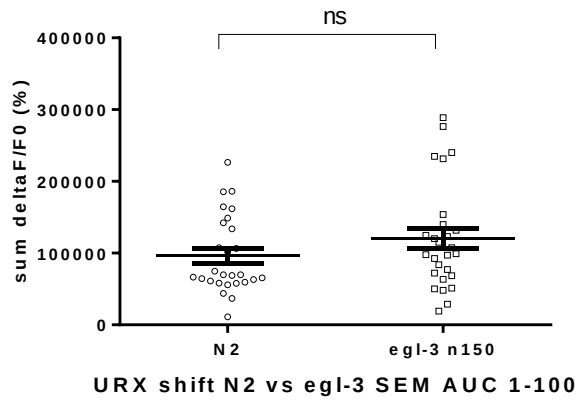
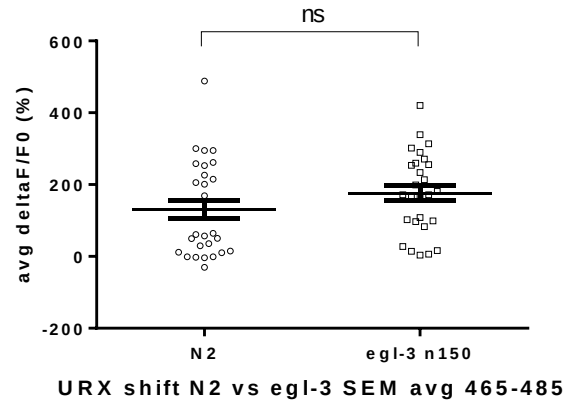
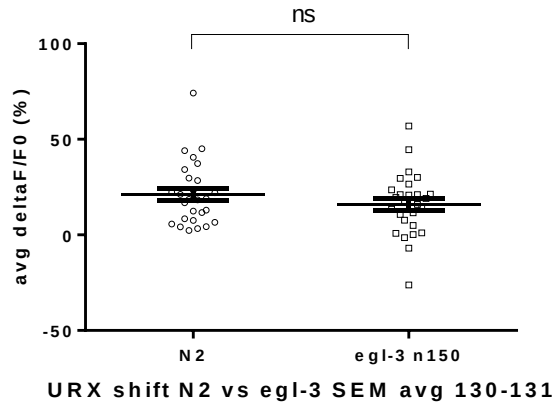
All of the following statistics were done using unpaired t-test. Error bars indicate standard error of the mean (SEM). P value < 0.05 ... *, P value < 0.01 ... **

BAG shift paradigm

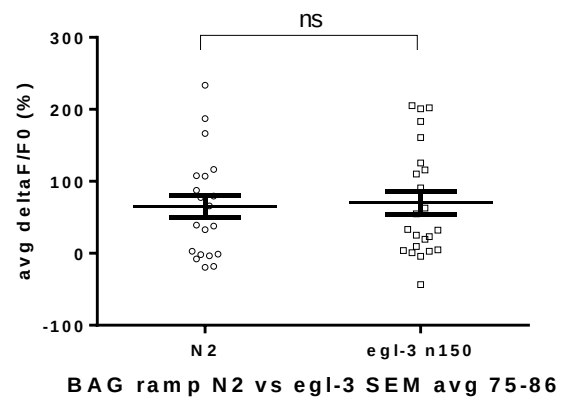
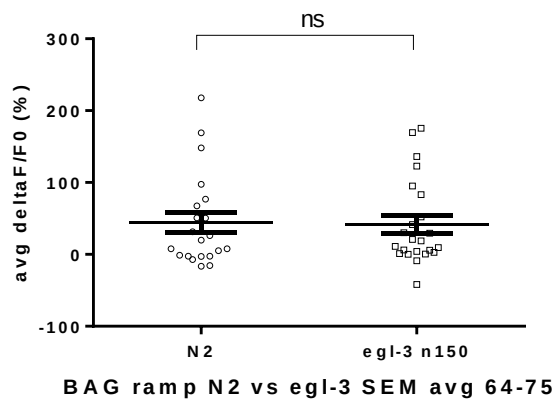
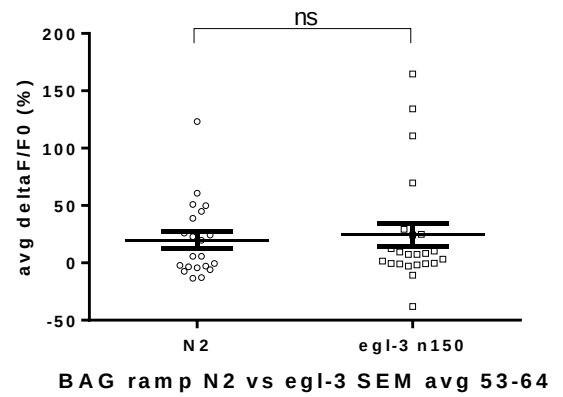
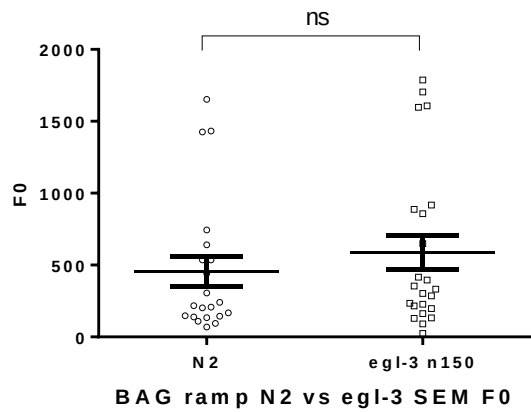


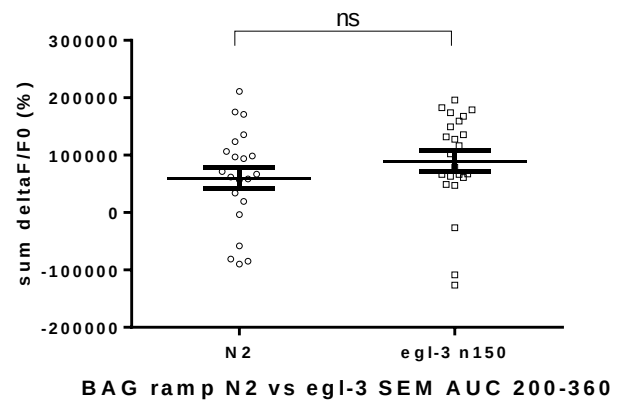
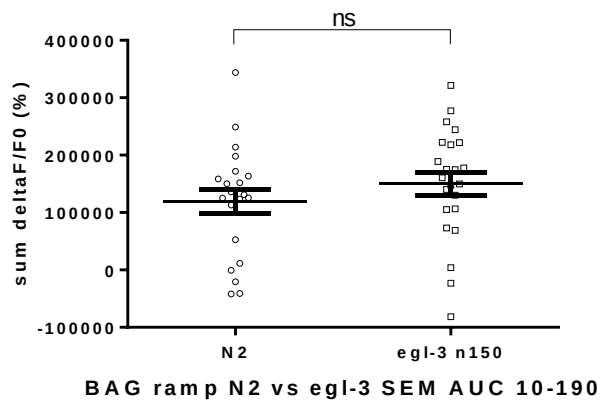
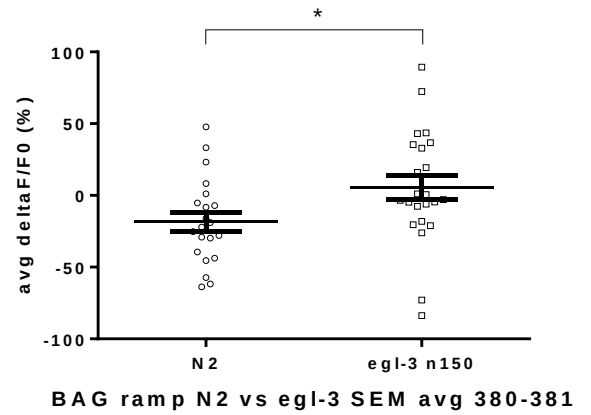
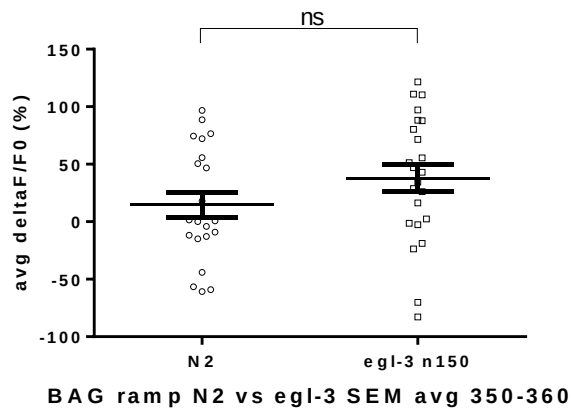
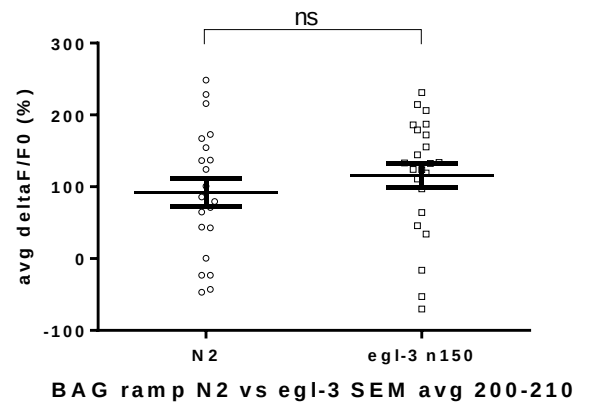
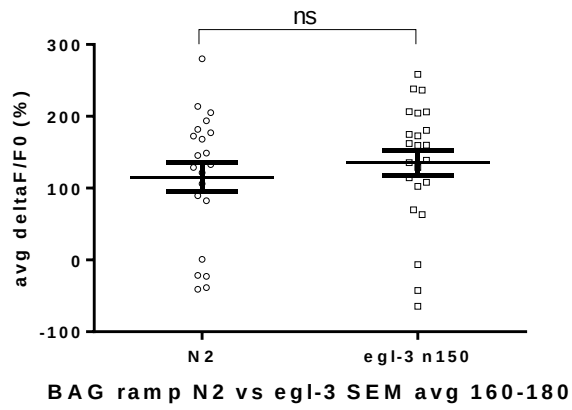
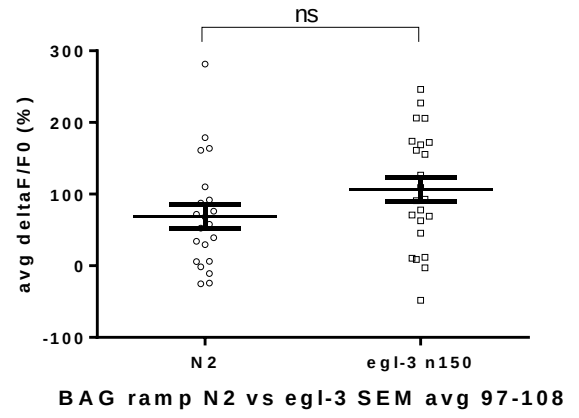
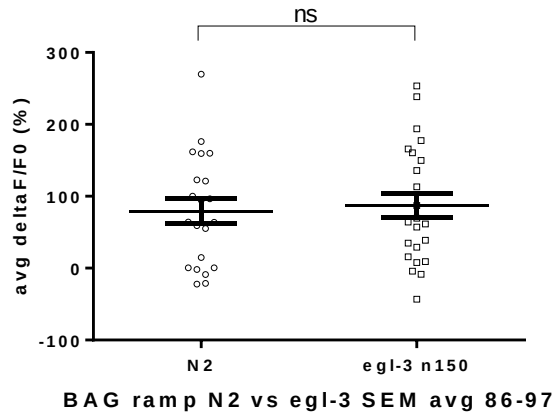
URX shift paradigm



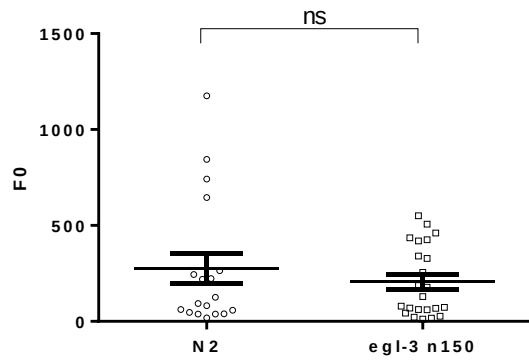


BAG ramp paradigm

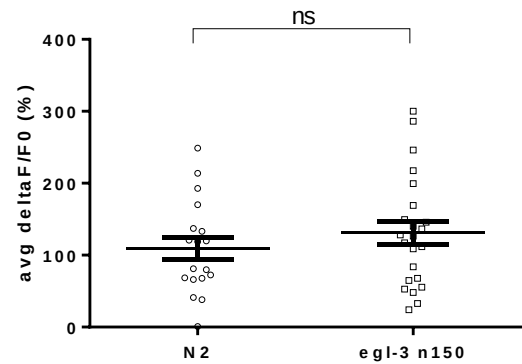




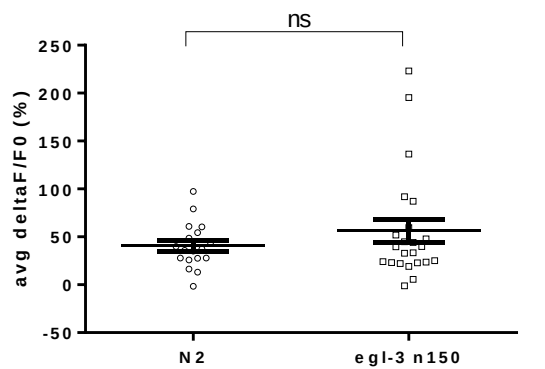
URX ramp paradigm



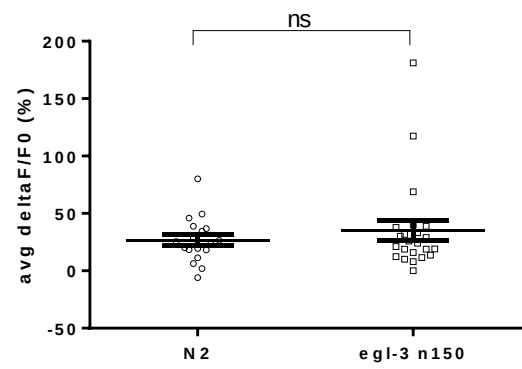
URX ramp N2 vs egl-3 SEM F0



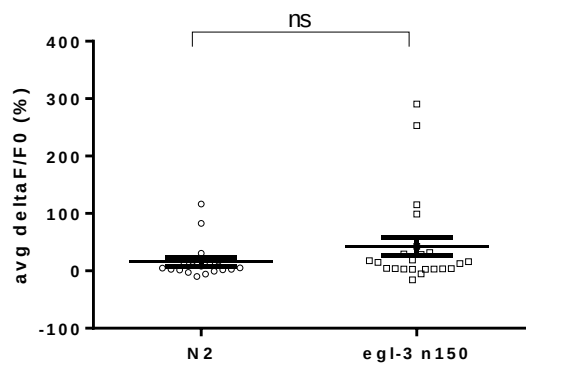
URX ramp N2 vs egl-3 SEM avg 10-20



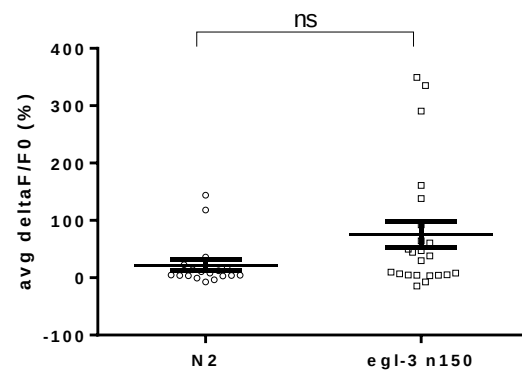
URX ramp N2 vs egl-3 SEM avg 53-64



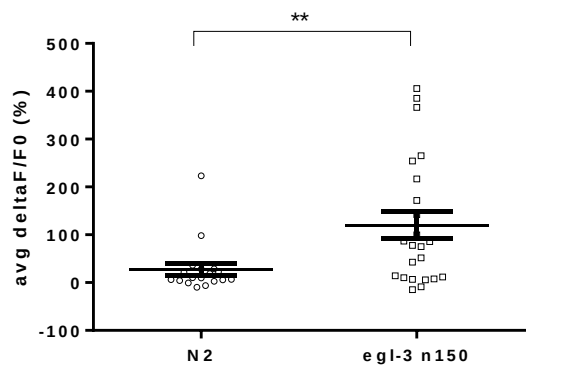
URX ramp N2 vs egl-3 SEM avg 97-108



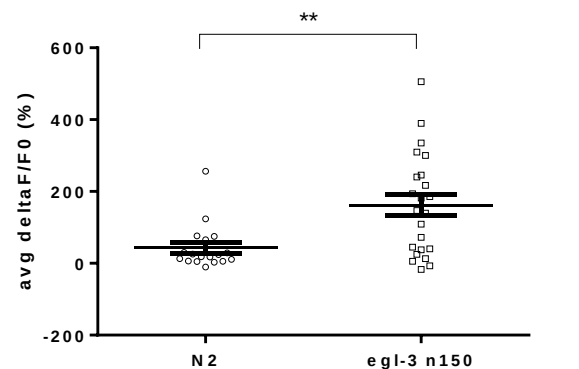
URX ramp N2 vs egl-3 SEM avg 476-487



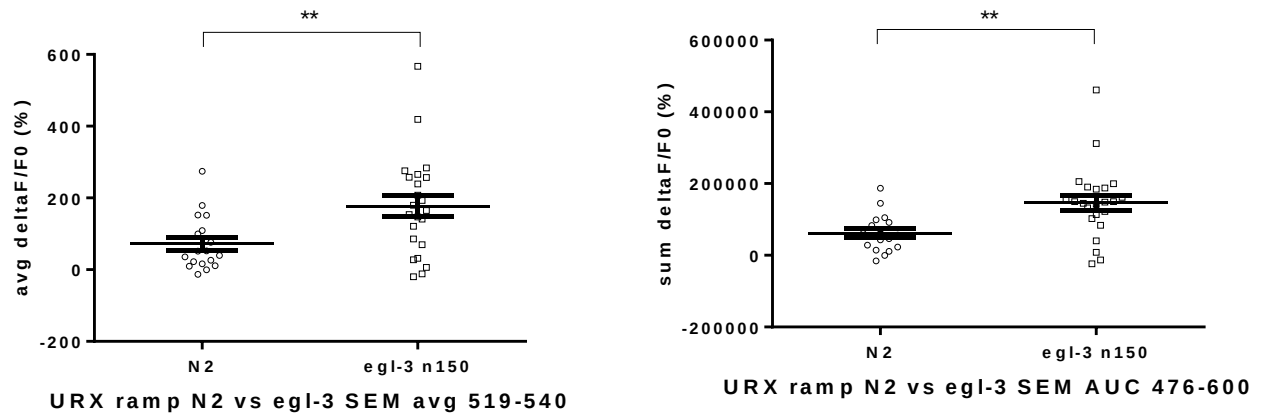
URX ramp N2 vs egl-3 SEM avg 487-497



URX ramp N2 vs egl-3 SEM avg 497-508



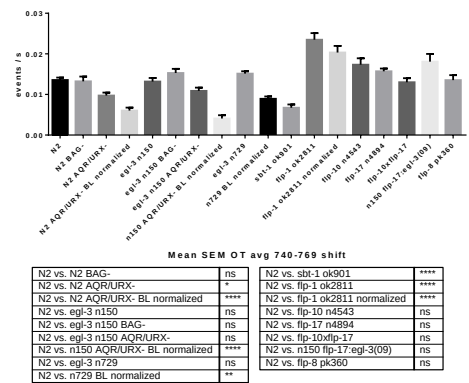
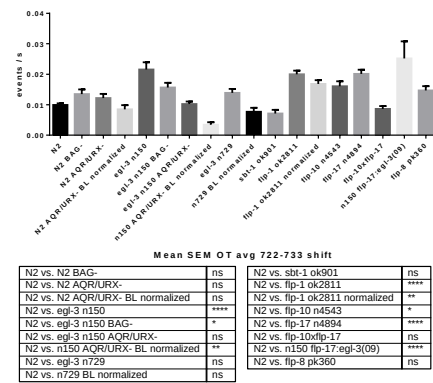
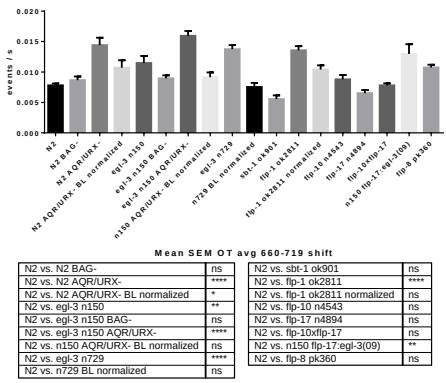
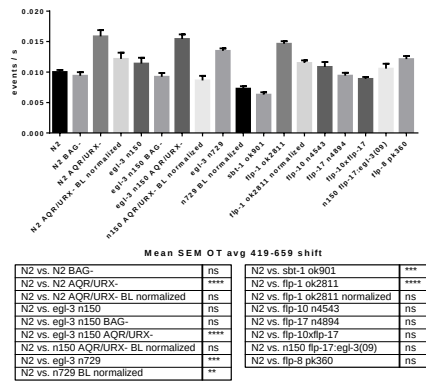
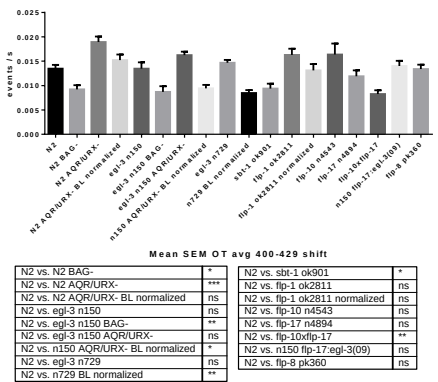
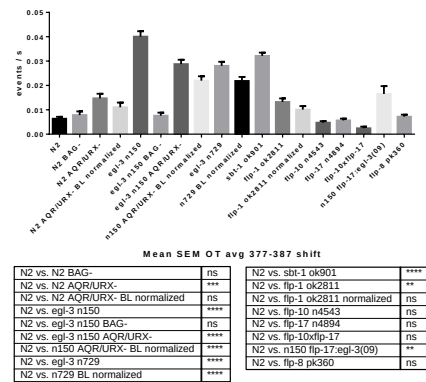
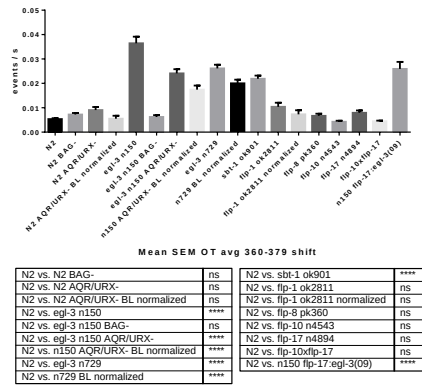
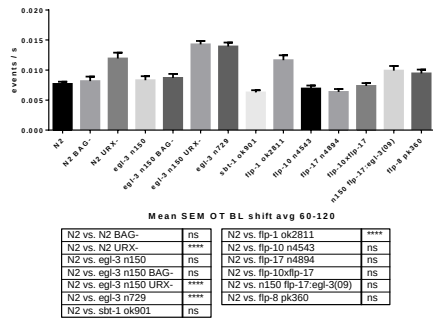
URX ramp N2 vs egl-3 SEM avg 508-519



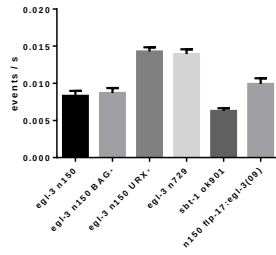
5.3.2 Behavior analysis

All of the following statistics were done using one way ANOVA followed by Dunnet's test for multiple comparisons. The significance levels relate always to comparisons to the first column. Error bars indicate standard error of the mean (SEM). Due to the extensive nature of the statistical analysis, significance levels can be found in a table below the bar graph plots.

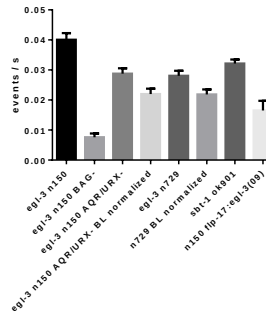
5.3.2.1 OTs in the shift paradigm – N2 vs. mutant strains



5.3.2.2 OTs in the shift paradigm – egl-3 n150 vs. mutant strains

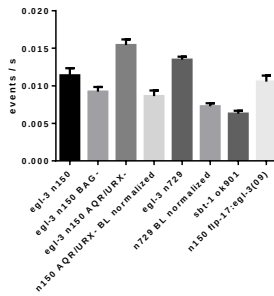


egl-3 n150 vs. egl-3 n150 BAG-	ns
egl-3 n150 vs. egl-3 n150 URX-	****
egl-3 n150 vs. egl-3 n729	****
egl-3 n150 vs. sbt-1 ok901	ns
egl-3 n150 vs. n150 flp-17:egl-3(09)	ns



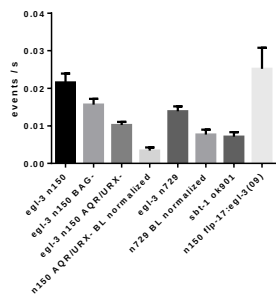
Mean SEM OT 360-379 egl-3 n150 vs mutants

egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	****
egl-3 n150 vs. n729 BL normalized	****
egl-3 n150 vs. sbt-1 ok901	*
egl-3 n150 vs. n150 flp-17:egl-3(09)	****



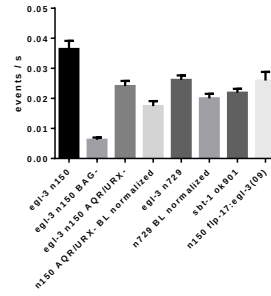
Mean SEM OT 377-387 egl-3 n150 vs mutants

egl-3 n150 vs. egl-3 n150 BAG-	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	***
egl-3 n150 vs. n150 AQR/URX- BL normalized	*
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. n729 BL normalized	***
egl-3 n150 vs. sbt-1 ok901	****
egl-3 n150 vs. n150 flp-17:egl-3(09)	ns



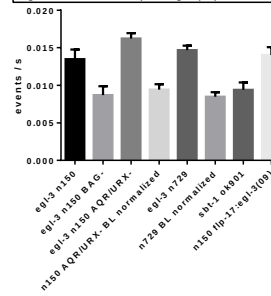
Mean SEM OT 400-429 egl-3 n150 vs mutants

egl-3 n150 vs. egl-3 n150 BAG-	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	****
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	****
egl-3 n150 vs. n729 BL normalized	****
egl-3 n150 vs. sbt-1 ok901	****
egl-3 n150 vs. n150 flp-17:egl-3(09)	ns



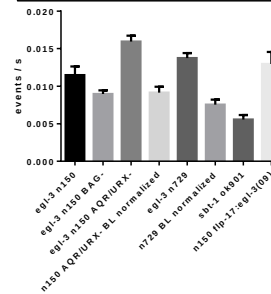
Mean SEM OT 660-719 egl-3 n150 vs mutants

egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	****
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	****
egl-3 n150 vs. n729 BL normalized	****
egl-3 n150 vs. sbt-1 ok901	****
egl-3 n150 vs. n150 flp-17:egl-3(09)	*



Mean SEM OT 722-733 egl-3 n150 vs mutants

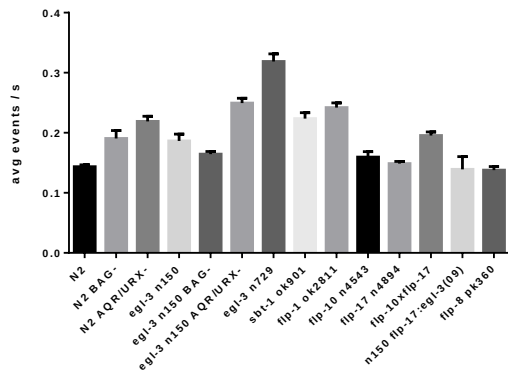
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	****
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	****
egl-3 n150 vs. n729 BL normalized	****
egl-3 n150 vs. sbt-1 ok901	*
egl-3 n150 vs. n150 flp-17:egl-3(09)	ns



Mean SEM OT 740-769 egl-3 n150 vs mutants

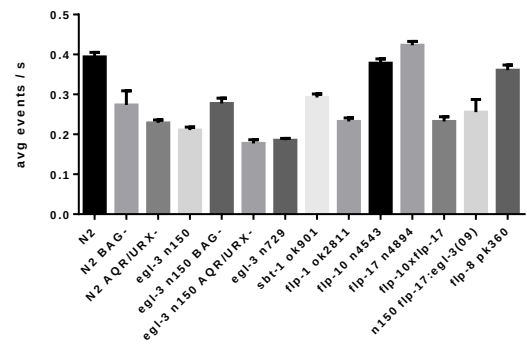
egl-3 n150 vs. egl-3 n150 BAG-	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	***
egl-3 n150 vs. n150 AQR/URX- BL normalized	ns
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. n729 BL normalized	**
egl-3 n150 vs. sbt-1 ok901	****
egl-3 n150 vs. n150 flp-17:egl-3(09)	ns

5.3.2.3 OT angles – N2 vs. mutant strains



Mean SEM normalized OT w/o rev turn angles 0-20 degrees 1-1080

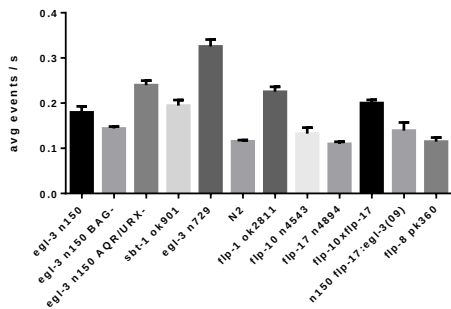
N2 vs. N2 BAG-	***	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	***	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	***
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sbt-1 ok901	****		



Mean SEM normalized OT w/o rev turn angles 120-180 degrees 1-1080

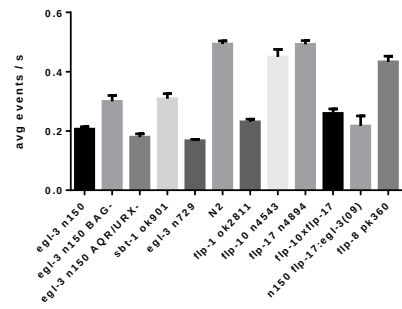
N2 vs. N2 BAG-	****	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-10xflp-17	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. n150 flp-17:egl-3(09)	****
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sbt-1 ok901	****		

5.3.2.4 OT angles – egl-3 n150 vs. mutant strains



Mean SEM normalized egl-3 OT angles 0-20 degrees 1-360

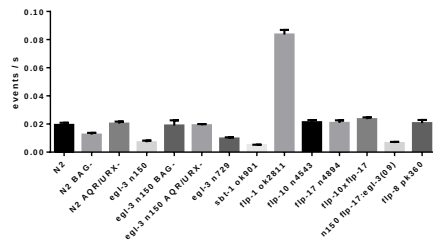
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-10 n4543	*
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-17 n4894	***
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. n150 flp-17:egl-3(09)	ns
egl-3 n150 vs. N2	****	egl-3 n150 vs. flp-8 pk360	***
egl-3 n150 vs. flp-1 ok2811	**		



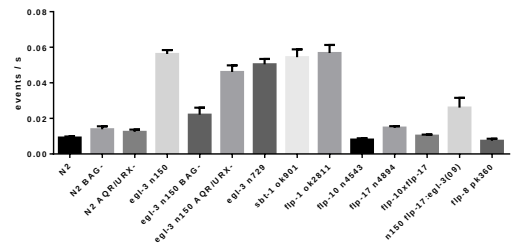
Mean SEM normalized egl-3 OT angles 120-180 degrees 1-360

egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-10 n4543	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns	egl-3 n150 vs. flp-17 n4894	****
egl-3 n150 vs. sbt-1 ok901	****	egl-3 n150 vs. flp-10xflp-17	*
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. n150 flp-17:egl-3(09)	ns
egl-3 n150 vs. N2	****	egl-3 n150 vs. flp-8 pk360	****
egl-3 n150 vs. flp-1 ok2811	ns		

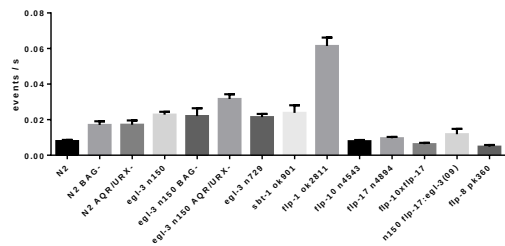
5.3.2.5 STs in the shift paradigm – N2 vs. mutant strains



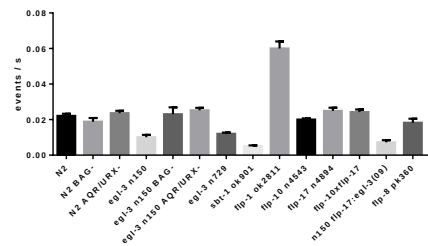
Mean SEM ST BL shift avg 60-120			
N2 vs. N2 BAG-	*	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. n150 flp-17:egl-3(09)	**
N2 vs. egl-3 n729	***	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



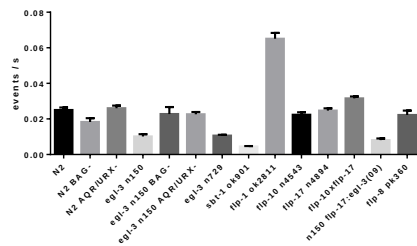
Mean SEM ST avg 377-387 shift			
N2 vs. N2 BAG-	ns	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	***	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	***	N2 vs. n150 flp-17:egl-3(09)	**
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



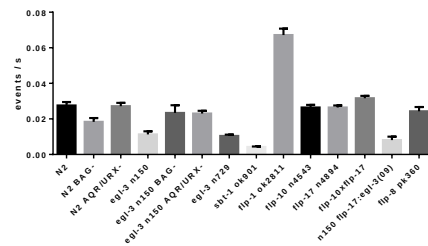
Mean SEM ST avg 377-387 shift			
N2 vs. N2 BAG-	*	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	*	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	***	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



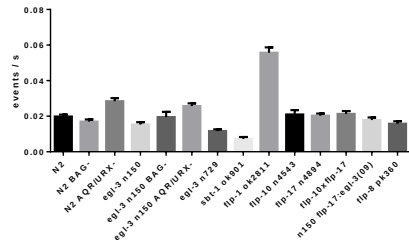
Mean SEM ST avg 400-429 shift			
N2 vs. N2 BAG-	ns	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. n150 flp-17:egl-3(09)	**
N2 vs. egl-3 n729	***	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



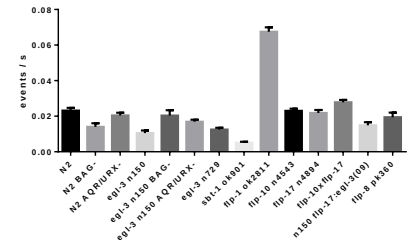
Mean SEM ST avg 419-659 shift			
N2 vs. N2 BAG-	ns	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. n150 flp-17:egl-3(09)	****
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



Mean SEM ST avg 660-719 shift			
N2 vs. N2 BAG-	*	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. n150 flp-17:egl-3(09)	***
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		

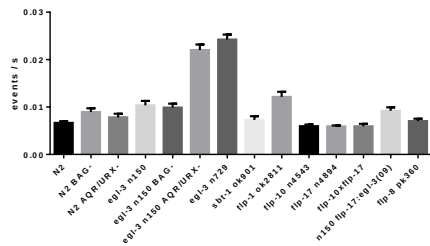


Mean SEM ST avg 722-733 shift			
N2 vs. N2 BAG-	ns	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	***	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	*	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	**	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		



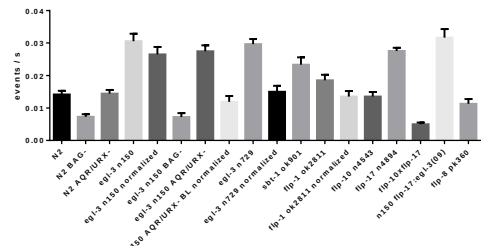
Mean SEM ST avg 740-769 shift			
N2 vs. N2 BAG-	**	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	*	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	****		

5.3.2.6 LRs in the shift paradigm – N2 vs. mutant strains



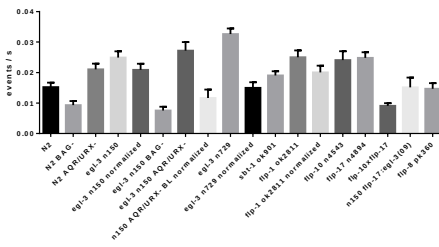
Mean SEM LR BL shift avg 60-120

N2 vs. N2 BAG-	ns	N2 vs. flp-1 ok2811	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150	**	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. sht-1 ok901	ns		



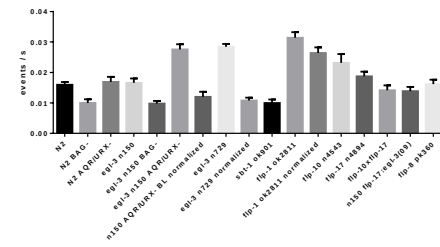
Mean SEM LR avg 360 - 379 shift

N2 vs. N2 BAG-	*	N2 vs. sht-1 ok901	**
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-1 ok2811	ns
N2 vs. egl-3 n150	****	N2 vs. flp-1 ok2811 normalized	ns
N2 vs. egl-3 n150 normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	***
N2 vs. n150 AQR/URX- BL normalized	ns	N2 vs. n150 flp-17:egl-3(09)	****
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n729 normalized	ns		



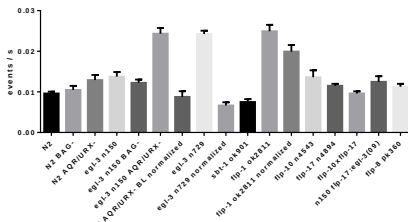
Mean SEM LR avg 377-387 shift

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-1 ok2811	**
N2 vs. egl-3 n150	***	N2 vs. flp-1 ok2811 normalized	ns
N2 vs. egl-3 n150 normalized	ns	N2 vs. flp-10 n4543	*
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. n150 AQR/URX- BL normalized	ns	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n729 normalized	ns		



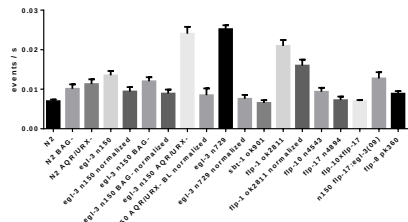
Mean SEM LR avg 400-429 shift

N2 vs. N2 BAG-	*	N2 vs. sht-1 ok901	*
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-1 ok2811	****
N2 vs. egl-3 n150	ns	N2 vs. flp-1 ok2811 normalized	****
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-10 n4543	*
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-17 n4894	ns
N2 vs. n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729 normalized	*	N2 vs. flp-8 pk360	ns



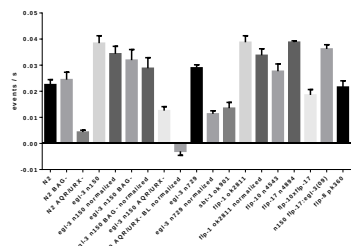
Mean SEM LR avg 419-659 shift

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-1 ok2811	****
N2 vs. egl-3 n150	ns	N2 vs. flp-1 ok2811 normalized	****
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 AQR/URX-	*	N2 vs. flp-17 n4894	ns
N2 vs. n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729 normalized	ns	N2 vs. flp-8 pk360	ns



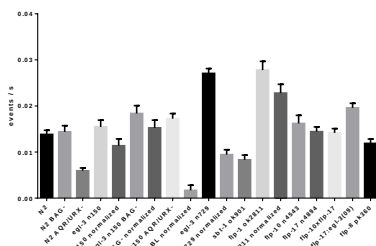
Mean SEM LR avg 660-719 shift

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	ns
N2 vs. N2 AQR/URX-	*	N2 vs. flp-1 ok2811	****
N2 vs. egl-3 n150	****	N2 vs. flp-1 ok2811 normalized	****
N2 vs. egl-3 n150 normalized	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. n150 AQR/URX- BL normalized	ns	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n729 normalized	ns		



Mean SEM LR avg 722-733 shift

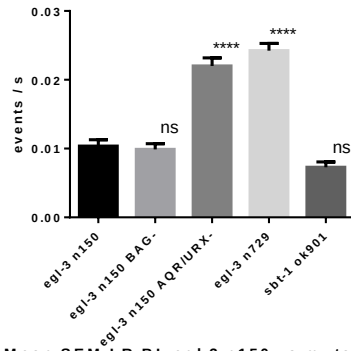
N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	ns
N2 vs. N2 AQR/URX-	****	N2 vs. flp-1 ok2811	****
N2 vs. egl-3 n150	****	N2 vs. flp-1 ok2811 normalized	****
N2 vs. egl-3 n150 normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-17 n4894	***
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. n150 AQR/URX- BL normalized	****	N2 vs. n150 flp-17:egl-3(09)	*
N2 vs. egl-3 n729	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n729 normalized	*		



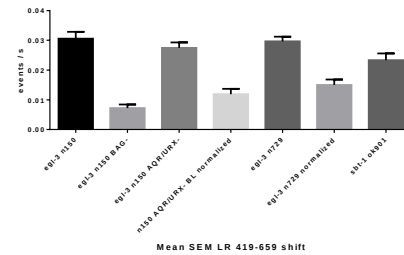
Mean SEM LR avg 740-759 shift

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	*
N2 vs. N2 AQR/URX-	****	N2 vs. flp-1 ok2811	****
N2 vs. egl-3 n150	ns	N2 vs. flp-1 ok2811 normalized	****
N2 vs. egl-3 n150 normalized	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. n150 AQR/URX- BL normalized	****	N2 vs. n150 flp-17:egl-3(09)	ns
N2 vs. egl-3 n729	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n729 normalized	ns		

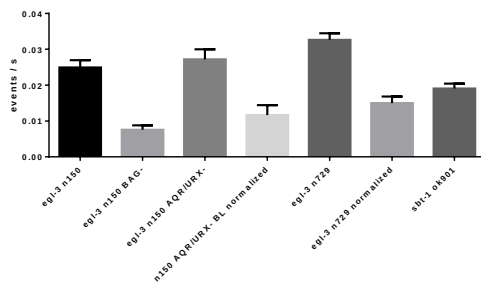
5.3.2.7 LRs in the shift paradigm – egl-3 n150 vs. mutant strains



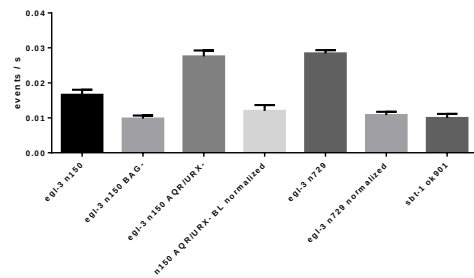
Mean SEM LR BL egl-3 n150 vs mutants



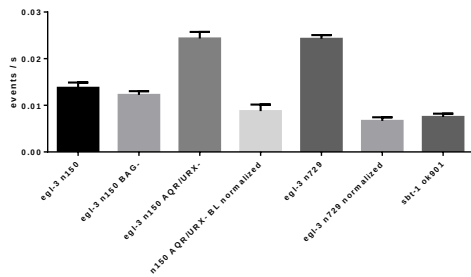
Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns



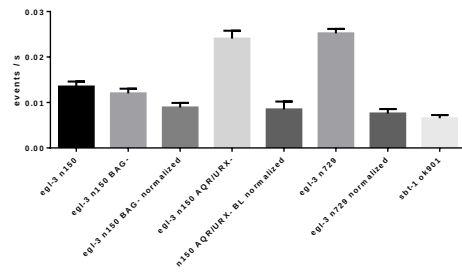
Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns



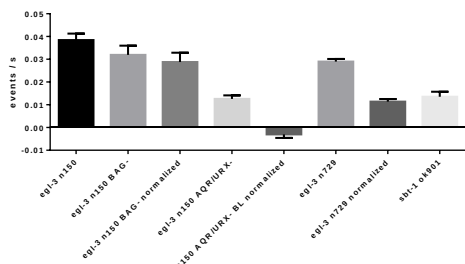
Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns



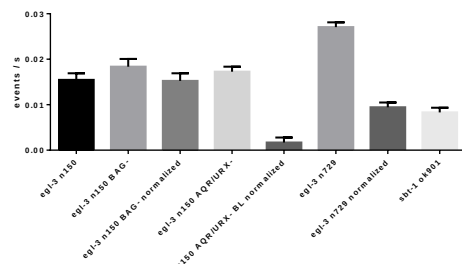
Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns



Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns

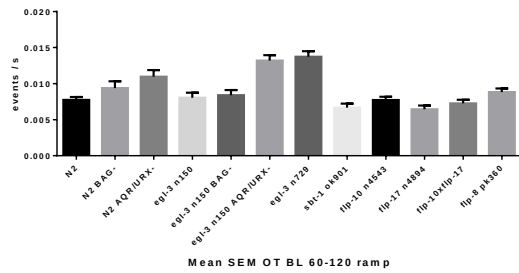


Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns

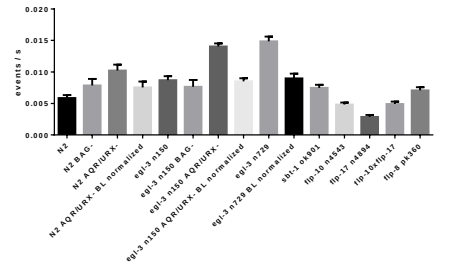


Mean SEM LR 419-659 shift	
egl-3 n150 vs. egl-3 n150 BAG-	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns
egl-3 n150 vs. n150 AQR/URX- BL normalized	****
egl-3 n150 vs. egl-3 n729	ns
egl-3 n150 vs. egl-3 n729 normalized	****
egl-3 n150 vs. sbt-1 ok901	ns

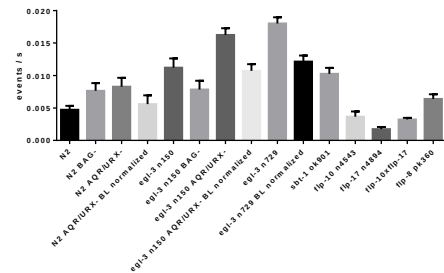
5.3.2.8 OTs in the ramp paradigm – N2 vs. mutant strains



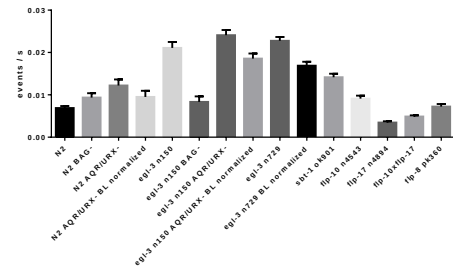
Mean SEM OT BL 60-120 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	***
N2 vs. egl-3 n150	ns
N2 vs. egl-3 n150 AQR/URX-	****
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



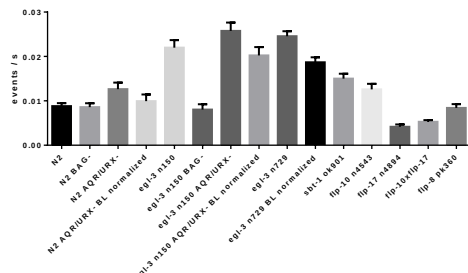
Mean SEM OT 181-224 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	****
N2 vs. egl-3 n150	ns
N2 vs. egl-3 n150 AQR/URX-	*
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	*
N2 vs. flp-10xflp-17	ns



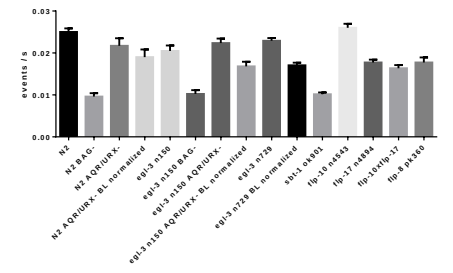
Mean SEM OT 212-244 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	*
N2 vs. egl-3 n150	ns
N2 vs. egl-3 n150 AQR/URX-	****
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



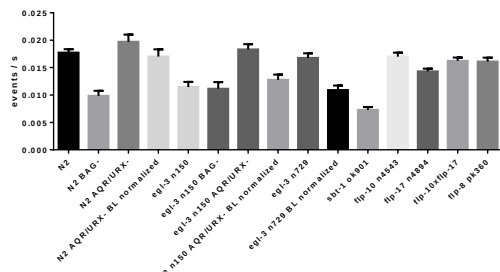
Mean SEM OT 224-267 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	***
N2 vs. egl-3 n150	ns
N2 vs. egl-3 n150 AQR/URX-	****
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



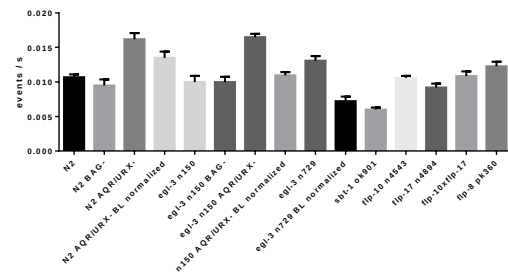
Mean SEM OT 245-267 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	ns
N2 vs. egl-3 n150	****
N2 vs. egl-3 n150 AQR/URX-	****
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



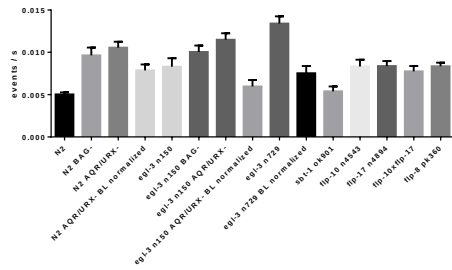
Mean SEM OT 300-360 ramp	
N2 vs. N2 BAG-	****
N2 vs. N2 AQR/URX-	ns
N2 vs. egl-3 n150	*
N2 vs. egl-3 n150 AQR/URX-	ns
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



Mean SEM OT 361-420 ramp	
N2 vs. N2 BAG-	****
N2 vs. N2 AQR/URX-	ns
N2 vs. egl-3 n150	****
N2 vs. egl-3 n150 AQR/URX-	ns
N2 vs. egl-3 n729	****
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns

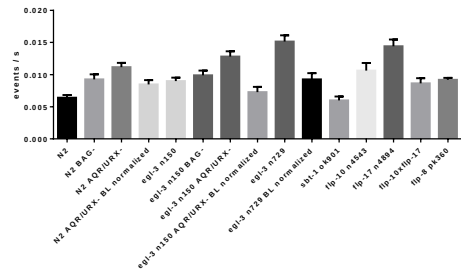


Mean SEM OT 480-540 ramp	
N2 vs. N2 BAG-	ns
N2 vs. N2 AQR/URX-	****
N2 vs. egl-3 n150	ns
N2 vs. egl-3 n150 AQR/URX-	****
N2 vs. egl-3 n729	ns
N2 vs. sht-1 ok901	ns
N2 vs. flp-8 pk360	ns
N2 vs. flp-10 n4543	ns
N2 vs. flp-17 n4894	ns
N2 vs. flp-10xflp-17	ns



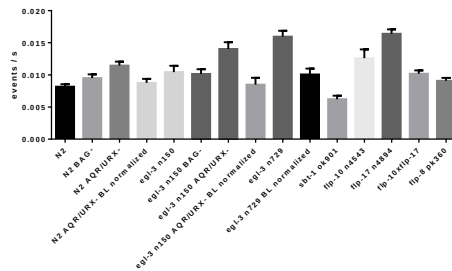
Mean SEM OT 585-630 ramp

N2 vs. N2 BAG-	****	N2 vs. egl-3 n729	****
N2 vs. N2 AQR/URX-	****	N2 vs. egl-3 n729 BL normalized	*
N2 vs. N2 AQR/URX- BL normalized	**	N2 vs. sbt-1 ok901	ns
N2 vs. egl-3 n150	**	N2 vs. flp-8 pk360	**
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-10 n4543	**
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-17 n4894	**
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	*



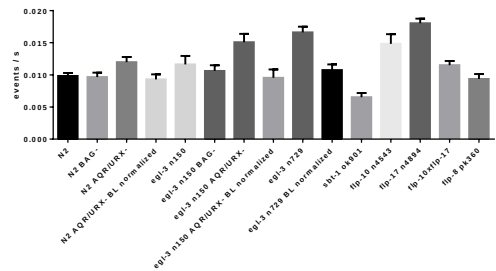
Mean SEM OT 635-657 ramp

N2 vs. N2 BAG-	*	N2 vs. egl-3 n729	****
N2 vs. N2 AQR/URX-	****	N2 vs. egl-3 n729 BL normalized	*
N2 vs. N2 AQR/URX- BL normalized	ns	N2 vs. sbt-1 ok901	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	*
N2 vs. egl-3 n150 BAG-	**	N2 vs. flp-10 n4543	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	ns



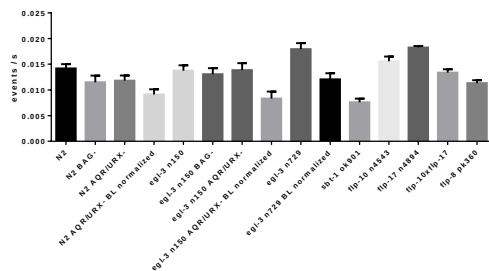
Mean SEM OT 635-689 ramp

N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 AQR/URX-	**	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX- BL normalized	ns	N2 vs. sbt-1 ok901	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10 n4543	***
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	ns



Mean SEM OT 657-698 ramp

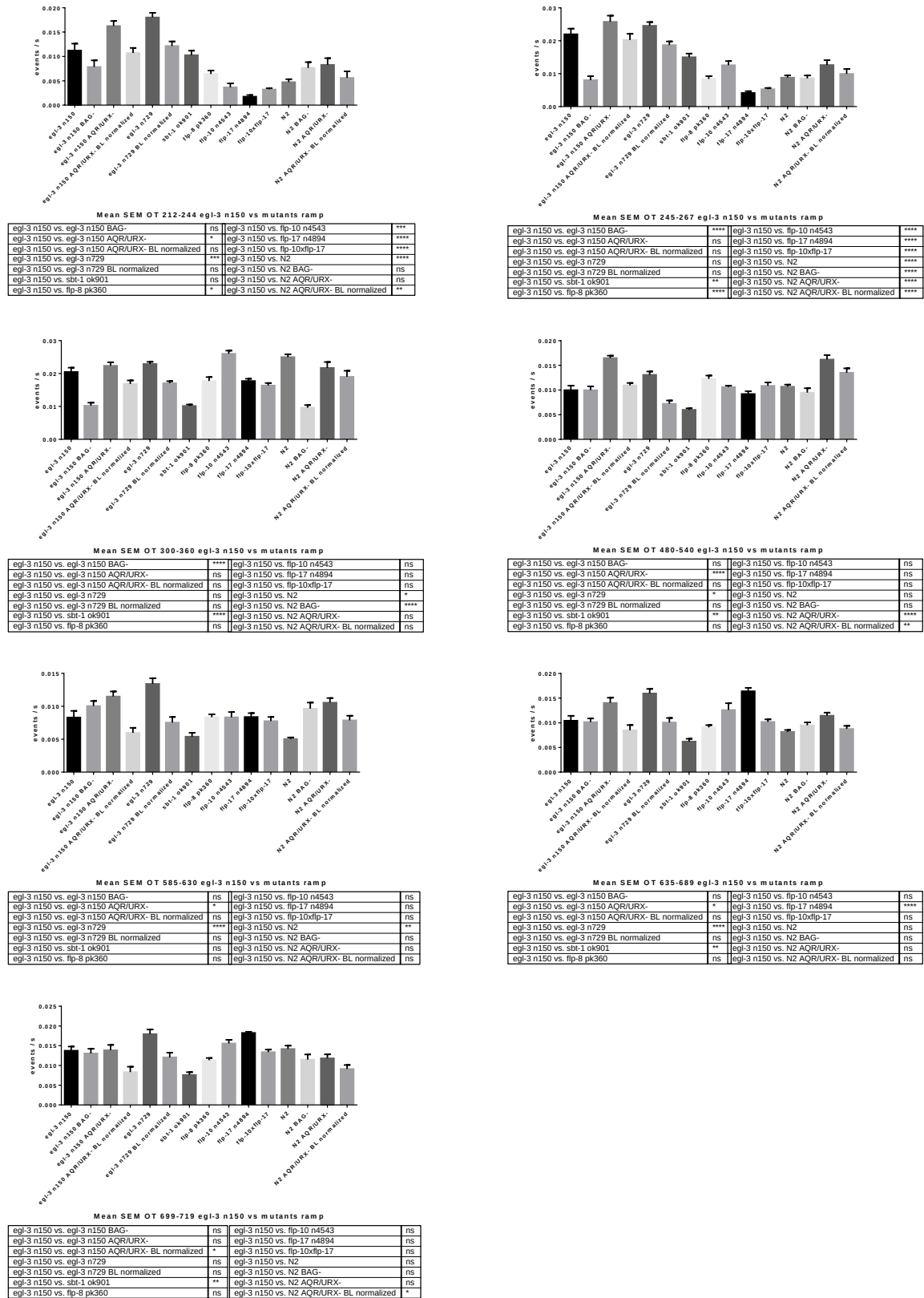
N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 AQR/URX-	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX- BL normalized	ns	N2 vs. sbt-1 ok901	*
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10 n4543	***
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns	N2 vs. flp-10xflp-17	ns



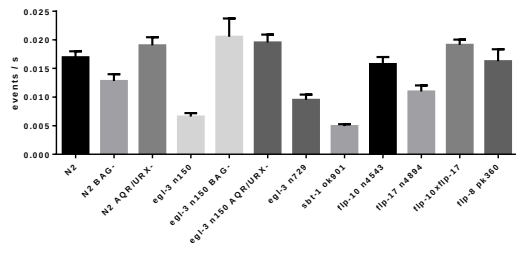
Mean SEM OT 699-719 ramp

N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	*
N2 vs. N2 AQR/URX-	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX- BL normalized	***	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	***	N2 vs. flp-10xflp-17	ns

5.3.2.9 OTs in the ramp paradigm – egl-3 n150 vs. mutant strains

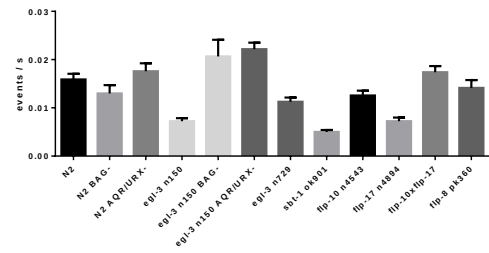


5.3.2.10 STs in the ramp paradigm – N2 vs. mutant strains



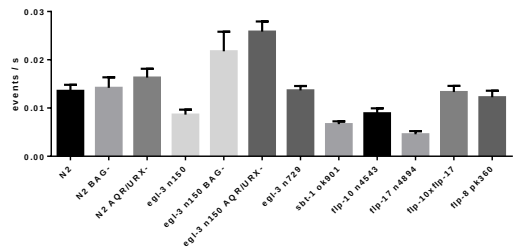
Mean SEM ST BL 60-120 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	*
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	***		



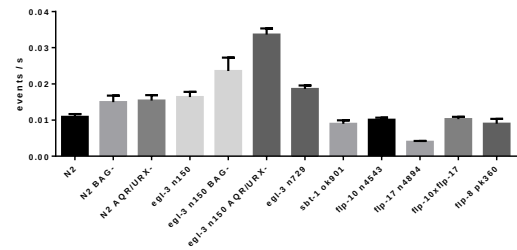
Mean SEM ST 181-224 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	***	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	**
N2 vs. egl-3 n150 AQR/URX-	*	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	ns		



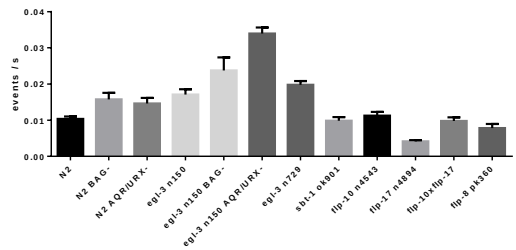
Mean SEM ST 212-244 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	*
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	**	N2 vs. flp-17 n4894	**
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	ns		



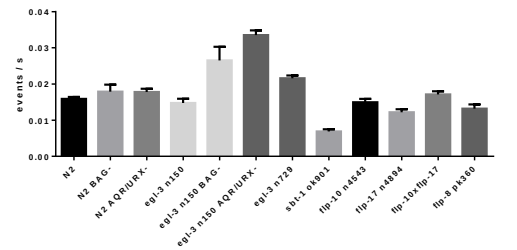
Mean SEM ST 224-267 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	*	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	*
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	***		



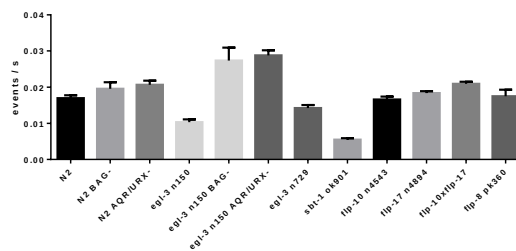
Mean SEM ST 245-267 ramp

N2 vs. N2 BAG-	*	N2 vs. sbt-1 ok901	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	**	N2 vs. flp-10 n4543	**
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	*
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****		



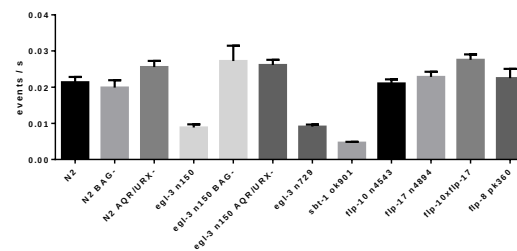
Mean SEM ST 300-360 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	*		



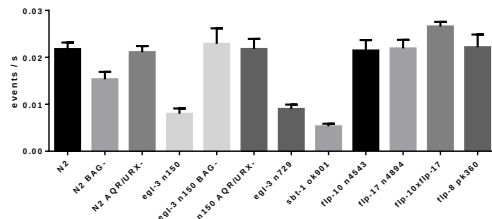
Mean SEM ST 361-420 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	**	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	ns		



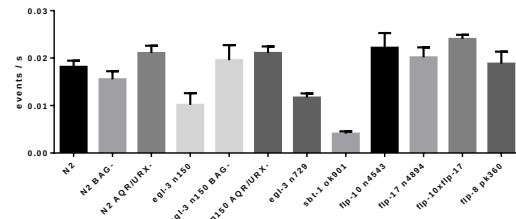
Mean SEM ST 480-540 ramp

N2 vs. N2 BAG-	ns	N2 vs. sbt-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****		



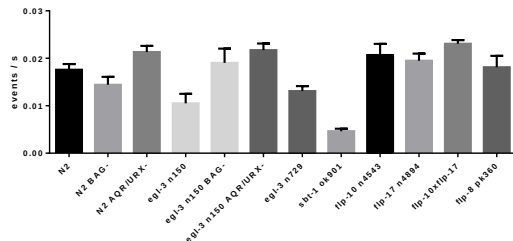
Mean SEM ST 585-630 ramp

N2 vs. N2 BAG-	*	N2 vs. sht-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****		



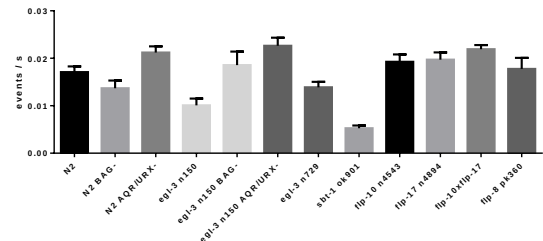
Mean SEM ST 635-657 ramp

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	**	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	*		



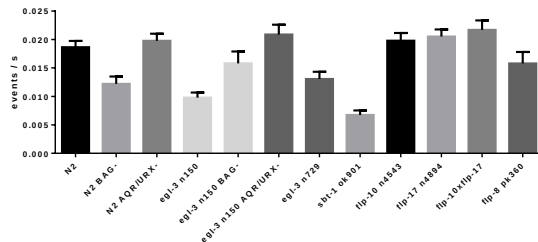
Mean SEM ST 635-689 ramp

N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	*	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	ns		



Mean SEM ST 657-698 ramp

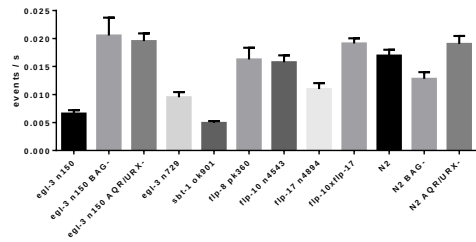
N2 vs. N2 BAG-	ns	N2 vs. sht-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	**	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	ns		



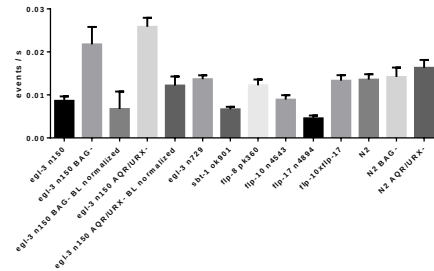
Mean SEM ST 699-719 ramp

N2 vs. N2 BAG-	**	N2 vs. sht-1 ok901	****
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	*		

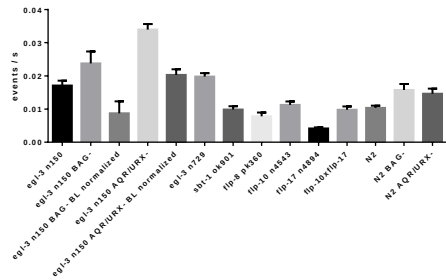
5.3.2.11 STs in the ramp paradigm – egl-3 n150 vs. mutant strains



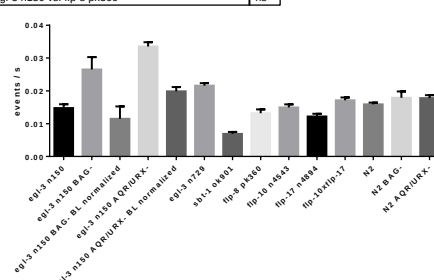
Mean SEM ST BL egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2	****
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. flp-8 pk360	***	egl-3 n150 vs. N2 AQR/URX-	****
egl-3 n150 vs. flp-10 n4543	**		



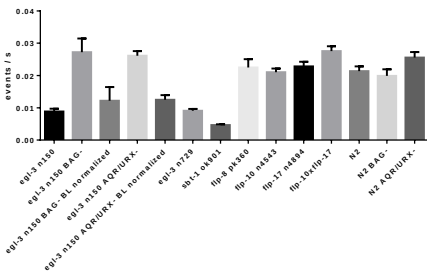
Mn SEM ST 212-244 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	***	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 AQR- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	ns
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	ns		



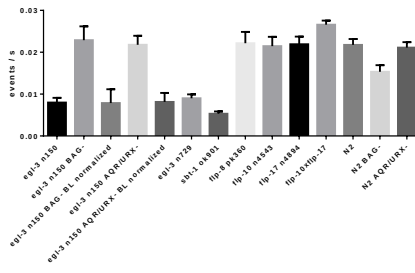
Mn SEM ST 245-267 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG- BL normalized	*	egl-3 n150 vs. flp-17 n4894	***
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	*
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	*
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	**		



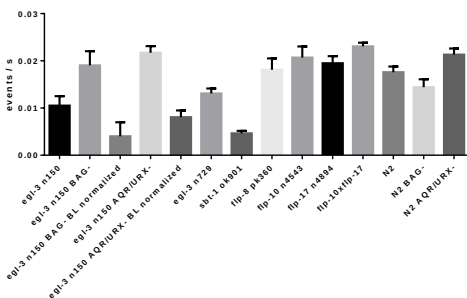
Mean SEM ST 300-360 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	ns
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	*	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	ns		



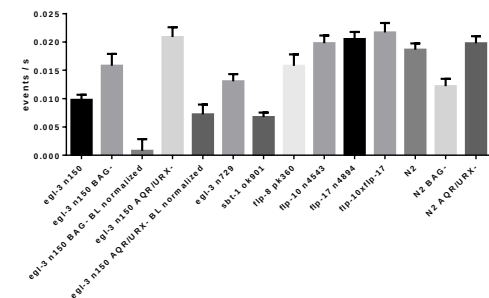
Mean SEM ST 480-540 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-10 n4543	**
egl-3 n150 vs. egl-3 n150 BAG- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	**
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	***
egl-3 n150 vs. egl-3 n1729	ns	egl-3 n150 vs. N2 BAG-	**
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	****
egl-3 n150 vs. flp-8 pk360	***		



Mean SEM ST 585-630 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-10 n4543	***
egl-3 n150 vs. egl-3 n150 BAG- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	***
egl-3 n150 vs. egl-3 n150 AQR/URX-	***	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	****
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	****
egl-3 n150 vs. flp-8 pk360	****		

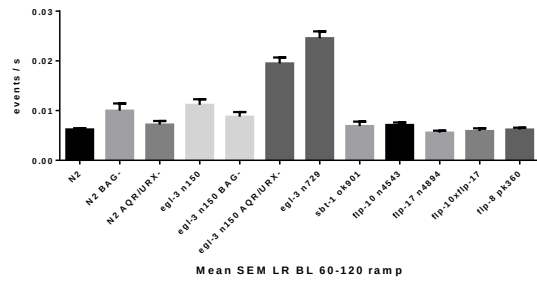


Mean SEM ST 635-689 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	*	egl-3 n150 vs. flp-10 m4543	**
egl-3 n150 vs. egl-3 n150 AQR- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	*
egl-3 n150 vs. egl-3 n150 AQR/URX-	***	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	*
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	***
egl-3 n150 vs. flp-8 pk360	*		

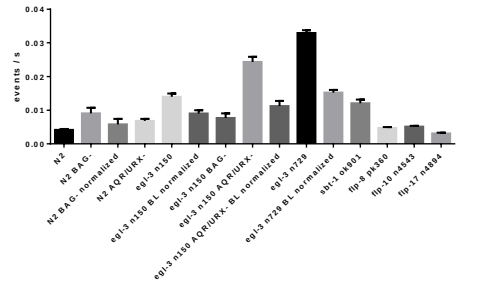


Mean SEM ST 699-719 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-10 n4543	**
egl-3 n150 vs. egl-3 n150 BAG- BL normalized	ns	egl-3 n150 vs. flp-17 n4894	***
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	ns
egl-3 n150 vs. egl-3 n729	ns	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	***
egl-3 n150 vs. flp-8 pk360	ns		

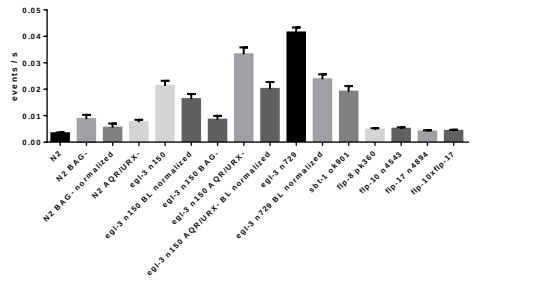
5.3.2.12 LRs in the ramp paradigm – N2 vs. mutant strains



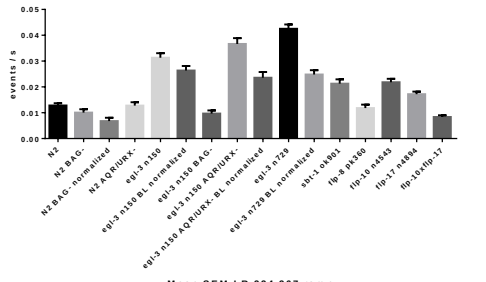
N2 vs. N2 BAG-	**	N2 vs. sbt-1 ok901	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n729	****		



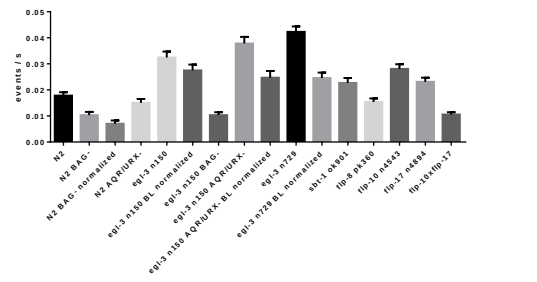
N2 vs. N2 BAG-	**	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	**	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****		
N2 vs. egl-3 n150 AQR/URX- BL normalized	****		



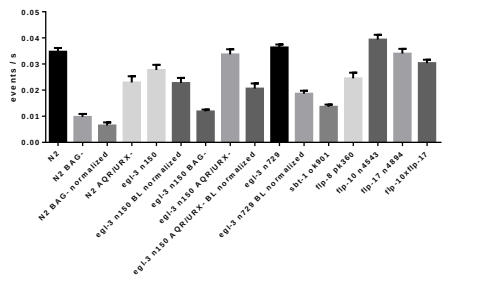
N2 vs. N2 BAG-	*	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	*	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	**	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	****		



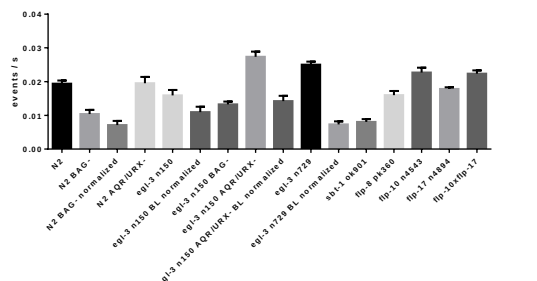
N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	*	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	****		



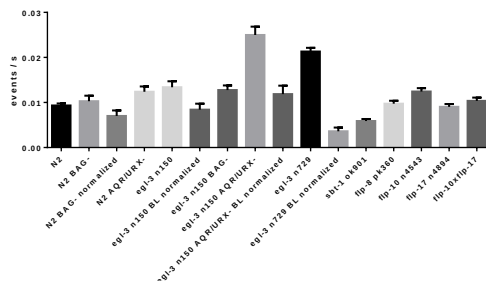
N2 vs. N2 BAG-	****	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	****	N2 vs. egl-3 n729 BL normalized	*
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	ns
N2 vs. egl-3 n150	****	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	****	N2 vs. flp-10 n4543	***
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	**	N2 vs. flp-10xflp-17	**
N2 vs. egl-3 n150 AQR/URX- BL normalized	*		



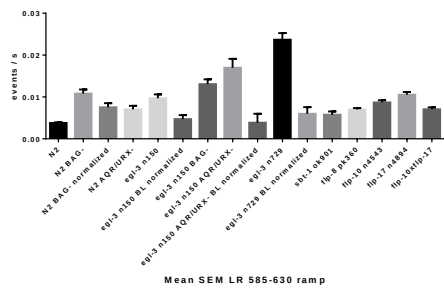
N2 vs. N2 BAG-	****	N2 vs. egl-3 n729	ns
N2 vs. N2 BAG- normalized	****	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	****	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	*	N2 vs. flp-8 pk360	****
N2 vs. egl-3 n150 BL normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	ns	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	****		



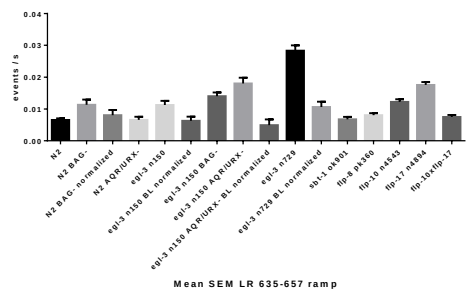
N2 vs. N2 BAG-	****	N2 vs. egl-3 n729	**
N2 vs. N2 BAG- normalized	****	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	****
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	****	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	**	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	*		



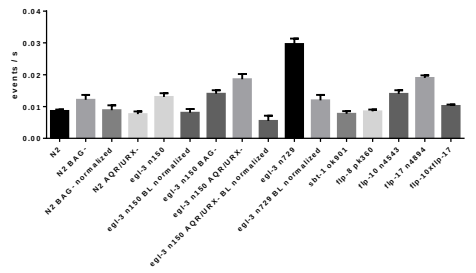
N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	****
N2 vs. N2 AQR/URX-	ns	N2 vs. sbt-1 ok901	ns
N2 vs. egl-3 n150	*	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns		



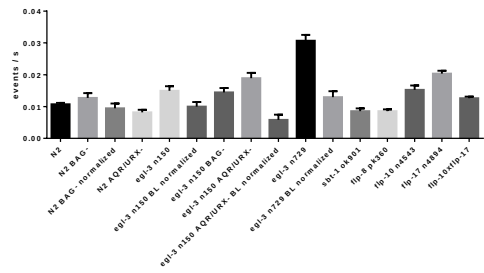
N2 vs. N2 BAG-	****	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	*	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. sht-1 ok901	ns
N2 vs. egl-3 n150	***	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	*
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	***
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns		



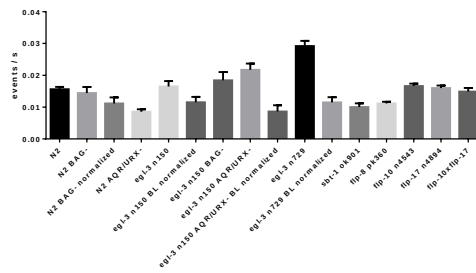
N2 vs. N2 BAG-	*	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. sht-1 ok901	ns
N2 vs. egl-3 n150	*	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	*
N2 vs. egl-3 n150 BAG-	****	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns		



N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. sht-1 ok901	ns
N2 vs. egl-3 n150	*	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	*
N2 vs. egl-3 n150 BAG-	**	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	ns		

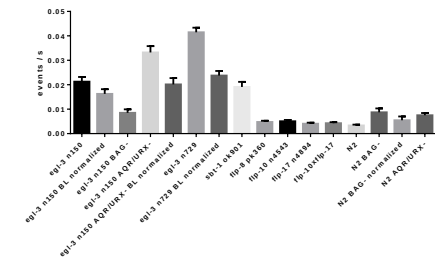


N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX-	ns	N2 vs. sht-1 ok901	ns
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	****
N2 vs. egl-3 n150 AQR/URX-	****	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	*		

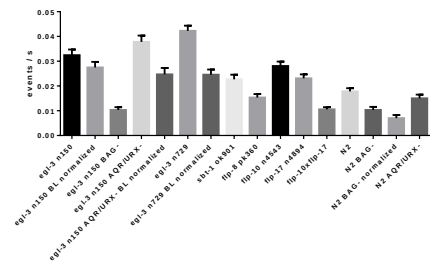


N2 vs. N2 BAG-	ns	N2 vs. egl-3 n729	****
N2 vs. N2 BAG- normalized	ns	N2 vs. egl-3 n729 BL normalized	ns
N2 vs. N2 AQR/URX-	***	N2 vs. sht-1 ok901	*
N2 vs. egl-3 n150	ns	N2 vs. flp-8 pk360	ns
N2 vs. egl-3 n150 BL normalized	ns	N2 vs. flp-10 n4543	ns
N2 vs. egl-3 n150 BAG-	ns	N2 vs. flp-17 n4894	ns
N2 vs. egl-3 n150 AQR/URX-	*	N2 vs. flp-10xflp-17	ns
N2 vs. egl-3 n150 AQR/URX- BL normalized	**		

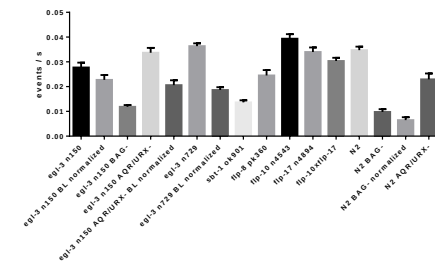
5.3.2.13 LRs in the ramp paradigm – egl-3 n150 vs. mutant strains



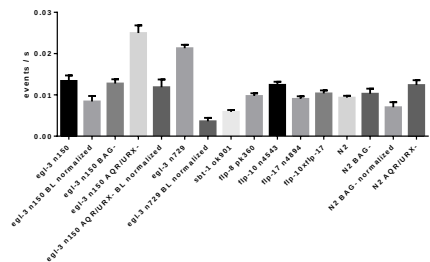
Mean SEM LR 212-244 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	ns	egl-3 n150 vs. flp-10 n4543	****
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-17 n4894	****
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	****
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. N2 BAG-	****
egl-3 n150 vs. egl-3 n729 BL normalized	ns	egl-3 n150 vs. N2 BAG- normalized	****
egl-3 n150 vs. sbt-1 ok901	ns	egl-3 n150 vs. N2 AQR/URX-	****
egl-3 n150 vs. flp-8 pk360	****		



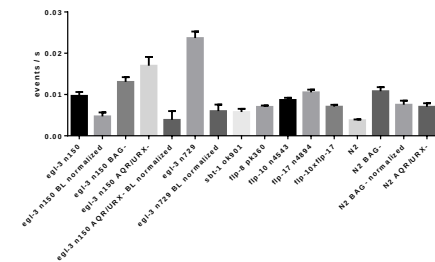
Mean SEM LR 245-267 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	ns	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-17 n4894	*
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns	egl-3 n150 vs. flp-10xflp-17	****
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	*	egl-3 n150 vs. N2	****
egl-3 n150 vs. egl-3 n729	**	egl-3 n150 vs. N2 BAG-	****
egl-3 n150 vs. egl-3 n729 BL normalized	*	egl-3 n150 vs. N2 BAG- normalized	****
egl-3 n150 vs. sbt-1 ok901	**	egl-3 n150 vs. N2 AQR/URX-	****
egl-3 n150 vs. flp-8 pk360	****		



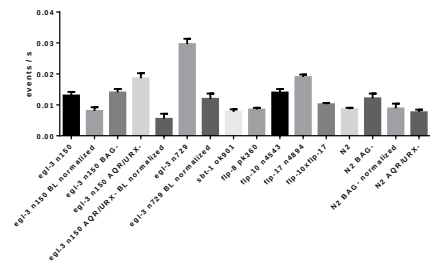
Mean SEM LR 300-360 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	ns	egl-3 n150 vs. flp-10 n4543	***
egl-3 n150 vs. egl-3 n150 BAG-	****	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	*
egl-3 n150 vs. egl-3 n729	**	egl-3 n150 vs. N2 BAG-	****
egl-3 n150 vs. egl-3 n729 BL normalized	**	egl-3 n150 vs. N2 BAG- normalized	****
egl-3 n150 vs. sbt-1 ok901	****	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	ns		



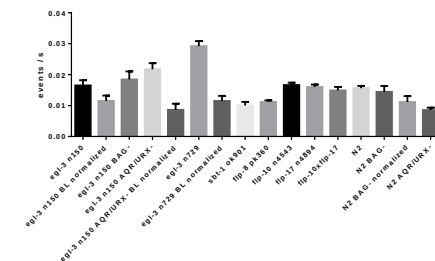
Mean SEM LR 480-540 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	*	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	****	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	ns	egl-3 n150 vs. N2	*
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. egl-3 n729 BL normalized	****	egl-3 n150 vs. N2 BAG- normalized	***
egl-3 n150 vs. sbt-1 ok901	****	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	ns		



Mean SEM LR 585-630 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	*	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	***	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	**	egl-3 n150 vs. N2	***
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. egl-3 n729 BL normalized	ns	egl-3 n150 vs. N2 BAG- normalized	ns
egl-3 n150 vs. sbt-1 ok901	****	egl-3 n150 vs. N2 AQR/URX-	ns
egl-3 n150 vs. flp-8 pk360	ns		



Mean SEM LR 635-689 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	*	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-17 n4894	*
egl-3 n150 vs. egl-3 n150 AQR/URX-	*	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	****	egl-3 n150 vs. N2	*
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. egl-3 n729 BL normalized	****	egl-3 n150 vs. N2 BAG- normalized	ns
egl-3 n150 vs. sbt-1 ok901	*	egl-3 n150 vs. N2 AQR/URX-	*
egl-3 n150 vs. flp-8 pk360	ns		



Mean SEM LR 699-719 egl-3 n150 vs mutants ramp			
egl-3 n150 vs. egl-3 n150 BL normalized	ns	egl-3 n150 vs. flp-10 n4543	ns
egl-3 n150 vs. egl-3 n150 BAG-	ns	egl-3 n150 vs. flp-17 n4894	ns
egl-3 n150 vs. egl-3 n150 AQR/URX-	ns	egl-3 n150 vs. flp-10xflp-17	ns
egl-3 n150 vs. egl-3 n150 AQR/URX- BL normalized	**	egl-3 n150 vs. N2	ns
egl-3 n150 vs. egl-3 n729	****	egl-3 n150 vs. N2 BAG-	ns
egl-3 n150 vs. egl-3 n729 BL normalized	ns	egl-3 n150 vs. N2 BAG- normalized	ns
egl-3 n150 vs. sbt-1 ok901	*	egl-3 n150 vs. N2 AQR/URX-	**
egl-3 n150 vs. flp-8 pk360	ns		

Curriculum Vitae

Education:

Sep 2009 – Jun 2014	University of Vienna Master Studies Molecular Biology Major Neuroscience, Minor Bioinformatics
Sep 2008 – Sep 2009	University of Vienna Baccalaureate Biology (Molecular Biology) Thesis at the Center for Integrative Bioinformatics Vienna
Sep 2004 – Sep 2008	University of Vienna Diploma Molecular Biology, Sep 2008 switch to baccalaureate
Sep 1994 – Jun 1999	TGM, Vienna, branch of study: Biomedical Engineering Final exams passed with distinction

Publications:

- Co-authorship "A neural circuit for head-body coordination mediates decision making in *C. elegans*", 2014, first author Ingrid Hums, paper in review, submitted to *Neuron*
- Bachelor's thesis "Bioinformatics analysis of interacting amino acids at the 2APro cleavage site of the Human Rhinovirus polyprotein"

Research and professional experience:

Feb 2014 – Jun 2014	Service contract with the research group of Manuel Zimmer at the Institute for Molecular Pathology (IMP), Vienna
Feb 2013 – Feb 2014	Master thesis at the research group of Manuel Zimmer at the Institute for Molecular Pathology (IMP), Vienna
Oct 2012 – Dec 2012	Internship at the research group of Manuel Zimmer at the Institute for Molecular Pathology (IMP), Vienna
Jun 2012 – Sep 2012	Service contract software project at the department of Computational Systems Biology (CUBE) of Thomas Rattei at the University of Vienna
Apr 2012 – Jun 2012	Internship at the department of Johannes Berger at the Center for Brain Research, Vienna
Feb 2012 – Apr 2012	Service contract software project at the department of Computational Systems Biology (CUBE) of Thomas Rattei at the University of Vienna
Jun 2011 – Aug 2011	Internship at the department of Computational Systems Biology (CUBE) of Thomas Rattei at the University of Vienna
Nov 2009 – Oct 2010	Service contract with the department of Michael Kiebler at the Center of Brain Research, Vienna
Jun 2009 – Sep 2009	Bachelors Thesis at the Center for Integrated Bioinformatics Vienna (CIBIV) of Arndt von Haeseler, Vienna
Jul 2007 – Aug 2007	Internship at the institute for Theoretical Chemistry (TBI) of Ivo Hofacker, Vienna
Jul 2001 - Sep 2004	LG Soft Software Solutions GmbH, Vienna Software development and project management
Nov 2000 – Jun 2001	Request Technology Education and Services GmbH, Vienna Software development for LG Soft Software Solutions

Zusammenfassung

Der Fadenwurm *Caenorhabditis elegans* ist in der Lage die Sauerstoffkonzentration seiner Umgebung wahrzunehmen. Er vermeidet sowohl atmosphärische wie hypoxische Sauerstoffkonzentrationen und sucht aktiv Konzentrationen um die 16%, nachdem diese mit seinen bakteriellen Nahrungsquellen assoziiert sind.

Bisher wurde nicht untersucht, wie *C. elegans* in der Lage ist, diese Konzentrationen zu identifizieren. *C. elegans* weist vier Hauptklassen an Neuronen auf, die für die Wahrnehmung von Sauerstoff zuständig und für aerotaktisches Verhalten notwendig sind. BAG Neuronen nehmen Abnahme, URX, AQR und PQR Neuronen Zunahme in der Sauerstoffkonzentration wahr. Die Öffnungen der Neuronen BAG, URX und AQR befinden sich am Kopf, die Öffnungen des Neurons PQR in der Schwanzregion des Fadenwurms.

C. elegans müsste dadurch in der Lage sein, auch Sauerstoffkonzentrationsänderungen entlang des Körpers und dadurch effizient Änderungen während seiner Fortbewegung wahrzunehmen, um eine ideale Sauerstoffkonzentration zu erkennen und sich dort hinzubewegen.

Verhaltensexperimente von Wurmpopulationen mit Wildtyp und Mutanten im Labor von Manuel Zimmer in zeigten, dass Neuropeptide eine essentielle Rolle im aerotaktischen Verhalten und dem Auffinden der bevorzugten Sauerstoffkonzentration spielen. Diese Experimente führten zu zwei grundsätzlichen Fragen. Erstens, ist die Aktivität der sensorischen Neuronen alleine ausreichend, um bevorzugte Sauerstoffkonzentrationen zu identifizieren. Zweitens welche Neuropeptide sind essentiell, um die Information von den sensorischen Neuronen durch das neuronale Netzwerk in sinnvolles Verhalten umzusetzen bzw. wo im Netzwerk sind diese Neuropeptide notwendig.

Calcium imaging der sensorischen Neuronen BAG und URX zeigte, dass die gemeinsame Aktivität dieser beiden Neuronen ausreichend ist, um die bevorzugte Sauerstoffkonzentration zu identifizieren. Weiters konnte gezeigt werden, dass Neuropeptide für diese Identifikation nicht notwendig sind.

Um die zweite Fragestellung zu beantworten, wurden Aufnahmen von Mutanten, Rescue und Cell Kill lines in Experimenten mit sich ändernder Sauerstoffkonzentration quantitativ ausgewertet. Die bis zu diesem Zeitpunkt verwendete Analysesoftware war für die Auswertung nicht geeignet und wurde daher teils optimiert, teils vollständig neu programmiert. Die Analyse der durchgeführten Verhaltensexperimente konnte zeigen, dass die unterschiedlichen Verhaltensmuster als Reaktion auf eine sich ändernde Sauerstoffumgebung auf einer Balance multipler Neuropeptide basiert wobei wiederum jedes Verhaltensmuster unterschiedliche Neuropeptide benötigt. Die Analyse war in der Lage, eine Liste an Neuropeptidkandidaten für die verschiedenen Verhaltensmuster zu liefern.

Zusätzlich zeigte die Studie, dass in Reaktion auf Änderungen in der Sauerstoffkonzentration der Umgebung zwei unterschiedliche Aktivitätsmuster im Nervensystem kodiert werden. Das erste Muster ist unabhängig von Neuropeptiden und führt nach einer Änderung in der Sauerstoffkonzentration zu einem sofortigen, starken Anstieg in Verhaltensmustern die mit Fluchtverhalten in Verbindung gebracht werden. In Anwesenheit von Neuropeptiden wird diese erste Reaktion überlagert, wenn die Änderungen in der Sauerstoffkonzentration nur gradueller Natur sind. Erst durch Neuropeptide sind Tiere in der Lage, graduell auf Änderungen in der Sauerstoffkonzentration zu reagieren und ihr Verhalten optimal zu regulieren. Die Interpretation der Ergebnisse legt nahe, dass es erst diese fein ausbalancierten Regulierungen durch Neuropeptide sind, die es *C. elegans* ermöglichen bevorzugte Sauerstoffkonzentrationen zu erreichen und dort zu verweilen.

Abstract

The soil nematode *Caenorhabditis elegans* is able to sense environmental oxygen. It avoids atmospheric as well as hypoxic oxygen levels and actively seeks intermediate levels of around 16% which are associated with the presence of microbial food sources. So far it has not been addressed, how *C. elegans* is able to identify and stay at these intermediate oxygen levels which is not as trivial as following a gradient to its source. *C. elegans* has four main classes of oxygen sensory neurons responsible for its oxygen dependent aerotactic behavior. The BAG neurons are sensing decreases, the URX neurons as well as the neurons AQR and PQR sense increases in oxygen. The sensory openings for AQR, BAG and URX are located in the head, while the sensory opening for PQR is located in its tail region. *C. elegans* should therefore be well equipped not only to sense absolute changes of oxygen concentrations along its path but should also be capable of sensing differences of oxygen concentrations along the length of its body and should therefore be well equipped to identify an optimal oxygen concentration. Behavior experiments of worm populations done in the laboratory of Manuel Zimmer using neuropeptide depleted *C. elegans* mutants revealed a severe defect in the behavioral response to changing oxygen concentrations regardless of the strength of the stimulus. These basic findings lead to the assumption, that neuropeptides play a significant role either in oxygen sensing or in the modulation of the corresponding activity in the neuronal circuit to elicit an appropriate behavioral response.

The findings so far posed two main questions. First, is the activity of the sensory neurons sufficient to provide animals with information concerning an optimal oxygen concentration. Secondly, which and where within the neuronal circuit of interest neuropeptides are required to transform the sensory input into appropriate behavior. Calcium imaging of the oxygen sensory neurons BAG and URX revealed that their combined activity pattern is sufficient to identify the concentration optimum. The experiments also showed, that neuropeptides are not required at the sensory level in respect to finding the preferred oxygen concentration range.

Quantitative recordings of worm population behaviors of *C. elegans* mutant, rescue and cell kill lines were used to address the second question. The existing informatics tool to analyse the recordings proved to be insufficient with the oxygen paradigm used and was partially rewritten and optimized accordingly. The results of the behavior analysis showed, that in a changing oxygen environment the transmission of sensory information from oxygen sensory neurons to motoneurons to elicit specific behavioral responses relies on a delicate balance of multiple neuropeptides and provided a candidate list for neuropeptides involved.

Furthermore the study revealed, that upon changes in the oxygen concentration, two different responses take place. The first is a response independent from neuropeptides. This response leads to a very strong but short lived increase in behavioral events after a drastic change in the oxygen concentration which is interpreted as a robust escape response to a harsh stimulus.

Neuropeptides efficiently mask this stereotypical escape response if changes in the environmental oxygen concentration are not associated with an immediate threat to the animal and mediate highly fine tuned responses to gradual oxygen changes. This fine tuned behavior supposedly enables *C. elegans* to efficiently sample the environment in response to gradual changes and is probably required to efficiently reach and stay at preferred oxygen concentrations.