Modelling valence learning in the olfactory circuit of the fruit-fly

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Abstract

The fruit fly memorises previous experiences of different smells along with information on whether the smell was associated with reward or punishment. The location of thism memory is the mushroom body (MB), an area comprised of thousands of neurons that has been implicated in olfactory memory and decision-making. More precisely it is thought to be held in the synapses unto a small group of so called "mushroom body output neurons" (MBONs) controls approach and retreat behaviour. They receive their input from other mushroom body neurons and are also affected by dopamine. Experimental evidence suggesting that dopamine controls both reward and aversive based learning (**?**). It is thus believed that MBONs form a “valence” coding space; depending on the combined firing rates of “retreat” and “approach” biasing MBONs, each odour will be considered to be either appetitive, aversive or neutral by the fly (**?**). In this vein, a recent study has shown that one such MBON decreases its activity after appetitive training and increases its activity after aversive learning (**?**).

To understand the mechanisms behind dopamine mediated learning of olfactory valence, we have developped a spiking model of plasticity in the olfactory circuit of the fruit fly. In our computational model we can reproduce experimental evidence that show bi-directional change of firing rate in MBONs. Furthermore we visualize the coding space formed by the firing rates of MBONs and observe how odours change their position in the valence coding space depending on the learning paradigm used. Finally we make experimentally verifiable predictions, proposing that if only the “retreat” biasing group of MBONs change their firing rates bi-directionally as current evidence suggests, aversive memories are more stable than appetitive memories. We also predict that bi-directional change of firing rate can enhance the discriminability between odours of different valences.

In the future, we propose to refine our olfactory valence learning model of the fruit fly by incorporating recent anatomical, behavioural and electrophysiological data available. With the help of state of the art computational models and close experimental collaboration, we hope to further our understanding of how reward and punishment activated dopaminergic neurons guide learning in the fruit fly, as well as general mechanisms that govern valence learning across different species.

Abbreviations

KC - Kenyon Cells, a group of neurons which reside in the Mushroom Body
MBONs - A group of 32 mushroom body output neuronsc that receive input from KCs
APL - Anterior Paired Lateral neuron, a global inhibitory neuron that inhibits all KCs
MVP2 - an MBON which biases for approach behaviour
M4β' - an MBON which biases for retreat behaviour
DAs- Dopaminergic Neurons, a group of neurons that become activate either to an appetitive or an aversive stimulus
PAM- a cluster of dopaminergic neurons (DAs) that provide positive reinforcement to MBONs
PPL - a cluster of dopaminergic neurons thast receive
CS+- *conditional stimulus*, an odour which is paired with reinforcement, the term CS+ is used both in the case of aversive and appetitive reinforcement
CS− an odour which is presented without reinforcement
$\alpha\beta, \alpha'\beta', '\gamma$ lobes- the three main anatomical subdivisions of the MB. α, α' are vertical lobes and β, β', γ are horizontal.

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Chapter 1

Introduction

Like all living organisms, the fruit fly uses sensory information to navigate its world. To a first approximation a fruit fly's behaviour in response to a sensory input can be described as a decision making process of approach or retreat. One such sense that the fly uses to decide whether to approach or retreat is the sense of smell, which is a well-studied function, employed to gather information about the environment, find food, mating partners, and detect dangerous conditions signaled by harmful odours. Flies are attracted and repelled to many odours innately (**?**), but also show ability to learn new odours (**?**).

When exposed to most odours, the response behaviour of flies depends on experience: if an odour is associated with a sweetened reward (CS+, or conditioned stimulus) and another odour offers no reward (CS- or unconditioned stimulus) the flies will show a preference for the rewarded odour in a binary choice test (*Fig.* 1.1 *A* top panel). However, if an odour is associated with a punishment (CS+) such as a shock, fruit flies can be conditioned aversively to odours (**?**) and will choose the neutral odour (CS-, *Fig.* 1.1 *A* bottom panel). Understanding how these memories of odours are generated and how they affect behaviour is an area of current research. Researchers are also beginning to understand how innate and learned olfactory behaviours interact with each other. However, until now there have been few computational driven approaches (**??**) that have

converted word models into mechanistic models that can explain mathematically the growing behavioural data, make predictions and testable hypotheses. We shell discuss these computational methods in Section 1.5.

Figure 1.1: Reinforcement learning experiment and circuit
A) In the learning paradigm the fly is shown a simple odour which is called the CS- (conditional stimulus without reinforcement) for 2 minutes in the appetitive training protocol. Afterwards, a fly is put into an empty chamber for 30 second where only air is present. Finally, an odour is shown that is paired with a reinforcement (CS+) which can be either sugar or shock. After that, the fly is tested for odour preference when given a choice between the CS+ and CS-. Note: In the case of aversive training, the reinforced odour (CS+) is shown first.
B) Normalized calcium imaging response in the $M4\beta'$ before and after learning shows a bidirectional change in firing rate with the calcium response increasing for aversive learning and decreasing after appetitive learning (averaged from ten flies).
C) (i) DA neurons representing aversive reinforcement. Studies suggest that neurons in PPL1 convey negative reinforcement and are strongly activated by shock (**?**). In this illustration a selection of DA neurons innervating MB zones and the MB-M3 neurons on the tip of the γ lobe are activated (red). (ii) DA neurons representing appetitive reinforcement. DA neurons in PAM innervate many discrete zones in the β, β' and γ lobes. Ca2+ imaging of sugar-evoked activity suggests only some zones receive appetitive reinforcement. Reproduced from **?**
D) The valence learning olfactory circuit is made of a group of approximately 2.000 KCs which are tasked with odour identification. There are two groups of dopaminergic neurons that signal either shock or sugar and depress connections between KCs and MBONs. M4 MBON biases towards retreat behaviour while MVP2 MBONs biases towards approach behaviour. The MVP2 MBON in this figure is show inhibiting the M4 retreat neuron (David Owald - personal correspondence). The DPM neuron decreases its activity during hunger and disinhibits KCs which are known to innervate the MVP2 Approach neuron.

1.1 Odour representation become concentration invariant in the early stages of olfactory processes

Flies detect odours using approximately 1,200 olfactory receptor neurons (ORNs) housed in their antennae. The tuning of each ORN is determined by a single odorant receptor gene, which controls whether the ORN is broadly or narrowly tuned to odours (**?**). Axons from the ORN expressing the same receptor always converge on the same glomerulus.

Their activity is picked up by inhibitory and excitatory projection neurons which perform normalization of the odour (?). Excitatory projection neurons deliver information to the calyces in the mushroom body while both inhibitory projection neurons and ePNs send information to the Lateral Horn. The Lateral Horn is associated with innate behavioral responses to odors, such as sex-specific responses to pheromones during courtship (Datta et al 2008), whereas the mushroom body is required for learned responses to odors (?).

1.2 The MB is the centre for associative learning in the fruit fly

The mushroom bodies are symmetrical structures with each mushroom body being composed of approximately 2,000 Kenyon Cells (?). Lesion experiments and synaptic blocking of mushroom bodies output have shown that that the mushroom body plays a critical role in memory formation and retrieval (?). While odour coding in the antennae was dense, the MB is believed to maintain a sparse and high-dimensional odour representation. Experimental studies have shown that the MB maintains a sparse representation of odours as each odour activates approximately 10% of KCs (approximately 150 - 200 neurons , ?).

1.2.1 Similar odours have overlapping representations in the MB

Previous experimental studies have shown that odours with similar chemical properties have overlapping representations in the MBs (?). Behavioural experiments have shown flies have the ability to both generalize similar odours and separate them depending on the training protocol. If we use an odour as a CS+ and train the fly to remember it as predicting reward the fly will showcase the same behaviour when exposed to a previously unexposed similar odour. However if the second odour is shown to the fly during training as the neutral odour (CS-), the fly will not show preference to it when compared to

another neutral odour (**?**). The fly appears to be able to be caable to both generalize similar odours and distinguish them depending on past experience.

1.2.2 The MB recurrent activity is critical for stabilizing odour memories

Experimental studies have shown that olfactory representations in the mushroom bodies are modulated by the anterior paired lateral (APL) neuron (**?**). Each mushroom body is innervated by a single "giant" APL neuron, which transmits inhibition to the mushroom body calyx as well as the vertical and medial lobes. It also appears that the APL receives input from KCs in the vertical and medial lobe and inhibits KCs at their dendritic terminals in the calyx. The APL releases GABA at a rate proportional to its level of depolarization and appears to play a role normalizing KC responses to odours and maintaining sparseness (**?**).

1.2.3 Anatomical subdivisions of the MB have unique role in odour processing

The dendrites of the KCs form the calyx of the MB, and their axons project anteriorly to form the peduncle before those axons terminate in one or more lobes, termed $\alpha\beta$ α', β', and γ lobe (Fig.1.2, **?**). The $\alpha\beta$ contains 1000 KCs while the α', β' contains 600 and the γ lobe 400 of KCs and each subdivision is believed to have unique roles in memory processing.

In a previous study it has been shown by blocking different anatomical subdivisions of KCs, that the $\alpha\beta$ neurons are needed during retrieval of aversive and appetitive memory whereas $\alpha'\beta'$ core neurons, when blocked only affect appetitive memory (**?**).

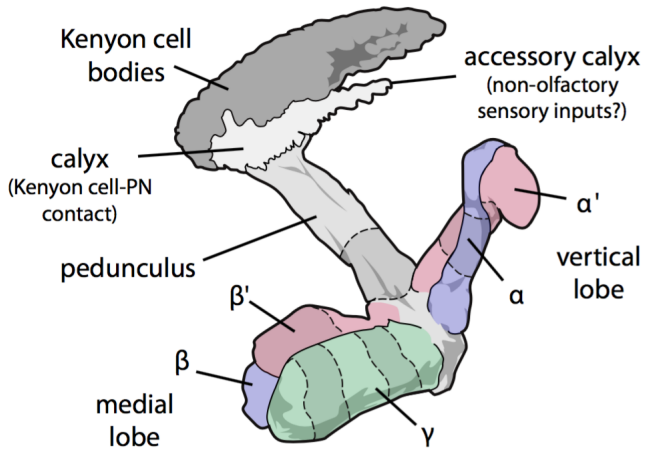

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Figure 1.2:
Gross anatomy of the mushroom body (reproduced from **?**). Dashed lines reflect the compartments defined by MBON dendrites. The α and γ lobes are each divided into three compartments, and β and β' are each divided into two, γ is divided into five compartments.

1.2.4 MBONs guide approach and retreat behaviours

Outputs from approximately 2,000 Kenyon cells of the mushroom body converge onto a population of 34 mushroom body output neurons (MBONs). The MBON dendrites divide the two lobes and the base of the pedunculus into a small number nonoverlapping compartments, within which the dendrite densely contacts either all KCs or an anatomically distinct subpopulation. KC axons in the α/α' lobes are clustered into anterior, middle, and posterior groups, in theβ/β' lobes into posterior, surface, and core groups, and in the γ lobe into main and dorsal groups. The MBONs innervating each compartment are genetically distinct, and each projects characteristically to other regions of the brain. MBONs project from the mushroom body to several regions. A small number of excitatory neurons innervating the α or α' lobes project to lateral horn, a region implicated in control of innate odor- related behaviors.

Every MBON projects to four neuropils just outside the mushroom body: the crepine, the superior medial protocerebrum (SMP) the superior intermediate protocerebrum and

the superior lateral protocerebrum where input from the approach and retreat MBONs is believed to be transformed into motor control.

We can classify MBONs according to the behaviour they bias towards, as two recent studies have shown by silencing and activating each of the 34 MBONs; they either promote approach or retreat behaviour (**??**).

For example, experiments for one retreat promoting MBON ($M4\beta'$) have shown bi-directional change in firing rate of the neuron controlled by the reinforcement used (appetitive or aversive). Calcium imaging before and after training have shown the response decreases compared to the naive response after appetitive learning and increased after aversive learning ($Fig.2B$). Furthermore, optogenetic activation of the $M4\beta'$ neurons changed a neutral behaviour into avoidance behaviour and blocking the neuron converted a naive odour approach into odour retreat in naïve flies (flies that had an innate repulsion to the odour without learning,**?**). Converseley, the MVP2 MBON neuron is known to promote approach behaviour. Interestingly, there is evidence that the $MVP2$ "approach" MBON inhibits the $M4\beta'$ retreat MBON ($Fig1.1D$, David Owald - private correspondence). It is not know whether any of the retreat MBONs inhibit approach MBONs. All though there are 32 MBONs, for our initial study we shall restrict our modelling efforts to only two that have been studied by our experimental collaborator: the $MVP2$ "approach" biasing neuron and the $M4\beta'$ retreat biasing neuron. Modelling these two neurons will allow us to reproduce the valence coding space.

1.3 DAs convey reward and punishment signals

Dopaminergic neurons (DAs) are the most prevalent modulatory neurons that innervate the mushroom bodies. There are distinct dopaminergic neurons that provide positive and negative value signals. The two major clusters where most DAs reside are the PPL and the PAM clusters ($Fig.1.1C$). Positive reinforcement are provided by subsets of the approximately 100 DAs in the PAM cluster (**?**). They predominately innervate nearby

zones in the β, β', γ lobes. Negative reinforcement such as from electric shock or bitter substances appears to be conveyed by DAs housed in the PPL cluster. Each PPL DA that innervates the mushroom bodies projects presynaptic terminals on the α or α' lobe(David Owald & Scott Waddell – in review). The dendrites of MBONs is restricted to few DA zones. For example for the "retreat" MBON we previously discussed ($M4\beta'$) experimental data exists to support that the axons from sugar rewarding dopaminergic neurons overlap with the dendrites of the MBON (**?**).

1.3.1 DAs convey reward and punishment signals in different areas of the mushroom body

Distinct DAs convey the effects of sugar and water reward as opposed to the same neurons representing subjective value such as in the case of negative reinforcing DAs (**?**). The sugar and water responsive DAs project to unique zones on the MB lobes, thus suggesting that learning-related plasticity is represented at different axon terminals along the lobes of the mushroom body. It is believed that since DAs reaches a subset of KCs axons, they modify KC output synapses onto MBONs in their respective zone. Thus water memories and sugar memories are predicted to have unique KC-MBON connections that represent them (**?**).

1.3.2 DA state control

Sugar memory is most robustly expressed in hungry flies and similarly, thirst promotes the expression of water memory. It appears that to form these memories, different zones of the MB provides the anatomical requirements in which state control be implemented. Interestingly, a group of DA neurons that provide the inhibitory constraint to the expression of sugar memory (**?**) occupy the same zones in the heel and peduncle of MB as the MVP2 approach neuron whose activity drives approach behaviour. Thus it seems likely that the internal state of hunger skews the balance of MBON pathways to favour

approach. It has not been yet shown whether the activation of thirst involves a similar layer of control. A current study has shown that the MVP2 "approach" neuron increases its response to an odour after it has been starved which biases the behaviour towards approach (David Owald - personal correspondence, *Fig*. 1.3). This adds another layer of complexity to the valence learning model which is state modulation of behaviour.

Figure 1.3: Normalized calcium imaging response of the MVP2 neuron to four different odours, averaged from ten flies.

1.3.3 DA modulated learning changes odour drive to KC-MBON synapses

A hypothesis of how valence learning is achieved, proposes that learning modifies subsets of KC-MBON pathways which in naive flies are balanced (**?**). Appetitive responding DAs promote odor approach by depressing odor drive to "avoidance" MBONs and possibly by strenghtening approach pathways. Conversely, aversive responding DAs promote depressing odour drive to "approach" MBONs (**?**). It is likely that a feedack loop from MBON to DAs influences learning as well. Blocking the $M4\beta'$ neuron induced a depression of the KC-$M4\beta'$ MBON connection (**?**), which could be because the $M4\beta'$inhibits some appetitive DAs.

1.4 Spike Timing Dependent Plasticity (STDP) as a candidate for plasticity at the KC-MBON synapses

A candidate mechanism for valence learning has been proposed in a recent study in the locust, a similar system to Drosophila (Cassenaer and Laurent 2012). They have shown that KC-MBON synapses usually obbey the spike-timing-dependent plasticity (STDP) rule. STDP can be seen as a spike-based variant of the Hebbian rule where synaptic change de-

pends on the relative timing of pre and postsynaptic action potential (**?**). During STDP, a synapse potentiates when the postsynaptic spike happens after the pre-synaptic spike within a time window of the order of tens of milliseconds and depresses when the order is reversed (**?**). Spike timing, however is not sufficient to explain how plasticity strengthens or weakens synapses. Neuromodulators such as dopamine can gate plasticity determining when learning can take place (**?**). Indeed, in the locust when octopamine was injected 1 s after spike pairing in the experimental protocol synapses were only observed to depress their strength (*Fig.*2.1*B*). In flies octopamine is known to act on the mushroom body indirectly, by activating PAM dopaminergic neurons (**?**). Therefore evidence from studying the locust supports the hypotheses of dopamine modulated STDP as the candidate mechanism for learning in the fruit fly.

1.5 Previous modelling attempts of olfactory learning

Previous modelling efforts of the fruit fly's olfactory circuit have mostly focused on the neurons implicated in olfactory processing before reaching the MBONs, since 3 landmark papers this year opened the path to modelling them (**??**), **?**).

However, there has been one study that reproduced the valence learning capacity of the olfactory circuit (**?**). They created a spiking model with dopamine modulated plasticity occurring at the synapses between KCs and MBONs and have show that the MBON can learn to distinguish between odours of opposite valences. In their model, contrary to current evidence dopamine potentiates the weights. Their model predicts the fly can also learn an odour mixture discrimination task where one half of the odour is paired with a negative reinforcement and another half is neutral which has been shown experimentally in the bee (**?**).

1.6 Improving previous models of valence learning in the fruit fly

The model that was previously discussed (**?**) have provided us with a good starting point, since it implemented a dopamine modulated learning rule that allows the model to classify odours of different valences witth a single MBON. However, we modelled two type of MBONs, which allows us to visualize the coding space for "retreat" and "approach" behaviours. Furthermore we have also studied competition between MBONs which code for opposite valences. Finally, we have studied for the first time in the fruit fly the functional consequences of bi-directional change of firing rate in MBONs.

Chapter 2

Methods

Experiments of the $M4\beta'$ and $MVP2$ MBONs were performed by David Owald at the CNCB and motivated the simulations which were created by the author. We decribe the first stage of our modelling efforts where we tested a plasticity learning rule that depresses the synapse in the presence of dopamine. The three main neuron types that we modelled heere are Kenyon Cells, MBONs and DAs.

2.1 Connectivity

We used experimental data that traces the connectivity between KCs and output neurons (**?**). The $M4\beta'$ neuron receives input from 500 KCs from the $\alpha'\beta'$ lobe and 400 KCs from the $\alpha\beta$ lobe. The MVP2 neuron receives input from 400 KCs from the γ lobe and 500 KCs from the $\alpha'\beta'$ lobe. We do not know the initial synaptic efficacies (weights) between KCs and MBONs, thus we make the assumption that the weights between KCs and MBONs have uniform random strengths at start. By doing that we found that we needed to tune our synapses with strengths between 0 nS and 2 Ns to achieve MBON firing rates of 50 Hz, whic is in accordance with experimental evidence (**?**).

2.2 MB Kenyon Cells model

When the fly is exposed to an odour ~200 KCs activate. The two MBONs sample approximately half of the total number of KCs, thus only a hundred odour responding KCs send an input MBON. We modelled the spike trains for 100 KCs in response to an odour based on experimental data (**?**). In our model an odour is represented as KC spikes. In our protocol we first select 100 KCs that will be active to an odour and send input to each MBON.

During spontaneous activity each KC fires at approximately ~0.1 Hz. Whole cell recording have shown that when an odour is shown KCs fire an average of 3 spikes per second. In normalized calcium imaging experiments, KCs are found to show a larger response in the first 200 ms of the odour exposure (**?**). We have used this information to generate KC spike trains with an average of 3 spikes per second and with a higher probability of a spike to occurr in the first 200 ms compared to the rest of time.

2.3 MBONs model

As a starting point we have modelled two MBONs, one for approach and one for retreat ($M4\beta'$ and $MVP2$). As previously discussed, each MBON samples half of the total number of KCs. The firing rate of the "approach" $MVP2$ neuron is determined entirely by input KCs. For each simulatuion the $M4\beta'$ neuron receives inhibition from the $MVP2$ MBON, which is accordance with experimental evidence (David Owald - unpublished work). We used a Linear Integrate and Fire (LIF) model with parameters that were taken from a recent modelling paper where whole cell recordings of MBONs was performed (**?**).

Parameter	Default Value	Symbol
MBON Membrane Time Constant	20 ms	τ_m
MBON Spiking threshold	-40 mV	V_{th}
MBON Resting membrane potential	-60 mV	V_{inh}
MBON Excitatory conductance	0 mV	E_{ex}
MBON Inhibitory conductance	-80 mV	E_{inh}

Table 2.1: MBON neuron parameters

2.4 LIF Neuron Model

We used the conductance based LIF model to model the MBONs. The LIF neuron equation is as follows:

$$\tau_m \frac{dV}{dt} = (V_{rest} - V(t)) + g_{ex}(E_{ex} - V(t)) + g_{inh}(E_{inh} - V(t)). \tag{2.1}$$

Here V is the membrane potential of the neuron as a function of time , $\tau_m$is the membrane time constant, $V_{rest}$is the resting membrane potential, $E_{ex}$is the excitatory reversal potential and $E_{in}$is the inhibitory reversal potential. The synaptic conductances are expressed by g_{ex}(excitatory) and g_{inh}(inhibitory). They are modelled according to the following equations:

$$\tau_{ex} \frac{dg_{ex}(t)}{dt} = -g_{ex}(t) \tag{2.2}$$

and

$$\tau_{inh} \frac{dg_{inh}(t)}{dt} = -g_{inh}(t), \tag{2.3}$$

where $\tau_{ex}$and $\tau_{inh}$are the synaptic time constants for the excitatory and the inhibitory conductance, respectively. When the neuron receives an action potential from a presynaptic cell the postsynaptic conductance increases by the following formulas: $g_{ex} \rightarrow g_{ex} + \Delta g_{ex}$ and $g_{inh} \rightarrow g_{inh} + \Delta g_{inh}$ for excitatory and inhibitory synapses, respectively.

2.5 The three-factor plasticity learning rule

We introduce an STDP (Spike-Timing Dependent Plasticity) learning rule into our model has been observed in the locust mushroom bodies (**?**). In this dopamine modulated STDP model,

Synaptic change in our model is the result of dopamine concentration value multiplied by synaptic tag value at each time point.

Synaptic weight change is gated by the presence of dopamine. Eq. 2.9 shows that weight change occurs when dopamine concentration is above 0. Change is negative according to experimental evidence so we multiply by -1.

$$\frac{\mathrm{d}w_{ij}(t)}{\mathrm{d}t} = D(t)c_{ij}(t) \tag{2.4}$$

We model the DA neuron as an integrate and fire neuron which determines the value of the final component of our learning rule. The neuron receives direct current input in the presence of a reward or a punishment depending on the training method use and fires regularly at a rate. The dopamine concentraftion (D(t) which modulates synaptic change is a global eligibility trace that increases each time the dopamine neuron fires,

$$D(t) = -\frac{D(t)}{\tau_d} + \sum_k \delta(t - t_k^*), \tag{2.5}$$

where $t_k^*$is the k-th DA spike and δ is the Dirac's delta. c_{ij} represents a temporal tag in synapses where pre-post firing occurs,

$$\frac{\mathrm{d}c_{ij}(t)}{\mathrm{d}t} = -c_{ij}(t)/\tau_c - A\_x_j(t)S_i(t) - A\_y_i(t)S_j(t), \tag{2.6}$$

where $A_$dtermines the amplitude of maximum depression that the synapse can undergo when presynaptic and postsynaptic spikes times occurr nearly at the same time. $S_i(t)$ is the spike train of the i-th neuron, given by

$$S_i(t) = \sum_k \delta(t - t^*_{i,k}), \tag{2.7}$$

where $t^*_{i,k}$ is the timing of the spike, where i is the i-th neuron and k is the index of the spike. The tag decreases when the postsynaptic neuron fires an action potential and depends on the timing of the last spike timings of the presynaptic neuron through the variable $x_{ij}(t)$ which evolves accordingly to:

$$\frac{dx_j(t)}{dt} = -\frac{x_j(t)}{\tau_-} + S_j(t). \tag{2.8}$$

Equally the tag also decreases when the presynaptic neuron fires an action potential, depending on the postsynaptic neuron activity through the variable $y_{ij}(t)$ given by

$$\frac{dy_i(t)}{dt} = -\frac{y_i(t)}{\tau_-} + S_i(t), \tag{2.9}$$

where τ_- is the characteristic time of the y_i and $x_i$eligibility traces.

Table 2

Parameter	Default Value	Symbol
STDP max Depression amplitude	0.005	A_-
STDP Depression time constant	20 ms	τ_-
Dopamine time constant	20 ms	τ_d

The STDP parameters we used were previously measured experimentally in vivo in the STDP learning paradigm (**?**). In our learning paradigm dopamine gates synaptic learning which is to say synaptic change can not occurr in the absence of dopamine. We have used a general model of dopamine gated STDP learning (**?**).

Figure 2.1: Learning components at the KC-MBON synapse
A) KC-MBON STDP learning rule, reproduced from Cassenaer and Laurent (**?**). In gray, the normal STDP rule in KC-MBON synapses, where δt is the time of the postsynaptic spike minus the presynaptic spike, and the y axis shows the percent change in KC-evoked EPSP size in MBONs following five trials in which pre- and postsynaptic spikes were paired at the given δt. In blue, the STDP rule observed when octopamine is injected 1s after pairing. B) Dopamine modulated spike-timing dependent learning rule: Near-coincident pre- and postsynaptic spikes induces synaptic depression. C) i) Raster plot showing KCs firing in response to an odour ii) $M4\beta'$ neuron membrane potential during appetitive training iii) Excitatory (green) and Inhibitory (currents) during appetitive training D) i) The relative timing of firings of the pre and post synaptic neurons induce changes in the synaptic 'tag' variable according to the dopamine modulated STDP learning rule. ii) The synaptic tag decays to zero (top), but if extracellular dopamine are present during the critical time window (middle) the maximal synaptic conductance w is modified (iii) The synaptic weight, here written as w depresses as there have been pre-post spike pairs and dopamine is present in the system

2.6 Odour exposure protocol

In our learning paradigm we require KCs and MBONs to spike, together with the presence of dopamine. Whole cell recordings have shown how KCs respond when exposed to an odour over 1 second, firing at approximately 3 Hz, compared to a spontaneous activity of approximately 0.1 Hz (**?**). We can not know if the KCs fire constantly at 3 Hz for the entire duration of appetitive or aversive conditioning or whether they adapt to the odour and return to spotaneous activity. It is however sufficient to show an odour for 2 seconds coincident with reward or punishment for learning to take place (Johannes Freisenbrug - personal correspondence). For this reason we showed the odour for 2 seconds in our training paradigm.

Chapter 3

Results

Our experimental collaborator has shown that when calcium imaging the $M4\beta'$ neuron, the activity changed both after appetitive and after aversive training. The $MVP2$ MBON is known to change its firing rate only after aversive training. We simulated the $M4\beta'$ and the $MVP2$ MBONs during these two scenarios to check the prediction (Fig . 3.2) and visualized the coding space the two MBONs form (*Fig.* 3.1).

3.1 Odours have a balance of approach and retreat drive before training

In our model the firing rate of the3 $M4\beta'$ neuron determines the retreat drive of an odour, while the firing rate of the $MVP2$ MBON determines the drive to approach. We visualized the valence coding space by plotting their firing rates on two different axes (*Fig.* 3.1). Using our protocol we generated two different odours by creating stereotypical KC responses. MBONs had a balanced drive of approach and retreat before training, which is to say the firing rate of the $M4\beta'$ and $MVP2$ neurons are approximately equal (*Fig.*3.1). Training an odour with appetitive reinforcement decreased their drive towards retreat while, training with aversive reinforcement both decreased their drive towards

approach and increased their retreat drive.

Figure 3.1: MBON coding space before learning
This figure shows the odor valence space coded by MBONs. The white line represents a perfect balance between retreat and approach drive corresponding to both MBONs populations firing at the same rate. The green area corresponds to a higher drive towards approach. Conversely, the red area corresponds to a higher retreat drive from MBONs.

3.2 Bi-directional change in firing rate can occur when dopamine depresses the KC-MBON synapses

We simulated an appetitive and aversive training protocols by showing odours for 2 seconds. In the presence of dopamine. We monitored the plasticity was active at the KCs-MBONs synapses and how this affected odour response before and after learning.

We observed that during appetitive training KC-$M4\beta'$ synaptic weights decreased ($Fig.\,3.2\,A$, ii). After learning, the response from the $M4\beta'$ neuron shows a decreased firing rate profile compared to the naive state ($Fig.\,3.2\,A$ iii). The firing rate decreased by 5 Hz compared to the initial state. Conversely, aversive learning increased the response of the $M4\beta'$ neuron compared to the naive state ($Fig.\,3.2\,B$ iii). This is due to the fact that the firing rate of the MPV2 neuron decreased ($Fig.\,3.2\,B\,iv$), thus increasing the ratio of excitation to inhibition (Fig .3.3).

To observe a change in the firing rate that qualitatively matched the experimental results ($Fig\,1.2\,B$) wee had to choose the strength of the inhibitory MVP2-$M4\beta'$ synapse that increased the ratio of excitation to inhibition ($Fig.\,3.4$). The MBONs we modelled receive input from 100 KCs which fire at a rate of 3 Hz. Every second they receive 300 excitatory spikes with an average weight of 1 nS. In comparison the $M4\beta'$ inhibiting MVP2 neuron fires at a rate of approximately 50 Hz. To obtain the increase in firing rate that qualitatively matches experimental results ($Fig\,1.2\,B$) we had to tune the weight of the inhibitory synapse to be twice as strong at the excitatory synapse.

The odour representation in valence space moves perpendicularly to the line of equal drive and thus has a strong affect, whereas appetitive training moves along a single axis. This can be explained due to the fact that aversive learning both decreases drive to the approach MBON and increase drive to the retreat MBON.

Figure 3.2: Responses of the MVP2 (approach) and$M4\beta'$(retreat) neurons
A) During appetitive training the dopamine modulated STDP rule targets the KC-$M4\beta'$ synapses i) In our simulation an odour is shown for 1 s while appetitive reinforcement is provided after 100 ms ii) A selection of 'weights' are shown that represent the efficacy of the KC-$M4\beta'$ synapses (blue) undergoing depression iii) Before (black) and after appetitive learning (light blue) $M4\beta'$ response to the odour iv) Before (black) and after (purple) appetitive learning response of the MVP2 neuron. B) During aversive training dopamine modulated STDP targets the KC-MVP synapse leading to disinhibition of the M4 neuron i) An odour is shown for 3 s paired with aversive reinforcement ii) A selection of 'weights' are shown that represent the efficacies of KC-MVP synapses (purple). iii) Before (black) and after aversive learning (light blue) $M4\beta'$ response to the odour iv) Before(black) and after (purple) aversive learning response of the MVP2 neuron.

Figure 3.3: MBON coding space after learning
A) The odour is shown before and after appetitive learning in valence coding space which shifts its position towards the green area of the valence space B) The odour is shown before and after aversive learning in the valence coding space which shifts its position towards the red area of the valence space.

Figure 3.4:
A) Synaptic current of the $M4\beta'$ neuron before aversive training B) Synaptic currents of the $M4\beta'$ neuron after aversive training

3.3 Inhibition between MBONs can enhance odour discrimination

To show that the MVP2-M4 inhibitory synapse is necessary and that it enhances dsicrimination we performed the simulation in two conditions, once with the MVP2-M4 inhibitory synapse active ($Fig.3.5A$) and once without ($Fig.3.5B$). The M4 MBON increases its firing rate only when there is an inhibitory synapse between the MVP2 and

M4 Furthermore, if we look at the valence coding space (Figure 9 bottom), we observe that the inhibitory synapse between the MBON increases discriminability by pushing the representation depeer into the reretreat area ($Fig.3.5B$ bottom).

Figure 3.5: Aversive learning with and without MVP2 - $M4\beta'$inhibition
To see the functional implications of lateral inhibition we pair an odour with aversive reinforcement and perform the simulation for 1 second with and without inhibition. A) Odour retrieval test without lateral inhibition In order: i) Response of approach neuron to odour after aversive training vs naive response ii) Response of retreat neuron to odour after aversive training vs. naive response iii) Odour before and after training in coding space B) Odour retrieval test with lateral inhibition. i) Response of retreat neuron to odor after aversive training vs. naive ii) Response of approach neuron after aversive training vs. naive; iii) Odor projected in valence space before and after training

3.4 Trained odour can change the valence of a similar untrained odour

Based on evidence that similar odour activates an overlapping subset of KCs in the MB we tested in our mnodel how learning affects untrained odours on our model.

We have selected two odours with 50% overlap in the identity of KCs activated two mimick similar odours that appear in nature. In the naive state, both of the odours have a neutral valence (Fig3.6). We subsequently trained, the first odour with both appetitive and aversive reinforcement. When we tested both of the two odurs for their response in the MBON code space we observed that both odours have changed the valence to that of the trained odour ($Fig.$ 3.6) which has been shown experimentally to occurr (**?**).

Figure 3.6: Training odours with overlapping representations
We have trained an odour with an appetitive (A) / aversive (B) stimulus (o1) and we visualize both the trained odour and a similar odour with overlap after o1 is trained. A) Bot the trained (o1) odour and the untrained (o2) have moved position in the valence space towards the approach area B) Both the trained (o1) and the untrained (o2) odour with overlapping representation havee moved their position in the valence space to the aversive area

3.5 Aversive memories are more stable than appetitive memories

We have shown that aversive learning leads to enhanced discriminability when compared to appetitive training in our model (*Fig* 3.4 *B* iii). This motivated us to test the following two scenarios. We have trained one odour with appetitive reinforcement first and aversive training aftewards to observe the change in the position it occupies in the odour valence space (*Fig* 3.5 *A*). Appetitive training for 2 seconds leads to a decrease in the firing rate of the M4 neuron (retreat) and converts the neutral odour into an odour that signals approach (*Fig* 3.5 *A*). When we induce aversive learning immediately afterwards, we observe a decrease in the firing rate of the MVP2 neuron (approach) and an increase in the firing rate of the M4 neuron. The bi-directional change of firing rate converts the odour from signaling approach to signaling retreat. Conversely in the following simulation we tested what happend when we chose to train the odour aversively first. The perpendicular movement in the coding space (*Fig* 3.5 *B*) due to bi-directional change in the firing rate ensured that the odour remained aversive even after we induced appetitive training. In our simulation we observed that his result depends on whether appetitive and aversive learning decrease the firing rate of the targeted neuron in an equal manner.

Figure 3.7: Training an odour with two types of reinforcement successively
A) We have trained a single odour first with an appetitive reinforcement and afterwards with aversive reinforcement. The odour first occupied a position in the approach area of the MBON valence space. After aversive training the odour moved into the area of the retreat valence space B) We have now trained an odour with an aversive reinforcement first and afterwards with appetitive reinforcement. The odour remained in the retreat area of the valence space after the two types of training.

Chapter 4

Discussion and future work

4.1 Conclusions from preliminary results

We have begun to explore how sparse odour information stored in a balanced spiking network can be read by the MBONs through a multifactor learning rule. We have used recent evidence that suggests that approach MBONs inhibit retreat MBONs (David Owald - personal communication) and have shown that MBON-to-MBON inhibition can lead to enhanced discrimination (*Fig*3.4). Enhanced discrimination is a property that is useful for a system classifying odours, thus it could be possible that there are retreat biasing MBONs that inhibit approach biasing MBONs. If this is true than both aversive and appetitive learning would induce bi-directional changes of firing rate into subsets of "approach" MBONs and "retreat" MBONs. In our model we tested bi-directional change that occurrs only during aversive learning. In such a scenario, we predict that training the same odour successively with both aversive and appetitive reinforcement would change the valence from appetitive to aversive if the odour was trained appetitive first and keep the valence aversive if aversive training occurred first. Our results suggest that in the fruit fly good odour memories with are easier to convert into bad memories and bad memories are more resilient.

We have visualized the coding space created by the firing rates of the MBONs create

in response to an odour. The valence space was inspired by experimental evidence that shows the highest degree of separation in terms of MBON firing rates is when odours have different valances rather than different identities (?). While MBONs may be very good at separating odours based on their valences it could be possible that other information could be stored in the spike times of the MBONs, such as information about odour identity or qualitative factors such as what kind of reward the odour predicts: water, food, mating. It is also worth noting that the experimental paradigm uses simple stimuli to train the fly which might restrict the ability of the neuron to learn complex interactions. For example we know that the flies are capable to associate an odour that predicts another odour that predicts reward which is called second ordour conditioning (?).

4.2 Future work

4.2.1 Learning over larger periods of time

We have modelled two dopaminergic neurons in our model one for each learning scenario: appetitive and aversive. The dopaminergic neurons create a temporal window in which plasticity can depress a synapse. The time window during which coincidence spikes and dopamine presence can depress a synapse is 20 ms in our model. However, there is evidence that the outcome of learning depends on the timing of the odour and the punishment over larger intervals of time (?) A fly that is shown an odour first and shocked 15 s later will learn to avoid the odour (?). If the sequence is reversed and a fly is shocked first the odour will become appetitive (?). Further more if a fly is shocked with a strong shock first paired with an odour and a milder shock later paired with a different odour the fly will learn to approach the second odour (?). Our model fails to replicate these results that belongs to the paradigm of relief learning because the shock signal always leads to depression of the approach pathway in our model. One possible solution to this problem could be that the activity of appetitive and aversive dopaminergic

neurons is relative to past experiences. In our model we can test a paradigm, where when we would stop a shock signal after a period of time, the aversive dopaminergic neurons will decrease their activity and the appetitive dopaminergic neurons will show an increase in their activity.

4.2.2 Second order conditioning

In our first model investigating plasticity in the MB we have used a learning rule that leads to synaptic depression when dopamine in present (**?**). However, the fly is able to learn to apparoach an odour that predicts another appetitively trained odour. This could be because during second order conditioning when the fly is exposed to the appetitive odour, appetitive dopaminergic neurons increase their activity which makes the fly susceptible to learn to consider the second odour as being appetitive. We would likje to explore how we can tune the activity of dopaminergic neurons in response to not only reward and punishment but also stimuli that were learned. A candidate mechanism that could explain this might be the feedback loop from MBONs to DAs.

4.2.3 Implementing the MBON - DA feedback loop

We have begun preliminary work on investigating the MBON - DA feedback loop. Recent anatomical work shown that the $M4\beta'$ "retreat" biasing MBON has axons projecting to appetitive DA neurons (**?**). We would like to examine the functional consequences that such a feedback loop has to learning and test different scenarios - what happens when the feedback loop is excitatory or inhibitory.

4.2.4 State dependent memory retrieval

Our experimental collaborator has recently shown that the approach MVP2 output neuron increases its response to an odour when the fruit fly is hungry (*Fig* 1.3). This implies that the olfactory circuit is modulated by the behavioural state of the organism. A plau-

sible mechanism which has been explored in cortex is inhibition, which has been linked to brain states such as attention (Harris and Thiele 2011, Stringer et al., 2015). We would like to explore how inhibition in the MB changes in response to motivational state. A candidate neuron mechanism might be APL state dependent inhibition, which is a neuron that inhibits the entire population of KCs (**?**).

Chapter 5

Timeline

Year	***2015***		***2016***			***2017***		
Term	*MT*	*HT*	*TT*	*Sum*	*MT*	*HT*	*TT*	*Sum*
Reproducing bi-directional change in M4 neuron in a network of integrate and fire neurons with STDP in a circuit with dopaminergic feedback loop.. *Refine simulations by adding information from Higue et al 2015 on output neurons and test learning rules.* *Make experimental verifiable predictions that rules out or validates learning mechanism* *Write paper on bi-directional valence learning*								
Model a recurrent mushroom body with state dependent memories								
Write second paper								
Investigate complex dynamics of dopaminergic neurons (i.e relief learning)								
DPhil write up								

Harris KD & Thiele A. 2011. Cortical state and attention. Nature Reviews Neuroscience 12, 509-523.

Stringer C, Pachitariu M, Hildebrandt KJ, Bartho P, Harris KD, Linden J, Latham P, Lesica J, & Sahani M. 2015. Changes in inhibition explain cortical variability and its role in sensory representations. Neural Coding, Computation and Dynamics (NCCD) Conference 2015. Bilbao, Spain.