Real time encoding of motion: Answerable questions and questionable answers from the fly's visual system

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In the past decade, a small corner of the fly's visual system has become an important testing ground for ideas about coding and computation in the nervous system. A number of results demonstrate that this system operates with a precision and efficiency near the limits imposed by physics, and more generally these results point to the reliability and efficiency of the strategies that nature has selected for representing and processing visual signals. A recent series of papers by Egelhaaf and coworkers, however, suggests that almost all these conclusions are incorrect. In this contribution we place these controversies in a larger context, emphasizing that the arguments are not just about flies, but rather about how we should quantify the neural response to complex, naturalistic inputs. As an example, Egelhaaf et al. (and many others) compute certain correlation functions and use the apparent correlation times as a measure of temporal precision in the neural response. This analysis neglects the structure of the correlation function at short times, and we show how to analyze this structure to reveal a temporal precision 30 times better than suggested by the correlation time; this precision is confirmed by a much more detailed information theoretic analysis. In reviewing other aspects of the controversy, we find that the analysis methods used by Egelhaaf et al. suffer from some mathematical inconsistencies, and that in some cases we are unable to reproduce their experimental results. Finally, we present results from new experiments that probe the neural response to inputs that approach more closely the natural context for freely flying flies. These new experiments demonstrate that the fly's visual system is even more precise and efficient under natural conditions than had been inferred from our earlier work.

1 Introduction

Much of what we know about the neural processing of sensory information has been learned by studying the responses of single neurons to rather simplified stimuli. The ethologists, however, have argued that we can reveal the full richness of the nervous system only when we study the way in which the brain deals with the more complex stimuli that occur in nature. On the other hand it is possible that the processing of natural signals is decomposable into steps that can be understood from the analysis of simpler signals. But even then, to prove that this is the case one must do the experiment and use complex natural stimuli. In the past decade there has been renewed interest in moving beyond the simple sensory inputs that have been the workhorse of neurophysiology, and a key step in this program has been the development of more powerful tools for the analysis of neural responses to complex dynamic inputs. The motion sensitive neurons of the fly visual system have been an important testing ground for these ideas, and there have been several key results from this work:

1. The sequence of spikes from a motion sensitive neuron can be decoded to recover a continuous estimate of the dynamic velocity trajectory (Bialek et al. 1991; Haag and Borst 1997). In this decoding, individual spikes contribute significantly to the estimate of velocity at each point in time.

2. The precision of velocity estimates approaches the physical limits imposed by diffraction and noise in the photoreceptor array (Bialek et al. 1991).

3. One or two spikes are sufficient to discriminate between motions which differ by displacements in the 'hyperacuity' range, an order of magnitude smaller than the spacing between photoreceptors in the retina (de Ruyter van Steveninck and Bialek 1995). Again this performance approaches the limits set by diffraction and receptor noise.

4. Patterns of spikes which differ by millisecond shifts of the individual spikes can stand for distinguishable velocity waveforms (de Ruyter van Steveninck and Bialek 1988), and these patterns can carry much more information than expected by adding up the contributions of individual spikes (de Ruyter van Steveninck and Bialek 1988, Brenner et al. in press).

5. The total information that we (or the fly) can extract from the spike train continues to increase as we observe the spikes with greater temporal resolution, down to millisecond precision (de Ruyter van Steveninck et al. 1997, Strong et al. 1998).

6. These facts about the encoding of naturalistic, dynamic stimuli cannot be extrapolated simply from studies of the neural response to simpler signals. The system exhibits profound adaptation (Maddess and Laughlin 1985, de Ruyter van Steveninck et al. 1986, Borst and Egelhaaf 1987, de Ruyter van Steveninck et al. 1996, Brenner et al. submitted), so that the encoding of signals depends strongly on context, and the statistical

structure of responses to dynamic stimuli can be very different from that found with simpler static or steady state stimuli (de Ruyter van Steveninck et al. 1997).

We emphasize that many of these results from the fly's visual system have direct analogs in other systems, from insects to amphibians to primates (Rieke et al. 1997).

In a series of recent papers, Egelhaaf and coworkers have called these results into question (Warzecha and Egelhaaf 1997, 1998, 1999; Warzecha et al. 1998). Several of these papers are built around a choice of a stimulus very different from that used in previous work. Rather than synthesize a stimulus with known statistical properties, they sample the time dependent motion signals generated by a fly tethered in a flight simulator. The simulator is operated in closed loop so that the fly, by producing a yaw torque which is measured electronically, moves a pattern on a CRT monitor, while the animal itself stays stationary. For experiments on the responses of the motion sensitive neurons these patterns and motions are replayed to another fly, again through a monitor. In their judgement these stimuli "are characteristic of a normal behavioral situation in which the actions and reactions of the animal directly affect its visual input" (Warzecha and Egelhaaf 1998).

For these stimuli, Warzecha and Egelhaaf claim that the timing of individual spikes has no significance in representing motion signals in the fly's motion sensitive neurons. Instead they suggest that the neuron's response should be averaged over time scales of order 40 to 100 ms to recover the essential information, and that timing of spikes within this averaging window is irrelevant. These claims are in conflict with points [1], [4], and [5] above. As part of their discussion of these points Warzecha and Egelhaaf make repeated references to the noisiness of the neural response, in apparent contradiction of points [2] and [3], although they do not address specifically the quantitative results of the earlier work. Finally, they suggest that there is no difference between the statistics of spike trains in response to steady state vs. dynamic stimuli, in contradiction of point [6].

Obviously the recent work of Egelhaaf and coworkers raises many different issues. In this contribution we try to focus on three problems of general interest. First, how do we define a meaningful "naturalistic stimulus," and does their "behaviourally generated" stimulus fall into this category? In particular, how do we reach an effective compromise between stimuli that occur in nature and stimuli that we can control and reproduce reliably in the laboratory? Second, how do we characterize the neural response to complex dynamic inputs? In particular, how do we evaluate all the relevant time scales in the sensory signal itself and in the spike train? Again, these are issues that we must face in the analysis of any neural system for processing of sensory information; indeed there are even analogous issues in motor systems. Thus the fly's visual system serves here as an example, rather than as an end in itself.

Before we begin our discussion of these two points, we must be clear that the first question—what is a natural stimulus?—is a question about the biology and

ecology of the animal we are studying, as well as a question about the design and constraints of a particular experimental setup. One might well disagree about the best strategy for generating naturalistic stimuli in the lab. On the other hand, our second question—how do we characterize the response to complex signals?—is a theoretical issue which is not tied to the particulars of biology. On this issue there are precise mathematical statements to be made, and we hope to make clear how these mathematical results can be used as a rigorous guide to the analysis of experiments.

The third and final question we address concerns the comparison between static and dynamic stimuli. Although we believe that the most interesting problems concern the way in which the brain deals with the complex, dynamic stimuli that occur in nature, much has been learned from simpler static stimuli and there are nagging questions about whether it really is 'necessary' to design new experiments that need more sophisticated methods of analysis. For reasons that will become clear below, the comparison of static and dynamic stimuli also is crucial for understanding whether many of the lessons learnt from the analysis of the fly's motion sensitive neurons will be applicable to other systems, especially the mammalian cortex.

2 What is a natural stimulus?

The fly's motion sensitive neuron H1 offers a relatively simple testing ground for ideas about the neural representation of natural signals. This cell is a wide field neuron, so rather than coding the motion of small objects or a component of the local velocity flow field, H1 is responsible primarily for coding the rigid body horizontal (yaw) motion of the fly relative to the rest of the world. Thus there is a limit in which we can think of "the stimulus" as being a single function of time, $\nu(t)$, which describes this angular velocity trajectory. It should be clear that this description is incomplete: the neural response is affected also by the mean light intensity, the spatial structure of the visual stimulus, and the area of the compound eye that is stimulated. Further, the system is highly adaptive, so that the encoding of a short segment of the trajectory $\nu(t)$ will depend strongly on the statistics of this trajectory over the past several seconds.

Traditional experiments on motion sensitive neurons (as on other sensory cells) have used constant stimuli (motion at fixed velocity), pulsed stimuli (stepwise motion), or have analysed the steady state behaviour in response to sinusoidal motion at different frequencies. In nature, trajectories are not so simple. Instead one can think of trajectories as being drawn from a distribution $P[\nu(t)]$ or "stimulus ensemble." A widely used example of stimulus ensembles is the Gaussian ensemble, in which the distribution of trajectories is described completely by the spectrum or correlation function. We can construct spectra and correlation functions so that there is a single characteristic stimulus amplitude—the dynamic range $\nu_{\rm rms}$ of velocity signals—and a single characteristic time τ_c in the dynamics of these signals. A reasonable approach to the study of naturalistic stimuli might then be to explore the coding of signals in H1 using stimulus

ensembles parametrized by $\nu_{\rm rms}$ and τ_c. Most of the results enumerated above have been obtained in this way.

In their recent papers (Warzecha and Egelhaaf 1997, Warzecha et al. 1998), as well as in their contribution to this volume, Warzecha and Egelhaaf argue that the stimulus ensembles used in experiments on H1 have been restricted unfairly to short correlation times. Put another way, the stimuli used in these experiments have included high temporal frequency components. Warzecha and Egelhaaf suggest that these high frequency components bias the response of the motion sensitive cells to artificially high temporal precision which is not relevant for the behaviourally generated stimuli that they use.[1] The question of whether timing precision is important under truly natural conditions is left open.

Independent of what is truly natural, one can argue that experiments with short correlation times have provided evidence on what the fly's visual system can do. Although we seldom sit in dark rooms and wait for dim flashes of light, such experiments led to the demonstration that the human visual system can count single photons (Hecht et al. 1942). In this spirit, studies of H1 using stimuli with short correlation times have revealed that the fly's nervous system can estimate velocity with a precision limited by noise in the photoreceptor array and that timing relations between neural responses and stimulus events can be preserved with millisecond precision, even as the signals pass through four stages of neural circuitry. It would seem strange that such impressive performance would evolve if it were irrelevant for fly behaviour.

Instead of choosing trajectories $\nu(t)$ from a known probability distribution, we could try to sample the trajectories that actually occur in nature. Here we have to make choices, and these will always be somewhat subjective: Dethier (1976) reports that female flies spend 12.7%, and male flies 24.3% of their time walking or flying. The other activities on Dethier's list are feeding, regurgitating, grooming and resting, during which information from the fly's motion sensitive cells presumably is not too relevant. So it seems the fly could live quite happily without its tangential cells most of its time. On the other hand, during periods of flight, the responses of its motion sensitive cells are strongly modulated. On top of that, the depth and speed of modulation may vary as the fly switches from periods of relatively quiet cruising to episodes of fast and acrobatic pursuit or escape, and back (Land and Collett 1974). Although it is not clear at the outset what portion of the total behavioural repertoire we should analyse, the thing that presumably tells us most about the "design" of the fly is the dynamics of neural signal processing during top performance. Correspondingly, Warzecha

[1] In fact Warzecha and Egelhaaf make two different arguments about high frequency stimuli. They make repeated references to the integration times and noise in the fly's visual system, all of which limit the reliability of responses to high frequency components in the input. These arguments generally are presented in qualitative terms, but Warzecha and Egelhaaf (1999) state explicitly that signals above 30 Hz are undetectable above the noise and hence can have no impact on the statistics of the spike train. On the other hand, Warzecha and Egelhaaf (1997) argue that the inclusion of high frequency components in the input causes an unnaturally tight locking of spikes to stimulus events, causing us to overestimate the significance of spike timing for the coding of behaviorally relevant stimuli. It should be clear that these two arguments cannot both be correct.

and Egelhaaf propose to use stimuli that are representative of the trajectories experienced by a fly in flight, and we agree that this is an excellent choice. There are still some difficulties, however. Warzecha and Egelhaaf propose that meaningful data can be obtained from "behaviourally generated" trajectories $\nu(t)$ recorded from flies that are tethered in a flight simulator apparatus in which the fly's measured torque is to move a pattern on a CRT monitor in the visual field of the fly. The combination of fly, torque meter, and moving pattern thus acts as a closed loop feedback system whose dynamical properties are determined both by the fly and by the gain and bandwidth of the mechanical and electronic components involved. The data presented by Warzecha and Egelhaaf (1997, 1998, and this volume) strongly suggest that the dynamics of the feedback system are dominated by the electromechanical properties of their setup, and not by the fly itself. This is most clearly seen from direct comparisons between the trajectories in the flight simulator and those observed in nature.

Trajectories during free flight were recorded in the classic work of Land and Collett (1974), who studied chasing behaviour in Fannia canicularis and found turning speeds of several thousand degrees per second. Wehrhahn (1979), Wehrhahn et al. (1982) and Wagner (1986a,b,c) report very similar results for the housefly Musca, and recent publications (Schilstra and van Hateren 1998, van Hateren and Schilstra 1999) report flight measurements at high temporal and spatial resolution, from Calliphora flying almost free. In their published dataset flies made about 10 turns per second, during which head velocities easily exceeded 1000°/s, while maximum head turning velocities were well over 3000°/s. If we compare the results of these studies to the motion traces used in the experiments by Warzecha and Egelhaaf (1997, 1998) we see that their traces are considerably smoother, and do not go beyond 100°/s. These differences are illustrated in Fig. 1, where we make an explicit comparison between free flight data obtained by Land and Collett (1974) and the motion traces data presented in Fig. 1 of Warzecha and Egelhaaf (1997). It is clear that there are dramatic differences in the frequency of alternation and, especially, in the amplitude of the motion signals. We are not sure how Egelhaaf and Warzecha can maintain their claim that "there are likely to be few instances in the normal world where visual motion encompasses a wider dynamic range than that which could be tested here" (Warzecha et al. 1998, p. 362). Simple theoretical arguments suggest that these differences between the flight simulator trajectories and true natural trajectories will have enormous consequences for the reliability of responses in the motion sensitive neurons. Warzecha and Egelhaaf (Fig 6 of their keynote paper in this volume) report estimates of the signal and noise power spectra in the graded voltage response of a motion sensitive cell. If we scale the signal to noise power ratio they present in proportion to the ratio between the power spectrum of natural motion and the velocity power spectrum they used, then the signal to noise ratio will increase so much that the natural trajectories will produce signal resolvable against the noise at frequencies well above 200 Hz. This would mean that events in natural stimuli will be localizable with millisecond precision.

There are other differences between the stimulus conditions studied by War-

zecha and Egelhaaf and the natural conditions of free flight. Outdoors, in the middle of the afternoon, light intensities typically are two orders of magnitude larger than are generated with standard laboratory displays (Land 1981). Further, the wide field motion sensitive cells gather inputs from large portions of the compound eye (Gauck and Borst 1999), which extends backward around the head to cover a large fraction of the available solid angle; rotation of the fly produces coherent signals across this whole area, and it is very difficult to reproduce this "full vision" in the lab with CRT displays. While it is difficult to predict quantitatively the consequences of these differences, the qualitative effect is clear: natural signals are much more powerful and "cleaner" than the stimuli which Warzecha and Egelhaaf have used.

We can take a substantial step toward natural stimulus conditions by recording from a fly that itself rotates in a natural environment along a trajectory representative of free flight. Preliminary results from such experiments will be analysed in more detail below, and a detailed account is forthcoming (Lewen et al. in preparation). A female wild fly (Calliphora), caught outdoors, was placed in a plastic tube and immobilized with wax. A small incision was made in the back of the head, through which a microelectrode could be advanced to the lobula plate to record from H1. The fly holder, electrode holder and manipulator were assembled to be as light and compact, yet rigid, as possible. In this way the fly and the recording setup could be mounted on the axle of a stepper motor (Berger-Lahr, RDM 564/50, driven by a Divi-Step D331.1 interface with 10,000 steps/revolution) and rotated at speeds of up to several thousand degrees per second. The motor speed was controlled through the parallel port of a laptop computer by means of custom designed electronics, and was played out at 2ms intervals. The data presented here are from an experiment in which the setup was placed outside on a sunny day, in a wooded environment not far from where the fly was caught. A simple, but crucial, control is necessary: H1 does not respond if the fly is rotated in the dark, or if the visual scene surrounding it rotates together with the fly. We can thus be confident that H1 is stimulated by visual input alone, and not by other sensory modalities, and also that electronic crosstalk between the motor and the neural recording is negligible. The motion trace $\nu(t)$ was derived from a concatenation of body angle readings over the course of the flight paths of a leading and a chasing fly as depicted in Fig. 4 of Land and Collett (1974). For technical reasons we had to limit the velocity values to half those derived from that figure, but we have no reason to believe that this will affect the main result very much. Translational motion components were not present, representing a situation with objects only at infinity. Padded with a few zero velocity samples, this trace was 2.5 seconds long. That sequence was repeated with the sign of all velocity values changed, to get a full 5 second long sequence. This full sequence was played 200 times in succession while spikes from the axon terminals of H1 were recorded as an analog waveform at 10 kHz sampling rate. In off line analysis spike occurrence times were derived by matched filtering and thresholding.

Before looking at the responses of H1, we emphasize several aspects of the stimulus conditions:

- The motion stimulus is obtained from direct measurement of flies in free flight, not from a torque measurement of a tethered fly watching a CRT monitor. As argued above, the electromechanical properties of the setup used by Warzecha and Egelhaaf are likely to have drastic effects on the frequency and amplitude characteristics of the motion.

- The field of view experienced by the fly in our setup is almost as large as that for a free flying fly. Most of the visual field is exposed to movement, with the exception of a few elements (e.g. the preamplifier) that rotate with the fly, and occupy just a small portion of the visual field.

- The experiment is done outside, in an environment close to where our experimental flies are caught, so that almost by definition we stimulate the fly with natural scenes.

- The experiment is performed in the afternoon on a bright day. ¿From dim to bright patches of the visual scene the effective estimated photon flux for fly photoreceptors under these conditions varies from 5×10^5 to 5×10^6 photons per second per receptor. Warzecha and Egelhaaf's experiments (as many experiments of ours) were done with a fly watching a Tektronix 608 cathode ray tube, which has an estimated maximum photon flux of about 10^5 photons per second per receptor.

Figure 2 shows the spike trains generated by H1 in the "outdoor" experiment, focusing on a short segment of the experiment just to illustrate some qualitative points. The top trace shows the velocity waveform $\nu(t)$, and subsequent panels show the spikes generated by H1 in response to this trajectory (H1+) or its sign reverse (H1−). Visual inspection reveals that some aspects of the response are very reproducible, and further that particular events in the stimulus can be associated reliably with small numbers of spikes. The first stimulus zero crossing at about 1730 ms is marked by a rather sharp drop in the activity of H1+, with a sharp rise for H1−. This sharp switching of spike activity is not just a feature of this particular zero crossing, but occurs in other instances as well. Further, the small hump in velocity at about 2080 ms lasts only about 10 ms, but induces a reliable spike pair in H1+ together with a short pause in the activity of H1−. The first spike in H1− after this pause (Fig. 2c) is timed quite well; its probability distribution (Fig. 2e) has a standard deviation of 0.73 ms. Thus, under natural stimulus conditions individual spikes can be locked to the stimulus with millisecond precision.

In fact the first few spikes after the pause in H1− have even greater internal or relative temporal precision. The raster in Fig. 2c shows that the first spike meanders, in the sense that the fluctuation in timing from trial to trial seems to be slow. This suggests that much of the uncertainty in the timing of this spike is due to a rather slow process, perhaps metabolic drift. To outside observers, like us, these fluctuations just add to the spike timing uncertainty, which even then is still submillisecond. Note, however, that to some extent the fly may be able to compensate for that drift. If the effect is metabolic, then different neurons might

drift more or less together, and the time interval between spikes from different cells could be preserved quite well in spite of temporal drift of individual spikes. Similarly, within one cell, spikes could drift together (Brenner et al., 2000), and this indeed is the case here. As a result the interval between the first spike and the next is much more precise, with a 0.18 ms standard deviaton, and it does not seem to suffer from these slow fluctuations (Fig. 2d). The timing accuracy of ensuing intervals from the first spike to the third and fourth, although becoming gradually less well defined, is still submillisecond (Fig. 2f). So it is clear that some identifiable patterns of spikes are generated with a timing precision of the order of a millisecond or even quite a bit better.

Although we have emphasized the reproducibility of the responses to natural stimuli, there also is a more qualitative point to be made. All attempts to characterize the input/output relation of H1 under laboratory conditions have indicated that the maximum spike rate should occur in response to velocities below about 100°/s, far below the typical velocities used in our experiments. Indeed, many such experiments suggest that H1 should shut down and not spike at all in response to these extremely high velocities. In particular, Warzecha and Egelhaaf (1998) claim that spike rates in H1 are essentially zero above 250°/s, that this lack of sensitivity to high speeds is an essential result of the computational strategy used by the fly in computing motion, and further that this behaviour can be used to advantage in optomotor course control. The outdoor experiment demonstrates that none of these conclusions are relevant to more natural conditions, where H1's response peaks at about 1000°/s (Lewen et al. in prep.) and responds robustly and reliably to angular velocities of over 2000°/s.

The arguments presented here rested chiefly on visual inspection of the spike trains, and this has obvious limitations. Our eyes are drawn to reliable features in the response, and one may object that these cases could be accurate but rare, so that the bulk or average behaviour of the spike train is much sloppier. To proceed we must turn to a more quantitative approach.

3 How do we analyse the responses to natural stimuli?

When we deliver simple sensory stimuli it is relatively easy to analyse some measures of neural response as a function of the parameters that describe the stimulus. Faced with the responses of a neuron to the complex, dynamic signals that occur in nature–as in Fig. 1–what should we measure? How do we quantify the response and its relation to the different features of the stimulus? The sequence of spikes from a motion sensitive neuron constitutes an encoding of the trajectory $\nu(t)$. Of course, this encoding is not perfect: there is noise in the spatiotemporal pattern of the photon flux from which motion is computed, the visual system has limited spatial and temporal resolution, and inevitably there is internal noise in any physical or physiological system. This may cause identical

stimuli to generate different responses. The code also may be ambiguous in the sense that, even if noise were absent, the same response can be induced by very different stimuli. Conceptually, there are two very different questions we can ask about the structure of this code. First, we can ask about the features of the spike train that are relevant for the code: Is the timing of individual spikes important, or does it suffice to count spikes in relatively large windows of time? Are particular temporal patterns of spikes especially significant? Second, if we can identify the relevant features of the spike train then we can ask about the mapping between these features of the response and the structure of the stimulus: What aspects of the stimulus influence the probability of a spike? How can we (or the fly) decode the spike train to estimate the stimulus trajectory, and how precisely can this be done?

There are two general approaches to these problems. One is to compute correlation functions. A classic example is the method of "reverse correlation" in which we correlate the spike train with the time varying input signal (see Section 2.1 in Rieke et al. 1997). This is equivalent to computing the average stimulus trajectory in the neighbourhood of a spike. Other possibilities include correlating spike trains with themselves or with the spike trains of other neurons. A more subtle possibility is to correlate spike trains that occur on different presentations of the same time dependent signal, or the related idea of computing the coherence among responses on different presentations (Haag and Borst 1997). All of these methods have the advantage that simple correlation functions can be estimated reliably even from relatively small data sets. On the other hand, there are an infinite number of possible correlation functions that one could compute, and by looking only at the simpler ones we may miss important structures in the data.

An alternative to computing correlation functions is to take an explicitly probabilistic point of view. As an example, rather than computing the average stimulus trajectory in the neighbourhood of a spike, as in reverse correlation, we can try to characterize the whole distribution of stimuli in the neighbourhood of a spike (de Ruyter van Steveninck and Bialek 1988). Similarly, rather than computing correlations among spike trains in different presentations of the same stimulus, we can try to characterize the whole distribution of spike sequences that occur across multiple presentations (de Ruyter van Steveninck et al. 1997, Strong et al. 1998). The probability distributions themselves can be difficult to visualize, and we often want to reduce these rather complex objects to a few sensible numbers, but we must be sure to do this in a way that does not introduce unwarranted assumptions about what is or is not important in the stimulus and response. Shannon (1948) showed that there is a unique way of doing this, and this is to use the entropy or information associated with the probability distributions. Even if we compute correlation functions, it is useful to translate these correlation functions into bounds on the entropy or information, as is done in the stimulus reconstruction method (Bialek et al. 1991, Rieke et al. 1997, Haag and Borst 1997, Borst and Theunissen 1999). Although the idea of using information theory to discuss the neural code dates back nearly to the inception of the theory (MacKay and McCulloch 1952), it is only in the last ten years

that we have seen these mathematical tools used widely for the characterization of real neurons, as opposed to models.

3.1 Correlation functions

Although we believe that the best approach to analyzing the neural response to natural stimuli is grounded in information theory, we follow Warzecha and Egelhaaf and begin by using correlation functions. From an experiment analogous to the one in our Fig. 2, Warzecha et al. (1998) compute the correlation function of the spike trains of simultaneously recorded H1 and H2 cells, $\Phi_{\mathrm{spikeH1-spikeH2}}(\tau)$, and also the average crosscorrelation function among spike trains from different presentations (trials) of the same stimulus trajectory, $\Phi_{\mathrm{crosstrialH1-H2}}(\tau)$. If the spike trains were reproduced perfectly from trial to trial, these two correlation functions would be identical; of course this is not the case. Warzecha and Egelhaaf conclude from the difference between the two correlation functions that the spikes are not "precisely time coupled" to the stimulus, and they argue further that the scale which characterizes the precision (or imprecision) of spike timing can be determined from the width of the crosstrial correlation function $\Phi_{\mathrm{crosstrialH1-H2}}(\tau)$. This is one of their arguments in support of the notion that the time resolution of the spike train under natural conditions is in the range of 40 to 100 milliseconds, one or two orders of magnitude less precision than was found in previous work.

The crosstrial correlation function obviously contains information about the precision of the neural response, but there is no necessary mathematical relation between the temporal precision and the width of the correlation function. To make the discussion concrete, we show in Fig. 3a the autocorrelation $\Phi_{\mathrm{spike-spike}}(\tau)$ and in Fig. 3b the crosstrial correlation function $\Phi_{\mathrm{crosstrial}}(\tau)$ computed for the outdoor experiment. We see that $\Phi_{\mathrm{crosstrial}}(\tau)$ is very broad, while $\Phi_{\mathrm{spike-spike}}(\tau)$ has structure on much shorter time scales, as found also by Warzecha and Egelhaaf. But the characterization of the crosstrial correlation function as broad does not capture all of its structure: rather than having a smooth peak at $\tau = 0$, there seems to be a rather sharp change of slope or cusp, and again this is seen in the data presented by Warzecha and Egelhaaf, even though the stimulus conditions are very different. This cusp is a hint that the width of the correlation function is hiding structure on much finer time scales.

Before analyzing the correlation functions further, we note some connections to earlier work. Intuitively it might seem that by correlating the responses from different trials we are probing the reproducibility of spike timing in some detail. But because $\Phi_{\mathrm{crosstrial}}(\tau)$ is an average over pairs of spikes (one from each trial), this function is not sensitive to reproducible patterns of spikes such as those we have seen in Fig. 2. In fact, the crosstrial correlation function is equal (with suitable normalization) to the autocorrelation function of the time dependent rate $r(t)$ that we obtain by averaging the spike train across trials. Thus the crosstrial correlation does not contain information beyond the usual poststimulus time histogram or PSTH, and the time scales in the correlation function just measure how rapidly the firing rate can be modulated; again, there is no

sensitivity to spike timing beyond the rate, and hence no sensitivity to spike patterns. Since the crosstrial correlation function is equal to the autocorrelation of the rate, the Fourier transform of $\Phi_{\mathrm{crosstrial}}(\tau)$ is equal to the power spectrum of the rate, which has been used by Bair and Koch (1996) to discuss the reproducibility of responses in the motion sensitive neurons of monkey visual cortex. If we Fourier transform both the crosstrial correlation function and the spike-spike correlation, their ratio is proportional to the crosstrial coherence considered by Haag and Borst (1997) in their analysis of H1.

Even granting the limitations of the correlation function as a probe of spike timing, we would like to reveal the finer time scale structure that seems to be hiding near $\tau = 0$. To do this we consider a simple model that can be generalized without changing the basic conclusions. Imagine that each spike has an "ideal" time $\langle t_{\mathrm{i}} \rangle$ relative to the stimulus, and that from trial to trial the actual arrival time of the ith spike fluctuates as $t_{\mathrm{i}} = \langle t_{\mathrm{i}} \rangle + \delta t_{\mathrm{i}}$. The meandering of spikes from trial to trial in Fig. 2c suggests that the δt_{i} and δt_{j} of nearby spikes i and j are correlated, and if these correlations extend over a sufficiently long time (roughly 10 ms is sufficient) then there is a simple approximate equation relating the crosscorrelation among trials to the autocorrelation and the distribution of time jitter, $P(\delta t_{\mathrm{i}})$:

$$\Phi_{\mathrm{crosstrial}}(\tau) = \Phi_{\mathrm{PP}}(\tau) \otimes \Phi_{\mathrm{spike-spike}}(\tau),$$

where $\otimes$ denotes convolution and $\Phi_{\mathrm{PP}}(\tau)$ is the autocorrelation of the distribution $P(\delta t_{\mathrm{i}})$. Thus $\Phi_{\mathrm{PP}}(\tau)$ can be computed from the measured correlation functions by deconvolution. For our outdoor experiment we find that $\Phi_{\mathrm{PP}}(\tau)$ has a width of 3.1 ms (Fig. 3c), so that a reasonable estimate for the width of the underlying jitter distribution is $\delta t_{\mathrm{rms}} = 3.1/\sqrt{2} \approx 2.2\,\mathrm{ms}$. This analysis shows that the difference between the crosstrial and the spike-spike correlation functions is consistent with jitter in the range of a few milliseconds, not the many tens of milliseconds claimed by Warzecha and Egelhaaf.[2]

Because the interpretation of correlation functions is a crucial issue, let us give an example from spatial vision, where it is clear that the width of the correlation function (correlation length) is not a good indicator of the precision required to read out a signal. It is well documented that natural scenes typically have broad spatial correlations, often associated with 1/f-like power density spectra (Srinivasan et al. 1982, Field 1987, Ruderman and Bialek 1994). Using the same reasoning that Warzecha and Egelhaaf apply to spike trains, one would conclude that the visual system should not bother to use high spatial resolution.

[2]There are further difficulties in the interpretation of correlation functions offered by Warzecha and Egelhaaf. One of their arguments for the irrelevance of high frequency stimuli is based on a comparison of the velocity spectrum with the spectrum of fluctuations in the time dependent rate (Fig. 2 of Warzecha and Egelhaaf 1997); the spectrum of the time dependent rate should be the Fourier transform of the crosstrial correlation function, as noted above. On the down going slope, across a decade of frequency the decline in the response spectrum is slower than the decline in the stimulus spectrum. If we define a transfer function by taking the ratio of the response and stimulus spectra, then the cell is amplifying the higher frequency components, not attentuating them as Warzecha and Egelhaaf claim. This is consistent with the experiments of Haag and Borst (1998) demonstrating that the motion sensitive neurons have active membrane mechanisms to achieve such amplification.

This would be true for environments with Gaussian statistics, where second order descriptions–the simplest correlation functions–are sufficient. But the world we live in definitely is not Gaussian. It is made out of objects that typically have well defined edges, and these edges are important to us, not least because they are often associated with rigid objects. The width of the spatial correlation function is defined, very roughly, by the apparent size of the objects in our visual field. But this width has nothing to do with the precision with which we can estimate the position of edges and hence the location of object boundaries. Just as for spatial edges, the location of temporal edges may also be important, and we can look at horse racing for an example: In Warzecha and Egelhaaf's interpretation we would not need to time horses any more precisely than the width of the "horse density" correlation function, which corresponds roughly to the time required for the entire horse to cross the finish line. Yet fortunes are won and lost over differences corresponding to a fraction of a horse's nose. What matters here is that we attach importance to features that are defined very sharply in time, and this temporal precision cannot be measured from the width of one simple correlation function. For precisely the same reason one cannot equate the relevant time scale of retinal image motion to spike timing precision, as Warzecha and Egelhaaf argue in this volume (Sect. 6). Let us then turn to an information theoretic approach.

3.2 Information

Looking at the responses to repeated presentations of a natural complex dynamic stimulus, as in Fig. 2, we see many different features, some of which have been noted above: there are individual spikes which are reproduced from trial to trial with considerable accuracy; there are patterns of spikes in which the intervals between spikes are reproduced more accurately than the absolute spike times, so that the patterns appear to 'meander' from trial to trial; there are trials in which spikes are deleted, apparently at random, and trials in which extra spikes appear. How are we to make sense out of this variety of phenomena? Specifically, we want to know whether the detailed timing of spikes is important for the encoding of naturalistic stimuli. How can we analyse data of this sort to give us a direct answer to this question about the structure of the neural code?

Intuitively, the sequence of action potentials generated by H1 "provides information" about the motion trajectory. If the response of H1 were always the same, independent of the trajectory, of course no information would be provided. Generally, then, the greater the range of possible responses the greater is the capacity of the cell to provide information: if we think of segments of the neural response as being like words in a language, then the ability of the neuron to 'describe' the input is enhanced if it has a larger vocabulary. On the other hand, it clearly is not useful to generate words at random, no matter how large our vocabulary, and so there must be a reproducible relationship between the choice of words and the form of the motion trajectory. These intuitive ideas have a precise formulation in Shannon's information theory (Shannon 1948): the size of the neuron's 'vocabulary' is measured by the entropy of the distribution of

responses, the (ir)reproducibility of the relation between stimulus and response is related to the conditional or noise entropy computed from the distribution of responses seen in multiple trials, and the information that the response conveys about the stimulus is the difference between the entropy and the noise entropy (see also de Ruyter van Steveninck et al. 1997). These measures from information theory are not just one of many possible ways of quantifying the neural response; Shannon proved that these are the only measures of variability, reproducibility and information that are consistent with certain simple and intuitively plausible constraints.

If we believe that the neural code makes use of a time resolution Δt, then we can describe the neural response in discrete time bins of this size. If Δt is very large this amounts to counting the number of spikes in each bin, while as Δt becomes small this description becomes a binary string in which we record the presence or absence of individual spikes in each bin. As our time resolution improves (smaller Δt) the size of the response 'vocabulary' increases because we are distinguishing as different responses that were, at larger Δt, lumped together as being the same. Quantitatively, the entropy of the responses is a function of time resolution, so that the capacity of the neuron to convey information is greater at smaller Δt, as first emphasized by MacKay and McCulloch (1952). The question of whether spike timing is important to the neural code is then whether neurons make efficient use of this extra capacity (Rieke et al. 1993, 1997). In the next section we address precisely this question in the context of the 'outdoor' experiment on H1, reaching conclusions that parallel closely those from our earlier work (de Ruyter van Steveninck et al. 1997, Strong et al. 1998). First we consider the results of Egelhaaf and coworkers, who have drawn nearly opposite conclusions.

Warzecha and Egelhaaf (1997) and Egelhaaf and Warzecha (1999) set out to study the dependence of information transmission on time resolution, along the lines indicated above. Specifically, they count spikes in bins of size Δt and then ask how much information this spike count on a single trial provides about the local firing rate, or "Stimulus Induced Response Component" (SIRC) computed as an average over many trials. Their information measure shows a peak for a window width of $\Delta t = 80\,\mathrm{ms}$ (Warzecha and Egelhaaf 1997, Fig. 3), from which they conclude that this is the time resolution at which signals are best represented by H1. It is not clear what measure of information Warzecha and Egelhaaf (1997) are using to find the optimum: the rate at which the spike train provides information about the stimulus must be a monotonic function of the time resolution. By marking spike arrival times more accurately we can only gain, and never lose, information. Thus a proper measure of information rate vs. time resolution cannot show the behavior reported by Warzecha and Egelhaaf.

In the present volume they substitute the information theoretical analysis by one in which they quantify the same difference (that is, between the SIRC and a running window average count of the single trial spike train) by a standard deviation. This standard deviation reaches a minimum for a window width Δt of about 50ms. This analysis, as their correlation function analysis, is based on a consideration of second order statistics, and is therefore subject to the same

shortcomings discussed before.

Both these approaches suffer from the same fundamental problem: Warzecha and Egelhaaf do not quantify the relation between the neural response and the stimulus, but instead between spike counts and the SIRC. Implicitly, then, they postulate that the stimulus is encoded exclusively in the time dependent firing rate, or the SIRC as they prefer to call it, and further that all information about the local rate can be "read out" by counting spikes.[3] As in the analysis of crosstrial correlation functions, this ignores by construction the possibility that temporal patterns of spikes may play a special role in the code, and their reasoning is therefore circular. For many investigators this issue of whether patterns are important is the question about the structure of the neural code, and in the case of H1 it is now more than a decade since de Ruyter van Steveninck and Bialek (1988) reported that patterns with short interspike intervals carry a considerable excess of information about the stimulus (see also Rieke et al. 1997 and Brenner et al. in press). The approach taken by de Ruyter van Steveninck et al. (1997) and by Strong et al. (1998) describes the neural response at fine time resolution as a binary string, marking the presence or absence of spikes in each small time bin, and hence all patterns of spikes are included automatically. This is the approach that we will use below for the analysis of the outdoor experiment.

In principle, the methods used by de Ruyter van Steveninck et al. (1997) and by Strong et al. (1998) are independent of any model for the structure of the neural code: we do not need to assume that we know which features of the neural response are relevant, nor do we need to assume which features of the stimulus are most important for the neuron. A number of results on information transmission by H1 have been obtained with a less direct method, in which we use the spike train to reconstruct the stimulus and then measure the mutual information between the stimulus and the reconstruction (Bialek et al. 1991, Haag and Borst 1997, 1998). Warzecha and Egelhaaf emphasize that errors in the reconstruction result only in part from noise, and they claim that one therefore cannot conclude anything about the reliability of neurons from the quality of reconstructions (Warzecha and Egelhaaf 1997; see also their contribution to this volume). The thrust of their argument is that there need be no conflict between their claim of imprecision in the coding of behaviourally relevant stimuli and previous work demonstrating precise reconstruction of the velocity waveforms, because the reconstruction doesn't really measure the precision of the neural system. But this discussion ignores the fact that the reconstruction method provides a lower bound on the performance of the neuron (Rieke et al. 1997, Borst and Theunissen 1999). Thus it is possible that reconstruction experiments underestimate the precision of neural coding and computation, but properly done the reconstruction method cannot overestimate neural performance.

[3]Even if the changing stimulus serves only to modulate the spike rate, it might be that different rates can be distinguished more easily because, for example, the shape of the interspike interval distributon changes as function of rate. This is known to occur in many cells. Mathematically, counting spikes is the optimal way of recovering rate information only if the spike train is a modulated Poisson process.

Since the reconstruction procedure is a bound on performance and not a direct measurement, it is reasonable to ask how tight this bound will be. Warzecha and Egelhaaf state that the reconstruction of velocity signals would underestimate the performance of the neuron if the cell is sensitive to derivatives of the velocity; specifically they claim that the coherence between the stimulus and reconstruction would be reduced if the neuron were sensitive to derivatives. In fact, the particular reconstruction procedure of Bialek et al. (1991) is invariant to linear transformations of the signal such as differentiation and integration, and the computation of coherence always is invariant to these transformations (Lighthill, 1958). Is there any independent way to assess the efficacy of the reconstruction method? One approach is to try different reconstruction algorithms (Warland et al. 1997). Another is to check for consistency among different measures of coherence (Haag and Borst 1997). Finally, we can compare the noise levels in the reconstructions with the noise levels that would be generated by an ideal observer who is limited only by noise in the photoreceptors. In the high frequency limit (of order 30 Hz), where the ideal observer's performance can be calculated from photoreceptor measurements, the ideal observer does not perform substantially better than the reconstruction (Bialek et al. 1991), which demonstrates that H1's response approaches ideal observer performance. This can only be true if the fly's visual brain makes efficient use of the information present in the array of photoreceptors, and does not add a substantial amount of noise to the computation of motion. This finding is confirmed by measurements of neural performance that do not depend on reconstructions (de Ruyter van Steveninck and Bialek, 1995). Of course, the accuracy and efficiency of the reconstructions also imply the functional correctness of the reconstruction algorithm. Criticism of the reconstruction algorithm itself cannot invalidate the demonstration of accurate reconstructions.

4 Information transmission with natural stimuli

In the following we use methods described in detail by Strong et al. (1998) to quantify information transmission in our natural motion experiment. Briefly, we analyse the statistics of firing patterns that H1 produces in response to the stimulus used in our experiment, and consider segments of the spike train with length T divided in a number of bins of width Δt, where Δt will range from very small (order of a millisecond) up to $\Delta t = T$. Each such bin may hold a number of spikes, and within a bin no distinction is made on where the spikes appear. However, two windows of length T that have different combinations of filled bins are considered to be different firing patterns, and are therefore distinct. From an experiment in which we repeat a reasonably long natural stimulus a number of times (here 200 repetitions of a 5 seconds long sequence) we get a large number of these firing patterns, and from that set we compute two entropies:

1. The total entropy, which characterizes the probability distribution of all spike firing patterns of length T that consist of n adjacent bins each Δt

wide (that is, $T = n\Delta t$). This entropy measures the richness of the 'vocabulary' used by H1 under these experimental conditions, hence the time of occurrence of the pattern within the experiment is irrelevant.

2. The noise entropy, which gives us an estimate of how variable the response to identical stimuli can be. We first accumulate, for each point in time in the stimulus sequence, the distribution across all trials of firing patterns that begin at that point. The entropy of this distribution measures the (ir)reproducibility of the response at each instant. Calculating this for each point in time and averaging all these values we obtain the average noise entropy.

The information contained in firing patterns of length T and resolution Δt is the total entropy minus the average noise entropy (Shannon 1948). One interesting measure is to estimate this information as we let T become very long, and Δt very short. This limit is the average rate of information transmission, as discussed by Strong et al. (1998). Here, instead, we will just calculate the information transmitted in constant time windows, $T = 30\,\mathrm{ms}$, as a function of Δt. We choose $T = 30\,\mathrm{ms}$ because that amounts to the delay time with which a chasing fly follows turns of a leading fly during a chase (Land and Collett, 1974); the end result, namely the dependence of information transmission on Δt, does not depend critically on the choice of T.

The data in Fig. 4a show that the information contained in a 30 ms window depends strongly on Δt, increasing from about 2 bits to about 5 bits when the resolution increases from $\Delta t = 30\,\mathrm{ms}$ to $\Delta t = 1\,\mathrm{ms}$. Although in the limit of arbitrarily fine time resolution ($\Delta t \to 0$), the information must reach a finite limit, we see no evidence for a plateau at $\Delta t = 1\,\mathrm{ms}$. For shorter time windows ($T = 12\,\mathrm{ms}$) we find that the information keeps on increasing up to $\Delta t = 0.25\,\mathrm{ms}$. This lack of a clear plateau makes sense: the motion stimuli themselves have a distribution of temporal features so it is not surprising that there is not a sharply defined single timescale in the response. We also note that, as in earlier work with less natural stimuli (Strong et al. 1998), the information rate is a bit more than half the total entropy, even at millisecond resolution (see Fig. 4b), so the neuron utilizes a significant fraction of its coding capacity even on this fine time scale.

The question of whether spike timing is important in the neural code has been debated for decades, and our present experiment addresses the importance of millisecond resolution in information transmission by a single cell. Ultimately one would like to connect the responses of neurons to animal behaviour. Thus, one way to demonstrate the importance of spike timing would be to search for experimental conditions in which the timing of just a few spikes would be correlated with a behavioral decision, in the spirit of the work by Newsome and colleagues (Newsome et al. 1995). Another approach is to look for other neurons that can "read" the temporal structure, for example along the lines of recent work from Usrey et al. (1998). Here we focus on the response of a single neuron, and ask if the precise timing of spikes carries information under natural stimulus conditions. The answer is yes.

5 Responses to static and dynamic stimuli

The measured precision of responses in H1 to dynamic stimuli seems to suggest that the behavior of the fly visual system might be very different from other systems, especially the mammalian cortex. Neurons in visual cortex, for example, commonly show a large variance in the responses across repeated presentations of the same visual stimulus (Tolhurst et al. 1983). To quantify this observation several groups have studied the variance in the number of spikes that are counted in a window of fixed size, and then manipulated the stimulus conditions to find the relation between the variance of the response and its mean. Typically, the variance in spike count is found to be close to or somewhat larger than the mean over a wide range of conditions; there is a tendency for the ratio variance/mean (the Fano factor) to be larger in larger time windows.

More recently several groups are investigating to what extent accurate spike timing, such as observed in H1, can be consistent with the variability of neural responses observed in cortex. Almost all experiments on the variability of responses in visual cortex had been done with static or slowly varying stimuli, while all the work indicating precise responses and the importance of spike timing in H1 had been done using complex, dynamic inputs. Newsome and collaborators studied the responses of motion sensitive neurons in the monkey visual cortical area MT using dynamic random dot stimuli, but their work focused on the connection of neural responses to the monkeys perception of coherent motion in the entire display (Newsome et al. 1995). Bair and Koch (1996) reanalysed some of these data to show that when the monkey saw exactly the same dynamic dot movies the neural response showed significant modulations on a time scale of 30 ms or less. Strong analysed the same data to show that the spike train of a cortical neuron could provide information about the movie at a rate of ~ 2 bits/spike, comparable to the results in H1 (see note 19 in Strong et al. 1998), and in unpublished analyses he found that the variance of the spike count in windows of 30 ms or less could be significantly less than the mean.

Mainen and Sejnowski (1995) found that they could produce irregular spike trains in a slice of cortex if they injected constant current into a neuron: after some time the cell 'forgets' the time at which the current was turned on and the spikes drift relative to the stimulus. With dynamic currents, however, there can be precise temporal locking of spikes to particular events in the input signal. Berry et al. (1997) found that ganglion cells in the vertebrate retina—which are known to generate irregular and highly variable spike trains in response to static or slowly varying images—generate highly reproducible spike trains in response to more dynamic movies. A hint in the same direction had been found earlier by Miller and Mark (1992), who showed that primary auditory neurons in the cat give less variable responses to complex speech stimuli than to pure tones. Finally, de Ruyter van Steveninck et al. (1997) showed explicitly that the low variance, reproducible response of H1 to dynamic stimuli coexists with a much more variable response to constant velocity inputs: studying a range of constant velocities that drove H1 to average firing rates up to about 70 spikes per second (which corresponds to the time average rate elicited by dynamic

stimuli in comparable stimulus conditions), mean counts and variances in 100 ms windows straddled the line at which variance is equal to mean, and fell well within a cloud of points obtained from experiments in visual cortex.

Taken together, all of these different results point to the conclusion that the statistical structure of the neural response to static stimuli may be very different from that in response to dynamic or naturalistic stimuli. The crucial conclusion is that we cannot extrapolate from the observation of highly variable responses under one set of conditions to reach conclusions about the structure of the neural code under more natural conditions. This fits very well with the ethological perspective that we introduced at the beginning of this contribution, and indeed many of the analysis methods that we have discussed here were developed to meet the challenges of quantifying the neural response to more naturalistic stimuli. From a more mechanistic point of view there is now considerable interest in understanding why neurons seem to respond so differently to static and dynamic inputs (Schneidman et al. 1999, Jensen 1998).

Against this background it came as a surprise when Warzecha and Egelhaaf (1999) claimed that the variance of H1's response to constant velocity is no different from that in response to dynamic stimuli. It would appear that they have done an experiment very similar to that described by de Ruyter van Steveninck et al. (1997) but reached the opposite conclusion: while Warzecha and Egelhaaf confirm the highly reproducible, low variance response to dynamic stimuli, they find similarly reproducible responses to constant velocities. There are many issues here, but we focus first on the explicit disagreement regarding the variability of responses to constant velocity. Warzecha and Egelhaaf themselves offer several possible explanations for the discrepancy, but they do not draw attention to the fact that the stimuli used in the two sets of experiments differ substantially; these differences exist along every stimulus dimension known to affect the response of H1—velocity, image contrast, spatial pattern, and size of the visual field. Further, in the crucial comparison of static to dynamic stimuli, it is not clear what is being held constant in the Warzecha and Egelhaaf experiments. In the dynamic experiments changes in spike rate are of course driven by variations in angular velocity, but in their static experiments they hold the velocity fixed and vary the image size. At best these experiments show that H1 responds with different statistics under different conditions, but we still find the discrepancies disturbing.

In an attempt to resolve the issue, we have gone back over several years of experiments to collect all the data which may be relevant to relation between variance and mean in static experiments, we have done new experiments that come close to the conditions of the Warzecha and Egelhaaf work, and we have designed new stimuli that highlight the differences between static and dynamic responses in a single experiment. In brief, studying 20 flies under a wide variety of static stimulus conditions, we find a broad distribution of variances at each value of the mean, but up to rates of about 100 spikes/s there is no overlap with the results of Warzecha and Egelhaaf (1999). Further, when we match the conditions of their experiments we cannot reproduce even the mean spike counts, let alone the variances. For example, their Fig. 4A in this volume shows

a mean spike count of about 6.5 in 100 ms windows for a high contrast large field ($91^\circ \times 7.5^\circ$) pattern moving at about 36°/s, and for the same experimental conditions Warzecha and Egelhaaf (1999) report a mean count of about 4. These values correspond to mean firing rates of 65 and 40 spikes/s respectively. In 8 flies tested under comparable conditions (contrast, velocity and stimulated area) we never get rates below 120 spikes/s, consistent with the findings of Lenting et al. (1984).

In Fig. 5 we show the response of H1 to a slowly varying velocity ramp, and contrast this response to that obtained with dynamic velocities. Computing the mean spike counts in 100 ms windows across 50 trials, we see that the static and dynamic stimuli give the same range of mean responses, yet when we compute the variances there are huge differences that are obvious to the eye. The count variance during quasistatic stimulation peaks for mean counts that are about equal to the average count during dynamic stimulation (that is, in the two places where the dashed line in Fig. 5b intersects the smooth curve). This is not just a coincidence of our choice of standard deviation of the dynamic stimulus; it turns out that the fly's visual system adapts such that the mean firing rate during dynamic stimulation is rather insensitive to the standard deviation of the dynamic stimulus (Brenner et al., submitted). For higher values of the mean count the variance decreases strongly, due to the effects of refractoriness (Hagiwara, 1954). So H1 has relatively low count variance both for low and high rates, but its count variance is high for intermediate rates. Loosely, one may think of the dynamic stimulus as switching the cell rapidly back and forth from a state of low rate and low variance to a state of high rate and low variance. By switching fast, the cell effectively bypasses the intermediate condition of high variance, so that its count variance for dynamic stimuli always remains low, as can be seen directly from Fig. 5c. Thus, if we match windows with the same mean count, up to a count of about 10 for 100 ms windows, we find that H1's count variance is lower in response to dynamic than to static stimuli, which was precisely the point of the original work by de Ruyter van Steveninck et al. (1997).

6 Conclusion

Most of what we know about the nervous system has been learned in experiments that do not even approach the natural conditions under which animals normally operate. Much of our recent work, and the core of the debate between our groups and Warzecha and Egelhaaf, concerns the structure of the neural code under natural conditions. We emphasize that this is not an easy problem, and by no means are the issues specific to flies or even the visual system; in many different sensory and motor systems we would like to design and analyse experiments on the coding and processing of more natural signals.

Our approach has been to break this large question into (hopefully) manageable pieces, and then to use information theory as a framework to pose these questions in a form such that suitable experiments should yield precise quan-

titative answers. In particular, we endeavour to make statements that do not depend on multiple prior assumptions, and to develop methods which can be used in analyzing many different kinds of experiments. Thus, we have used stimulus reconstruction techniques to give lower bounds on the performance of fly motion sensitive neurons, and we have been able to measure the average information carried by single spikes, patterns of spikes, and continuous segments of the spike train, all without assumptions regarding the "important" features of the stimulus or neural response. Many of the results obtained in this way point clearly toward a picture of the fly's visual system as close to optimal in extracting motion information from the photoreceptor cell array, and then encoding this signal efficiently in the timing of action potentials of motion sensitive neurons. In contrast to the view developed over the past decade, the recent papers from Egelhaaf and coworkers, including their contribution to this volume, make the explicit claim that the system is very noisy and that meaningful information is contained only in averages over time windows containing many spikes. In many cases these claims are introduced with plausible qualitative arguments. As emphasized long ago by Bullock (1970), however, the challenge is to quantify the degree of noisiness or precision in the nervous system, and there is a danger that a neuron may appear noisy because we have an incomplete understanding of its function. Thus we have grown skeptical about qualitative or even semiquantitative arguments for the imprecision of neural responses. The interpretation of correlation functions, discussed above, provides a good example: Although there may be an obvious "correlation time" in one correlation function, the hint that other time scales are relevant is hidden in the cusp of the correlation function at short times. More detailed analysis shows that the relations among correlation functions are consistent with temporal precision on scales a factor of 30 smaller than the nominal correlation time. Further, this measure of temporal precision is consistent with the results of a rigorous information theoretic analysis.

Because this paper is intended (by the editors) as a response to the contribution of Warzecha and Egelhaaf, we have tried to understand how they have reached conclusions so nearly opposite from our own. As emphasized at the outset, there are two different questions. First there is the problem of constructing an approximately natural stimulus, and then there is the problem of analyzing the response to such a complex signal. Although there is a whole generation of quantitative observations on insect flight trajectories, Warzecha and Egelhaaf present as the stimulus they analyse a signal that is substantially impoverished both in amplitude and in frequency content, as is clear from Fig. 1. Further, their visual stimulus is very dim compared to daytime natural conditions, and has a visible area much smaller than what is experienced by a free flying animal. They repeatedly stress that motion induced responses depend on many stimulus variables in addition to velocity, but never discuss critically the extrapolation from their experiments to natural behaviour, in spite of the large differences in many of the crucial variables. Similarly, although there is now a decade of papers concerning the quantitative information theoretic analyses of neural spike trains, and of the cell H1 in particular, Warzecha and Egelhaaf do not present their results on information transmission in absolute units (bits);

closer examination suggests that there are more basic mathematical problems in their approach, as outlined above.

In this paper we have presented the results from a new experiment (Lewen et al. in preparation) which brings us much closer to the natural conditions of fly vision. Visual inspection of the responses of H1 under these conditions indicates that individual spikes are reproducible on a millisecond time scale, and aspects of temporal pattern in the spike train can be reproducible on a substantially submillisecond time scale. This impression is borne out by the quantitative demonstration that the spike train conveys information with nearly constant efficiency down to millisecond time resolution; indeed, the information provided by the spike train shows no sign of saturation as we approach millisecond resolution. These responses to natural stimuli thus are even more precise than suggested by our earlier work.

In the early 1960's Reichardt and his coworkers started working on flies, with a special emphasis on motion detection (Reichardt, 1961). One of their motivations was that motion detection in flies represents a good compromise between a reasonable complexity of information processing properties and an amenability to quantitative analysis (Reichardt and Poggio 1976). Over the years this intuition has proved to be very fruitful, and the fly has turned out to be a system in which many issues could be studied, often with unprecedented quantitative detail. In particular, the fly's motion sensitive neurons have been an important testing ground for ideas about the neural code, especially in the ongoing effort to characterize the coding of more natural stimuli. Approached with proper mathematical tools, the fly visual system can continue to provide answers to many fundamental and quantitative questions in real time neural information processing.

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Figure Captions

Figure 1. Comparison of the rotational velocity traces reported from free flying and tethered flies. a: Rotation velocity of a fly (Fannia canicularis) in free flight, derived from video recordings by Land and Collett (1974). b: Rotation velocity of a pattern in a flight simulator, derived from torque signals measured from a tethered fly, as reported by Warzecha and Egelhaaf (1997). c: The data from a and b plotted on the same scale.

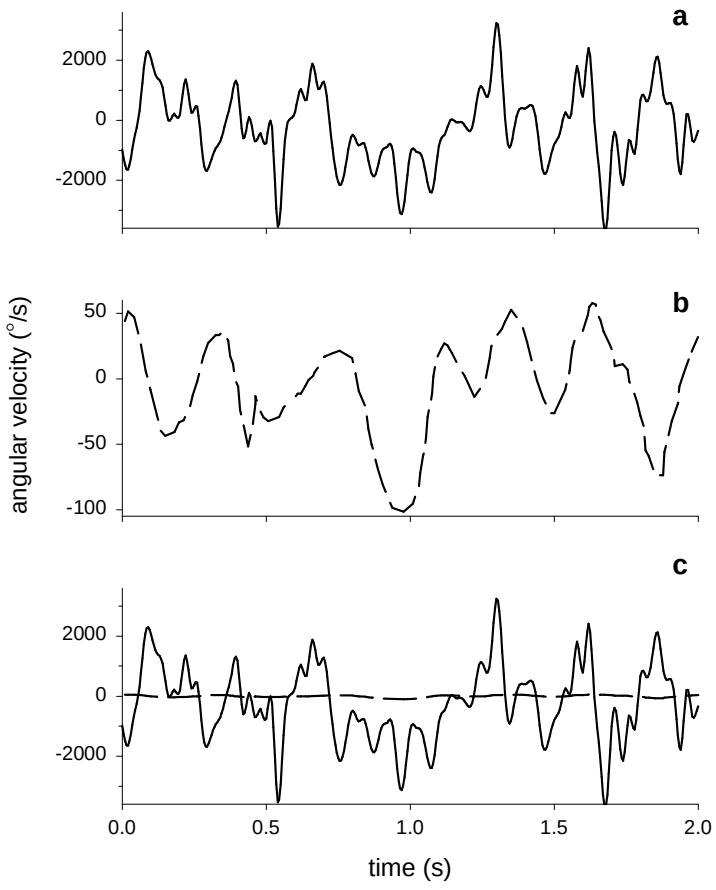
Figure 2. Direct observations of H1 spike timing statistics in response to rotational motion derived from Land and Collett's (1974) free flight data (see Fig. 1a). a: A 500 ms segment of the motion trace. b: Top: raster plot with 25 traces representing spike occurrences measured from H1. Bottom: raster plot of 25 traces of spike occurrences from the same cell, but in response to a velocity trace that was the negative of the one shown in a. For ease of reference we call these traces H1+ and H1− respectively. c: Raster plot of 25 samples of the occurrence time of the first spike fired by H1− after time $t = 2080$ ms in the stimulus sequence (indicated by the dashed line connecting the axis of b to panel c). d: Raster plots of 25 samples of the interval from the spike shown in c to the first (filled circles), second (open circles), third (filled triangles), and fourth (open triangles) spike following the spike shown in c. Note the time axes: The rasters in c and d are plotted at much higher time resolution than those in b. e: Probability density for the timing of the spike shown in c. The spread is characterized by $\sigma = 0.73$ ms, where σ is defined as half the width of the peak containing the central 68.3% of the total probability. If the distribution were Gaussian, then this would be equivalent to the standard deviation. Here we prefer this definition instead of one based on computing second moments. The motivation is that there can be an occasional extra spike, or a skipped spike, giving a large outlier which has a disproportionate effect on the width if it is calculated from the second moment. Filled squares represent the experimental histogram, based on 200 observations, while the solid line is a Gaussian fit. f: Probability densities for the same interspike interval shown in d. The definition of σ is the same as the one in e.

Figure 3. Correlation functions for H1 during stimulation with natural motion, all computed at 0.2 ms resolution. a: The spike–spike autocorrelation $\Phi_{\mathrm{spike-spike}}(t)$, normalized as a conditional rate. There are strong oscillations in the conditional rate, due to neural refractoriness. b: The cross-trial correlation function $\Phi_{\mathrm{crosstrial}}(t)$, computed as the correlation function of the estimated time dependent rate minus a contribution from $\Phi_{\mathrm{spike-spike}}(t)$ scaled by $1/N$ (N is the number of trials) to correct for intratrial correlations. c: Autocorrelation of the assumed underlying distribution of spike jitter times, computed by deconvolving the data in b by those in a. See text for further explanation.

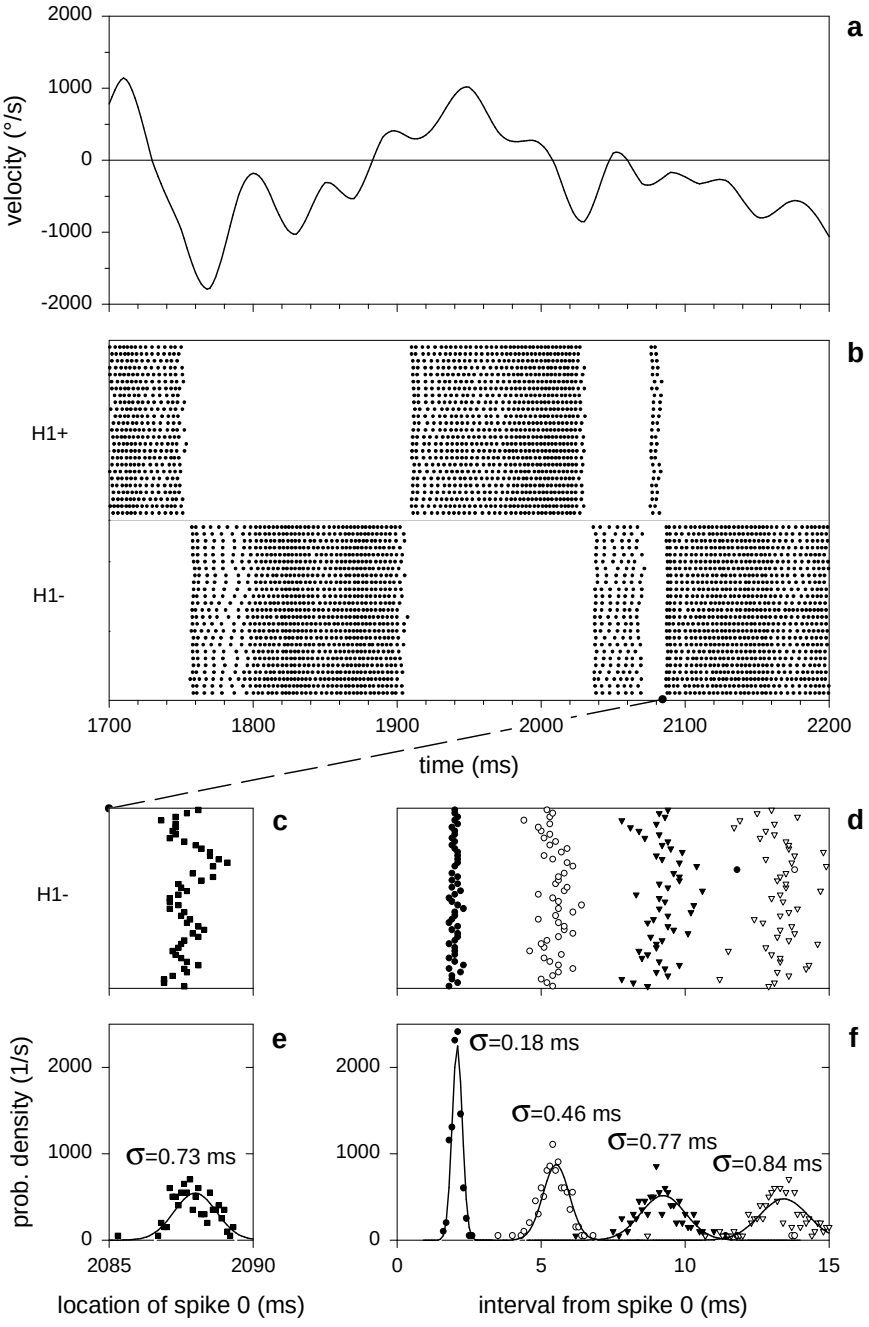
Figure 4. Information and coding efficiency in firing patterns for naturalistic motion stimuli (see legend for Fig. 2). a: Total entropy, noise entropy and infor-

mation in an observation window of $T = 30\,\mathrm{ms}$, as a function of time resolution, Δt. From the trends observed in partitioning the finite dataset we estimate first and second order extrapolations to the entropies for an infinite dataset. Filled symbols are first order, open symbols are second order extrapolations. The deviation between first and second order extrapolations is small, indicating that systematic errors in the entropy estimates are small (for details see Strong et al. 1998). Statistical errors were estimated from the spread in the different partitions of the original dataset. These errors are smaller than the size of the symbols. b: Coding efficieny (information divided by total entropy) as a function of Δt.

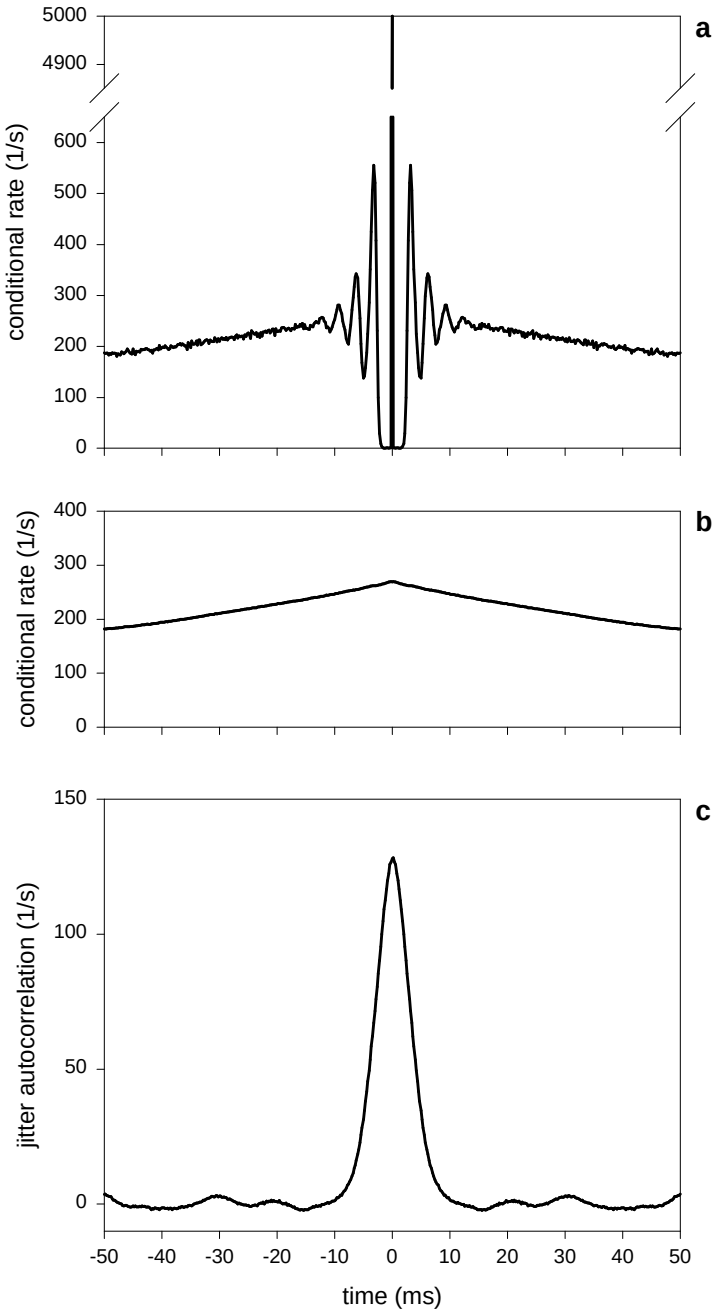
Figure 5. Mean count and variance compared for quasistatic and dynamic velocity stimuli. a: Stimulus. for the first 48 s the velocity is slowly ramped up and down. From 50 to 72 seconds the stimulus is dynamic with a standard deviation of 100°/s, and a cutoff frequency of 250 Hz. Note that the vertical scales (left for the quasitationary and right for the dynamic stimulus) are different. The peak at 50 s is a reset phase in which the pattern is moved at maximum speed so as to bring the stimulus pattern into exact register on every trial. b: Trial average spike count in 100 ms windows, as a function of time. The dashed line represents the time averaged count in response to the dynamic stimulus. c: Spike count variance in 100 ms windows, as a function of time.



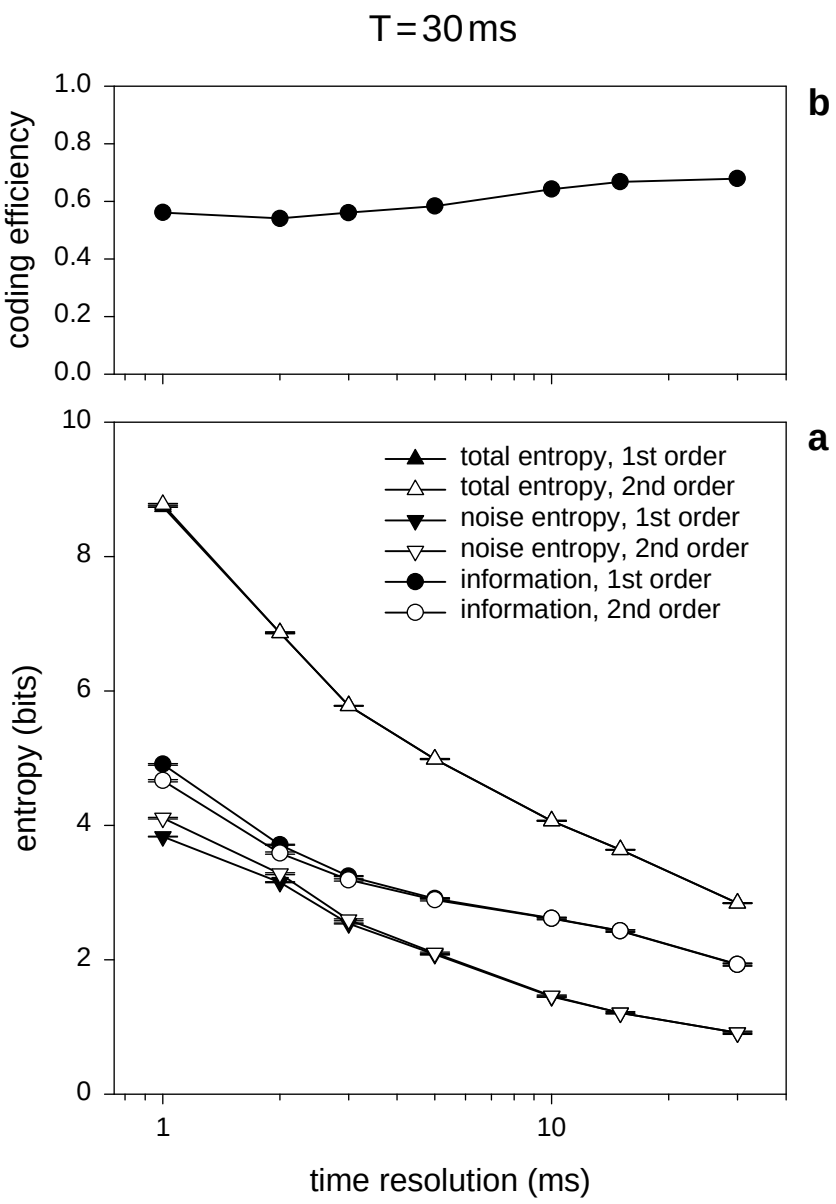
de Ruyter et al. FIG 1



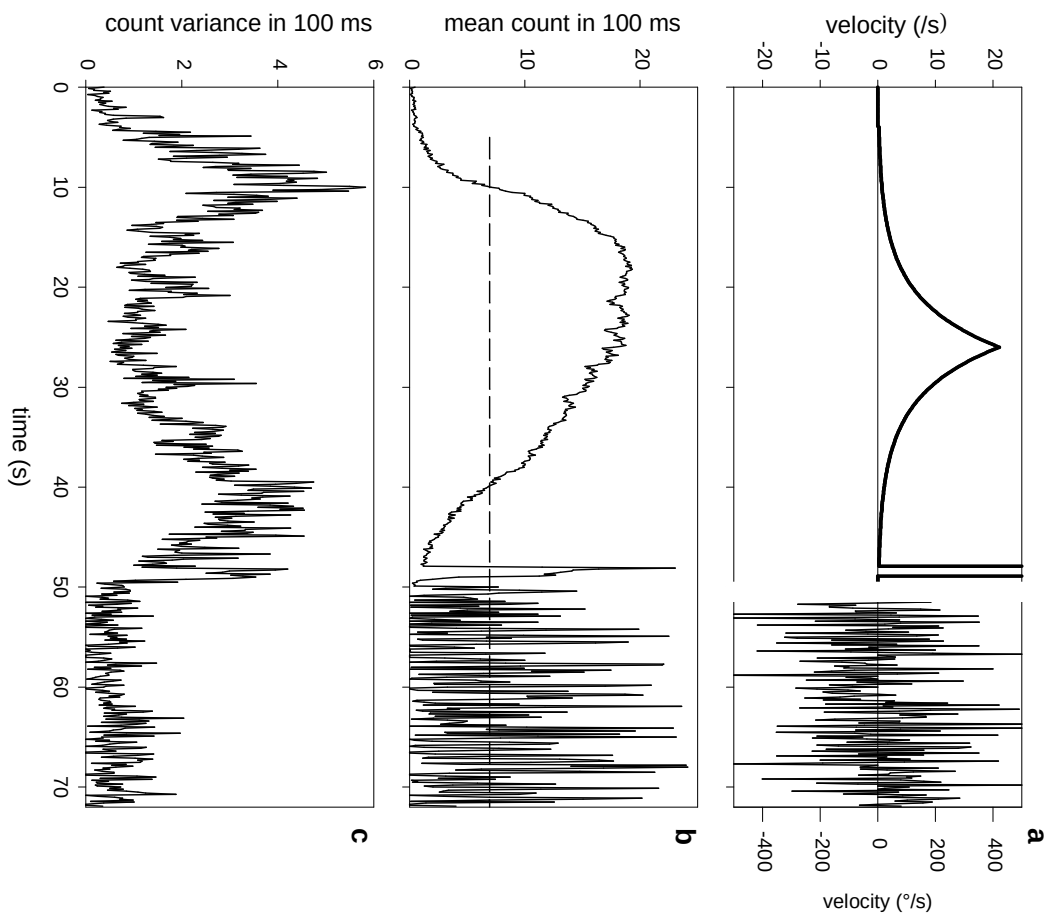
de Ruyter et al. FIG 2.



de Ruyter et al. FIG 3.



de Ruyter et al. Fig. 4



de Ruyter et al. FIG 5