Electrophysiological and Neuroethological Investigations of the Teleostean Optic Tectum

by

Craig G McDonald
BSc University of Victoria, 1989
MSc University of Victoria, 1994

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We accept this dissertation as conforming
to the required standards

Dr CW Hawryshyn, Supervisor (Department of Biology)

Dr DH Paul, Departmental Member (Department of Biology)

Dr NJ Livingston, Departmental Member (Department of Biology)

Dr ME Corcoran, Outside Member (Department of Psychology)

Dr RW Turner, External Examiner (Departments of Cell Biology and Anatomy, Physiology and Biophysics, University of Calgary)

Supervisor: Dr Craig W Hawryshyn

ABSTRACT

Although colour-opponent neurons appear to subserve colour vision, precisely how these cells encode hue is still not clear. Single-unit, extracellular recordings from the rainbow trout optic tectum were made in order to examine the possible role of action potential timing in coding chromatic stimuli. I found that colour-opponent units can exhibit differences in response latency which are a function of wavelength and response sign, with the Off response exhibiting the shorter response latency. I also found that units often responded with spike bursts characterized by early and late spikes separated by a silent period, with the relative proportion of early and late spikes varying as a function of wavelength. This type of discharge pattern appears to be a result of inhibitory, colour-opponent processes. I suggest that complete inhibition of early spikes may be the mechanism underlying the observed latency differences. These findings suggest a role for action potential patterning in coding chromatic stimuli.

To further explore chromatic processing in the trout tectum, I recorded tectal evoked potentials (TEPs) from the tectal surface. I found that TEP waveforms show distinct variation as a function of wavelength. In addition, my findings indicate that the On and Off channels of the tectum each possess distinctly different wavelength dependent properties. Middle wavelength stimulation typically evoked a waveform similar to that reported for another anamniote vertebrate, the toad. For both the On and Off response, this waveform was comprised of two negative waves, N1 and N2, which were interrupted by a positive wave, P2. The N2 wave was followed by a final positive wave, P3. Principal components analysis revealed that the N2/P3 wave sequence of the On response became significantly more pronounced as a function of increasing wavelength. In contrast, the N2/P3 wave sequence was most pronounced at middle

wavelengths for the Off response. The N1 wave was relatively invariant with respect to wavelength. Should colour-opponent tectal units provide a substantive contribution to the TEP, it is probable that its wavelength-dependent properties indicate underlying neural processes that facilitate colour discrimination.

Despite considerable study of the visual behaviour of fish, neurophysiological studies that examine the nature of perception of realistic ethologically relevant stimuli are lacking. The second goal of this dissertation, therefore, was to investigate how an ethological stimulus, the agonistic display of *Betta splendens*, is perceived by conspecifics. To this end, I made multi-unit recordings from the optic tectum of *Betta splendens* while they viewed the agonistic display of conspecifics. I have found that the discharge pattern of tectal units is strongly modulated by dynamic movement associated with agonistic display. Moreover, the tectum appears to be attuned to a feature of the display that is known to be of ethological significance, the onset of full display. It is hoped that the work presented here will encourage future neurophysiological investigators to utilize natural stimuli to explore visual sensation in animals.

Examiners:

Dr CW Hawryshyn, Supervisor (Department of Biology)

Dr DH Paul, Departmental Member (Department of Biology)

Dr NJ Livingston, Departmental Member (Department of Biology)

Dr ME Corcoran, Outside Member (Department of Psychology, University of Saskatchewan

Dr RW Turner, External Examiner (Departments of Cell Biology and Anatomy, Physiology and Biophysics, University of Calgary)

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AON	accessory optic nuclei
Dc	area dorsalis centralis
EPSP	excitatory postsynaptic potential
GABA	γ-amino butyric acid
IPSP	inhibitory postsynaptic potential
L	long wavelength sensitive
M	middle wavelength sensitive
MURF	multi-unit receptive field
ND	neutral density
NI	nucleaus isthmi
NMDA	N-methyl-D-aspartate
OKN	optokinetic nystagamus
OPM	optomotor response
OT	optic tectum
POA	preoptic area
PTec	pretectum
RF	receptive field
RGC	retinal ganglion cell
S	short wavelength sensitive
SAC	stratum album centrale
SFGS	stratum fibrosum et griseum superficiale
SGC	stratum griseum centrale
SM	stratum marginale
SO	stratum opticum
SPV	stratum periventriculare
Thal	thalamus
TL	torus longitudinalis
TS	torus semicircularis
Tub	tuberculum
TEP	tectal evoked potential
UV	ultraviolet sensitive

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To my parents,

George and Gisela McDonald

Chapter 1

An overview of electrophysiological properties of the teleostean optic tectum, with neuroethological implications

1. Introduction

The optic tectum is the largest external feature of the brain of many teleost fishes (Figure 1), and is the primary visual centre in all anamniote vertebrates. This suggests that the teleostean tectum has a substantially greater role in mediating visual behaviour than does its homologue in mammals, the superior colliculus, or the optic tecta of birds (Guthrie 1990). Thus, physiological investigations of the optic tectum, in addition to providing a better understanding of how stimuli are encoded, bring us closer to a general understanding of the causal mechanisms of visually guided behaviour.

Although this review will emphasize the role of the optic tectum as a visual centre, it should be noted that this structure also receives other sensory inputs (e.g. auditory, lateral line). Moreover, these multisensory inputs are organized as maps of the external world, and these maps are in register with each other (Butler and Hodos 1996). In this chapter, I will focus on studies that have examined the role of the tectum in processing visual input, particularly chromatic stimuli. I will also describe other detector properties which provide insight (albeit indirect) into behavioural function, as well as consider how neural elements within the tectum contribute to its physiological properties.

2. Tectal organization

The optic tectum is a paired structure forming the roof of the midbrain. It is the primary recipient of the output neurons of the retina, the retinal ganglion cells (RGCs), the majority of which terminate in the contralateral tectal hemisphere. The cell bodies of neurons within the optic tectum are organized into a layered pattern. These layers, from the outer layer to the deepest, are: the stratum marginale (SM), stratum opticum (SO), stratum fibrosum et griseum superficiale

(SFGS), stratum griseum centrale (SGC), stratum album centrale (SAC) and stratum periventriculare (SPV) (Figure 2).

The SO contains the majority of retinotectal fibres (retinal ganglion cell axons), which terminate predominantly in the SFGS. A relatively sparse, deep retinotectal projection terminates in the SAC and/or SPV. This projection pattern is remarkably consistent across most teleost species, with the exception that, in some groups, an additional projection terminates in the SGC (von Bartheld and Meyer 1987). There is a point-to-point correspondence between the location of adjacent retinal ganglion cell bodies in the retina and the termination points of their axons within the tectum. This innervation pattern gives rise to a retintotectal map of visual space (Schwassmann and Kruger 1965; Schwassmann 1968). Although visual input to the tectum is primarily from retinotectal fibres, there are also significant indirect visual inputs from other brain structures.

2.2 Non-retinal visual projections to the tectum

In most teleost fishes, the retina projects bilaterally to the diencephalon and optic tectum, though the ipsilateral projections are comparatively sparse. The projection to the tectum is considerably larger than that to the diencephalon. Within the diencephalon, retinal fibres terminate in a number of regions, including the preoptic area, thalamus, posterior tuberculum, prectectum and accessory optic nuclei. One or more nuclei within each of these regions project, in turn, to the ipsilateral tectum (Pinganaud and Clairambault 1979; Butler and Saidel 1993). Although there are few data regarding the diencephalic projection patterns, it appears that fibres from at least two regions, the pretectum and thalamus, terminate primarily in the SO and SFGS (Meek 1990).

The tectum also receives visual input from brain centres located in the telencephalon, mesencephalon and rhombencephalon (Figure 3). In the telencephalon, a central region composed of large cells, termed the area dorsalis centralis, projects to the tectum. The area dorsalis centralis receives its visual input from the retinorecipient preoptic area and thalamus

(Echteler and Saidel 1981). In addition, the nucleus preglomerulosus of the diencephalon projects to the telencephalon. As this nucleus receives projections from the torus semicircularis, its sensory input might be expected to be visual (Murakami *et al.* 1983; Ito *et al.* 1986). Most of the telencephalotectal projection fibres terminate at the midlevel of the SGC (Vanegas and Ebbesson 1976; Ito and Kishida 1977).

In the mesencephalon, the torus longitudinalis is the primary source of tectal afferents. (Grover and Sharma 1981; Luiten 1981). Fibres from this structure project topographically to the SM of the ipsilateral tectum. These fibres make en passant synapses on the dendritic trees of tectal pyramidal cells (Vanegas *et al.* 1979). A reciprocal projection from the tectum makes contact with large cells in the dorsal torus longitudinalis. These cells are photically responsive, increasing their activity to dimming stimuli but decreasing their activity when luminance is increased (Northmore *et al.* 1983). It is conceivable that projections from these cells could provide visual input to the ipsilateral tectum. The primary input to the torus longitudinalis, however, is from the cerebellum. This input appears to generate bursting activity in the ventral torus longitudinalis associated with saccadic eye movements, suggesting that the torus longitudinalis relays information about saccadic movements from the cerebellum to the tectum (Northmore 1984).

The torus semicircularis is another mesencephalic structure that projects to the tectum. This nucleus projects ipsilaterally to the SAC and its boundary with the SGC (Grover and Sharma 1981; Murakami *et al.* 1986). It is reciprocally connected with the tectum and may also receive visual input via the accessory optic centre of the thalamus (Beaudet 1997). The torus semicircularis is a multimodal sensory centre, receiving, in addition to visual input, acoustic and lateral line input (Knudsen 1977; Wolf *et al.* 1983; Echteler 1984). In the weakly electric fishes, the torus semicircularis is large and well differentiated for processing electrosensory information (Bell and Szabo 1986). In gymnotids, this structure makes a topographic projection to the tectum, terminating primarily in the SAC. The electrosensory and visuotopic maps are in register with each other (Bastian 1982).

The contralateral tectum is also a source of tectal afferents. The tectal lobes are reciprocally connected via the intertectal commissure and the postchiasmatic commissure. The intertectal fibres terminate in the lower region of the SGC as well as the SAC (Meek 1990).

In the dorsolateral mesencephalic tegmentum, a structure termed the nucleus dorsolateralis tegmenti makes bilateral projections to the tectum. These projections are not radially localized, having terminations that span from the SPV to the SFGS. A tectal input appears to be the only source of afferents to the nucleus dorsolateralis tegmenti, although cells in this nucleus have been shown to respond to electrical stimulation of the optic nerve and rhombencephalon (Niida and Ohno 1984).

Lastly, the nucleus isthmi, a structure located in the transition zone between the mesencephalon and rhombencephalon, is reciprocally connected with the ipsilateral tectum. In at least one species (Novodon), the nucleus isthmi also receives input from the pretectum (Ito *et al.* 1981). Isthmotectal fibres terminate in the boundary region between the SGC and SFGS (Vanegas and Ito 1983). Northmore and Gallagher (pers. comm.) have recently found that the nucleus isthmi responds selectively to looming stimuli.

3. Chromatic Processing

Of the single-unit studies that have examined chromatic processing in the tectum, the majority involved recordings made from the superficial tectal layers or the root of the dorsal optic tract. As a result, it is uncertain whether these recordings indicate activity of retinotectal fibres or intrinsic tectal cells (Vanegas *et al.* 1984; Guthrie 1990). Recall that the cell bodies and axon terminals of RGCs constitute retinal and tectal elements, respectively. It is therefore evident that studies that have specifically examined colour-coding at the retinal level provide insight relevant to understanding tectal processing, and should be included here.

3.1 Retinal circuitry mediating colour-opponency

At an early point in visual processing, input from different spectral classes of cone photoreceptor combine in an antagonistic, or opponent, fashion. In teleost fishes, colour-opponency arises at the level of second order retinal neurons, namely the horizontal and bipolar cells. Bipolar cells are the first cells in the visual pathway with a centre-surround receptive field organization, with the surround response presumably generated via negative feedback from the horizontal cells to the cone photoreceptors (Kamermans and Spekreijse 1999). It is also at the bipolar cell level where spatially dependent colour-opponency originates. Two types of colour-opponent bipolar cells, termed single and double opponent, have been described. (Figure 4). Single colour-opponent cells show chromatic antagonism between the centre and surround receptive field. By contrast, double colour-opponent cells exhibit chromatic antagonism both within and between each region of the receptive field (Kaneko and Tachibana 1981, 1983; Shimbo *et al.* 2000). Before proposing a mechanism mediating colour-opponency in bipolar cells, it is first necessary to review the nature of colour-opponency in horizontal cells.

Horizontal cells are interneurons that contact the photoreceptors. They possess large receptive fields and respond with slow hyperpolarizing or depolarizing responses. They receive sign-preserving glutamatergic input from the photoreceptors and feedback to the cones via a sign-inverting GABAergic pathway. This pathway presumably produces the surround responses of the bipolar cells (Kamermans and Spekreijse 1999). There are three classes of horizontal cell: monophasic, biphasic and triphasic. Monophasic horizontal cells (MHCs), as the name implies, hyperpolarize to all wavelengths of stimulation. That is, they are not colour-opponent. Biphasic horizontal cells (BHCs) are colour-opponent, hyperpolarizing to green light and depolarizing to red light. Triphasic horizontal cells (THCs) are also colour-opponent, hyperpolarizing to red and blue light, but depolarizing to green light (Djamgoz and Yamada 1990).

To account for the response properties of the different horizontal cell types, Stell *et al.* (1975) formulated a model involving a hierarchical set of feed forward and feedback interactions

between the horizontal cells and the cone photoreceptors (Figure 5). The model proposes that MHCs receive sign-preserving input from red cones, resulting in a monophasic response, but send sign-inverting signals to all cone classes. Sign-inverting feedback from MHCs to green cones, and subsequent feed forward onto BHCs, which were presumed not to make connections with red cones, would result in the generation of a biphasic response. Similarly, sign-inverting feedback from BHCs onto blue cones would result in a triphasic response in THCs. Note that, with this model, each horizontal cell type receives direct sign-preserving input from only a single cone class.

More recently, Kamermans *et al.* (1991) have modified Stell's model in a fashion that they suggest is more consistent with available anatomical and physiological data (Figure 5). This model is based on the assumptions that all contacts between horizontal cells and cones are both feed forward and feedback and that all horizontal cell types contact all cone types. In general agreement with Stell *et al.* (1975), Kamermans *et al.* (1991) also suggest that MHCs are dominated by red cone input, BHCs by green cone input and THCs by blue cone input.

Kamermans and Spekreijse (1995) have also created a qualitative model of a retinal circuit capable of endowing bipolar cells with colour-opponent receptive field properties. For the sake of clarity the proposed circuit includes only the red and green cone mechanisms (although the circuit can be extrapolated to include the blue cone mechanism and THCs). The double colour-opponent cell will be considered first. The model suggests that a double opponent bipolar cell with, for example, a red hyperpolarizing and a green depolarizing centre response, receives direct sign-preserving red cone input and direct sign-inverting green cone input.

The upper panel in Figure 6 provides a schematic of how the surround responses of a double opponent bipolar cell might be generated. Stimulation of the surround with red light will depolarize the central red cones due to feedback from the MHCs to these cones. This depolarization will be attenuated somewhat owing to the weaker feedback from the MHCs to the green cones. Thus, feedback from the MHC via red and green cones is antagonistic. Conversely, feedback from BHCs to the red and green cones will respectively attenuate and strengthen the depolarizing surround response of the bipolar cell. On a broader level, it can be

seen that the model predicts that feedback through green cones always opposes feedback through red cones.

A response to green light in the centre will be depolarizing owing to direct sign-inverting green cone input, although it will be attenuated by the direct sign-preserving red cone input. Surround stimulation with green light depolarizes the green cones in the centre due to negative feedback from the MHCs to the green cones, resulting in a hyperpolarizing surround response. However, feedback from the MHCs to the red cones counters the hyperpolarizing surround response. Again, feedback via the green and red cones is antagonistic. Feedback from the BHCs serves to strengthen the hyperpolarizing surround response to green light.

The model can also generate the receptive field properties of single colour-opponent bipolar cells. Though, in the case of this cell class, the MHCs and the BHCs act antagonistically in the red part of the spectrum, but synergistically in the green part of the spectrum (Figure 6, bottom). Note that for both single and double colour-opponent cells, colour-coding is mediated by combined input from MHCs and BHCs. This argues against a unique role for colour-opponent horizontal cells in generating bipolar cell colour-opponent receptive fields.

It is important to note that the colour-opponent properties of bipolar cells are virtually identical to those of retinal ganglion cells (Djamgoz and Yamada 1990), an observation consistent with Naka's (1977) finding of sign-preserving synaptic transmission from bipolar cells to RGCs. This raises the question of the functional consequence of RGC receptive field characteristics mimicking those of bipolar cells. In this context, it appears that the primary function of RGCs is simply to convert the graded potentials of bipolar cells into spike trains. It should be noted, however, that some RGCs, termed complex cells, receive substantial amacrine cell input, and that these cells possess asymmetric receptive field characteristics (Lasater 1982). Whether complex RGCs are colour-opponent remains to be determined.

3.2 Single unit studies of retinal ganglion cells and retinotectal fibres

Wagner *et al.* (1963) and Jacobson (1964) provided the earliest evidence for colour-opponent RGCs in the goldfish visual system. Wagner *et al.* recorded the activity of RGCs, while Jacobson made recordings from the superficial tectum, which likely resulted in inclusion of retinotectal fibres in his sample. Daw (1968), who recorded from RGCs in the goldfish retina, confirmed and extended the findings of these workers to include a number of additional colour-opponent cell types. Of particular significance was his discovery of cells with single and double colour-opponent centre-surround configurations. Double colour-opponent cells showed spectral opponency, both within and between the centre and surround RF, while single colour-opponent cells showed only centre-surround antagonism. About half the cells that Daw described (referred to as type O) were double colour-opponent. A few cells showed colour opponency in the RF centre only (type P). That is to say, these cells possessed a spatially coextensive colour-opponent centre RF, but did not exhibit a surround RF. Another group of cells (type Q) showed response features similar to those of type O cells; however, these cells were unique in that responses to middle wavelength (green) stimuli could only be evoked by using high intensity stimulation or after bleaching the long-wavelength sensitive (red) visual pigment. Red On/green Off centre cells were most abundant in Daw's study, although cells with blue On/red Off centres were also observed. Interestingly, Daw also noticed that the green surround component of double colour-opponent cells often showed exceptionally long latencies on the order of ~400 ms. Despite this early observation, few attempts have been made to further explore the temporal response features of colour-opponent neurons.

Relatively recent efforts to determine the nature of the input of the blue cone mechanism to goldfish RGCs have yielded contradictory results. Spekreijse *et al.* (1972), recording from isolated goldfish retinae, found that cells with blue-sensitive centres also receive red and green input, and report that blue is usually complementary to red but antagonistic to green. They also report that cells with a blue centre possess no detectable surround RF. Conversely, Beauchamp and Lovasik (1973), who recorded from RGC axons in the optic nerve, found that in cells with a

blue centre, blue was always complementary to green and antagonistic to red. Moreover, they found that all three chromatic inputs contributed to a double colour-opponent RF organization. Mackintosh *et al.* (1987), also recording from isolated retinae, found units with response properties similar to those encountered by both Spekreijse *et al.* (1972) and Beauchamp and Lovasik (1973). Mackintosh *et al.* suggested that Beauchamp and Lovasik might not have found units similar to those encountered by Spekreijse *et al.* because these units may have been difficult to isolate when recording from the optic nerve. An alternate explanation may relate to the fact that cone photoreceptors possess a secondary sensitivity peak at a considerably shorter wavelength than the primary sensitivity peak. This peak is known as the β band absorption peak. In the case of the red cones, the β band absorption peak is in the UV-blue region of the spectrum. Thus, it is possible that the trichromatic cells described by Spekreijse *et al.* were in fact driven by green- and red-sensitive cones and that the response to blue light was evoked by stimulating the β band of the red cone mechanism. Experiments that employ chromatic adaptation to alter the magnitude of the different cone photoreceptor inputs would help to resolve this conundrum.

Although a number of teleosts are now known to possess an ultraviolet-sensitive (UV) cone mechanism (Harosi and Hashimoto 1983; Hawryshyn and Beauchamp 1985; Neumeyer 1985; McFarland and Loew 1994; Tovee 1995), the nature of this cone mechanism's input to colour-opponent neurons has received little attention. Coughlin and Hawryshyn (1994a,b) have recently examined colour-opponent cells in the visual system of an ultraviolet sensitive salmonid, the rainbow trout. They found that the majority of colour-opponent units in the optic nerve and tectum (recordings from superficial layers) had properties similar to goldfish RGCs described by Spekreijse *et al.* (1972). That is, cells with blue centres that receive antagonistic green input and complementary red input. Ultraviolet input to colour-opponent units in the optic nerve and tectum was not common, but when it was encountered, its sign (almost always an On response) was the same as that of the blue input. Coughlin and Hawryshyn did, however, find that a large proportion of units in a subtectal region, the torus semicircularis, received UV input. The RF surround was not examined in these studies.

3.3 Single-unit studies of intrinsic tectal units

Guthrie (1981) studied the spectral properties of intrinsic tectal cells in the perch. Half of the cells Guthrie encountered were luminosity detectors. Some cells (approximately 16 %) showed preferential wavelength sensitivity, but were not colour-opponent. Eighteen percent of the cells sampled showed colour-opponency in their receptive fields. These cells tended to have red On/blue-green Off centre RFs and blue-green On surround RFs. Schellart *et al.* (1979) found that some goldfish tectal cells displayed 'hidden' colour-opponency. In these cells, colour opponency was only evident if the red On centres were chromatically adapted to reveal the presence of a central green component.

3.4 Functional and ecological considerations

The shape of the spectral sensitivity curves of retinal ganglion cells and tectal cells is worth commenting on. Generally, spectral sensitivity of colour-opponent neurons roughly agrees with the spectral sensitivity of the three (or four if an UV mechanism is present) cone classes. However, there are two primary differences. First, spectral sensitivity curves of colour-opponent units are often characterized by narrower peaks and troughs than would be predicted by the corresponding photopigment absorption curves (Coughlin and Hawryshyn 1994a; Gibbs and Northmore 1998). Second, the spectral sensitivity peaks of colour-opponent units do not always coincide with the sensitivity peaks of the cone photoreceptors. These differences indicate that a critical function of colour-opponent processing is to further filter the chromatic inputs of the cone photoreceptors. Such processing plays an integral role in tuning spectral sensitivity of the cone mechanisms (Gouras and Zrenner 1981). Spectral tuning is thought to play an important role in maintaining wavelength-dependent response properties under a variety of photic conditions.

Although the single-unit studies discussed above provide ample evidence for extensive colour processing in the teleostean visual system, it is difficult to draw any unifying conclusions. As a number of taxa were studied, varying ecological constraints can be expected to have

selected for different colour-opponent processes. It follows that the functional significance of the chromatic response properties of the visual systems of these species will only become apparent when their visual ecology is better understood. It is also difficult to comment on the role of the tectum itself in chromatic processing. So little work is definitively associated with intrinsic tectal cells, that one can only say that the types of chromatic processing observed at the retinal level can be observed at the tectal level (Guthrie 1990). Future studies should endeavor to determine whether the tectum substantially modifies retinal inputs or simply relays information to other processing centres.

4. Tectal Evoked Potentials

4.1 Interpretation of waveform structure

Electrical stimulation of the optic nerve evokes a field potential at the tectal surface comprised largely of a high amplitude negative deflection (PS1 wave) followed by a relatively broad positive (PS2 wave) deflection (Figure 7a; nomenclature after Sajovic and Levinthal 1983). The PS1 and PS2 waves are postsynaptic phenomena derived from intrinsic tectal cells oriented radially with respect to the tectal surface (Schmidt 1979; Leung 1990). A series of one or more low amplitude waves, representing presynaptic processes, typically precede the PS1-PS2 wave sequence. Photic stimulation evokes rather similar waveforms, although the waves are broader and a second negative-positive wave sequence is sometimes observed (see below and chapter 3). Negative deflections recorded at the tectal surface are indicative of a current sink generated by either superficial excitatory input or deep inhibitory input. Conversely, positive surface waves represent a current source generated by either superficial inhibitory input or deep excitatory input. To differentiate between these possibilities, as well as ascertain the possible cellular elements mediating the postsynaptic response, one must determine the spatial distribution and polarity of the synaptic inputs. This necessitates depth profile recordings in combination with neuropharmacological investigation.

Depth profile recordings and current source density analyses have revealed that the postsynaptic waves, PS1 and PS2, reach maximal amplitude at the level of the SFGS, but reverse polarity at the SFGS/SGC boundary. Thus, one can conclude that the current sink associated with PS1 and the current source associated with PS2 originate in the SFGS. Application of the GABA blocker bicuculline results in abolition of the PS2 wave at all recording depths, strongly suggesting that it represents an inhibitory postsynaptic process (Sajovic and Levinthal 1983; Manis and Freeman 1988). Also consistent with this interpretation is the marked increase in time course of the PS1 wave. Prolongation of the PS1 wave has also been observed during in vitro incubation with Cl^- deficient media (Matsumoto and Bando 1981). It is worth noting here that EPSPs cut short by IPSPs have been recorded intracellularly from tectal cells of carp (Matsumoto *et al.* 1983) and goldfish (Freeman and Norden 1984).

Application of the excitatory amino acid antagonist kynurenic acid eliminates the postsynaptic response, indicating that retinotectal transmission is most likely glutamatergic (Langdon and Freeman 1986, 1987). An antagonist selective for the N-methyl-D-aspartate (NMDA) receptor, 2-amino-5-phosphonovalerate, has little effect, suggesting that a glutamate receptor subtype other than NMDA mediates excitatory retinotectal transmission (Langdon and Freeman 1986). It follows that the current sink associated with the PS1 wave is indicative of excitatory synaptic input.

I will next consider which tectal elements might be expected to contribute to the physiological responses just described. If a neuron is to contribute to the tectal evoked potential (TEP) it should be vertically elongated, course through the SFGS and SGC and possess an axon that branches from the primary neurite at or near the SFGS. Three cell classes fulfill these criteria: pyramidal neurons (type I cells), fusiform neurons (type XII cells) and piriform neurons (type XIV cells) (Vanegas *et al.* 1984). Of these cell classes, only pyramidal and piriform neurons have been shown to be responsive to photic stimulation (Niida *et al.* 1980; Guthrie and Sharma 1991).

Pyramidal cells have extensively branched apical dendrites terminating in the SM and cell bodies located in the SFGS (Figure 8). As pyramidal cells receive their primary input from the

marginal fibres of the torus longitudinalis, it is possible that they are visually stimulated via a reciprocal connection with this structure. It is more likely, however, that the marginal fibre pathway carries an efference copy that nullifies reafferent input due to saccadic eye movements (see above). Direct retinal input to pyramidal cells is probably slight as they receive sparse innervation by optic nerve fibres (Meek 1983). Although Niida *et al.* (1980) recognized a number of pyramidal sub-types, they did not find a correlation between morphology and physiological response properties.

Piriform cells possess radially oriented dendrites located primarily in the SFGS, although there are also dendritic terminations in the SO and SGC. Their cell bodies are located in the SAC and SPV (Figure 8). The location of piriform cell dendritic terminals is certainly consistent with the notion that this cell class receives strong retinal input (Meek 1983). Moreover, as piriform neurons dominate the tectum numerically (they are 2 to 3 orders of magnitude greater in number than any other cell type), it seems reasonable to suggest that they are the primary contributors to the TEP. Their pharmacological properties (see below), in addition to their morphology and numerical superiority support this contention. In Figure 9, I present a putative tectal circuit capable of generating the excitatory and inhibitory processes that can be expected to give rise to the TEP waveform.

Two salient findings lend support to the proposed circuit. First, it has been shown that a number of piriform neurons send axon collaterals to the SFGS (Ito *et al.* 1981; Meek and Schellart 1978). These axon collaterals most likely either make recurrent connections or contact neighbouring piriform cells (pictured). Second, there is evidence that some piriform neurons are GABAergic, suggesting that their collateral terminals are capable of generating inhibitory synaptic inputs (Villani *et al.* 1981). Thus, it is conceivable that the PS1 wave is derived from excitatory retinal input to the piriform cells and that the PS2 wave is due to either lateral or feedback inhibition.

4.2 TEPs in response to visual stimulation

Konishi (1960a) and Buser (1955), working on carp and catfish, respectively, found that an incremental stimulus evoked an On response comprised of a brief initial negative-going wave, followed by a slower second wave (Figure 7b). Konishi termed these waves PSP1 and PSP2, as they represent excitatory postsynaptic processes. I shall use a terminology that has been adopted by more recent studies of TEPs (Schwippert *et al.* 1996; Bullock *et al.* 1991). In these studies, waveforms are identified according to their response polarity and order of occurrence. This terminology is useful in that it accounts for the fact that the TEP is comprised of a number of distinct positive- (P) and negative-going (N) waves. Konishi's PSP1 and PSP2 waves (Figure 7) might be equivalent to the N1 and N2 waves observed in the toad tectum by Schwippert *et al.* (1996) (but see chapter 3). Guthrie (1981) reported the occurrence of a third negative-going wave in the perch, N3. He also found that the N2 wave was less sustained in the perch. Although the positive-going waves provide a conspicuous contribution to the TEP (at least in toads), they have received little mention in studies of the fish tectum (Buser 1955; Konishi 1960a; Guthrie 1981; but see Bullock *et al.* 1991). The characteristics of the Off response also remain largely unexplored. In fact, in the majority of Konishi's (1960a) experiments, the stimulus duration was set too short (Figure 7) to reveal the presence of a separate Off process (Bullock *et al.* 1991).

5. Feature Detection

A neuron is considered a feature detector if it responds preferentially to a specific stimulus configuration. While all sensory neurons can be thought of as feature detectors in some sense, they are generally only designated as such when they selectively respond to particularly complex stimulus configurations or stimuli that are known to be of ethological significance. Two well studied examples of the latter are the prey recognition neurons found in the optic tectum of toads (Ewert 1985; 1997) and the face recognition cells found in the temporal cortex of primates

(Sugase *et al.* 1999). The studies by Ewert represent the most extensive work to date addressing feature recognition in the tectum of an anamniote.

Despite Ewert's success in elucidating the feature detector properties of the toad tectum, there have been no attempts to apply similar approaches to other anamniote vertebrates. Indeed, virtually no data are available regarding visual feature detection by the fish tectum. I shall therefore focus on studies that have examined in some detail the complex receptive field structure that can be seen in some intrinsic tectal neurons. Units with complex RFs can be expected to show greater stimulus specificity than units with relatively simple RFs, and are therefore likely to act as feature detectors. Movement sensitivity of tectal units has received somewhat more attention and will also be considered here.

5.1 Receptive field properties of tectal neurons

A number of investigators have endeavored to determine the RF structure of intrinsic tectal neurons (Guthrie and Banks 1974; Schellart and Spekreijse 1976; Guthrie and Banks 1976, 1978; Guthrie 1983; Schellart *et al.* 1979; Niida *et al.* 1980). These workers are in agreement that the RFs of tectal cells are often quite large (30-160°) and that although regular concentric RFs can be observed, asymmetrical and multi-centre RFs with complex spatial organization are more frequently encountered.

Guthrie and Banks (1976) made some of the most intriguing discoveries. Using a scanning raster technique (see Figure 10 for details), they found tectal units in the perch with RFs that showed distinctive low and high spiking areas. The most striking of these units were those with vertical bar-shaped features forming the RF (Figure 10a). In a subsequent study, Guthrie and Banks (1978) found that adjacent units often had RFs with complementary excitatory regions. They suggest that this finding provides evidence for functional assemblies of tectal cells. Schellart *et al.* (1979), working on goldfish, found units with adjacent On and Off areas (as opposed to a concentric centre–surround arrangement), as well as units with spatially separated multi-centre

On RFs flanking an Off region (Figure10b). They also observed units with spatially co-extensive On and Off receptive fields.

Interestingly, the distinct vertical barring patterns within the RFs of some tectal units (Guthrie and Banks 1976; 1978) suggest a strong resemblance, in terms of RF structure, to the complex cells of the primate striate cortex (Hubel and Wiesel 1962). It is conceivable that the RF properties of these units is a result of hierarchical processes similar to those believed to be responsible for shaping the RF structure of complex cells. Figure 11 provides an illustration of a hypothetical scheme similar to that proposed by Hubel and Wiesel (1962) that may account for the properties of the neurons described by Guthrie and Banks. In this scheme, a number of RGCs with concentric RFs converge onto a first order tectal cell. Should the RGCs be displaced linearly along the retina, their convergent input to the first order tectal neuron can be expected to generate an elongated or bar shaped RF. A multiple bar pattern can in turn be generated by convergent input from a number of first order tectal neurons onto a second order tectal cell.

The complexity of RF organization of intrinsic tectal units certainly suggests that they subserve a feature detection function. However, studies that employ more complex stimulus regimes are necessary to confirm this. Stimuli that resemble natural features of the animal's visual environment might be expected to be most informative. Natural stimuli would provide insight to the functional constraints that shaped the RF properties of tectal cells, and would enhance our appreciation of the visual world of fishes.

5.2 Movement detection

There is some evidence that a primary function of the tectum is to enhance movement sensitivity. Riemslag and Schellart (1978) found that units recorded from the optic chiasma of goldfish seldom showed strong responses to movement, although movement sensitivity was common in intrinsic tectal units. Similarly, Guthrie (1990), working on perch, found that only twelve percent of units recorded from the optic nerve showed notable movement sensitivity, while the majority of intrinsic tectal cells sampled showed this property. Given that tectum is known to

facilitate localization of objects in visual space (Northmore 1981), acute movement sensitivity can be expected to enrich the capacity to attend to and track the dynamic movements of ethologically significant stimuli (e.g. prey, predators or conspecifics).

Despite the apparent role of the tectum in enhancing movement sensitivity, it is important to note that there is compelling evidence for movement sensitivity in retinal neurons. Lasater (1982) observed that a number of goldfish RGCs show large and asymmetric RFs with movement and directional sensitivity. Cells with these properties, termed complex RGCs, receive substantial amacrine cell input, attesting to the latter cell type's involvement in facilitating movement sensitivity.

As with studies of chromatic processing, much evidence regarding movement sensitivity in the tectum is derived from recordings primarily from retinotectal fibres, possibly axons of complex RGCs. Thus, it can be assumed that in the majority of studies discussed here (excepting those mentioned above), recordings were likely predominantly from retinotectal afferents.

Cronly-Dillon (1964) and Jacobson and Gaze (1964) were the first to study movement and direction sensitivity of afferent fibres in the goldfish tectum. They found that most units responded preferentially to temporo-nasal movement. Riemslag and Schellart (1978), who recorded visual evoked potentials and unitary responses from the tectum, also report temporo-nasal dominance, but indicate that the tendency was weak. Galand and Liege (1975) report similar findings for another teleost, the brown trout. Conversely, O'Benar (1976), working on goldfish, found that naso-temporal movement was most effective in modulating unit activity, albeit in an inhibitory fashion. A common finding among these studies is that units sensitive to horizontal movement were considerably more numerous than units sensitive to vertical movement. Regarding directional responses of vertically sensitive units, it is worth noting that Guthrie and Banks (1978) found a preferential response to dorso-ventral movement in perch.

The RF sizes of movement sensitive units appear to vary widely. Cronly-Dillon (1964) and Ormond (1974) report RF sizes of 2° and 5°, respectively for goldfish. Movement sensitive units in the pike also had small receptive fields of 2 to 12° (Zenkin and Pigarev 1969). By

contrast, Jacobson and Gaze (1964) found units in the goldfish tectum with considerably larger RFs on the order of 15 to 40°. Kawasaki and Aoki (1983) found both narrow-field and wide-field units in another cyprinid, the Japanese dace, suggesting that two classes of movement detector exist in the goldfish as well.

Although tectal units with velocity dependent response properties are prevalent in a number of teleosts, it appears that tuning of most units is not stringent. Rather, tectal units appear to respond equally well to a broad range of target velocities. For example, the majority of units in the perch tectum showed a preferred range of velocities spanning10 to 50°/sec. Zenkin and Pigarev (1969) found units in the pike with two preferred velocity ranges. Some units in their sample responded to slow velocities (0.5-5°/sec), while others responded preferentially to a broad range of higher velocities (3-40°/sec). A proportionately smaller number of units with narrower tuning curves have also been described. For example, Wartzok and Marks (1973) found units in the goldfish tectum with sharp sensitivity peaks ranging from 8 to 20°/sec.

A unique property of some movement sensitive tectal units is the tendency toward rapid habituation (O'Benar 1976; Sutterlin and Prosser 1970; Kawasaki and Aoki 1983). Units of this kind were believed to be intrinsic tectal neurons, and became unresponsive to stimuli for up to 30 seconds after initial stimulus presentation. It has been suggested that these units facilitate detection of novel stimuli. It would be interesting to employ a variety of configurational stimuli to determine whether these units can be dishabituated with stimulus configurations that differ from those which initially induced habituation.

6. Behavioural studies

Some workers have studied tectal function in the goldfish by carrying out behavioural discrimination tests following tectal ablation. Springer *et al.* (1977) examined five visually mediated behaviours following ablation of the optic tectum. Three behaviours disappeared: the optomotor response (swimming with the direction of vertical stripes on a rotating drum), food pellet localization and deceleration of respiration induced by looming stimuli. The dorsal light

reflex and optokinetic nystagamus (movement of the eyes with the stripes on a rotating drum) persisted. The finding that the optomotor response (OPM), but not optokinetic nystagamus (OKN) depends on an intact tectum was surprising. Both OPM and OKN were elicited by the same stimulus, and both responses serve the same function, namely to stabilize visual images on the retina. Thus, it appears that the tectum serves a pre-motor function in addition to its sensory role, and that OKN must be driven by visual input from a non-tectal processing centre.

The finding of Springer *et al.* (1977) of a loss in ability to localize and strike food pellets in lesioned animals is supported by a similar study by Yager *et al.* (1977). Moreover, like Springer *et al.*, Yager *et al.* also found insensitivity to looming stimuli, suggesting that the tectum plays an important role in the shadow escape reflex (Northmore 1973). These findings are consistent with neuroethological studies of amphibians that have shown the tectum to be integrally involved in prey localization and predator avoidance (Ewert 1985; Ingle and Hoff 1990).

Localized electrical stimulation of the tectum elicits eye and/or body movements directed towards appropriate points in visual space, indicating that the visuotopic and motor maps of the tectum are in register (Northmore 1981). For example, ocular convergence movements can be elicited by stimulation of the rostral tectum, which receives the projection of the rostral visual field (Akert 1949; Meyer *et al.* 1970; Ali-Akell *et al.* 1986). Meyer *et al.* (1970) found that forward body movements often accompanied electrically evoked ocular convergence movements. Such responses resembled food-searching behaviour. Thus, it appears that elevated localized activity within the tectum is sufficient to drive specific motor programmes capable of directing attention towards visual stimuli.

7. Rationale and objectives of dissertation

7.1 Chromatic Processing

It is evident from this overview that there has been extensive study of the chromatic and spatial properties of colour-opponent tectal units. Considerably less is known of the temporal response features of colour-opponent units. Although Daw (1968) noted that the RF surround of double colour-opponent units exhibited substantially longer latencies than the RF centre, little systematic study of the temporal response features of colour-opponent neurons has followed this initial observation. This is unfortunate, as our understanding of the nature of colour processing in fish in particular, and vertebrates in general, is far from complete.

Study of temporal response properties of tectal neurons may prove especially rewarding in light of recent experimental (Wehr and Laurant 1996; Stopfer *et al.* 1997) and theoretical work (Hopfield 1995; Northmore and Elias 1997) suggesting that temporal patterning of neural activity may play an important role in encoding sensory information. To date, the strongest evidence for a temporal code that signifies the qualitative nature of sensory inputs comes from studies of olfactory processing in the antennal lobe of locusts and bees. Wehr and Laurent (1996) have found that the spike activity of projection neurons within the antennal lobe is temporally patterned in a stimulus-specific fashion. In subsequent work, Laurent and co-workers (Stopfer *et al.* 1997) have demonstrated that by altering the pattern, but not the frequency, of neural activity, it is possible to impair behavioural discrimination of odours. This work provides compelling evidence that information temporally encoded by the projection neurons can be decoded by other regions of the brain, thus facilitating odour discrimination. In contrast, the possibility that temporal coding plays a role in visual perception has received less attention. Singer and co-workers (Fries *et al.* 1997), working on the cat visual cortex, have shown that greater synchrony in neural assemblies serves as a predictor of perception. However, whether individual neurons within a particular neural assembly fire with singular temporal patterns in relation to different stimulus configurations has not been determined.

The first goal of this dissertation was to determine whether chromatic stimuli evoke temporally patterned neural activity in the optic tectum. To this end, I employed both single-cell (chapter 2) and population level recording techniques (chapter 3). In chapter 2, I examined response latencies and discharge patterns of colour-opponent tectal units. Chapter 3 comprises an investigation of the chromatic properties of the rainbow trout TEP. In this study, I used *principal components analysis* to search for wavelength-dependent waveform characteristics. Although the work presented here represents a phenomenological approach, the findings have direct implications in terms of addressing the hypothesis that temporal structure in neural activity encodes the qualitative properties of sensory stimuli. Specifically, my work provides evidence consistent with the intriguing possibility that colour is encoded by the temporal discharge patterns of tectal neurons.

7.2 Exploring visual perception with natural stimuli

Traditional approaches to studying visual perception have involved the use of simple geometric stimuli. It is generally believed that by using these kinds of stimuli, it is possible to predict how the visual system would respond to more complex or natural stimuli. However, whether such extrapolation is valid has rarely been tested. Indeed, there is some evidence to suggest that this assumption can not be made. For example, Dan *et al.* (1996) found that neurons within the cat lateral geniculate nucleus show different response properties depending on whether they are stimulated with a natural visual scene characterized by smooth spatial transitions or an artificial stimulus characterized by abrupt transitions. Thus, it seems logical that much could be learned of how neural networks generate visual percepts by employing the natural stimuli that likely shaped the detector properties of the visual system in question. When considering visual processing in non-humans, the use of natural stimuli also provides a means to determine which features of an animal's visual environment are ethologically relevant. This is of particular importance in light of the fact that it is not always possible to employ behavioural

methods to study the perception of biologically significant stimuli. Despite the merits of natural stimuli, they are rarely employed in vision research.

The final goal of this dissertation is to examine how the teleostean tectum responds to a realistic, ethological stimulus, the agonistic display of the Siamese fighting fish (*Betta splendens*). The tectum is responsible for localization of objects in visual space, and for shifting gaze and attention towards those objects. Thus, as the agonistic display can only be effective if it elicits the attention of conspecifics, it is a necessary (and probably sufficient) condition that the display excites the tectum of potential viewers. Moreover, as the optic tectum is the primary centre for integrating visual input, recording from this structure should provide a holistic neurological correlate of visual sensation. I have chosen to use live conspecifics as stimuli to provide the utmost realism possible. The work presented in chapter 4 represents the first step of a novel approach towards determining how species-specific behaviours are perceived by conspecifics.

Figure 1 Lateral view of the brain of the rainbow trout. Abbreviations: c, cerebellum; ob, olfactory bulb; on, optic nerve; ot, optic tectum; t, telencephalon. Adapted from Davis and Northcutt (1983).

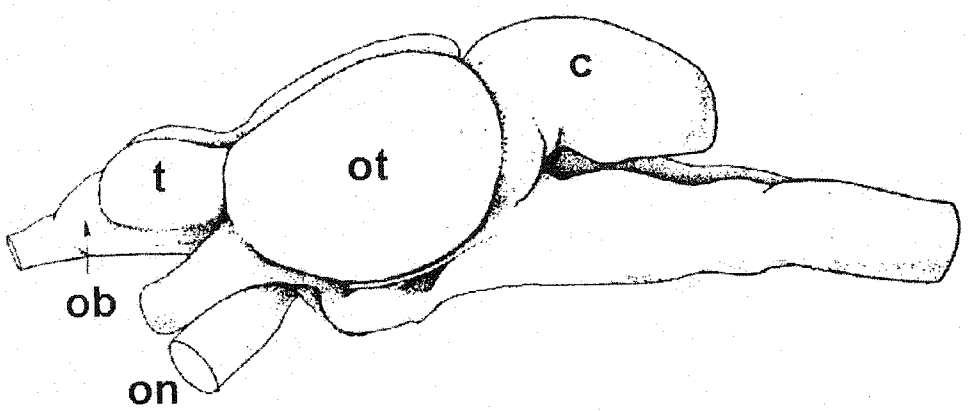

c
t
ot
ob
on

Figure 2 Cross section views of the optic tectum of the goldfish. Top, tectum shown at low magnification; bottom, tectal layers revealed with hematoxylin-eosin. Abbreviations: TL, torus longitudinalis; TS, torus semicircularis; TTB, tractus tectobulbaris; SM, stratum marginale; SO, stratum opticum; SFGS, stratum fibrosum et griseum superficiale; SGC, stratum griseum centrale; SAC, stratum album centrale; SPV, stratum periventriculare. Adapted from Meek (1990).

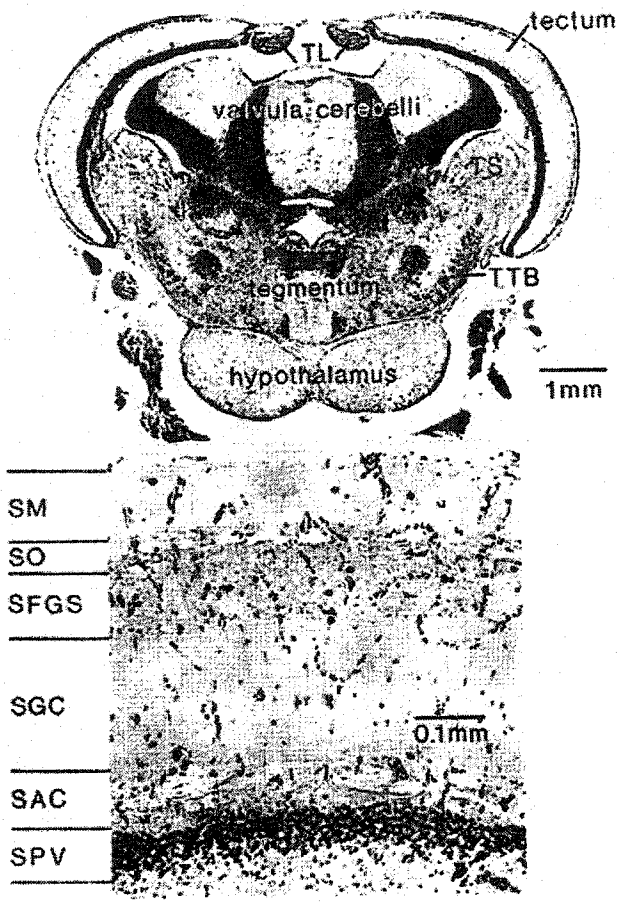

tectum
TL
valvula cerebelli
TS
TTB
tegmentum
hypothalamus
1mm
SM
SO
SFGS
SGC
SAC
SPV
0.1mm

Figure 3 Schematic illustration of the primary non-retinal visual projections to the optic tectum. The solid lines indicate tectal inputs from retinorecipient structures, while dotted lines indicate input from non-retinorecipient structures. The inset shows the approximate location of the various structures described in the main diagram. Abbreviations: AOC, accessory optic centre; Dc, area dorsalis centralis; NI, nucleus isthmi; OT, optic tectum; POA, preoptic area; PTec, pretectum; Thal, thalamus; TL, torus longitudinalis; TS, torus semicircularis; Tub, tuberculum. Modified after Beaudet 1997.

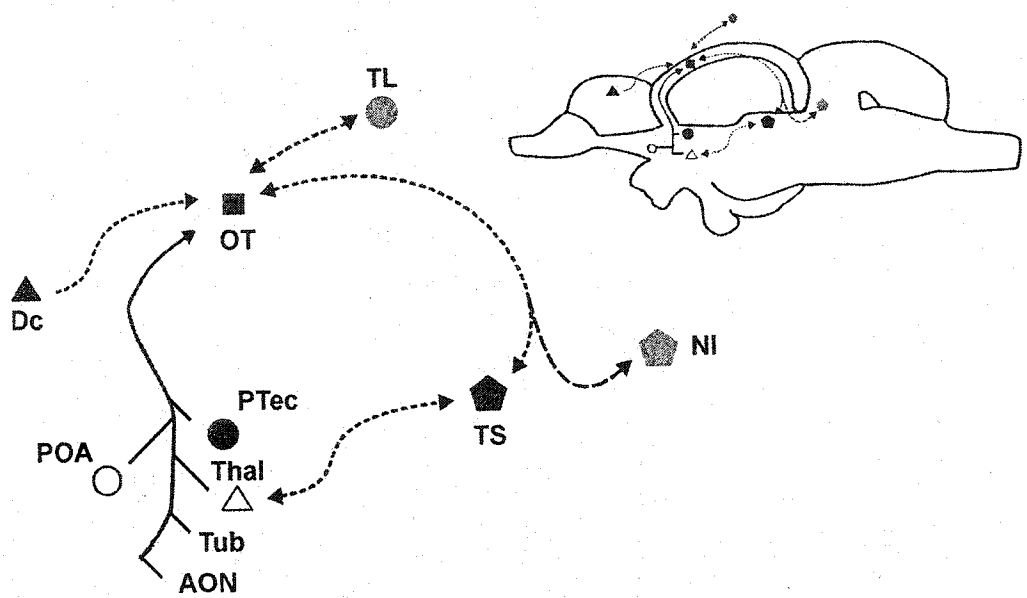
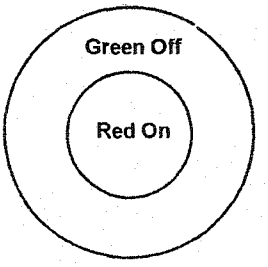

TL
OT
Dc
NI
PTec
TS
POA
Thal
Tub
AON

Figure 4 Receptive field organization of colour-opponent bipolar cells. Note that with double colour-opponent bipolar cells there is opponency within and between the receptive field centre and surround.

Single colour-opponent cell



Double colour-opponent cell

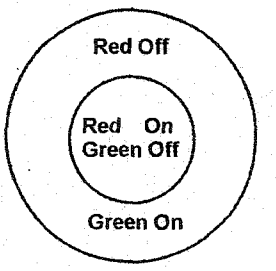
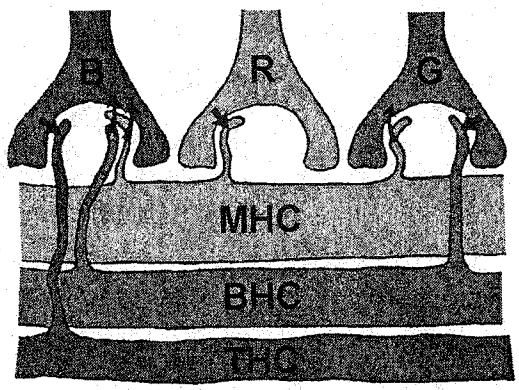


Figure 5 Functional connectivity between horizontal cells and cones as proposed by Stell *et al.* (1975) (A) and Kamermans *et al.* (1991) (B). Synapses are indicated with black arrows. The synapses that are proposed to be most instrumental in generating the spectral response functions are indicated by the larger arrows. R, G and B indicate red, green and blue cone photoreceptors, respectively. MHC, BHC and THC indicate mono-, bi- and triphasic horizontal cells, respectively.

a



b

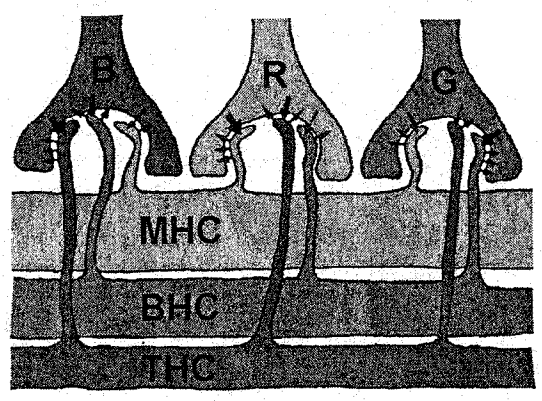


Figure 6 A qualitative model proposed by Kamermans and Spekreijse (1995) for generation of the surround responses of bipolar cells. Left: schematic representation of the relevant pathways for generation of bipolar cell surround responses. Open and closed arrows indicate sign preserving and sign inverting inputs, respectively. Right: spatial profiles of HCs and bipolar cells when only the surround processes are considered. Dashed lines: contribution of feedback from HCs via the R-cones to the bipolar cell surround. Dotted lines: contribution of feedback from horizontal cells via the G-cones to the bipolar cell surround. Top and bottom panels illustrate single and double opponent cells, respectively. The plus sign represents summation of the HC contributions. Modified after Kamermans and Spekreijse (1995).

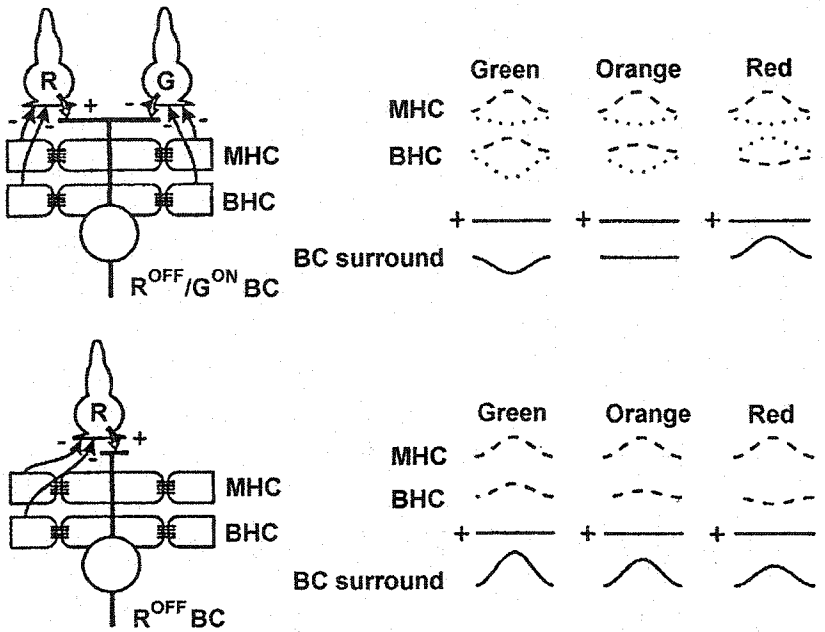

R
G
MHC
BHC
R^OFF/G^ON BC
Green
Orange
Red
MHC
BHC
BC surround
R
MHC
BHC
R^OFF BC
Green
Orange
Red
MHC
BHC
BC surround

Figure 7 Tectal evoked potentials in response to electrical stimulation of the optic nerve (A) and light flash (B-C). A: Responses recorded from the surface of the goldfish tectum. sa, stimulus artifact. Modified after Manis and Freeman (1988). B: Visually evoked responses recorded from the surface of the carp tectum. Note that with the longer stimulus duration a separate Off response is evident. Bars indicate stimulus duration. Modified after Konishi (1960a). C: On and Off responses recorded from the surface of the toad tectum. Three superimposed traces are shown. Distinct negative- (N) and positive-going (P) waves are evident. From Schwippert *et al.* (1996).

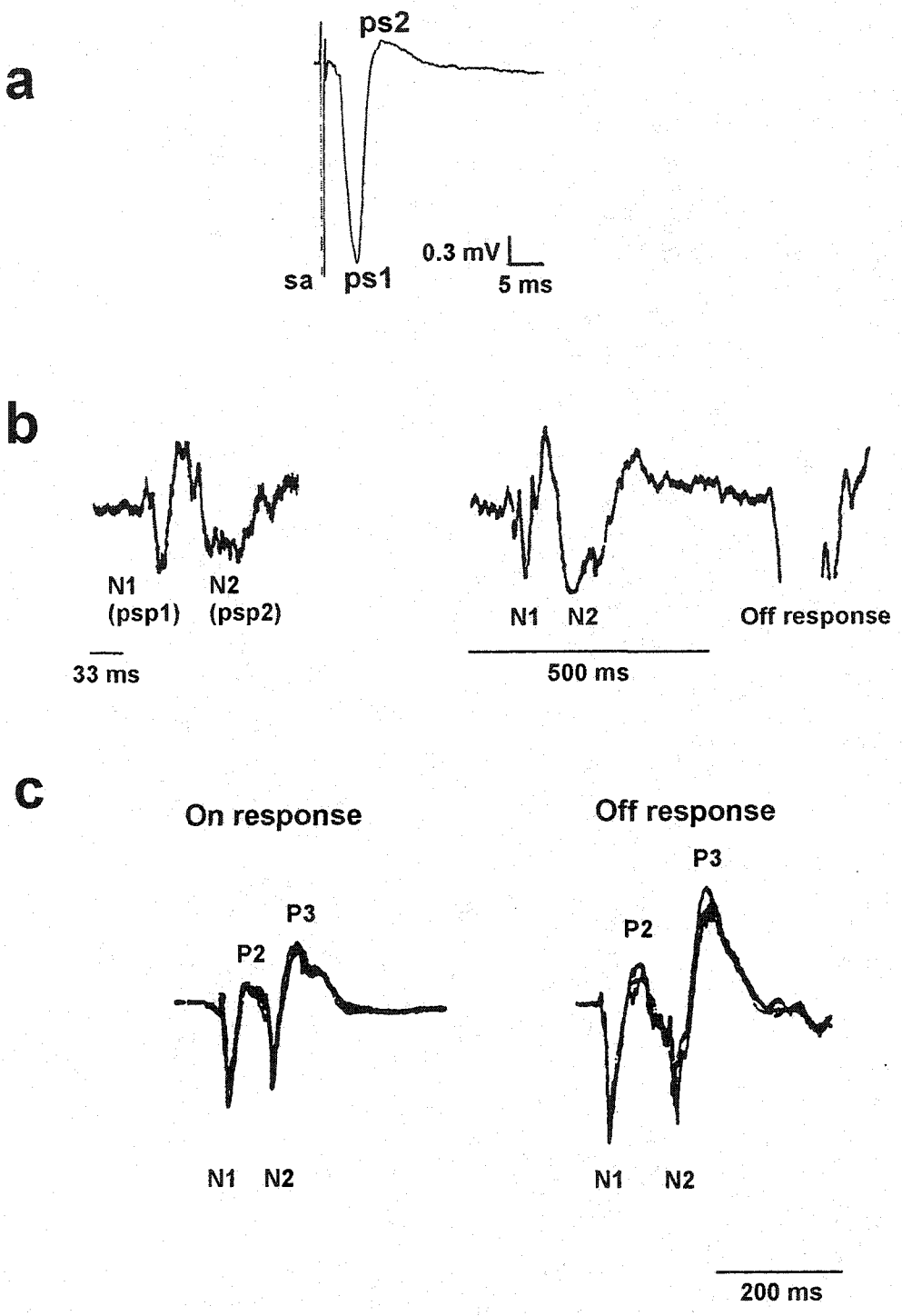

a
ps2
sa
ps1
0.3 mV
5 ms
b
N1
(psp1)
N2
(psp2)
33 ms
N1
N2
Off response
500 ms
c
On response
P3
P2
N1
N2
Off response
P3
P2
N1
N2
200 ms

Figure 8 Visually sensitive neurons in the optic tectum of a teleost (*Eugerres plumieri*). a and b are piriform neurons; c is a pyramidal neuron. Adapted after Vanegas *et al.* (1974).

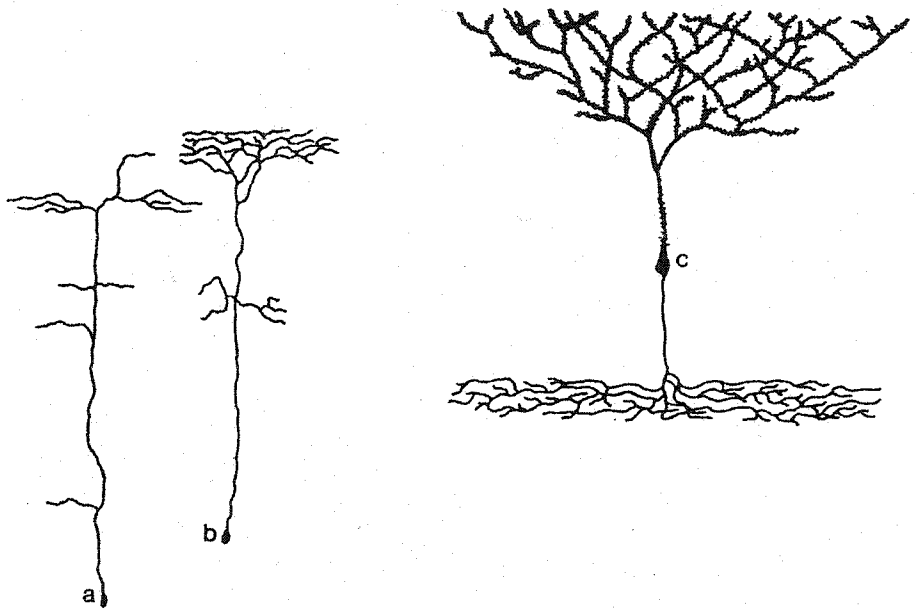

a
b
c

Figure 9 Schematic diagram of a putative tectal inhibitory feedback circuit. Open triangles represent excitatory synaptic input, while the closed circle represents inhibitory synaptic input. Abbreviations: sm, stratum marginale; so, stratum opticum; sfgs, stratum fibrosum et griseum superficiale; sgc, stratum griseum centrale; sac, stratum album centrale; spv, stratum periventriculare; GABA, γ amino-butyric acid

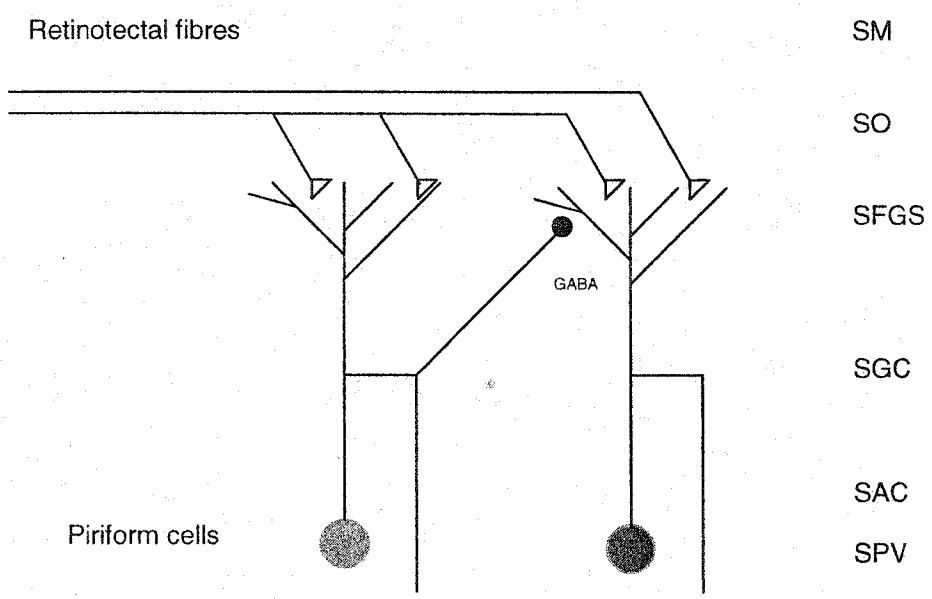
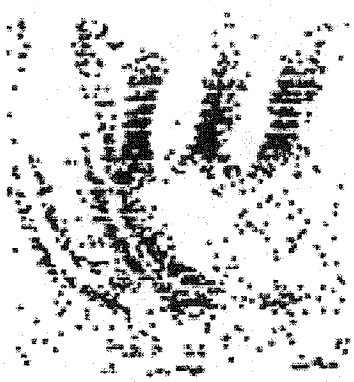

Retinotectal fibres
SM
SO
SFGS
GABA
SGC
SAC
Piriform cells
SPV

Figure 10 Receptive field patterns of cells in the perch (A) and goldfish (B) tectum. To obtain the response patterns shown here, a small spot of light was scanned horizontally at different vertical levels across a flat screen placed in front of the eye. In A, the triple bar feature to the right and the two double bars to the left are derived from two adjacent cells. Data are presented in the form of raster plots of spike activity. Each dot represents an action potential. From Guthrie and Banks (1978). In B, a tectal cell with separated On and Off fields is shown. The solid lines represent the iso-response contours at which the response is 71% and 50% of the response magnitude recorded at the most sensitive point (black dot) of the On field. Dashed lines give the contours of the Off field. From Schellart *et al.* (1979).

a



b

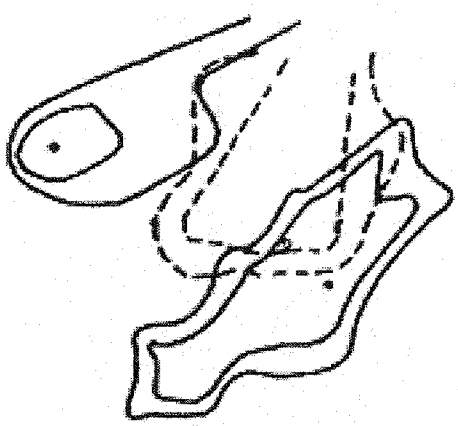
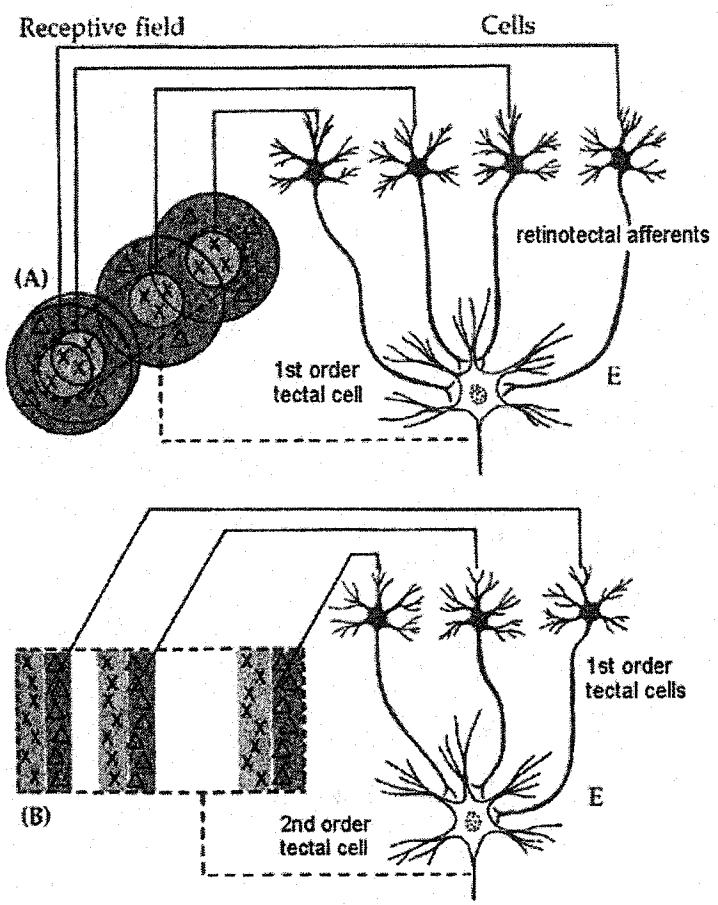


Figure 11 Hypothesis based on a scheme devised by Hubel and Wiesel to explain the receptive field properties of the tectal neurons shown in Figure 10a. A: With this hypothesis, the receptive field of a first order tectal neuron is derived from convergence of a number of retinal ganglion cells with concentric receptive fields. They must be arranged in a straight line on the retina in accordance with the orientation of the receptive field of the first order tectal cell. B: In the next level of the hierarchy, a number of first order tectal neurons with displaced receptive fields of similar orientation converge onto a second order tectal neuron. X, excitatory portion of the receptive field, Δ, inhibitory portion. Modified after Hubel and Wiesel (1962).


Receptive field
Cells
retinotectal afferents
(A)
1st order
tectal cell
E
1st order
tectal cells
E
(B)
2nd order
tectal cell

Chapter 2

Latencies and Discharge Patterns of Colour-Opponent Neurons in the Rainbow Trout Optic Tectum

A version of this manuscript has been published in Vision Research 39 (1999) 2795-2799

Introduction

The criterion for a colour-opponent neuron is that its response sign vary as a function of the wavelength of stimulation (Daw 1968). While this property suggests that colour-opponent units are involved in coding chromatic stimuli, it could also be argued that colour-opponency simply serves a filtering function which narrows spectral sensitivity of the cone mechanisms (Gouras and Zrenner 1981; Neumeyer 1984). A recent resurgence of research addressing the functional significance of the timing of neural activity (Koch 1997) has prompted me to consider the possibility that temporal structure in spike discharges of colour-opponent neurons may play a role in coding chromatic stimuli. Surprisingly, relatively few studies have considered the implications of action potential timing for coding of chromatic stimuli (Gur and Purple 1979) and further study of this possibility is certainly warranted.

Recent work has shown that a large proportion of units in the optic nerve and tectum of rainbow trout exhibit colour opponency (Coughlin and Hawryshyn 1994a), indicating that the visual system of this species is suitable for studying aspects of colour coding. My main objective here was to determine whether the temporal response properties of colour-opponent neurons varied in a wavelength dependent fashion which might provide a basis for neural computation.

Material and Methods

Rainbow trout (*Oncorhynchus mykiss*) were acquired from the Fraser Valley Trout Hatchery at Abbottsford, British Columbia. The fish were held a minimum of 8 weeks prior to experimentation. They were maintained under a 12:12 hr light cycle at 15°C. Experiments were carried out at the same water temperature. Lighting in the holding facility was provided by fluorescent bulbs at an average intensity of 33.54 +/-14.39 $\mu W/cm^2$.

Before each experiment, fish were anesthetized with Tricaine methanosulfonate (MS 222, 0.1g/L) and immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide, 10mg/Kg body weight). To maintain anesthesia during surgery, fish were respired with aerated water containing MS 222 (0.038g/L). Once the fish reached complete anesthesia, the right optic tectum was surgically exposed using a narrow gage electric drill (Fine Surgical Instruments). After surgery, the MS 222 anesthesia was halted. During the course of each experiment, fish were maintained under mild anesthesia with an intramuscular injection of Marinil (3mg/Kg body weight). Fish health was assessed by examining blood flow through the capillaries on the surface of the OT. The animals were held out of water in a neoprene-lined holder, and respired with aerated water throughout the experiment. All procedures were in accordance with the guidelines for the Canadian Council for Animal Care.

Extracellular, single-unit recordings were made from the dorso-central optic tectum of juvenile rainbow trout (6 to 12 cm total length). Recordings were made with resin-coated tungsten microelectrodes (5-12 MOhm, A-M Systems and Frederick Haer & Co.). A reference electrode was inserted into the epithelium of the right nares. Signals were differentially amplified (5 000-10 000x), bandpass filtered (0.3KHz-3KHz) and fed into a variable level window discriminator for the isolation of single units.

The recording electrode was initially placed on the tectal surface, then inserted using a motorized microdrive (Frederick Haer & Co.). The electrode was advanced incrementally into the tectum, with frequent pauses during which the eye was stimulated and the response examined. Recordings were made at depths (100 - 150 μm) corresponding to the SO and SFGS (Coughlin and Hawryshyn 1994a; Beaudet 1997). The numerous retinotectal fibres which course through the SO, and thereafter terminate in the SFGS, are likely the primary contributors to unit responses (Guthrie 1990). However, it should be noted that the apical dendrites of a number of tectal cell types are also located in these layers (Meek 1990). Given the seemingly high prevalence of active dendrites (Turner pers. comm.), it is conceivable that some of the recorded units were intrinsic tectal cells (but see results).

The response features of colour-opponent units were examined with a three channel optical system, permitting independent control of the stimulus and background illumination. The three light channels entered the Faraday cage via three liquid light pipes. The ends of the light guides were positioned such that light from each pipe was aimed at a single spot on a small diffusing screen positioned 1 mm from the eye. This stimulus arrangement provided full field stimulation of the eye.

The relative sensitivity of the different cone mechanisms was altered by using interference and Inconel coated neutral density (ND) filters. Weak (3 ND) and moderate (2 ND) intensity yellow (500 nm longpass) backgrounds were used to attain different levels of adaptation of the middle wavelength-sensitive (M) and long wavelength-sensitive (L) cone mechanisms (Coughlin and Hawryshyn, 1995). In addition, several units were recorded under background conditions intended to mimic the light environment of a shallow clear water lake (Novales Flamarique, Hendry and Hawryshyn 1992). For this background, a 400 nm longpass filter, a 650 nm shortpass filter and a 3 ND filter were placed in one background channel (Novales Flamarique and Hawryshyn 1996). This background condition and the weak yellow background resulted in approximately equal (or 'balanced') sensitivity of the short wavelength-sensitive (S), M and L cone mechanisms. The moderate intensity yellow background diminished sensitivity of the longer wavelength cone mechanisms, resulting in partial isolation of the S cone mechanism. Stimulus duration was set at one second.

Spectral sensitivity curves were determined using the increment-threshold technique. Threshold responses were measured at up to 12 wavelengths ranging from 340 to 720 nm. At a given wavelength, a stimulus series was begun at least 0.5 log units below threshold intensity. Intensity was increased by 0.1 log unit increments until two consecutive threshold responses were detected at a given wavelength intensity combination. The inter-trial interval was set at 20 seconds to avoid habituation. A response was classified as above threshold if the firing rate was 3.0 standard deviations or greater above the mean pre-stimulus firing rate (Barlow and Levick 1969). A number of units exhibited very low spontaneous activity (< 1 Hz), making it necessary to determine their thresholds aurally using an audio monitor. Latency to the first action potential of a

threshold spike burst was determined for stimulus onset (On response) and/or offset (Off response) at each wavelength sampled.

A consequence of the technique used to determine threshold is that only responses from the receptive field centre could be determined. Specifically, this is due to the fact that a full field stimulus was used and that the receptive field centre is up to 2 orders of magnitude more sensitive than the receptive field surround (Daw 1968; Beauchamp and Daw 1972; Spekreijse, Wagner and Wolbarsht 1972).

All statistical comparisons were made using the Wilcoxon Signed-Ranks test.

Results

It should be noted that while all recordings were made from the optic tectum, afferent fibres are difficult to distinguish from intrinsic tectal neurons using extracellular recording techniques (Guthrie 1990). However, a number of response properties of the units recorded suggest that they are retinotectal afferents. First, spontaneous activity of the recorded units was low (< 2 Hz). Studies on various teleost species indicate that only a small proportion of retinotectal fibres show appreciable spontaneous activity (Jacobson and Gaze 1964; Sajovic and Levinthal 1982; Guthrie 1990). By contrast, intrinsic tectal cells in goldfish and rainbow trout have been shown to have spontaneous firing rates of 5-10 Hz (Guthrie 1990).

Second, unit responses were characterized by short, phasic bursts terminating ~ 100-150 ms after stimulus onset. This time course is comparable to that of retinal ganglion cells of anamniote vertebrates (Daw 1968; Roska *et al.* 1998). Moreover, the nature of the temporal discharge patterns reported here (see below) is similar to that of colour-opponent retinal ganglion cells in the kissing gourami (Sakai *et al.*1997).

A total of 28 units (recorded from 27 animals) were sufficiently characterized for inclusion in the present study. Units were included if they were sampled at five or more wavelengths and showed wavelength-dependent changes in response sign. The majority of colour-opponent units

encountered (n = 23) exhibited a triphasic response pattern, with On inputs from the L and S cone mechanisms and Off input from the M cone mechanism. Such units were coded as S+M-L+. This finding is in accordance with previous work (Coughlin and Hawryshyn 1994a).

I recorded from five units with a response pattern not previously encountered. This type of unit was termed biphasic, and showed an On response to short wavelength stimuli and an On-Off response to longer wavelength stimuli. These units were coded as S+ML+/-

Triphasic units recorded under balanced adaptation conditions (n=19) or under a moderate yellow background (n=4) showed similar wavelength-dependent response latency profiles, and were therefore combined for statistical analysis. The M- component of triphasic units exhibited significantly shorter response latencies than the L+ ($p < 0.001$; $n = 23$) and S+ ($p < 0.001$; $n = 23$) components (Figures 12 and 13). The latency of the L+ component was significantly shorter than that of the S+ component ($p < 0.001$; $n = 23$).

For biphasic units, the Off response of the ML+/- component of two units (out of five) exhibited substantially shorter response latencies (~30-50 ms) than the On response (Figure 14). Note that, as with triphasic units, the S+ component has the longest latency.

The relatively long latency of the S+ component of both triphasic and biphasic units (Figures 12, 13 and 14) may be a function of differences in adaptation state of the different cone mechanisms. Indeed, I found that the S+ component of triphasic units had a significantly lower response threshold than the M- ($p < 0.001$; $n = 23$) and L+ ($p < 0.001$; $n = 23$) components (Figure 13a). However, I also found that the response threshold of the fast M- component was slightly but significantly lower than that of the slower L+ component ($p = 0.001$; $n = 23$).

During the course of the latency study, I noticed that the discharge patterns of colour-opponent units were often characterized by an initial spike burst which was interrupted by a silent period and then followed by a second series of spikes. This discharge pattern appeared to occur primarily at middle wavelengths. This observation prompted me to record spike discharge patterns in some of the latter experiments (n = 5 units), with the goal of determining whether there is a relationship between response latencies of colour-opponent units and action potential patterning.

Figure 15 shows a representative example of how the temporal structure of On and Off responses can vary as a function of wavelength and response sign. Note that the Off response to middle and long wavelength stimuli consists of either an early and late burst separated by a silent period or only an *early* burst. In response to the same stimuli, the On response tends to exhibit an early burst only when the Off response is weak or absent. This is best illustrated by responses to the long wavelength stimuli.

Early spikes constitute a progressively larger proportion of the On response at 620 and 660 nm, respectively (Figure 15). Note that the progressive increase in relative frequency of early spikes corresponds to abatement of the Off response, suggesting that the spike distribution of the On response may be the result of inhibitory influences.

Discussion

The main findings of this study are that colour-opponent units in the optic tectum exhibit differences in latency and discharge pattern which are a function of wavelength and response sign. In both triphasic and biphasic units, the Off response was found to exhibit the shorter response latency.

Differences in the adaptation state of cone mechanisms can affect their response latencies; shorter latencies are a function of the magnitude of chromatic adaptation of a given cone mechanism (Beaudet 1997). This may explain, in part, why the S+ component of both triphasic and biphasic units had the longest response latency. Alternatively, it is possible that the S cone mechanism is intrinsically slower than the M and L cone mechanisms (Perry and McNaughton 1991). Here it is important to note that despite having a lower response threshold, the M- component of triphasic units exhibited significantly shorter latencies than the L+ component. Thus, a mechanism other than chromatic adaptation is likely responsible for the observed latency differences, at least at longer wavelengths.

Wavelength dependent action potential patterning was observed in the form of spike bursts characterized by early and late spikes separated by a silent period, with the proportion of early and late spikes differing as a function of wavelength. Similarly, bimodal spike frequency distributions have been observed in colour-opponent retinal ganglion cells of the kissing gourami (Sakai *et al.* 1997).

The discharge patterns of colour-opponent units may be due to inhibitory processes. If so, inhibition of early spikes could be the mechanism underlying the observed latency differences. Inhibition may arise at the level of the bipolar cell, where it has been reported that the centre hyperpolarizing component of double colour-opponent Off bipolar cells responds with substantially shorter response latencies than the center depolarizing component (Kaneko and Tachibana 1981; Shimbo *et al.* 2000). Alternatively, inhibitory processes may originate in higher order retinal neurons such as amacrine cells.

Interestingly, it has recently been shown that amacrine cells can influence the timing of retinal ganglion cell activity in amphibians (Roska *et al.* 1998). Specifically, Roska *et al.* (1998) provide evidence that two classes of amacrine cell, one glycinergic and the other GABAergic, form an inhibitory feedback circuit that truncates bipolar-to-ganglion cell transmission. Their findings suggest that the glycinergic amacrine cells respond first, inhibiting the GABAergic amacrine cells for ~150 msec. The GABAergic amacrine cells appear to provide inhibitory feed back to bipolar terminals, but only after the 150 msec delay, allowing for excitation of retinal ganglion cells for the first 150 msec. It is possible that dynamic, wavelength-dependent changes in the proposed circuit could alter the discharge pattern of colour-opponent units. For example, delayed excitation of the glycinergic amacrine cell population can be expected to result in a corresponding increase in latency at the retinal ganglion cell level.

My findings provide evidence that the temporal response features of colour-opponent units recorded in the tectum may play a role in coding chromatic stimuli. It is conceivable that signals from colour-opponent units provide input to a recognition cell (i.e. a coincidence detector) which monitors the relative timing of chromatic inputs. Signals associated with a particular hue arriving in a precise temporal sequence at the recognition cell might be expected to evoke a

maximal response (Hopfield 1995). Alternately, temporal separation of On and Off responses may serve to 'bind' incremental and decremental stimuli, respectively (Gawne *et al.* 1996).

Finally, it is important to note that one of the adapting backgrounds employed in the current study closely matched the photopic underwater light environment of the rainbow trout (Novales Flamarique *et al.* 1992; Novales Flamarique and Hawryshyn 1996). Thus, it appears that the temporal response properties of tectal units reported here can be expected in natural aquatic environments.

Figure 12. Spectral sensitivity (log [1/photons • cm^{-2} • nm^{-1} • s^{-1}]) and latency plot of a triphasic (S+M-L+) tectal unit recorded under a 'balanced' adapting background. Note that the latency of each component of the response is relatively constant. Open and closed circles indicate On and Off responses, respectively. Triangles indicate latency.

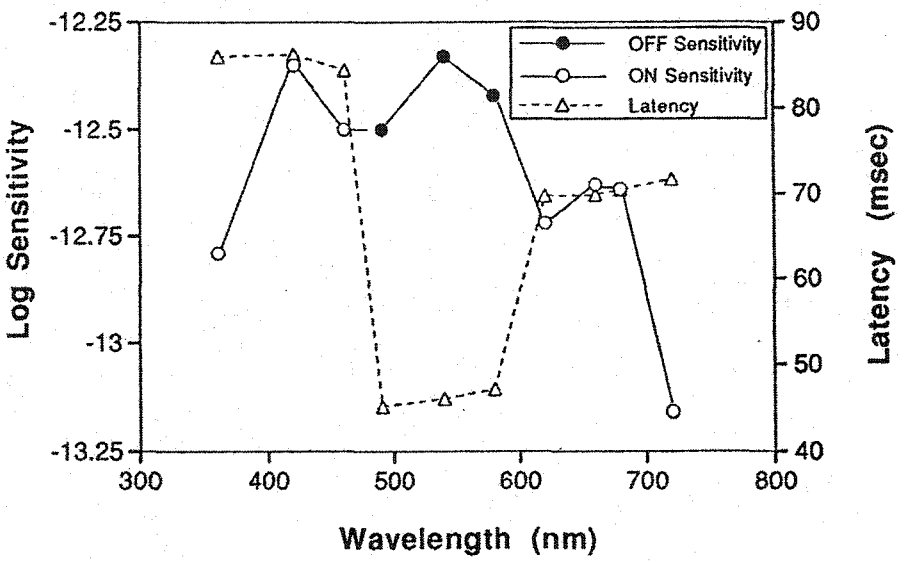

-12.25
-12.5
-12.75
-13
-13.25
Log Sensitivity
90
80
70
60
50
40
Latency (msec)
300
400
500
600
700
800
Wavelength (nm)
OFF Sensitivity
ON Sensitivity
Latency

Figure 13 A: Average latency at threshold of triphasic (S+M-L+) units (n=23). Mean latency values were calculated at 420 nm (S+), 540 nm (M-) and 620 nm (L+). Sensitivity (log [1/photons • cm^{-2} • nm^{-1} • s^{-1}]) is plotted on the second ordinate axis. Error bars indicate +/- one standard error of the mean. B: Histogram of the latency distributions of the S+ (white), M- (black) and L+ (gray) components of all triphasic units. Bin width is 4 ms.

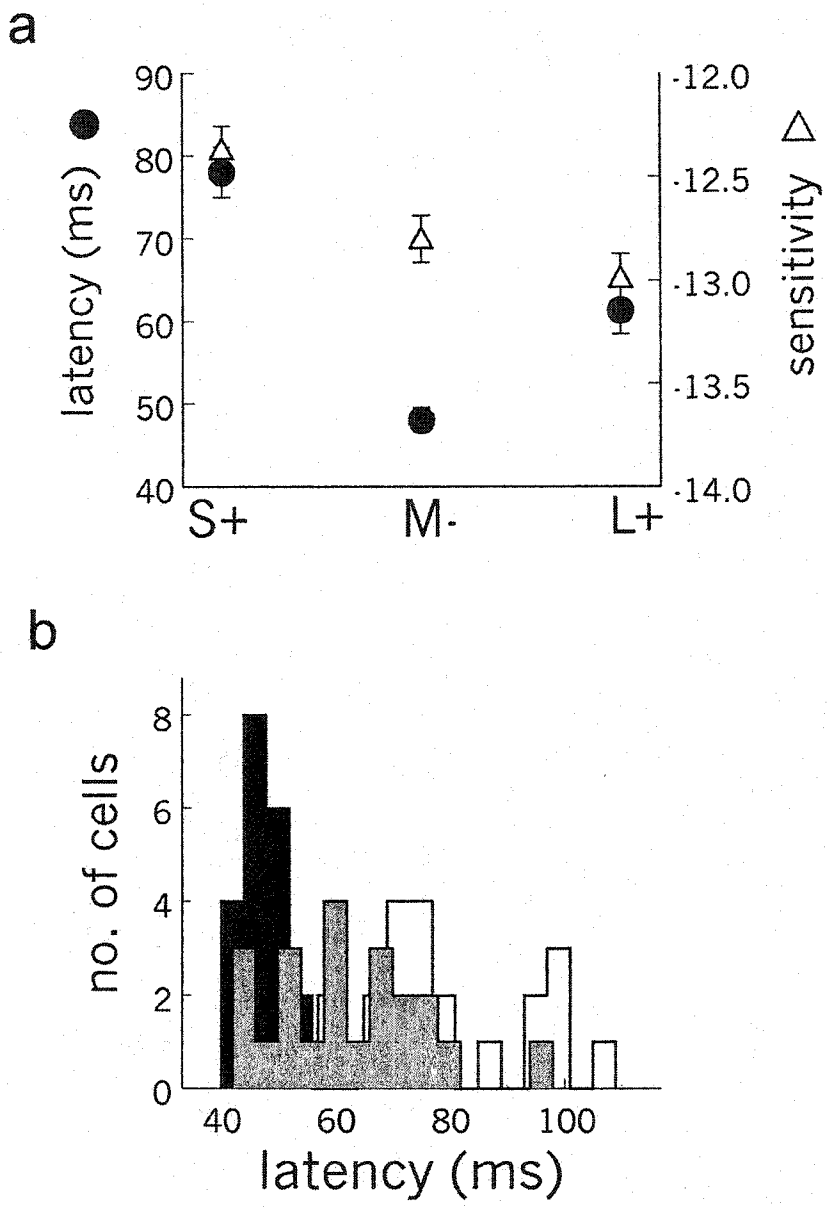

a
latency (ms) ●
sensitivity △
90
80
70
60
50
40
-12.0
-12.5
-13.0
-13.5
-14.0
S+
M-
L+
b
no. of cells
8
6
4
2
0
40
60
80
100
latency (ms)

Figure 14. Spectral sensitivity and latency plot of a biphasic tectal unit (S+ML+/-) recorded under a moderate intensity 'yellow' background. Open and closed circles indicate On and On-Off responses respectively. Open triangles indicate On response latencies and closed triangles indicate Off response latencies.

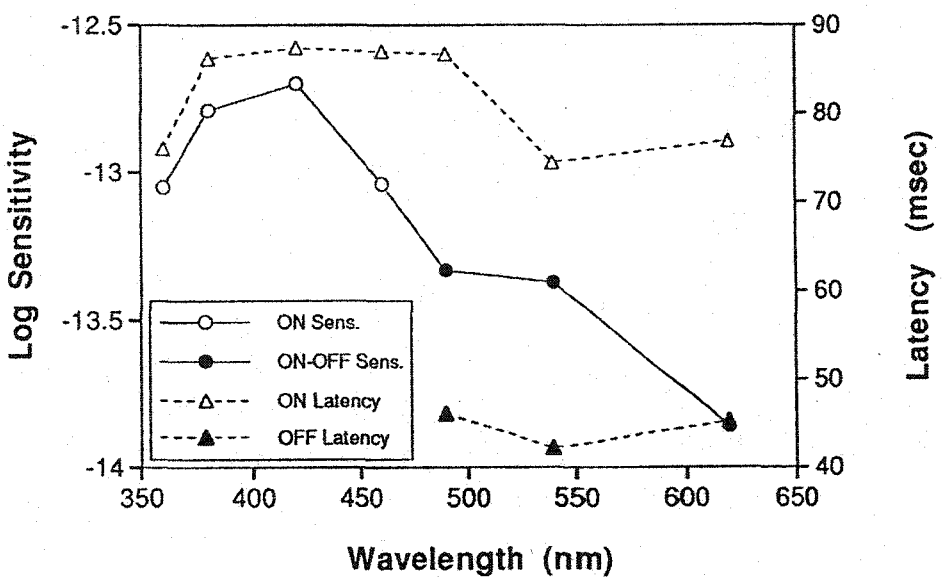
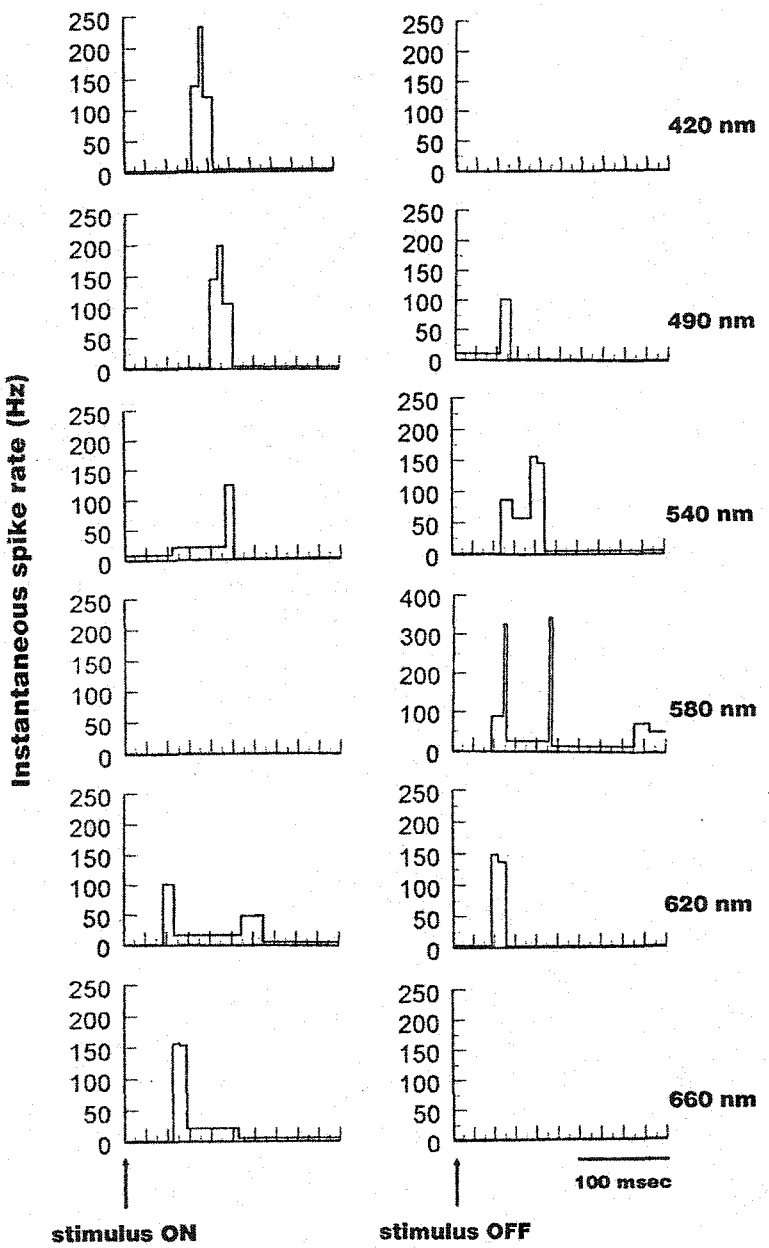

-12.5
-13
-13.5
-14
90
80
70
60
50
40
350
400
450
500
550
600
650
Log Sensitivity
Latency (msec)
Wavelength (nm)
ON Sens.
ON-OFF Sens.
ON Latency
OFF Latency

Figure 15. Instantaneous spike frequency plot showing a representative threshold response of a triphasic tectal unit recorded under a 'balanced' adaptation background. Instantaneous spike rates are calculated as 1000/inter-spike interval. Scale bar is 100 msec.


Instantaneous spike rate (Hz)
420 nm
490 nm
540 nm
580 nm
620 nm
660 nm
100 msec
stimulus ON
stimulus OFF

Chapter 3

Chromatic properties of tectal evoked potentials in rainbow trout

Introduction

Evoked potentials represent the summed activity of organized populations of cells. They provide signs of neural processing that cannot be predicted from single-unit recording and, in some cases, can exert influence on neighbouring cells (Bullock 1997). When studying visual processing in the optic tectum, tectal evoked potentials (TEPs) offer a particular advantage in that their most prominent components represent activity postsynaptic to the retinotectal fibres (Schmidt 1979). This is significant in light of the fact that the majority of single-unit studies to date have been unable to distinguish whether responses originate from intrinsic tectal neurons or presynaptic elements (Guthrie 1990). In view of these considerations, it appears that study of TEPs provides an excellent opportunity to add to our limited knowledge of chromatic processing in the teloestean optic tectum.

A small number of studies suggest that TEPs recorded from teleost fishes show wavelength dependent properties (Buser 1955; Konishi 1960a). Konishi (1960a) reported that the postsynaptic components of the TEP in carp comprise primarily two large negative-going waves, the N1 and N2 waves, and that there is differential attenuation of these components as a function of wavelength. More specifically, he observed that the N1 wave tended to predominate at longer wavelengths, while the N2 wave was only evident at middle and short wavelengths.

It is important to note, however, that these studies were carried out prior to the realization that the retinae of most teleost fishes possess different spectral classes of photoreceptors. Indeed, in an effort to provide equal quantal effectiveness across the spectrum, Konishi (1960a) adjusted stimulus intensity according to the absorption spectrum of a single visual pigment, visual violet. Such compensatory adjustments would have inadvertently resulted in inappropriately high stimulation at short and long wavelengths. Thus, as the form of the TEP and the amplitude of its various components are influenced by stimulus intensity (Bullock *et al.* 1991; Prosser and Nagai 1968), it is possible that the previous findings do not indicate exclusively wavelength dependent properties. Another important consideration is that, in the majority of Konishi's experiments, the stimulus duration was too short to evoke separate On and Off potentials (Bullock *et al.* 1991).

The properties of the TEPs described in these experiments were therefore most likely determined by input from both the On and Off channels. Given that the On and Off channels are independent, excitatory pathways (Wheeler 1982), it would be informative to examine the properties of each alone. In the current study, I have recorded TEPs in response to On and Off stimulation, with the goal of seeking wavelength-dependent waveform characteristics.

Material and Methods

Juvenile (6-12 cm total length) rainbow trout (*Oncorhynchus mykiss*) were acquired from a local fish farm and the Fraser Valley Trout Hatchery. The fish were held a minimum of 8 weeks prior to experimentation. They were fed daily with Biodiet Grower pellets (Bioproducts Inc.) and maintained under a 12:12 hr light cycle at 15°C. Experiments were carried out at the same water temperature. Fluorescent bulbs provided broad spectrum ambient illumination at an intensity of 33.54+/-14.39 $\mu W/cm^2$.

Before each experiment, fish were anesthetized with Tricaine methanosulfonate (MS 222) at a dosage of 0.1g/L and immobilized with an intramuscular injection of Flaxedil (gallamine triethiodide) at a dosage of 10mg/Kg body weight. During the course of each experiment, fish were maintained under mild anesthesia with an intramuscular injection of Marinil (3mg/Kg body weight). All procedures were in accordance with the guidelines for the Canadian Council for Animal Care.

Tectal evoked potentials (TEPs) were recorded from the surface of the central region of the exposed tectum. Recordings were made with teflon-coated, chlorided silver electrodes (250 μm diameter). A reference electrode was inserted into the epithelium of the right nares. Signals were differentially amplified (2 000-10 000x) with a Grass P5 amplifier, bandpass filtered (3Hz-300Hz) and fed into an oscilloscope. Waveforms were digitized with a 16 bit A/D board (National Instruments, AT-MIO-16XE-50) at a sampling rate of 1 kHz and stored on a personal computer.

A three channel optical system was used to present a light stimulus to the left eye of test fish. Two channels were used to manipulate the background illumination, while the third channel

was used for stimulation. The three light channels entered the Faraday cage via a trifurcated, quartz fibre optic cable (Fiber Optic Systems, Inc). Light passed from the common end of the fibre optic (5 mm diameter) to a small, quartz diffusing screen positioned 4 mm beyond the tip of the cable. The distance from the diffusing screen to the eye was 5 mm. This stimulus arrangement provided full field stimulation of the eye.

The light source for the stimulus was a 300 W Xenon lamp (Oriel). An Inconel coated neutral density wedge (Optikon) was used to control stimulus intensity and a monochromator (SA Instruments) was used to manipulate stimulus wavelength. Illumination for the background channels was provided with tungsten-halogen bulbs.

Novales Flamarique and Hawryshyn (1996) have shown that an adapting background approximating the photopic light environment of a clear, fresh water system results in roughly equal sensitivity of the short-wavelength sensitive (S), middle-wavelength sensitive (M) and long-wavelength sensitive (L) cone mechanisms. Thus, as in the single-unit study (chapter 2), 'balanced' adapting backgrounds were utilized to help ensure that the observed TEP waveforms could be expected under natural viewing conditions. The 'white' background of Novales Flamarique and Hawryshyn (1996) and the weak yellow background were both used.

Stimuli were presented over an intensity range of 1.6 to 2.0 log units, typically beginning at a subthreshold level. Stimulus duration, which was controlled by an electronic shutter (Uniblitz), was set at 500 msec. Waveform comparisons were made at high but submaximal stimulus intensities. All comparisons were made at stimulus intensities providing equal quantal effectiveness at each wavelength (14.1-14.3 log quanta $\bullet$ cm^{-2} $\bullet$ s^{-1}). The slope of the descending phase of the first negative deflection (N1 wave) served as the criterion for evaluating quantal efficacy. Two responses were averaged at each wavelength-intensity combination. Principal Components Analysis was used to characterize TEPs recorded in response to retinal stimulation at wavelengths corresponding to the sensitivity peaks of the S (420 nm), M (540 nm) and L cone mechanisms (620 nm).*

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* Although juvenile rainbow trout possess an UV cone mechanism, UV projections to the tectum are relatively sparse (Coughlin and Hawryshyn 1994a). This observation, in addition to the finding that the UV and S cone mechanisms

Principal Components Analysis of TEPs.

Traditionally, studies of evoked potentials have involved characterizing waveforms with only one or two points, one example being the points used to delineate peak-to-peak amplitude. Such simple measures are insensitive to differences in wave shape. Moreover, when presented with relatively complex waveforms, choosing which parameters to measure becomes increasingly difficult. PCA, on the other hand, provides a relatively simple, objective method for quantifying the shape of an entire waveform (Chapman and McCrary 1995; Kisley and Gerstein, 1999).

Each TEP waveform (U_i) can be represented as a series of discrete values:

$$U_i = u_{ij}\{j=1,2,\ldots,m\},$$

where time between samples is 1 msec, and m is the number of points per waveform (250). I applied PCA considering time points as variables and TEP traces as cases. PCA was applied separately to On and Off responses. For each analysis, responses to all wavelengths were grouped.

Principal components were computed from the eigenvectors of the signal covariance matrix by the method described in Glaser and Ruchkin (1976). As principal components of a covariance matrix are not invariant with respect to the scaling of the original variables, waveforms were normalized relative to the N1 wave (Chapman and McCrary 1995). Respective eigenvalues describe the amount of variance explained by each principal component.

PCA allows for partial reconstruction of a waveform from the principal components (also known as basis functions) that account for the greatest amount of variance in the data. Because the first three components, F_1, F_2 and F_3, accounted for ~ 90% of the data variance in the current study, all waveforms were represented with only these three components:

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provide qualitatively similar input to colour-opponent tectal units, prompted me to focus on analysis of the longer wavelength sensitive cone mechanisms.

$$F_1 = f_{1j}\{j=1,2,...m\} \quad F_2 = f_{2j}\{j=1,2,...m\} \quad F_3 = f_{3j}\{j=1,2...m\}$$

First, second and third principal component scores were computed by convoluting each TEP waveform with the first, second and third principal components, respectively:

$$S_{xi} = 1/m \sum_{j=1}^{m} (f_{xj} * u_{ij})$$

where x = 1, 2 and 3 denote the three principal components used. Each score represents the signal power accounted for by that principal component.

Data analysis was performed with customized software (developed by Theodore Haimberger) using 'Interactive Data Language', as well as the statistical analysis package SPSS.

Results

General considerations

Evoked potentials represent the summed activity of populations of neurons in the neighbourhood of the recording electrode. According to field potential theory (Vanegas *et al.* 1984; Leung 1990), evoked potentials are generated by neurons oriented radially with respect to the surface of a cortical structure. The properties of the numerous, radially arranged piriform cells (type XIV cells) suggest that they provide the greatest contribution to the TEP (see chapter 1). Since the dendritic trees of the piriform neurons are located primarily in the superficial layers (stratum opticum and stratum fibrosum et griseum superficiale) and their somata in deeper layers (stratum album centrale and stratum periventriculare), synaptic input leads to electric dipoles arranged perpendicularly to the tectal surface. The polarity of TEPs is dependent on whether current flow in the vicinity of the recording electrode is inward (towards a current sink) or outward (from a current source). Excitatory synaptic input to the apical dendrites of radially arranged

tectal cells leads to inward current flow. Conversely, inhibitory input results in outward current flow. Thus, negative waves recorded from the tectal surface represent excitatory postsynaptic processes, while positive waves represent inhibitory postsynaptic processes (chapter 1; Leung 1990; Schwippert *et al.* 1996).

TEP components

Figure 16 shows TEP records obtained from the surface of the optic tectum of a rainbow trout. Responses to middle wavelength stimulation (540 nm) are shown, as the complete TEP pattern was typically readily observed in this region of the spectrum. On and Off responses began with a small positive component, P1, followed by two large negative waves, N1 and N2. The initial P1 wave represents presynaptic activity (Schwippert *et al.* 1996), and did not emerge in all records. The N1 and N2 waves were interrupted by a second positive wave, P2, and the response terminated with a final positive wave, P3. Greater complexity in the TEP pattern was sometimes observed. For example, additional deflections superimposed on the basic pattern or oscillations that persisted after termination of the initial response sometimes occurred.

Modulation of TEPs by different wavelengths of stimulation

I found that, when stimulated with the three test wavelengths, the wave shapes of the TEP differed substantially. The grandmean waveforms and averaged traces from individuals both show wavelength-dependent variation of the N2/P3 wave sequence. This basic phenomenon was the same for both On and Off responses, although wavelength-dependence of these TEP components differed for On and Off responses (Figures 17 and 18). The On response showed an incremental increase in magnitude of the N2/P3 wave sequence as a function of increasing wavelength (Figure 17). By contrast, the N2/P3 wave sequence of the Off response was most pronounced at 540 nm, occurred infrequently at 420 nm and not at all at 620 nm (Figure 18).

Inter-subject variation of both On and Off TEPs was lowest at 620 nm. This can be seen from inspection of individual traces, as well as the coefficient of variation plots (Figures 17b,c and 18b,c). Variability of the On response was greater at 540 nm than 420 mn, while variability in the Off response was similar at these wavelengths (Figures 17 and 18).

To further characterize and quantify the variability in TEP wave shape, I used PCA. It must be emphasized that only those elements of a data-set which vary will be measured as a PCA component (Chapman and McCrary 1995). In the current study, the data were normalized relative to the N1 wave, making it possible to use PCA to search specifically for variation in the N2/P3 wave sequence. Accordingly, the first three principal components computed for both On and Off responses represent differences in shape and amplitude of the N2/P3 wave sequence (Figures 19a and 20a).

On response

Figure 19 shows the results of PCA on 26 TEPs recorded from 10 animals. The first, second and third components account for 67.0%, 15.0% and 7.7% of the variance in the data set, respectively (total = 89.7%). The results of computation of the principal component scores are presented in a three-dimensional scatter plot (Figure 19b). Three clusters of response type are evident. Although the clusters do not segregate perfectly as a function of wavelength, statistical analysis of principal component scores confirmed the initial interpretation, based on grandmean waveforms (Figure 17), of wavelength-dependent TEP properties (Table 1). At least one principal component contributed to statistical significance in all pairwise comparisons. The TEP wave shapes recorded in response to stimulation at 420 nm and 620 nm were significantly different with respect to the second and third components. By contrast, the wave shapes of the 540 nm and 620 nm responses differed with respect to only the first principal component. The third principal component contributed to significant differences between responses to the 420 nm and 540 nm stimuli.

Off response

As with the On response, the first three principal components accounted for almost 90% of the total variance (Figure 20) in the data set (i = 10; n = 28). Although the principal components of the Off response are qualitatively similar to those of the On response (Figure 20a), wavelength-dependence in the TEP was distinctly different. Specifically, only the 420-540 nm and 540-620 nm pairwise comparisons yielded statistically significant differences. Moreover, just the first principal component contributed to significance in these cases (Table 1). The segregation of the 540 nm responses as a function of the first principal component is evident in Figure 20b, and indicates the prominence of the N2/P3 wave sequence at this wavelength. Also note the similarity in principal component scores for the 420 nm and 620 nm responses (evidenced by extensive overlap of the data points). These findings are concordant with the observed wave shapes of the grandmean TEPs (Figure 18).

Discussion

I have shown that TEPs of rainbow trout show distinct wavelength-dependent waveform characteristics. In addition, my findings indicate that the On and Off channels of the trout tectum each possess distinctly different wavelength dependent properties. The N2/P3 wave sequence of the On response became more pronounced as a function of increasing wavelength, while the Off response typically exhibited this component of the TEP only at middle wavelengths. The N1 wave was relatively invariant with respect to wavelength.

It is difficult to compare my findings with those of Konishi (1960a), whose study of the carp tectum provides the most comprehensive description prior to mine of the chromatic properties of the TEP. The short stimulus duration (~ 33 ms) employed in his study can be expected to have resulted in incomplete separation of On and Off potentials (Bullock *et al.* 1991). In contrast, I employed a significantly longer stimulus duration in order to evoke separate On and Off responses. Although Konishi states that Off responses were seldom observed, it is difficult to understand why this would be. The evidence for an independent, excitatory Off pathway in

cyprinids is not in doubt (Wheeler 1982). Indeed, Konishi presents a trace where a distinct Off response can be seen superimposed on the descending phase of the On response. Moreover, separate On and Off responses can also be seen in a second trace where a longer stimulus duration was used (chapter 1, Figure 7b).

With these considerations in mind, it is important to note that the shapes of the waveforms presented by Konishi are somewhat different than those described in the current study. For example, the broad P3 wave reported here is not evident in Konishi's records. Additionally, in Konishi's study, the N2 wave is significantly broader than the N1 wave. I have found no such difference between N1 and N2. One could argue that differences in shape of the TEP indicate species differences. However, this is doubtful given that the waveform of the TEP of the rainbow trout is strikingly similar to that of the toad (Schwippert *et al.* 1996). I therefore suggest that the characteristics of the waveforms shown by Konishi were determined by combined input from the On and Off channels. Thus, the TEPs recorded here and those described by Konishi (1960a) most likely do not reflect equivalent neural processes, and consequently are not directly comparable.

It is tempting to suggest that the distinct wavelength dependence in the TEP waveform is indicative of a temporal code for colour. However, without a more comprehensive knowledge of the neural circuitry underlying this phenomenon, it is difficult to assign a definitive functional role for the differences in TEP waveform. At present, the number of retinal ganglion cell classes and the abundance of these classes relative to each other are not known, making it difficult to determine the role of presynaptic axonal inputs in shaping the TEP. Single-unit studies do suggest, however, that a large proportion of units in the rainbow trout tectum are colour-opponent (Coughlin and Hawryshyn 1994a, chapter 2). Should these units provide a substantial contribution to the TEP, it is conceivable that its waveform characteristics provide a sign of underlying processes that enable colour discrimination. Here it is interesting to note that Guthrie (1983) found little evidence of wavelength dependence in the TEP of the perch, a species with a small proportion of colour-opponent tectal units.

Origin of evoked components

It has been convincingly demonstrated that negative and positive deflections recorded at the tectal surface represent excitatory and inhibitory postsynaptic processes, respectively (Sajovic and Levinthal 1983; Manis and Freeman 1988). Electrical stimulation of the optic nerve typically results in a TEP comprised of an excitatory postsynaptic process followed by an inhibitory one. This finding is consistent with intracellular recordings from the carp (Matsumoto *et al.* 1983) and goldfish (Freeman and Norden 1984) tectum that often showed EPSPs cut short by IPSPs. Thus, it is probable that visually evoked negative-to-positive wave sequences are generated by the same processes. This leaves open the question of the mechanism responsible for creating a second, delayed negative-to-positive wave sequence.

It is conceivable that the TEP waveform is a function of both retinal and tectal properties. The early and late TEP components (N1/P2 and N2/P3 wave sequences) could be generated by serial excitation of a tectal circuit. This possibility is supported by evidence that the tectum receives temporally segregated excitatory input from retinotectal afferents. For example, Beaudet *et al.* (1993) and Demarco and Powers (1991) found that compound action potentials (CAPs) recorded from the optic nerve of rainbow trout and goldfish were frequently made up of more than one wave. This phenomenon was observed for both On and Off responses. In addition, the latency difference between the CAP wave peaks is consistent with that observed between the N1 and N2 waves (~20-40 ms), suggesting a retinal origin of the N2 wave. The single-unit study (chapter 2) also suggests that colour-opponent intraretinal interactions could generate sufficiently large differences in time of arrival at the tectum.

The most parsimonious explanation for the origin of the IPSPs that follow each of the excitatory events is the presence of an inhibitory feedback loop. This contention is consonant with intracellular recordings of tectal cells showing EPSPs cut short by IPSPs (Matsumoto *et al.* 1983). Moreover, a simulation model comprised of two consecutive population EPSPs, each immediately followed by a population IPSP, provides a good fit to the experimental data (Figure 21; Appendix A). Given the possibility of monosynaptic feedback (see below), IPSP onset was

set rather late (6 ms latency) relative to onset of the EPSPs. However, similar results can be expected upon shortening of the delay period.

A number of properties of one tectal cell class, the piriform cells, suggest that they contribute to a prominent inhibitory feedback circuit. Their morphology, spatial orientation and numerical superiority all suggest that they are likely the primary contributors to the TEP (see results and chapter 1). Interestingly, many piriform cells send axon collaterals to the SFGS that contact either their own dendrites or those of neighbouring piriform cells (Vanegas *et al.* 1974; Meek and Schallart 1978). Most importantly, there is evidence that some piriform cells are GABAergic, suggesting that their collateral terminals are capable of providing recurrent inhibition (Villani *et al.* 1981). A schematic of the proposed circuit is provided in chapter 1 (Figure 9). Serial activation of this circuit by temporally separated excitatory inputs can be expected to give rise to the complete TEP waveform. An inhibitory feedback loop could function to preserve or possibly even embellish the temporal structure of neural signals originating at the level of the retina. If this is indeed the primary function of the proposed circuit, then it would seem to support the notion that temporal patterning of neural activity has significant functional consequences.

An alternative possibility is that the N2/P3 wave sequence is generated by an excitatory, polysynaptic pathway intrinsic to the tectum. Indeed, some electrophysiological studies of the tecta of goldfish and carp report a long latency excitatory wave elicited by electrical stimulation of the optic nerve (Konishi 1960b; Manis and Freeman 1988; King and Schmidt 1991a,b). In three of these studies (Manis and Freeman 1988; King and Schmidt 1991a,b), recordings were made from isolated tecta, confirming a tectal origin for the long latency component. King and Schmidt (1991b) provide evidence suggesting that this phenomenon is due to recurrent cholinergic excitation of retinal terminals through a polysynaptic tectal pathway. Note that, with this scenario, the proposed inhibitory feedback loop would still give rise to negative-to-positive wave sequences.

It should be noted that, if both retinal and tectal elements were to contribute multiple excitatory inputs, one would expect a rather more complex visually evoked TEP. In the simplest case, one would predict more than two excitatory TEP components. Pharmacological

investigations designed to alter the patterning of retinal potentials would aid in determining the origin of the late components of the visually evoked TEP.

Table 1 Statistical analysis of principal component scores for On (n=8) and Off (n=9) responses. Standard deviations of mean principal component scores are given in parentheses.

	ON			OFF		
Wavelength	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
620 nm	8.68	4.91	10.31	-2.39	-1.40	2.57
	(2.00)	(1.70)	(3.73)	(2.34)	(1.47)	(1.68)
540 nm	3.39	3.21	7.45	6.92	-1.50	2.48
	(2.06)	(1.60)	(3.18)	(7.47)	(3.38)	(4.12)
	p = 0.006	p = 0.081	p = 0.081	p = 0.005	p = 0.429	p = 0.339
620 nm	8.68	4.91	10.31	-2.39	-1.40	2.57
	(2.00)	(1.70)	(3.73)	(2.34)	(1.47)	(1.68)
420 nm	1.85	0.40	0.33	-0.98	-1.38	3.27
	(4.88)	(2.19)	(4.17)	(3.13)	(3.12)	(2.89)
	p = 0.018	p = 0.009	p = 0.009	p = 0.055	p = 0.476	p = 0.339
540 nm	3.39	3.21	7.45	6.92	-1.50	2.48
	(2.06)	(1.60)	(3.18)	(7.47)	(3.38)	(4.12)
420 nm	1.85	0.40	0.33	-0.98	-1.38	3.27
	(4.88)	(2.19)	(4.17)	(3.13)	(3.12)	(2.89)
	p = 0.200	p = 0.025	p = 0.009	p = 0.014	p = 0.476	p = 0.429

Statistical comparisons were made using a Wilcoxon Signed Ranks test.
Significance was set at 0.0167 (0.05/3) to account for multiple (three) pairwise comparisons.

Figure 16 Main components of the TEP of the rainbow trout in response to On and Off stimulation at 540 nm: P1, N1, P2, N2 and P3. For explanations see text. Positive deflections of the field potential are upward.

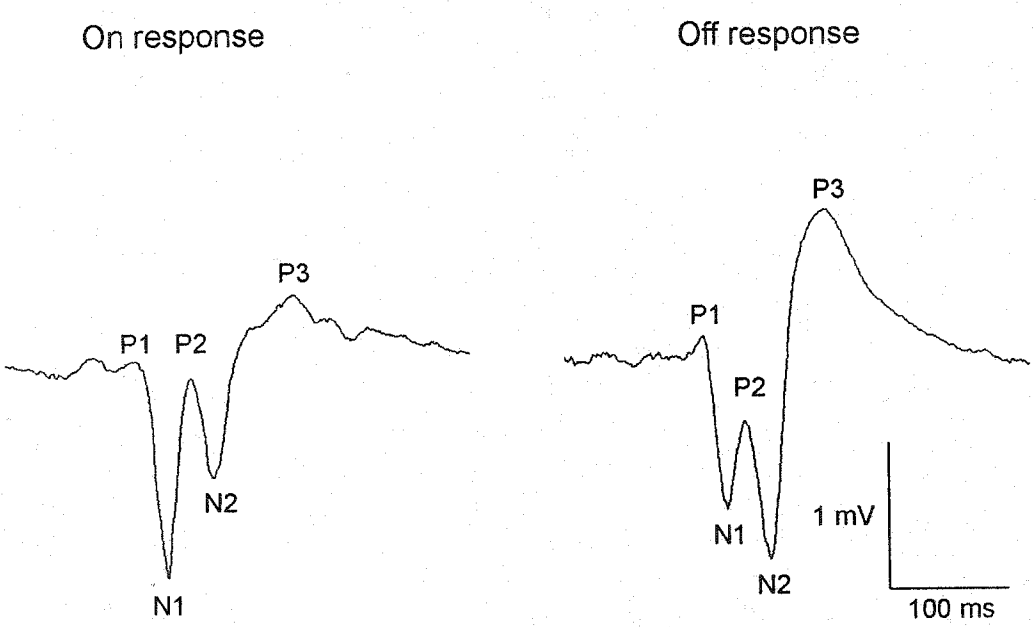

On response
Off response
P1
P2
P3
N1
N2
P1
P2
P3
N1
N2
1 mV
100 ms

Figure 17 Modulation of the On response by different wavelengths of stimulation. A, Grandmean of normalized waveforms. Grandmeans were calculated form the averaged responses of 8-10 individual animals. Stimulus onset occurs at time = 0 msec. Positive deflections of the field potential are plotted upward. B, Responses of individual animals to 420 nm (n = 8), 540 nm (n = 10) and 620 nm (n = 8) stimuli. C, Coefficient of variation (C_v = SD/mean) as a function of wavelength.

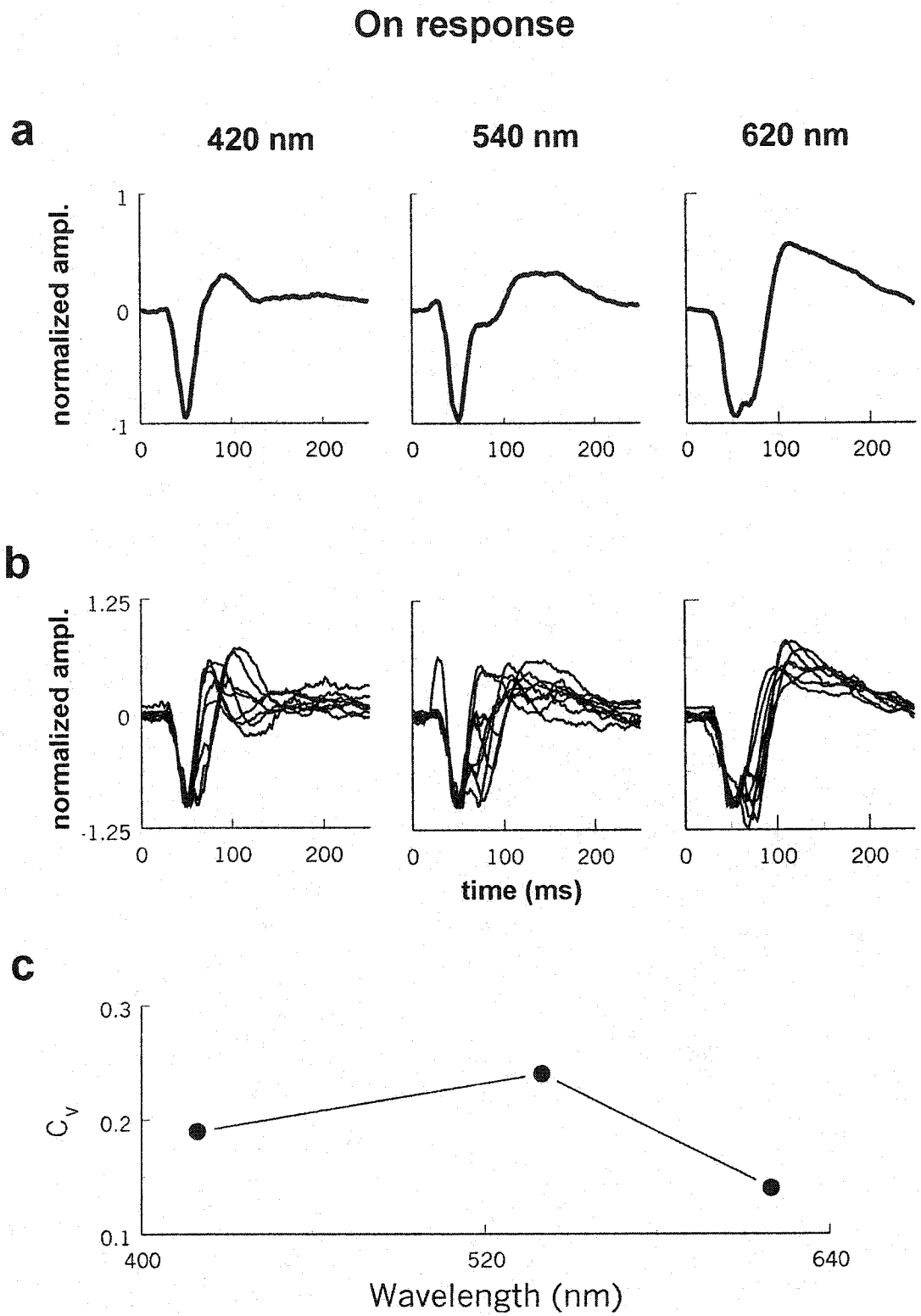

On response
a
420 nm
540 nm
620 nm
normalized ampl.
1
0
-1
0
100
200
b
normalized ampl.
1.25
0
-1.25
time (ms)
c
C_v
0.3
0.2
0.1
400
520
640
Wavelength (nm)

Figure 18 Modulation of the Off response by different wavelengths of stimulation. A, Grandmean of normalized waveforms. Grandmeans were calculated form the averaged responses of 9-10 individual animals. Stimulus offset occurs at time = 0 msec. Positive deflections of the field potential are plotted upward. B, Responses of individual animals to 420 nm (n = 10), 540 nm (n = 9) and 620 nm (n = 9) stimuli. C, Coefficient of variation (C_v = SD/mean) as a function of wavelength.

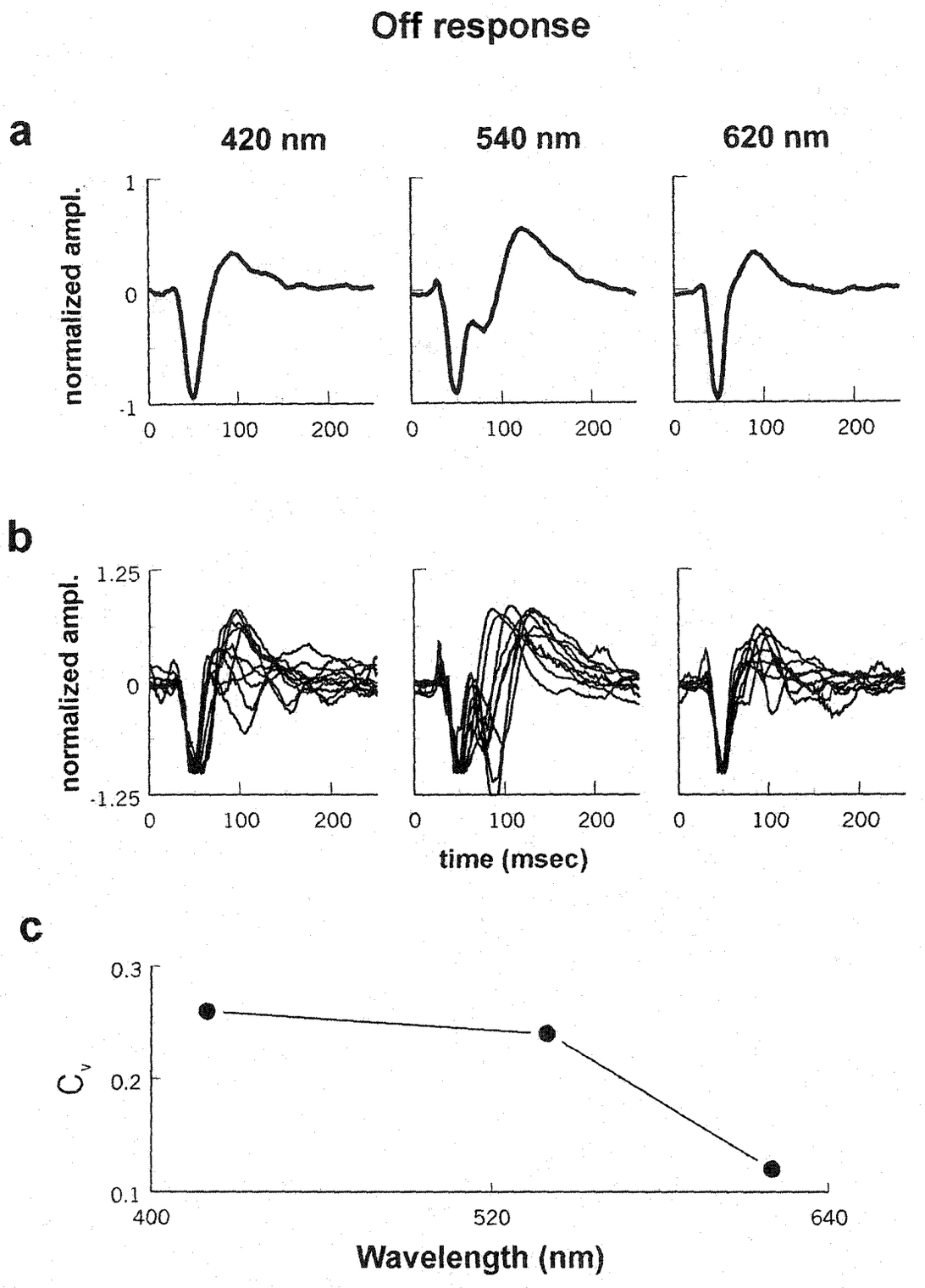
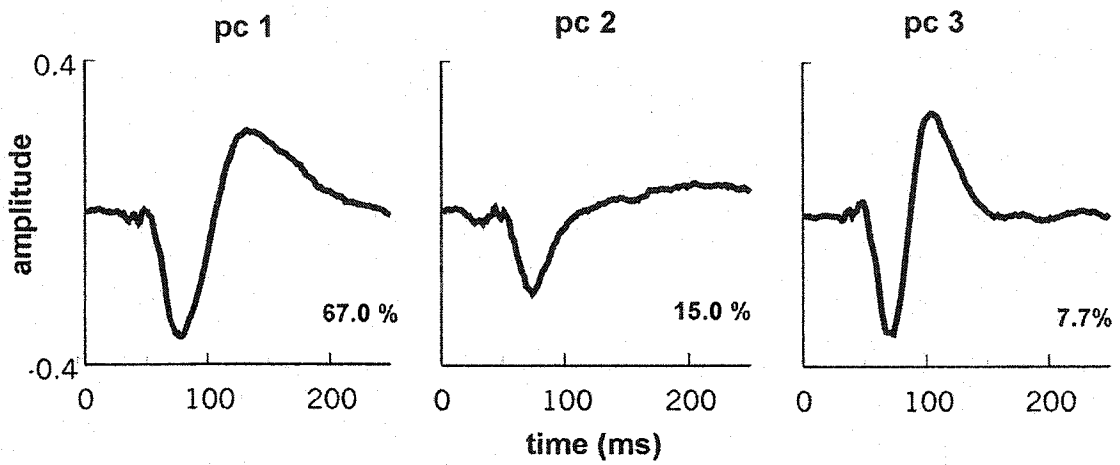

Off response
a
420 nm
540 nm
620 nm
normalized ampl.
1
0
-1
0
100
200
b
normalized ampl.
1.25
0
-1.25
time (msec)
c
C_v
0.3
0.2
0.1
400
520
640
Wavelength (nm)

Figure 19 A, First three principal components for the On response computed from the data presented in Figure 17. Data from all wavelengths were grouped for the analysis. The value in the bottom right of each plot corresponds to the percentage of total data variance accounted for by that component. B, Three-dimensional scatter plot of principal component scores. 420 nm, black circles; 540 nm, gray squares; 620 nm, white circles.

On response

a



b

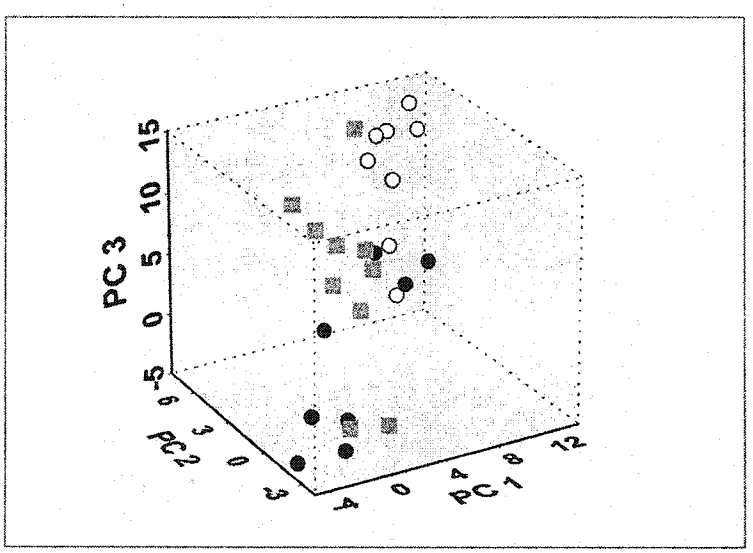
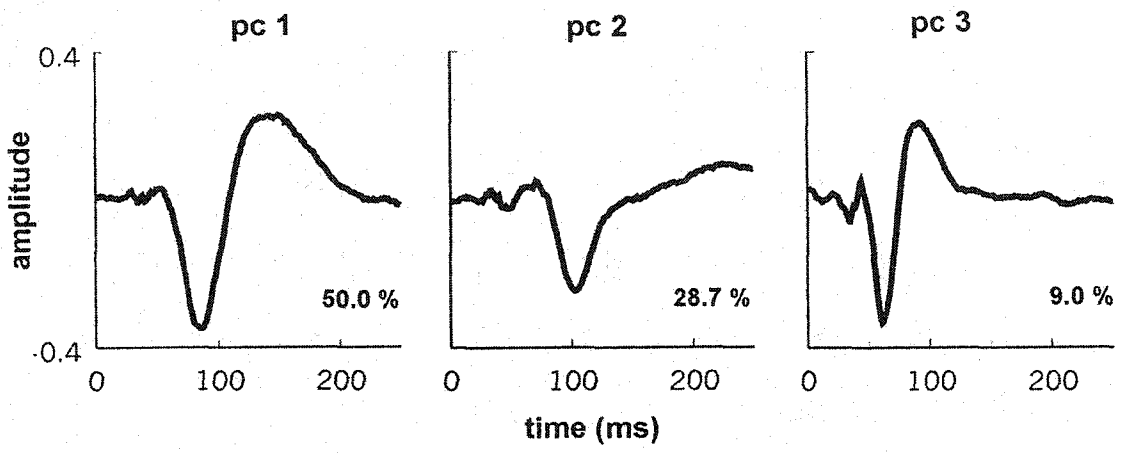


Figure 20 A, First three principal components of the Off response computed form the data presented in Figure 18. The value in the bottom right of each plot corresponds to the percentage of total data variance accounted for by that component. B, Three-dimensional scatter plot of principal component scores. 420 nm, black circles; 540 nm, gray squares; 620 nm, white circles.

Off response

a



b

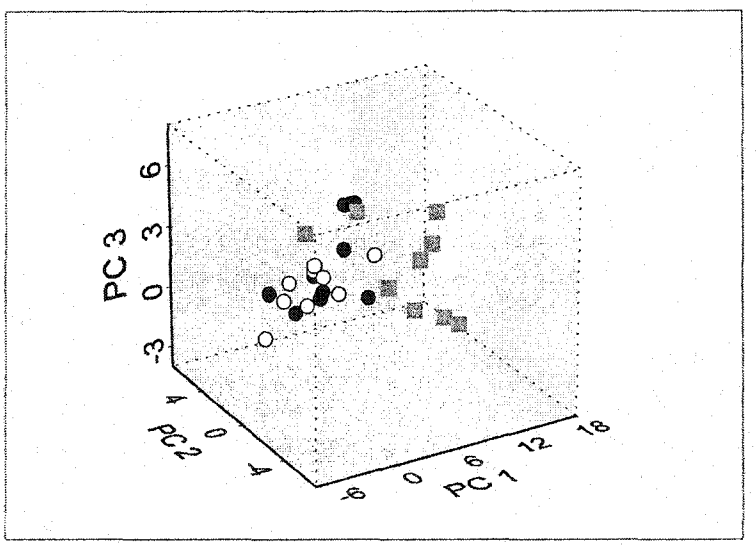
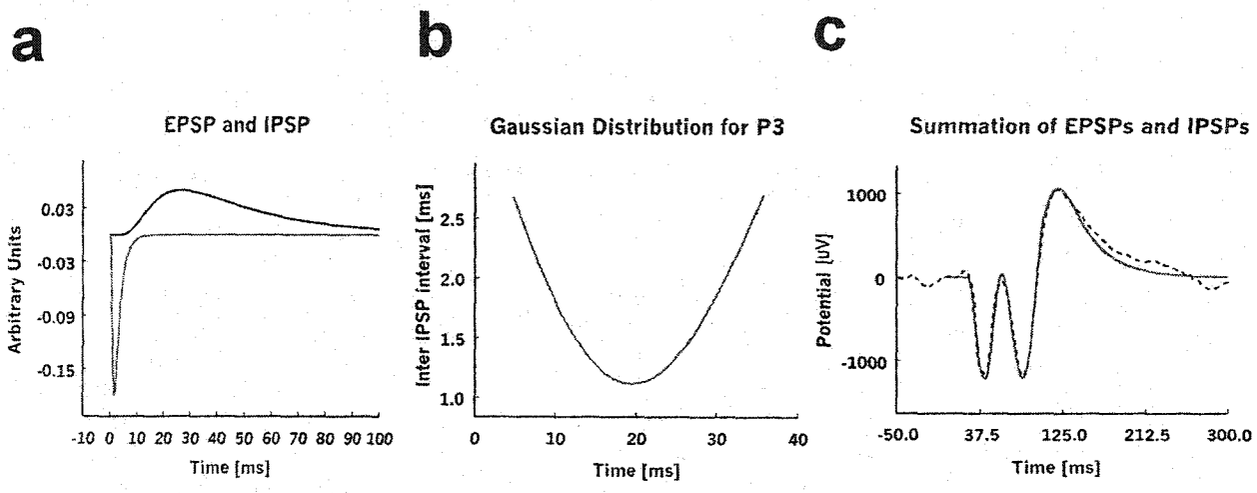


Figure 21 The experimental data were modeled as a combination of population EPSPs and IPSPs. Individual elements were summated over time using a Gaussian distribution. A middle wavelength Off response showing the complete TEP waveform was selected for modeling. A: EPSP (gray line) and IPSP (black line) waveforms utilized for generation of population postsynaptic potentials (see appendix A). The relatively long duration of the IPSP is in accordance with the time course of IPSP-like deflections recorded extracellularly from the teleostean tectum (Vanegas 1974). B: An example of a Gaussian distribution utilized for temporal summation of individual neural elements. The Gaussian distribution shown in B was used for summation of IPSPs in order to model the P3 wave. Gaussian widths and widths of postsynaptic potentials are provided in Appendix A. C: Model of the TEP using an alternating sequence of two population EPSPs and two population IPSPs. Experimental data are represented by the dashed line. The numbers of individual neural elements used is presented in Appendix A.


a
EPSP and IPSP
Arbitrary Units
0.03
-0.03
-0.09
-0.15
-10 0 10 20 30 40 50 60 70 80 90 100
Time [ms]
b
Gaussian Distribution for P3
Inter IPSP interval [ms]
2.5
2.0
1.5
1.0
0 10 20 30 40
Time [ms]
c
Summation of EPSPs and IPSPs
Potential [uV]
1000
0
-1000
-50.0 37.5 125.0 212.5 300.0
Time [ms]

Chapter 4

Visual responses to agonistic display of *Betta splendens*

Introduction

In *The Study of Instinct*, Tinbergen emphasized that in order to understand behavioural reactions to external stimuli it is essential that the perceptual capabilities of the animal under study are realized. Tinbergen (1951) made this abundantly clear by paraphrasing von Uexkull (1921), who stated that 'each animal has its own *Merkwelt* (perceptual world) and that this world is different from its environment as we perceive it, that is to say, from our own *Merkwelt*.' Tinbergen also realized that knowledge of the capacities of sensory systems is not adequate to discover the nature of stimuli that are sufficient to elicit a behavioural response. This is because an animal does not react to all changes in its environment that it is capable of perceiving, but rather reacts to a subset of its perceptual world.

Based on Tinbergen's founding principles, it would seem logical, when trying to understand the neural basis of behaviour, to undertake neurophysiological studies that employ ethologically relevant stimuli; that is to say, stimuli that are known to elicit behavioural responses. However, such an approach, particularly when studying visual behaviour, has rarely been adopted (but see Herzog *et al.* 1993). Indeed, visual studies have traditionally employed simple geometric stimuli rather than more natural stimuli such as would likely have shaped the evolution of the neural networks of visual systems (Guthrie 1990).

A few researchers have made considerable progress in understanding the neural basis of visual behaviour by employing stimuli of ethological relevance. Most notable in the literature on anamniote vertebrates, are the studies by Ewert (1985; 1997) on the neuroethology of prey capture in frogs and toads. Ewert has provided a comprehensive notion of how retinal, tectal and thalamic neurons interact to facilitate prey recognition. But, by and large, neuroethological studies that employ natural stimuli to understand causal mechanisms of visually guided behaviour are lacking. Particularly lacking are studies on perception of social stimuli. The absence of neurophysiological studies contrasts the abundance of ethological studies of visually mediated social behaviour. This is especially true of fish, where vision is often the primary sensory modality utilized for social communication (Guthrie and Muntz 1993).

The primary goal of the present study is to gain insight into how a social behaviour, the agonistic display of Siamese fighting fish (*Betta splendens*), is perceived by conspecifics. *B. splendens* is well known for its spectacular agonistic display. Males use the display to establish territories where they build nests and court passing females (Simpson 1968; Evans 1985). Interestingly, all elements of agonistic display also occur during courtship, and can be performed by both males and females (Simpson 1968). The ease with which the display can be elicited in the laboratory provides a unique opportunity to study this behaviour pattern in relation to its efficacy in generating visual sensation.

To investigate the nature of perception of the agonistic behaviour of *B. splendens*, I have chosen to record multi-unit responses from the optic tectum (OT), the primary visual centre of teleost fish. In many teleostean fish species, including *B. splendens*, the OT is the largest external feature of the brain, and what is known of its response properties suggests a significant role in producing visually guided behaviours (Vanegas *et al.* 1984; Guthrie 1990). However, to date the nature of perception and/or recognition by the OT of realistic, behaviourally relevant stimuli can only be inferred by extrapolating from physiological studies that have employed artificial stimuli. At best, such extrapolations can provide only a limited perspective of visual sensation. Moreover, their validity remains to be determined. In this work, I examine directly how the teleostean OT responds to an ethological stimulus, the agonistic display of an unrestrained conspecific.

Material and Methods

Siamese fighting fish (*B. splendens*) were acquired from a local pet supplier. The fish were held in two litre opaque aquaria and fed daily with Tetramin betta flakes. Water temperature in both the holding tanks and the experimental set-up was 20-22°C.

Before experimentation, fish were anesthetized with Tricaine methanosulfonate (MS 222, 0.1g/L) and immobilized with an intramuscular injection of Flaxedil (gallamine thriethiodide,

10mg/Kg body weight). To maintain anesthesia during surgery, fish were respired with aerated water containing MS 222 (0.038g/L). Once the fish reached complete anesthesia, the right optic tectum was surgically exposed. After surgery, fish health was assessed by examining blood flow through the capillaries on the surface of the OT. Handling of experimental animals was in accordance with Canadian Council on Animal Care Guidelines.

To compensate for the myopic nature of the eyes of fish in air, a water filled 'eye cell' was attached to the head such that it covered the left eye. The eye cell consisted of a small plastic cylinder to which a glass cover slip was affixed. This device formed a water-tight chamber. A similar apparatus has been successfully employed by other researchers (Zenkin and Pigarev 1969; Guthrie and Banks 1978). Fish were then moved into a Faraday cage for electrophysiological recording.

Multi-unit recordings were made from the dorso-central OT of male *B. splendens* using resin-coated, tungsten microelectrodes (2-4 MOhm, Frederick Haer & Co.). A reference electrode was placed in the olfactory epithelium of the right nares or near the site of the surgical incision. Signals were differentially amplified (5 000-10 000x), bandpass filtered (0.3-3KHz) and fed into a variable level window discriminator.

The recording electrode was initially placed on the tectal surface, then inserted using a motorized microdrive (Frederick Haer & Co.). The electrode was advanced incrementally into the tectum, with frequent pauses to search for visually-evoked unit activity. A small black rectangle (1 cm^2) affixed to the end of a thin, wooden dowel was used as a stimulus probe. Recordings were made at relatively shallow depths (50-100 μm) corresponding to the SO and SFGS. The location of these layers was confirmed with histological examination of tectal cross sections.

Stimulus animals were induced to display to their mirror image with a mirror-type beam splitter (Edmund Scientific) placed adjacent to the front wall of the test aquarium. The aquarium was back-lit with a 75 watt tungsten bulb. Illumination from the bulb was diffused with sheets of albanene tracing paper that covered the back wall of the Faraday cage. Light reflected from the beam splitter allowed the stimulus animal to see its own reflection, while light transmitted through the beam splitter permitted the experimental animal to view the stimulus fish. The front wall of the

aquarium was centred relative to the left eye of the experimental animal and positioned at a distance of 7 cm. This distance is well within the inter-subject distance (≤15 cm) necessary for the release of agonistic display (Bronstein 1981). The experimental animal was positioned such that its left eye was parallel to the front wall of the experimental aquarium.

The experimental aquarium (21x15x14 cm) was constructed with clear plexi-glass. The floor, lateral walls and back wall were rendered opaque to avoid extraneous reflections. The front wall of the aquarium was left clear to permit a direct view of the stimulus animal by the experimental animal.

The display of stimulus fish and associated spike time histories of the experimental fish were recorded with an 8 mm video camera. Action potentials, as determined by the window discriminator, were transformed into single pulses and fed into the audio channel of the video camera (Figure 22). Timing of spike activity in relation to movement of the stimulus animals was determined by frame-by-frame analysis of video segments. Audio output of an editing VCR is maintained when analyzing video frame-by-frame, making it possible to analyze spike activity by feeding the audio channel of the VCR into an oscilloscope. I toggled through each frame of a selected sequence to count the number of spikes per frame (1/30 sec). The audio track of some video segments was also exported to a personal computer via the window discriminator and an A/D board to digitize spike trains of interest.

For most experiments animals were filmed from a top view. To avoid introducing the camera into the Faraday cage, I used a mirror positioned above the experimental chamber oriented at an angle of 45 degrees from vertical. In some preliminary experiments, the stimulus animals were filmed from an oblique, frontal view.

A detailed analysis of the display behaviour of some stimulus animals was carried out using Peak Motus, a computer system designed for quantification of movement patterns. Using this system, a spatial model of the animal was created to track movement of anatomical features of interest, as well as relevant features of the external environment. The spatial model of the fish consisted of points delimiting the rostrum, the base of the head, the anterior base of the dorsal fin and the caudal peduncle. The base and tip of the left operculum were also included in the spatial

model to enable recording of gill-cover erection. The position of the beam splitter was recorded to provide a frame of reference for whole body movements (Figure 23).

Results

I will first provide a brief description of the behaviour patterns that are of primary interest with respect to the neurophysiological data. The display behaviour of *Betta splendens* is composed of two primary components, lateral and frontal display. Typical behavioural sequences comprise a series of alternations between lateral display and frontal display. Both displays are accompanied by spreading of the non-paired fins. The transitory period leading from lateral to frontal display is termed *turn to face*. The most striking feature of frontal display is gill-cover erection, where the gill covers (opercula) are flared outward until they reach (and sometimes exceed) right angles to the body axis (Simpson 1968). Gill-cover erection is often virtually synchronous with turn to face and reaches its fullest extent upon completion of the act of turning to face (Figure 24a). I term this point in the behavioural sequence full display. The period leading up to full display is typically characterized by a reduction in swimming speed, followed by a rapid flexion of the body associated with turning to face (Figure 24b).

Experiments which provided the most useful information were those in which a stimulus animal swam in a regular pattern back and forth along the front wall of the test aquarium, alternating between frontal and lateral display. Animals that moved in this fashion repeatedly passed through the multi-unit receptive field (MURF) of the experimental fish, making it relatively simple to determine its extent. Moreover, animals moving in such a pattern tended to exhibit the same type of behaviour in similar spatial locations. MURFs ranged from ~ 20 to 35°. Stimulus animals, when performing lateral display, subtended approximately 70-80° in the horizontal plane.

Movement of a stimulus animal through the MURF of an experimental animal resulted in brisk discharge of tectal units. Spontaneous activity was consistently low (less than 1 Hz), allowing unambiguous association of spike discharge with movements of the stimulus animals. Multi-unit activity evoked by movement of stimulus animals was typically characterized by

arrhythmic bursts that persisted until the animal passed entirely through the MURF. Discharge frequencies within bursts frequently transiently reached ~500 Hz (Figure 25).

In all experiments, movement appeared to be necessary to evoke tectal responses. In some cases, stimulus animals ceased movement after entering the MURF, resulting in a drop in firing rate to background levels.

Viewing of video records revealed that movement associated with the transition from lateral to frontal display clearly modulated the pattern of multi-unit tectal activity. I therefore chose to focus on turn to face for a more detailed analysis. This behaviour proved to be amenable to quantitative description, and is known to be of particular ethological significance (Simpson 1968). In addition, the distinct start and end point of turn to face allowed for precise temporal alignment between the behaviour and associated neural activity.

A frame-by-frame analysis of sequences where turn to face occurred within the MURF revealed a consistent, high frequency burst coinciding with full display (Figures 26 and 29). Typically, a series of relatively low frequency bursts preceded the high frequency burst, which always occurred within 0-66.67 ms (0-2 frames) of full display (Figures 26 and 29). The spike frequency dropped markedly immediately after the onset of full display. Although males were used as stimulus animals for the majority of the experiments, female stimulus animals evoked similar responses (Figure 27).

Given that turn to face is a dynamic behaviour involving the movement of various anatomical structures, it is difficult to attribute neural responses to the movement of a specific anatomical feature. However, I did observe responses attributable to gill-cover erection in two instances. Figure 28 shows a sequence where gill-cover erection lags behind turn to face. In this sequence, gill-cover erection evoked a strong response, while the preceding turn to face failed to evoke a response.

Discussion

The present study has revealed that multi-unit activity in the OT *of B. splendens* is strongly modulated by dynamic movement associated with the agonistic display of conspecifics. Moreover, these findings indicate that the OT is attentive to a behavioural event known to be of ethological significance, the onset of full display. Indeed, Simpson (1968) has shown that the frequency of full display performed by a combatant in turn influences the frequency of full display performed by its opponent, providing behavioural confirmation that this motor pattern is attended to during encounters.

My findings are consistent with behavioural studies that have shown movement to be an important cue that plays a substantial role in eliciting agonistic behaviour in *B. splendens*. Johnson and Johnson (1973) compared the efficacy of a number of heterospecific species and conspecifics in influencing display behaviour in *B. splendens*. They found that, provided the heterospecific species responded with agonistic display, they were as effective as conspecifics in eliciting responses. The only heterospecific species tested that elicited a significantly weaker response (*Corydoras fasciata*) failed to react to the display of *B. splendens*. Johnson and Johnson also carried out experiments with models of different shapes and failed to find any particular shape critical for elicitation of display. Based on these findings, they suggested that the social behaviour of an opponent is more crucial than any particular visual stimulus. It should be noted, however, that *Corydoras* is the most phylogenetically distant (and morphologically most dissimilar) of the stimulus species used. All other stimulus animals were from the family *anabantidae*. Thus, one cannot rule out the possible significance of sign stimuli.

A study by Thompson (1963) also suggests the importance of movement. Thompson (1963) trained *B. splendens* to carry out an instrumental response to obtain the visual image of another male. He found that the frequency of instrumental responses was significantly higher when a live conspecific or a moving model rather than a stationary model was used as the visual reinforcer. It certainly appears that the current neurophysiological study and previous behavioural

studies are complementary. Taken together, they provide strong evidence for movement as an important visual stimulus attended to in agonistic encounters.

Although it appears that the OT is attentive to dynamic movement associated with display, the postural configuration of the stimulus animal can also be expected to influence tectal discharge. It is also important to note that the apparent configuration of the animal would vary as a function of the perspective from which it is viewed. If the orientation of the stimulus animal were to influence tectal discharge, it would suggest a form of feature recognition. From the present study, however, it is difficult to comment on the role of changes in body form in evoking tectal responses. Further studies should attempt to resolve this issue by employing stimuli that consist of the same type of dynamic motion presented from different perspectives. The use of video imaging holds promise for generating appropriate stimuli (see below).

Comparative studies using either heterospecific stimulus fish or heterospecific experimental fish would be useful when investigating the role of the OT in mediating recognition of conspecifics. Given the ample behavioural evidence for conspecific recognition in a number of teleost fish and the importance of the OT for visual processing in this group (Guthrie and Muntz 1993), it would be surprising if this brain structure were not integrally involved. The current study suggests that the distinctive motor patterns associated with social behaviours should be taken into consideration when searching for evidence of conspecific recognition.

One caveat of the present study was the inability to present identical stimuli in a systematic fashion. Although useful information can be gleaned by using live conspecifics as stimuli, such an approach can be laborious and time consuming. As mentioned above, a useful follow-up to the present study will be to determine the nature of neural responses to video images of agonistic displays. The use of video imaging would afford the opportunity of presenting realistic stimuli in a systematic fashion. That fish recognize and respond to video images has been well established (McDonald *et al.* 1995; McKinnon 1995; Rowland *et al.* 1995; Trainor and Basolo 2000). Moreover, recent work has shown that *B. splendens* in particular responds vigorously to computer animations (Allen and Nicoletto 1997). The data presented here will

provide a useful comparative baseline when attempting to determine the appropriate properties of video stimuli.

I would like to emphasize that there were no grounds for making an *a priori* prediction of how the tectum would respond to a natural stimulus such as the one employed here. Although previous studies have shown that the tectum is highly sensitive to movement, they have all utilized artificial stimuli (chapter 1). Therefore, I was unsure of whether responses could be detected from single trials. Furthermore, some workers have found that natural scenes induce neurons in the mammalian lateral geniculate nucleus to become quiescent, while artificial stimuli, such as a flickering checkerboard (white noise), evoke brisk unit responses (Dan *et al.* 1996). Thus, it was a necessary first step to explore the efficacy of natural stimuli in evoking neuronal responses in the OT of fish.

The present study has demonstrated that studies employing realistic visual stimuli have the potential to provide novel information relevant to understanding behavioural processes, as well as visual processing. By adopting neuroethological approaches similar to the one described here, it will be possible to determine precisely which features of the visual environment are attended to, providing a new window to the perceptual world of animals.

Figure 22 Set-up for filming stimulus animals and recording multi-unit tectal activity of experimental animals. The schematic provides a camera view perspective of the stimulus fish as viewed via a mirror (not shown) positioned above the aquarium at an angle of 45 degrees from vertical.

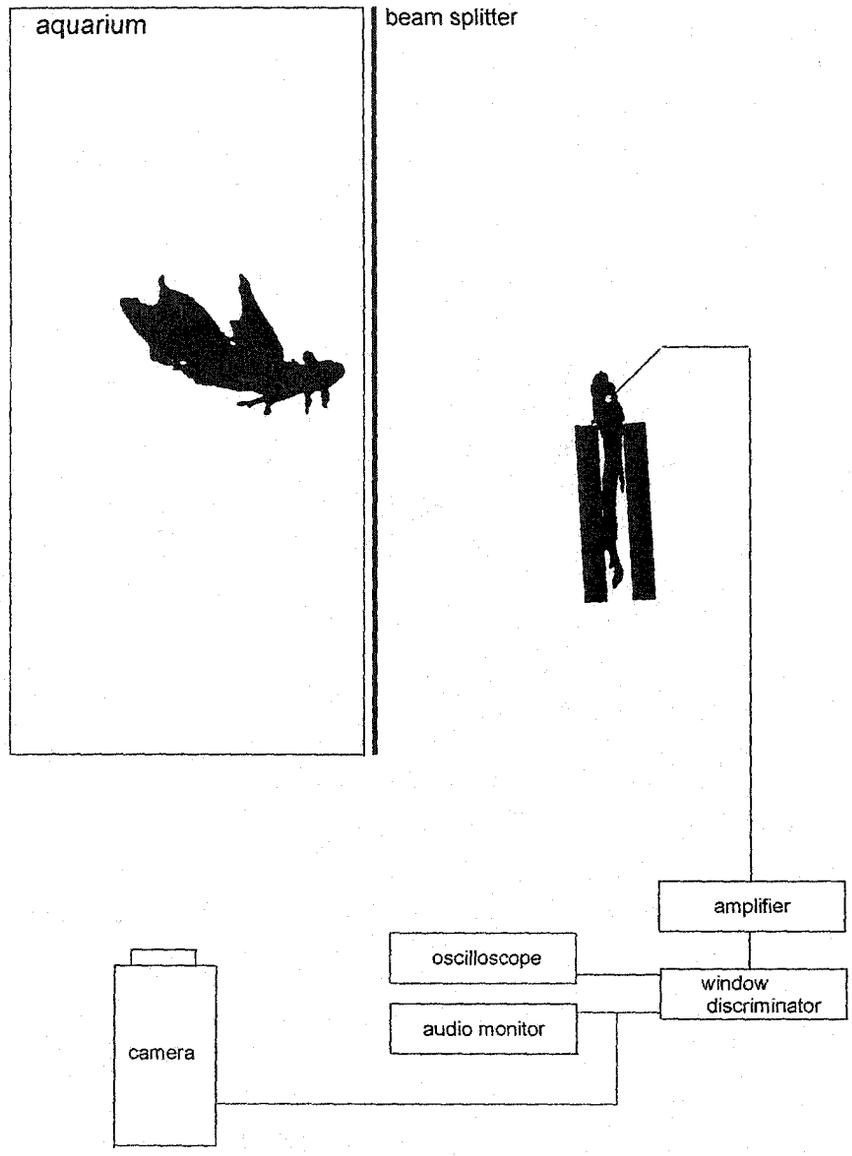
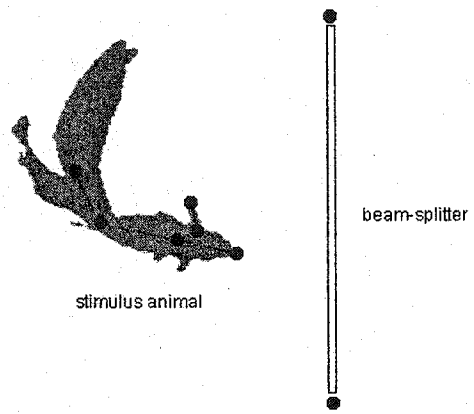
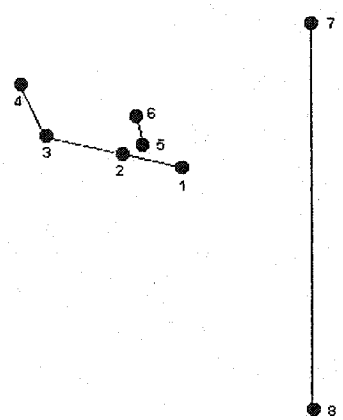

aquarium
beam splitter
amplifier
oscilloscope
window
discriminator
audio monitor
camera

Figure 23 Spatial model of stimulus fish. The upper panel shows the anatomical features that were monitored using Peak Motus. The lower panel shows a 'stick figure' representation of the animal as well as the position of the beam-splitter. Each of the anatomical features is represented by a closed circle, and corresponds to the following: 1, rostrum; 2, base of head; 3, anterior base of dorsal fin; 4, caudal peduncle; 5 and 6, base and tip of left operculum, respectively. The ends of the beam-splitter are represented by points 7 and 8. To monitor the relative position of anatomical features with respect to each other, as well as the external environment, a number of angular relationships were determined. These included the angle of the operculum relative to the longitudinal axis of the head (as determined using points 1 and 2), the angle of the head relative to the longitudinal axis of the beam-splitter and the angle of the posterior of the animal (determined by points 3 and 4) relative to the head. All angles were determined in an anti-clockwise direction.



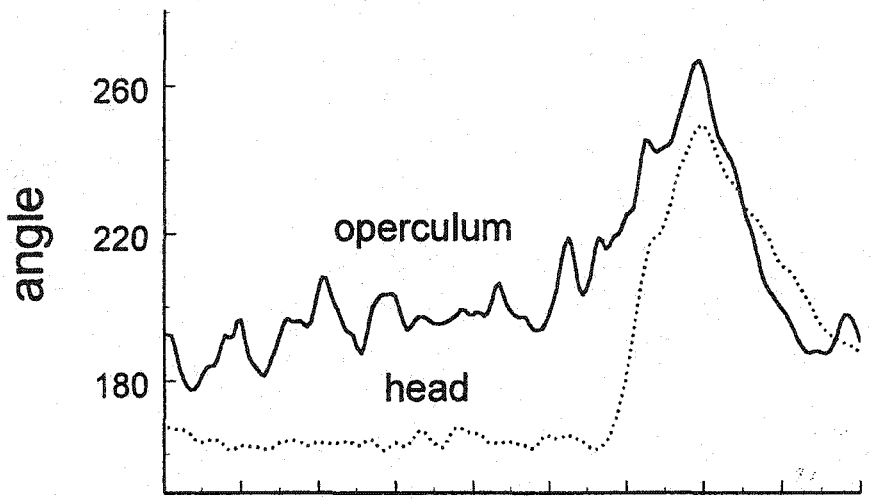
stimulus animal



spatial model

Figure 24 Quantitative representation of movement associated with display. A: Illustration of turn to face as evidenced by the rapid change in angular orientation of the head relative to the beam-splitter (dashed line). Note the concomitant occurrence of gill-cover erection (solid line). B: Illustration of dynamic changes in linear velocity (dashed line) associated with turn to face. As in A, Turn to face is represented by the change in angular orientation of the head (solid line). Scale bar indicates one second.

A


angle
260
220
180
operculum
head

B

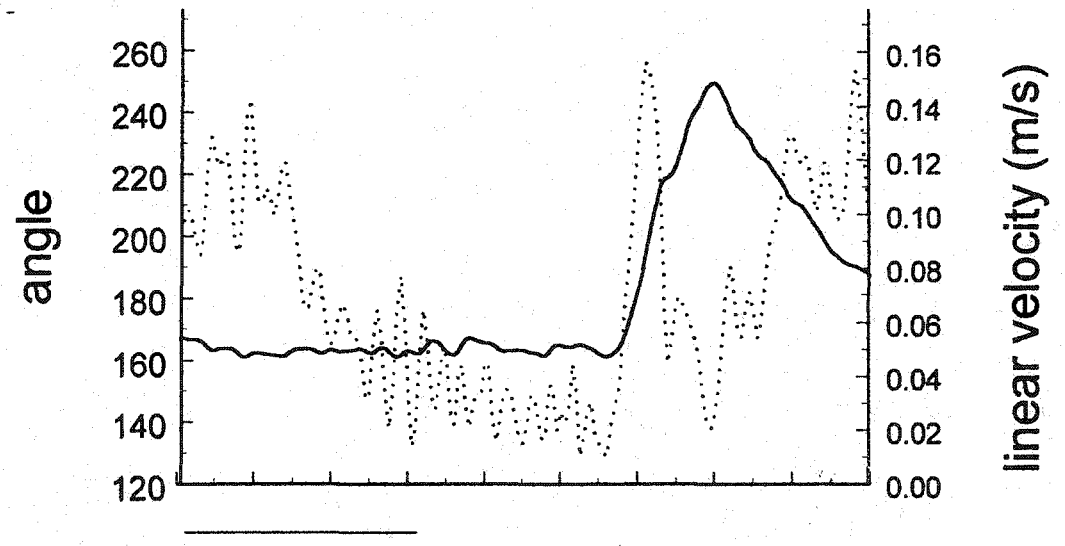

angle
260
240
220
200
180
160
140
120
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
linear velocity (m/s)

Figure 25 Multi-unit tectal activity evoked by a stimulus animal engaged in lateral display. Two representative examples from two experiments are shown. Multi-unit activity is expressed in the form of instantaneous spike rate plots. Instantaneous spike rates are calculated as 1000/inter-spike interval (in milliseconds). The arrows denote when the stimulus animal entered and exited the multi-unit receptive field of the experimental animal. Note the temporally modulated discharge pattern. Scale bar indicates one second.

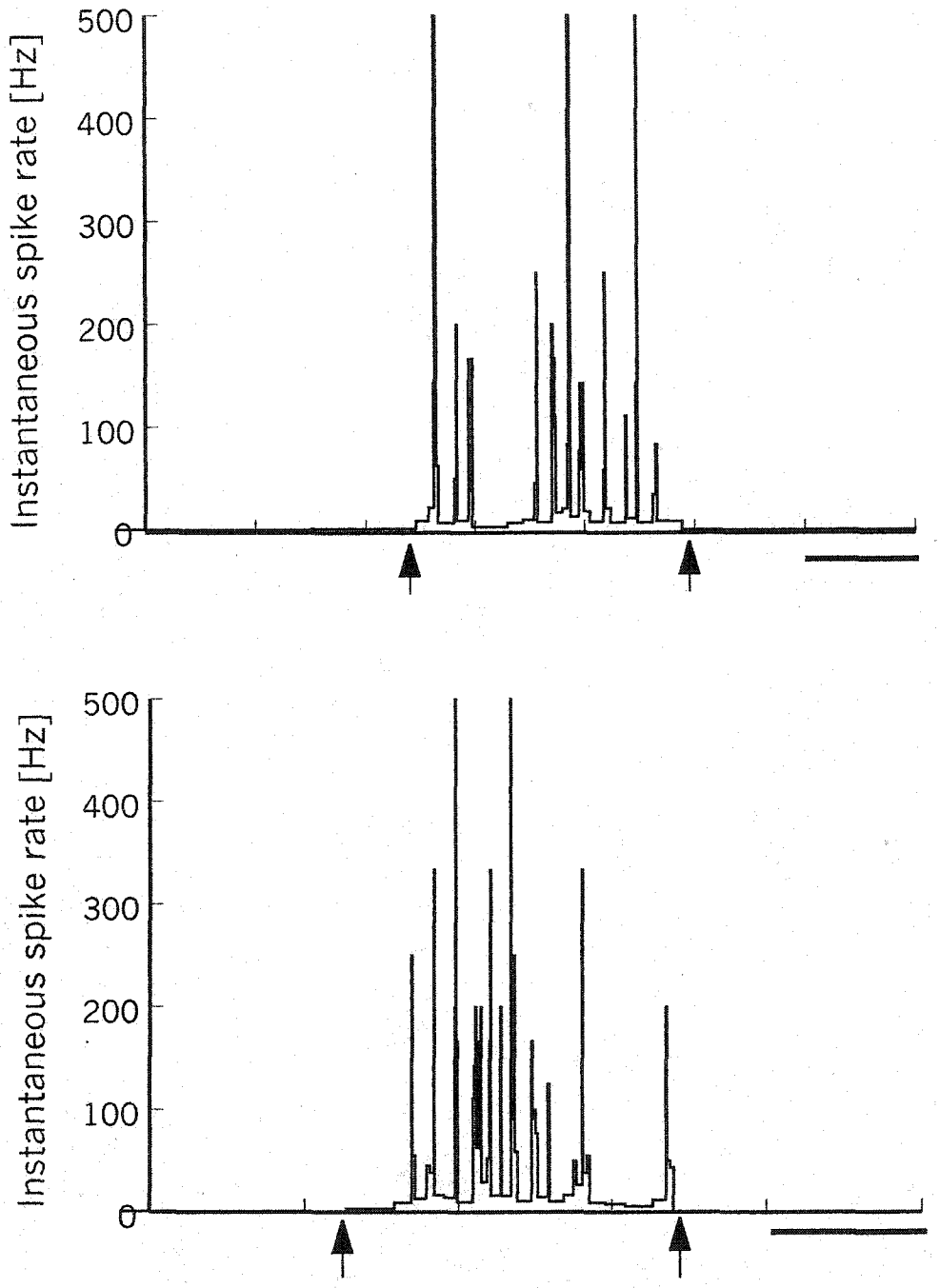

Instantaneous spike rate [Hz]
500
400
300
200
100
0
Instantaneous spike rate [Hz]
500
400
300
200
100
0

Figure 26 Multi-unit tectal responses evoked by turn to face. Responses from two separate experiments are shown. In A and C, spike histograms and head angles are plotted. As in Figure 24, turn to face is evidenced by the rapid change in angular orientation of the head. Bin width is 1/30 sec (1frame). Scale bar indicates one second in A and 0.5 seconds in C. In B and D, turn to face is illustrated with sequences of consecutive video frames. Spike counts per frame are plotted below. The image of the stimulus animal in each frame is coloured on a gray-scale to provide an alternate representation of spike rate.

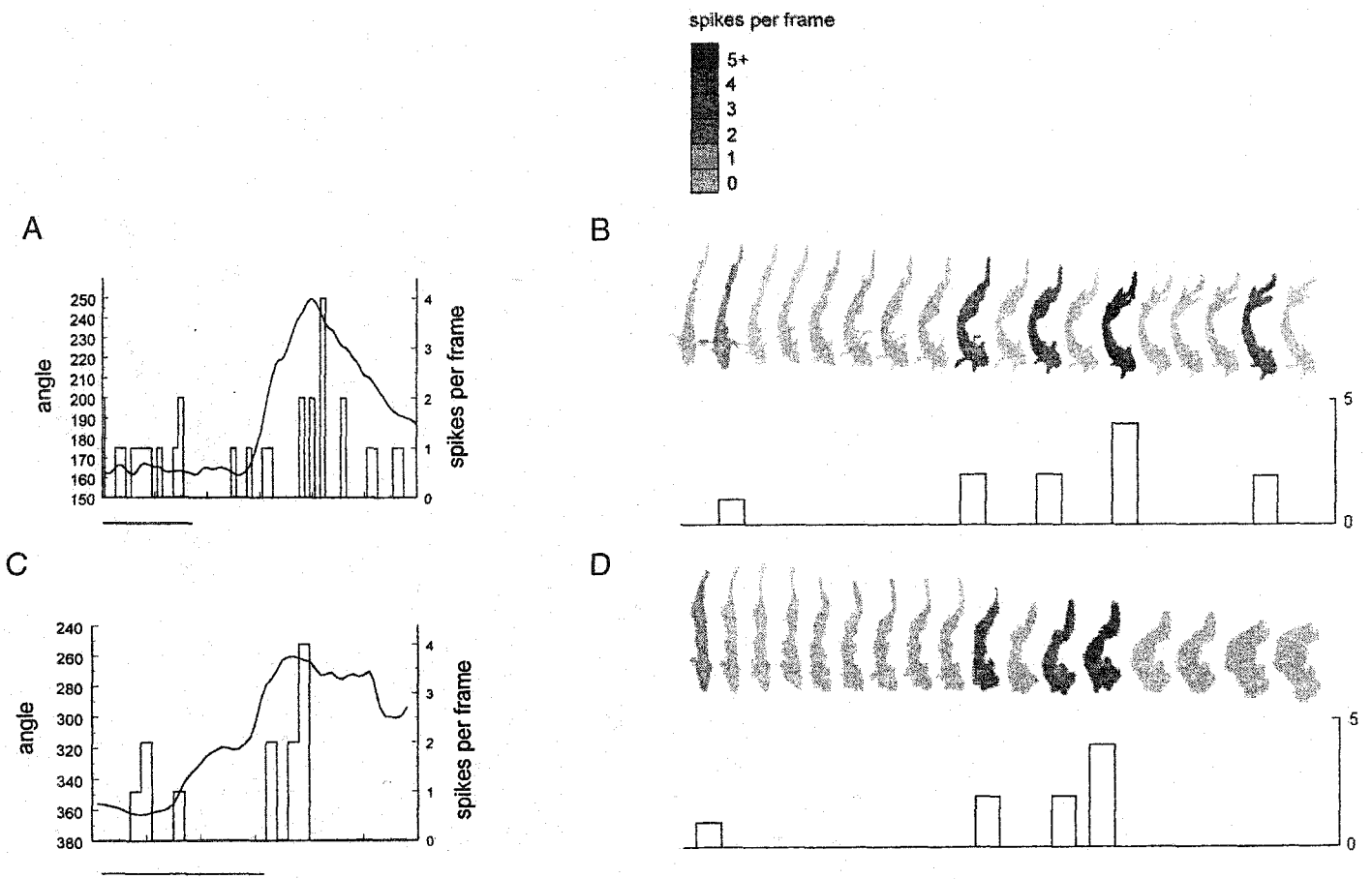

spikes per frame
5+
4
3
2
1
0
A
B
C
D
angle
spikes per frame
250
240
230
220
210
200
190
180
170
160
150
4
3
2
1
0
5
0
240
260
280
300
320
340
360
380

Figure 27 Multi-unit tectal response evoked by a displaying female showing turn to face. Scale bar; 1 second. Data are presented as in Figure 26.

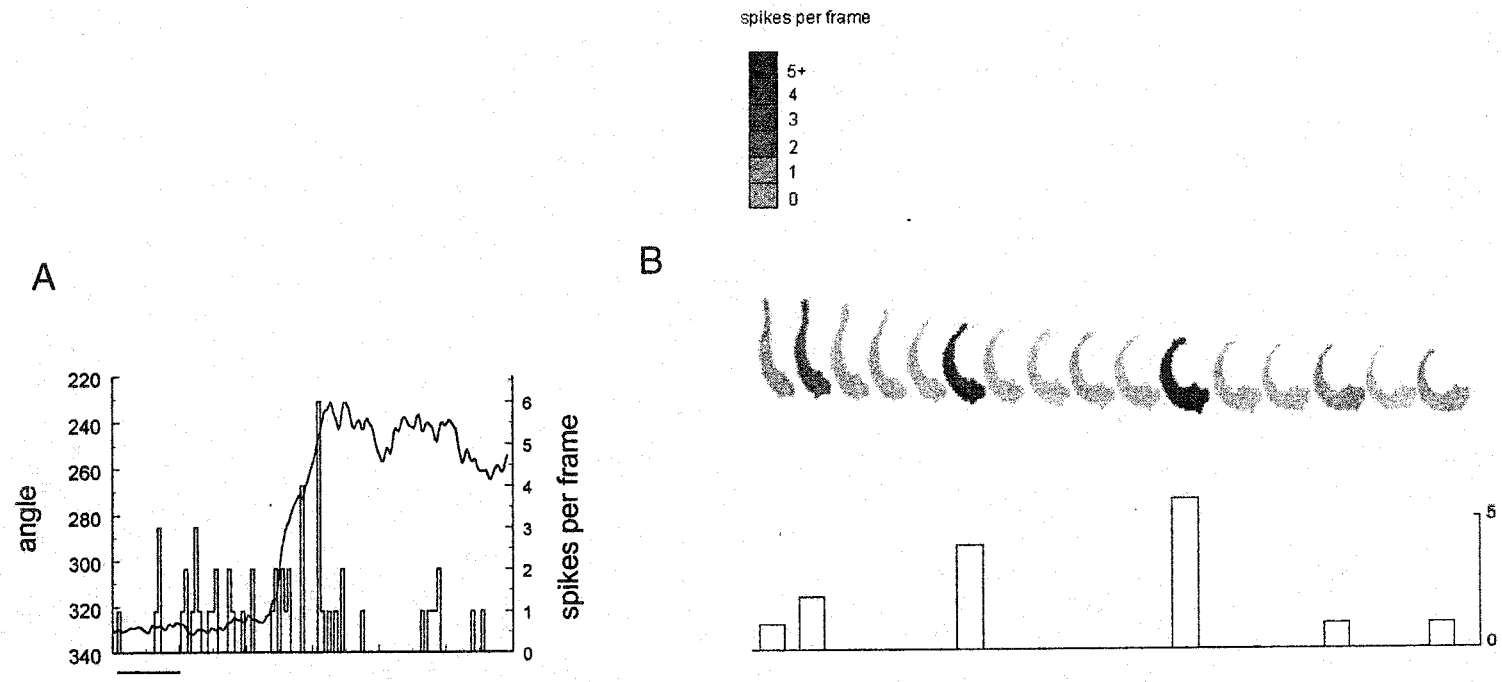
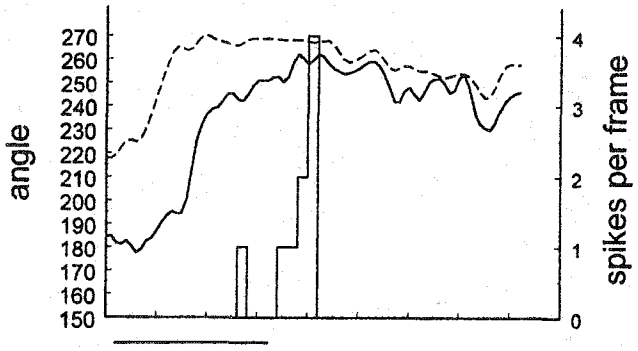

A
B
spikes per frame
5+
4
3
2
1
0
angle
220
240
260
280
300
320
340
spikes per frame
6
5
4
3
2
1
0
5
0

Figure 28 Tectal response to gill-cover erection. Data are presented as in Figure 26, with the exception that in A, gill-cover erection (solid line) is plotted in addition to head angle (dashed line). Scale bar; 1 second.

A


angle
270
260
250
240
230
220
210
200
190
180
170
160
150
spikes per frame
4
3
2
1
0

B

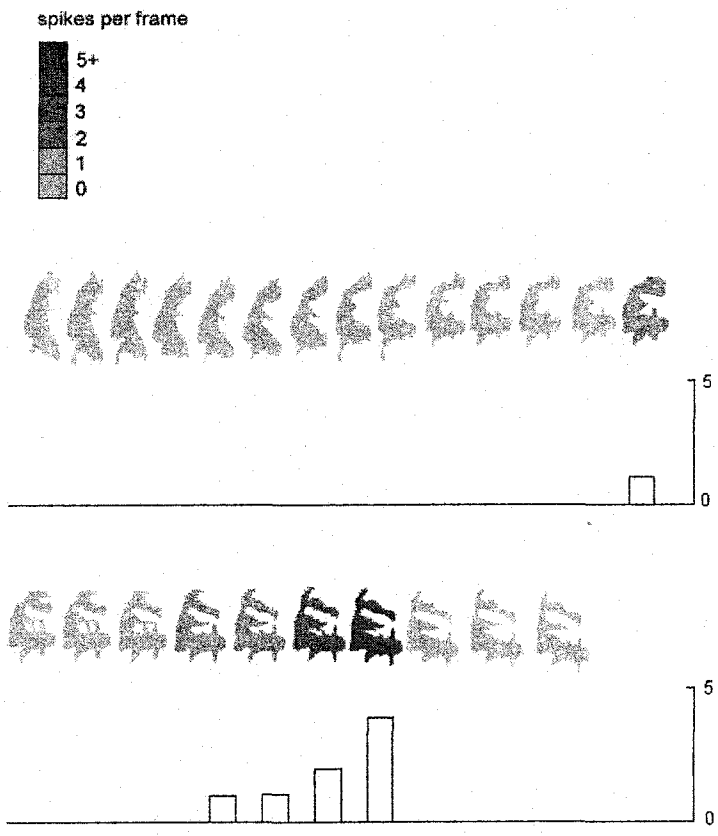
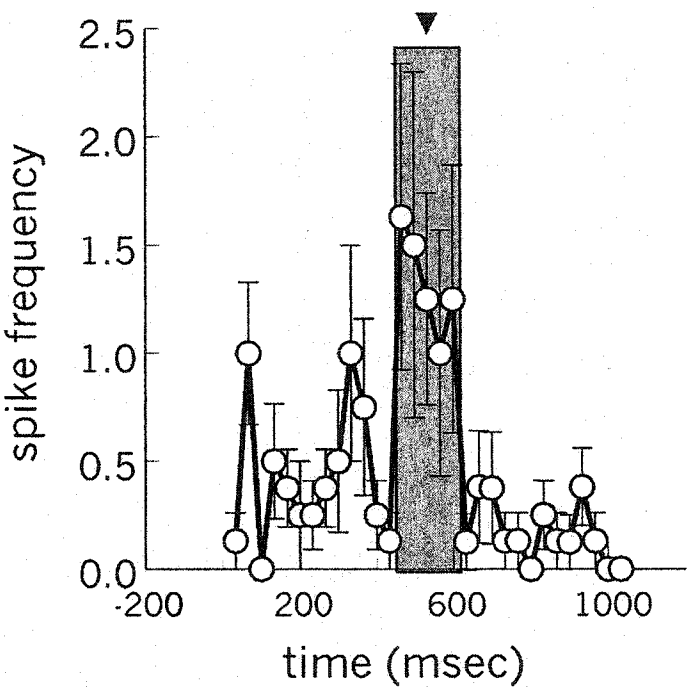

spikes per frame
5+
4
3
2
1
0
5
0
5
0

Figure 29 Average spike frequency plotted relative to the onset of full display. Bin width is 33.33 msec (1 frame). Data are from sequences one second in length. The time at which full display occurs is indicated by the arrowhead. The gray rectangle indicates the time epoch within which maximal spike frequency occurred in all animals. The mean was taken from single trials of four animals and four trials of a fifth animal (i = 5; n = 8). Error bars indicate +/- one standard error of the mean.


2.5
2.0
1.5
1.0
0.5
0.0
spike frequency
-200
200
600
1000
time (msec)

Chapter 5

Epilogue

Chromatic Processing

The data presented in chapters 2 and 3 provide compelling evidence for wavelength-dependent temporal properties of tectal units and cell populations. The next logical step would be to test the hypothesis that the observed temporal patterning of neural activity is indicative of a code for colour. To this end, both electrophysiological and behavioural studies must be employed. Specifically, it would be informative to use neuropharmacological agents in an attempt to alter the temporal structure of tectal potentials. One candidate would be the GABA blocker bicuculline, which can be expected to abolish the inhibitory components of the TEP. Care will need be to taken to ensure that the pattern, but not the power, of the responses is altered by pharmacological treatment. The next step would be to carry out behavioural colour discrimination experiments, preferably using the same animals whose tectal response properties have been physiologically characterized. This approach would be technically difficult, but would provide strong evidence in support of the role of temporal patterning in colour discrimination.

Future investigation would also benefit from employing simultaneous recording of unit activity and evoked potentials. Given that the properties of evoked potentials can not always be predicted from the pattern of unit activity, it is evident that concurrent unit and evoked potential recordings will provide information that would otherwise remain undetected (Bullock 1997). This approach would be particularly useful when studying the optic tectum, as intracellular recording is often difficult, owing to the small size of tectal neurons (Rowe 1980). Although intracellular recording will ultimately be required to confirm neural circuitry, simultaneous unit and evoked potential recordings can help to narrow the focus of intracellular investigations. Perhaps the greatest asset to be exploited is the use multi-electrode arrays to explore how neural assemblies interact to generate functional outputs. Multi-electrode recording and current source density analysis have recently been successfully employed to understand the mechanism underlying the temporal modulation of spatially separated neuronal populations in the turtle visual cortex (Prechtl

et al. 2000). The same approach can certainly be expected to provide novel information about the significance of temporally patterned neural activity within the tectum.

It is hoped that the work presented here has illustrated that new properties of colour-opponent neurons and neural assemblies likely remain to be discovered, and that the often overlooked methodology of evoked potential recording (generally discarded in favour of unit recording) can be valuable in uncovering new properties of the CNS. Although unable to provide definitive evidence of neural mechanisms, evoked potential recording offers an approach that can provide the initial evidence for new phenomena.

Natural stimuli in vision studies

Natural stimuli have so rarely been employed in vision studies that a necessary first step must be to determine their efficacy in evoking neural responses. Only then can one continue with more detailed studies. My investigation of visual perception of the agonistic display of *Betta splendens* has shown that it is feasible to use natural stimuli for vision research. I suggest that two primary avenues of research will benefit from this approach: (1) study of properties of sensory systems and (2) investigation of the perceptual world of animals.

When studying the organization of a sensory system one would expect that employing the kinds of stimuli that played a role in shaping its characteristics would provide considerable information. For example, receptive field mapping with natural stimuli may reveal unexpected, salient RF properties. It has been shown that the RF properties of individual neurons within the primary visual cortex of cats can differ as a function of general brain state (Worgotter *et al.* 1998). It seems reasonable to assume that ethologically relevant stimuli would evoke different brain states than simple, geometric stimuli, and that altered brain states must in turn signify modified response properties of individual cells. It is also important to consider that some neurons within visual processing areas remain silent when stimulated with the usual battery of artificial stimuli (Barinaga 1998). It is tempting to suggest that these neurons are 'waiting' for natural stimuli to cross their receptive fields.

One of the primary difficulties associated with natural stimuli is that they are typically complex. Consequently, it is difficult to attribute neuronal responses to the specific stimulus elements that evoke them. In this regard, an approach that involves deconstruction of a complex stimulus into simpler elements would be advantageous (Tanaka 1993). Using this method, the critical stimulus features responsible for evoking a response can be determined. These constituent stimulus elements may also prove to be appropriate for RF mapping studies.

Although some workers have recently employed the use of stimuli such as natural visual scenes to explore visual function (Vinge and Gallant 2000), natural, behaviourally relevant stimuli have rarely been adopted with the goal of determining the *Merkwelt* of an organism (chapter 4). Although behavioural studies can provide insight into what kinds of stimuli are biologically significant, a neuroethological approach employing natural stimuli can potentially offer insight regarding *how* visual percepts of these stimuli are generated. It is my hope that future investigators will increasingly adopt the use of natural stimuli, keeping in mind questions of both 'how' and 'what' stimuli are represented by the visual system.

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Appendix A: Parameters used in the simulation model presented in chapter 3.

The following function, taken from Nicholson and Llinas (1971), was used to model the waveforms of individual neural elements:

$$A(T) = \frac{-\alpha K}{2\sqrt{\pi T^3}} \cdot e^{-T} \cdot e^{\frac{-K^2}{4T}}$$

where K is a dimensionless constant and α is an arbitrary constant.

T= time (t)/ time constant (τ).

To model an EPSP, K was set at 1.60; the time constant (τ) was set at 6 ms. The EPSP waveform was inverted, reduced in amplitude and lengthened to represent an IPSP. Amplitude and time course were altered by reducing α and increasing τ, respectively.

Population postsynaptic potentials were modeled by linear summation of individual neural elements (generated with the Nicholson-Llinas function) over time using a Gaussian distribution. Temporal spacing of inter-PSP intervals was varied according to the Gaussian, allowing for manipulation of the shape of the population PSPs. The characteristics of the Gaussian distributions used are provided below. The experimental data were modeled using an alternating sequence of two population EPSPs and two population IPSPs. That is to say, two consecutive population EPSPs, each immediately followed by a population IPSP.

Table A Properties of individual and population postsynaptic potentials (PSPs).

	EPSP/IPSP	PSP width (ms)*	Number of PSPs summated	Population PSP width (ms)*	Gaussian width (ms)
N1	EPSP	2.9	34	19	20
N2	EPSP	2.9	47	23	30
P2	IPSP	43	1	NA	NA
P3	IPSP	43	23	46	37.6

*width at half amplitude