3 Results

Here we analyse the task-specific information provided by visually responsive ring neurons by simulating their responses during well-known behavioural experiments To do this we use data from [6] who used calcium imaging to examine the receptive fields (RFs) of ring neurons whose cell bodies are in specific glomeruli in the lateral triangle. As the RFs of glomeruli are remarkably consistent across flies [6], we combine them to reduce measurement error and obtain sets of ‘canonical’ RFs. Though this averaging process will produce RFs that are more regular than those given for individual flies, we feel this process is better than using RFs from a specific fly as, firstly, some of the irregularity in the RFs presumably derives from measurement error, and secondly, it is unlikely that smoother edges on the RFs give an ‘unfair advantage’ in the tasks we examine. This process (for details, see Section 5.2) gave us a set of 28 R2 and 14 R4d filters. To investigate the information these cells encode, we calculate outputs for a given visual stimulus by convolving it with the averaged ring neuron filters. This gives a population code whereby the outputs of the set of filters is the encoded ‘representation’ of the current visual stimulus. We interrogate these encodings to understand the information they contain focusing on the relationships to specific behaviours.

3.1 Orientation towards bar stimuli

We first consider experiments in which flies are presented with bar stimuli as flies are known to spontaneously orient towards black bars [11], aiming for the centres of narrow bars and the edges of wide bars [25]. We thus examine the response of simulated ring neurons to bars of different widths (Figure 1A and B). The summed output of the ensembles of ring neurons show peaks to the bars of different widths which broadly match experimental results (Figure 1B). For instance, R2 neurons respond maximally to the inside edges of large bars, while peak activity in R4d neurons occurs at bar centres and also at roughly ±90◦. While we do not know the details of mechanisms downstream of the ring neurons and hence how their activity is transformed into behaviour, the simulation is an existence proof that the information needed to control the observed behaviour is present in the sparse ring neuron code.

We further demonstrate this point by closing the loop between sensory systems and behaviour using an agent based model of a fly viewing a bar in which the fly’s heading is controlled by the difference between the summed activation of left and right ring neurons (Figure 1C; see Methods section XX for details). The simulated fly approaches the bar from different distances, demonstrating bar fixation when far from a bar and fixation of the edges when nearer and the bar’s apparent size is greater (Figure 1D). Through this example, we can see how the informationpresent in this small population of visually responsive ring neurons can control a specific behaviour.More generally this shows how we can link the outputs of these sensory cells to a particular behaviour. We now turn to a more complex behaviour, pattern discrimination.

3.2 Pattern discrimination in flies and ring neuron population codes

The standard paradigm for testing pattern discrimination in Drosophila [7, 13, 14, 26], involves tethering a fly in a drum with a pair of alternating patterns on the inside wall of the drum (Figure 1A). When the fly attempts to rotate about the yaw-axis, the pattern on the drum is rotated by a corresponding amount in the opposite direction, giving closed-loop control. Conditioning is aversive: orientation towards a quadrant containing one of the patterns of the pair is punished with heat from a laser. Hence, if the fly can discriminate the patterns, it will learn to orient towards the non-punished pattern. The ability to discriminate patterns in such an assay requires R2 neurons, specifically synaptic plasticity afforded by rutabaga in these neurons is sufficient and necessary [7, 13, 14, 27]. We therefore investigate the responses of Ring neurons in simulations of the classic pattern discrimination paradigm.

To recreate the visual information perceived by flies in such experiments, we simulated a typical experimental flight arena with a fly tethered in the centre. We then examined the output of the ensembles of ring neurons for a fly rotating in the drum and looked at the difference in the activation code when facing the different patterns of a pair. Our logic is that if the codes were identical, it would be impossible for the patterns to be discriminated by interrogating the outputs alone. Similarly, the greater the difference in activation codes when looking at the pattern pairs, the easier they would be to discriminate (Figure 1F and G, see Section 5.3 for details). Our difference measure is the root mean square (r.m.s.) difference between ensemble outputs when the fly faces different azimuths in the drum. As can be seen in Figure 1, comparing the ensemble output for a simulated fly oriented at 0◦ (i.e. view centered on one pattern) and the ensemble output when the ‘fly’ is oriented at other azimuths, we can see how the code changes. The difference to the view at 0◦ rises as the fly rotates in the drum, peaking as it faces the space in between the patterns and dropping to a minimum when facing the centre of the next pattern (Figure 1F and G). For some pairs of patterns, there is still an appreciable r.m.s. difference between the codes when facing the centres of each pattern enabling their discrimination. However, in the example of Figure 1F, if we displace the patterns vertically, we see a drop in the r.m.s. difference between activation codes for when the fly fixates the patterns. This isdespite the fact that to the human eye, the patterns still appear very different. Interestingly, the second pattern-pair is also harder to discriminate for flies.

In this way, we can use the difference between ensemble codes when flies face either pattern to re-examine the discriminability of pattern pairs tested with flies. One illustrative example is Set (9) from [REF], which contains pairs of ‘triangles’ (either a filled equilateral triangle, or a long and short bar arranged on top of one another), one facing up and the other down. Flies are able to discriminate these pattern pairs when they are aligned along the top and bottom, but not when aligned about the vertical centres of mass [13]. Looking at the placement and form of the R2 RFs allows us to determine where this difference comes from (Figure 3). The excitatory regions of the RFs fall roughly across the middle of triangles that are not aligned about their vertical centre of mass the difference in width at this point will lead to differences in activation. If the triangles are offset (Figure 3) so as to be aligned about their vertical centres of mass their width will be similar for the regions of peak R2 coverage and the difference in activation will be lower. Thus the failure to be able to discriminate features with an equivalent vertical centre of mass can be explained by the shape of the RFs interacting with the patterns rather than the system somehow extracting and comparing the vertical centres of mass of the patterns.

Similarly, Set (2) in Figure 2 gives examples of pattern pairs that are not discriminable by flies and also give only small differences in the outputs of R2 filters. This may seem surprising, given that these patterns appear quite different to human observers and are also very dissimilar if compared retinotopically. Thus we can see how the Drosophila R2 ring neuron encoding is informationally sparse. Whilst the human V1 region of human visual cortex contains neurons representing a full range of orientations all across the visual field, R2 neurons have large RFs and poor orientation resolution. Hence, a pattern pair consisting of a diagonal line facing left and a diagonal line facing right, for example, have only a small difference in R2 outputs in our simulation and are also not discriminable by flies. This could, in the light of behavioural experiments alone, be interpreted as evidence that flies do not discriminate patterns on the basis of orientation. A more parsimonious explanation, however, is that the flies are failing because the form of the RFs means that the activation triggered by each of the patterns is roughly the same.

To emphasise the independence of apparent similarity of patterns and the visual encoding from R2 cells, we designed shape pairs (Section 5.4.1) that appear similar to humans, but are easily discriminable to the R2 encoding (Figure 3D and white bars in 3F), as well as shape pairs that are considered similar by the R2 network but not to human observers (or in terms of retinotopic overlap; Figure 3E and black bars in 3F). Despite the similarity between the pairs of patterns, the first is readily discriminable, especially from the outputs of glomeruli 1, 3, 5 and 11, while the second pair – which we easily see as having a different orientation – have very low overall differences throughout the glomeruli. This shows that counterintuitive results can arise from the irregular RF shapes..

If there is sufficient information information available to the pattern learning/discrimination systems of a fly once passed through the ring neurons, we should see a close relationship between the r.m.s. difference in simulated R2 output for a pattern pair and the flies’ ability to learn to discriminate that pair. We thus examined the difference in the outputs of the R2 filters between patterns from pairs drawn from [13] (Figure 2)In general, the pattern pairs for which flies show a significant learned discrimination have a greater r.m.s. difference in R2 population activity [13]. [THIS IS WHERE WE PUT THE Rank sum test] All of the pattern pairs where flies show significant learning (n = 8) have R2 r.m.s. differences above the overall mean/median[which?] (Figure 2A and B), whereas 13 out of 18 patterns that flies found more difficult to learn had below average r.m.s. differences. (There were nine pattern pairs for which a significance level was not given that were excluded.) Across all pattern pairs, we find a significant correlation between the strength of the learning index reported for flies in [13] and the r.m.s. difference we found in R2 activation (Spearman’s rank, n = 30, ρ = .420, p < .05).

Of course, these differences could simply result from the appearance of the patterns. As a control comparison we therefore quantified the similarity of patterns within a pair based on the degree to which the patterns overlap in a pixel-by-pixel manner (Methods, section X). For this difference, there was no significant correlation with the flies’ learning index over the pattern pairs (Spearman’s rank, n = 32, ρ = −.068, p = n.s.). We additionally looked at the relationship between our two visual encodings (R2 population code and the retinotopic encoding) and the degree to which flies’ show a spontaneous preference (i.e. before any conditioning procedures have commenced) for one of the patterns within a pair (Figure 2D and E). For both retinotopic encoding and R2 population codes there was no significant correlation. This is in keeping with research showing that R2 neurons alone are critical for learned pattern differences [13], but not spontaneous preferences which, by contrast, seem to result from activity across all subsets of ring neurons [28].

There are, however, some discrepancies where the learning performance of flies for a pattern pair does not match the r.m.s. difference of our R2 population code. In some cases flies are better at discriminating pairs of patterns that discriminate along the vertical rather than horizontal axis (Set (3) vs Set (4), and the pairs in Set (12), marked with red Xs in Figure 2). In contrast, the r.m.s. difference in the R2 population code discriminates horizontal and vertical patterns equally. This may be because while our R2 filters are being presented with static stimulus pairs to simulate a fly oriented to the centre of a pattern, for the flies the patterns were moving horizontally but fixed in the vertical axis (as noted in [13]) making it harder for flies to resolve horizontal information.

Overall, we have shown that the behavioural performance of flies on a pattern discrimination task is approximated by a simple difference metric applied to the population activity of a small number of simulated R2 cells. However it is curious that both flies and our R2 population are bad at a variety of seemingly simple pattern discriminations which begs the question of why evolution has not optimised the visual system for this task. A simple thought experiment is helpful in considering the purpose of the visual code provided by the R2 neurons. If we double the number of R2 neurons in our population by inserting additional RFs of the same forms at random points on the visual field, then the r.m.s. difference for centre of mass-aligned triangles increases to levels similar to those for pattern pairs easily discriminated by flies (data not shown). We can therefore see how the pattern discrimination ability of an R2-like neuronal population could easily have been improved over evolutionary time without need for any radical architectural changes, simply through the addition of more R2 cells. We propose that, given how easily improved performance could have been achieved through evolution, there must be little selection pressure specifically for a specialised pattern recognition module in flies.

3.3 What information is preserved in this simple neural code?

Having such a small number of cells providing a visual encoding is a sensory bottleneck with information from 3000 ommatidia condensed onto 28 R2 or 14 R4d ring neurons. We have shown that this code provides sufficient information to discriminate some pattern pairs, but that, as performance could be improved with the addition of more ring neurons, general purpose pattern recognition seems unlikely to be the purpose of the Ring neuron system. The question thus arises as to what information this system is tuned to extract. Examining the pattern pairs which flies and the R2 population were able to discriminate we see that certain pattern parameters are implicitly coded for in the R2 population. Sets (6) and (9) (Figure xxx) suggest that, for instance, shape size and vertical centre of mass are parameters that can be recovered from the R2 population code after the sensory bottleneck.

Here we therefore address in more general terms the shape information implicitly conveyed in the ring neuron population code. To do this, we generated large sets of ellipse-like ‘blobs’ that varied in size (specified by major-axis length), position (azimuth and elevation) and orientation. The blob generation procedure was also stochastic so that the precise shape of each blob was random and different to all others (see Methods, Section 5.4.1). We then trained an artificial neural network (ANN) to recover this shape information from either a raw image of the shape (a control condition) or from the output of the R2/R4d population. Here we use ANNs as statistical engines interrogating the output of the ring neuron population code to determine the shape information that is implicit to the code and has therefore made it through the sensory bottleneck.

We first examined whether ANNs could be trained to extract positional information (elevation and azimuth) of randomly-generated blobs with orientation, θ, and major-axis length, a, held constant (θ = 0◦, a = 30◦). There were 100 possible azimuths and 100 possible elevations, giving a total of 10,000 stimuli. Of these, 4000 were used for training and 6000 for testing. Performance on the test set (Figure 4) shows that ANNs are indeed able to extract information about elevation and azimuth based on any of the input types (raw, R2 or R4d). Performance was better with parameter values near the middle. At the extremes, portions of the stimuli lay outside the visual field of the simulated fly meaning stimuli begin to disappear ‘off the edge’ of the visual field (Figure 4A and B), making the task a harder one (i.e., is it a large object projecting outside the visual field, or a smaller one just on the edge of the field of view?). While performance was best when raw views were input (Figure 4C and D), position information could still be reliably extracted from ring neuron outputs. The R2 code performs better than the R4d and the addition of R4d RFs to the R2 code, while adding dimensionality, does not improve performance, suggesting that either an R2-like encoding is sufficient to extract positional information, or that the information in the two codes is redundant. Thus small populations of ring neurons retain positional information.

We next trained ANNs to decode information about shape orientation and size. The stimuli were again randomly generated blobs at different positions but this time ten different orientations and sizes were used, giving a total of 1000 stimuli, of which 400 were used for training and 600 for testing. The ANNs were again able to extract this shape information from raw images and the ring neuron outputs (Figure 5). Orientation was the parameter with the highest error score, possibly because its calculation necessitates using a second-order statistic (the covariance of the shape). Nonetheless, both parameters could be simultaneously estimated by an ANN neural network fed with ring neuron outputs.

In summary, we have shown that information about a number of shape properties passes through the bottleneck created by the small number of ring neurons. This indicates that such information is available downstream of the ring neurons for the guidance of behaviour.

4 Discussion

A general problem in neuroscience is understanding how sensory systems organise information to be at the service of behaviour. Computational approaches have always been important in this endeavour, as they allow simulation of the sensory experience of a behaving animal meaning that one can investigate how this information is transformed by populations of neurons into behaviour. In this way, we can relate the details of neural circuitry to theories about the requirements of behaviour. Work by [6], showing the forms of visual receptive fields for ring neurons projecting to the ellipsoid body of flies, gave us the opportunity to investigate how these neurons transform information and how this information relates to specific behaviours. In particular, we have shown that despite the size of the code, the outputs of the ring neurons could subserve both bar following and limited pattern recognition and implicitly convey information about shape paramters. We now discuss the implications of our findings.

4.1 Are flies performing pattern recognition?

R2 cells are critical for conditioning in a pattern learning task [14] Now that we know the visual characteristics of these cells, we can investigate how pattern discrimination is implemented. Previously, it has been suggested that pattern recognition relies on distinguishing visual patterns on the basis of higher-order properties, such as size, orientation and elevation [13, 14], however it has been found that at the R2 synapses the encoding is independent of any single parameter [7]. Both of these views are consistent with our analysis. Size and position information are preserved in the R2 population code but do not need to be extracted by specific sub-populations of cells.

Going one step further we found that the patterns of activity in the R2 population code are a good fit with the learning index of flies for pattern pairs, as shown in [13]. We also found, however, that these cells do not appear to be specifically ‘pattern recognition’ cells as performance is increased by simply having more cells or spreading the RFs out more widely. Any selection pressure on flies’ ability to discriminate patterns (as bees need to do for instance) would surely have led to a larger R2 population or, more likely, visual input to the Mushroom Body, and therefore we can be confident that ring neurons have not been tuned for arbitrary pattern recognition. We therefore suggest caution if research on flies is used to understand the neural basis of pattern recognition.

Interestingly, flies’ spontaneous preference for patterns, which does not involve R2 neurons [13], was not correlated with the values obtained in our simulation. This fits with work showing that flies’ preference for novelty involved the ellipsoid body but did not require any one of the R1, R3, R2/R4m or R3/R4d subsets of neurons specifically [28].

4.2 Short-term memory for object position in flies’

One striking feature of the ring neuron receptive fields is that they are in general tuned to vertically oriented objects. We know that fruit flies are strongly attracted to vertical bars, a finding that has been leveraged across a range of behavioural paradigms (bar fixation: [8]). In one, single flies are placed into a virtual-reality arena, with two vertical stripes shown 180◦ apart. In this scenario, flies typically head back and forth between the two bars. Occasionally, however, the bars would disappear when a fly crossed the arena’s midline and a new bar appears at 90◦ to the old ones. Flies respond by reorienting to this new target, which then also disappears, whereupon flies will resume their initial heading, even though the original bars are no longer there. This indicates that directional information is stored in short-term memory and updated. Work by [8] has shown that R4 (and R3) ring neurons are involved in a spatial orientation memory for bars.

Accordingly, we examined the responses of the ring neuron filters to vertical bars and what role they could play in a spatial orientation task. We found that both R2 and R4d neurons were responsive to vertical bars of varying widths, particularly to the edges of larger bars and the centres of narrower ones, mirroring fly behaviour [25]. We also showed that the cells provide sufficient information to guide homing towards a large vertical object, as with a spatial orientation task [8]. Finally, we showed that information about the azimuth of stimuli makes it through the sensory bottleneck and whilst the population code does not perfectly maintain orientation information, it is a good detector of vertical bars.

The sensory information provided by these cells could be used in a variety of ways and there are suggestions that R4d neurons could form part of a ‘path integration’ system [8] or be analogous to mammalian head-direction cells [29]. Indeed, there is evidence that, as with path integration and head direction cells, these cells also integrate idiothetic information [30]. This raises the intriguing possibility that ring neurons play a role in a short-term memory system in order to facilitate complex behavioural responses which require integration of multiple sources of information over time, rather than simpler reflexive or classically conditioned behaviours. The fly could be remembering the position of a stimulus, the history of its own movements, or both [29]. Work by [31] indicates that R3/R4d neurons, but not R2/R4m, play a role in learned spatial orientation to stimuli other than simple vertical bars. Flies tethered in a drum were conditioned to fly toward either the left or right of a visual pattern (such as an inverted ‘T’); it was found that the absence of these neurons prevented conditioning. This suggests a role for R4d cells in remembering the position of a stimulus with respect to the fly’s own movements, as would be required in a path integration system.

4.3 Summary

We have given here a novel insight into the functions and organisation of the Drosophila visual system, and additionally raised issues regarding neural coding in insects more generally. In particular, we challenge the idea that a complex behaviour must be supported by a discrete cognitive module to extract abstract features or properties of stimuli. Drosophila’s limited ability to discriminate patterns using abstract properties seems to be the by-product of a simple visual system tuned to provide information to guide specific behaviours. In the future, a combined approach – behavioural research that incorporates insights and predictions from computational models – could help pave the way to a mechanistic, quantitative account of insect behaviour and its relation to sensory information.