

## Automated retinotectology

The retinotectal projection has been the principal battleground, since the 1930s, for the experimental investigation of the factors determining specific neural connections. Since the late 1950s, the electrophysiological determination of the retinotectal map has been the dominant experimental method. Many of the conclusions derived with this method have been questioned (by other investigators using the same method), and some of these disputes remain unresolved even today. Recently, David Northmore and his associates have developed two automated methods for assessing retinotectal function which are described below. In different ways, these methods correct some of the deficiencies of the old method.

There are at least two difficulties with the standard retinotectal mapping procedure. The first is that the microelectrode in the tectum records electrical activity which, as everyone agrees, does not necessarily originate from tectal cells. This is not a fatal flaw; even if the electrical activity is assumed to be presynaptic, it reveals where the axons are arborized, and this is not trivial. Even so, one would like to know if synapses are established. The second problem is the low signal to noise ratio in most experimental tecta. This is probably a consequence of the fact that regenerated and sprouted axons are physically smaller than the original processes, and therefore give smaller action currents. Many of the points in a map may have been decided by the experimenter with some uncertainty, but none of this doubt comes across in the published map. In other words, the experimental results are in fact well digested and filtered by the observer, and so readers of the work, who may well have a different set of biases, suspect that they might have obtained a different map, under the same conditions.

The first of these difficulties is a technical one, and could be overcome by the development of a method sensitive only to postsynaptic activity, an automated method of behavioral perimetry is one answer<sup>1</sup>. The second difficulty is more a matter of personal intuition and mistrust, about the only technical fix here is to display the results in a less digested form, or else to make the decision-making unquestionably objective. Northmore and Masino<sup>2</sup> have opted for the latter.

Behavioral perimetry has been used to assess the retinotectal projection for many years. The behavioral signs of vision, such as the optomotor response to a rotating striped drum, or the orientation response to a prey object, establish that vision has returned, but they indicate nothing about where in

the brain the signalling fibers have projected. Sperry<sup>3</sup> combined experimental surgery with the orientation response to establish that the retina's projection to the tectum was the important one, and he showed that it was spatially ordered, what the order was, and that the regenerated order

resembled the original order. He did this by ablating either the caudal, dorsal, or rostral quadrant of the tectum in frogs (The ventral quadrant was surgically inaccessible.) Following this surgery, the animal was then presented with prey, and was found to have regional blind spots. For example, after the caudal lesion, the frog oriented to prey presented everywhere except in the posterior field of the eye contralateral to the surgery. This was interpreted to mean that the retinal axons with receptive fields in the posterior field – those in nasal retina – no longer contacted the tectal cells responsible for the orientation response. Analogous results were obtained for different parts of the visual field after the other lesions. When similar lesions were made in frogs with

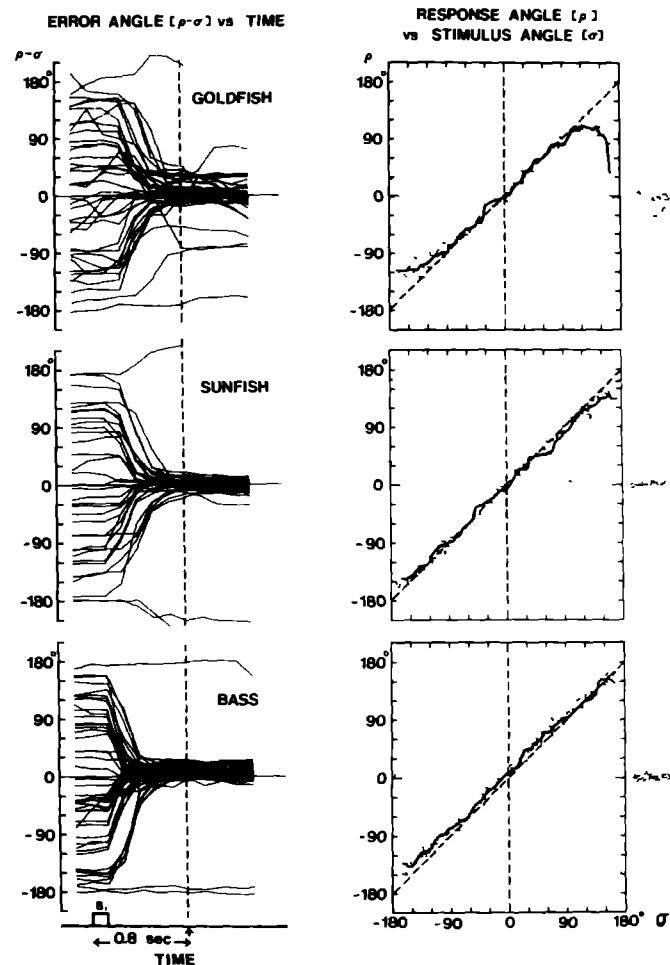


Fig. 1 The left column shows the error angle, the difference between the fish's orientation and the target, as a function of time. By convention, the initial error is positive for stimuli in the left field, negative in the right. The right column shows the final response angle (vertically) vs the stimulus angle (horizontally). Each point is one trial. The dashed diagonal lines show perfect behavior. The solid line is a running weighted median of the data. The points to the right of the boxes show the results of dummy trials, which were conducted, without previous stimuli, when the fish was centered. (Reproduced, with permission, from Ref. 1)

regenerated optic nerves, the same lesions produced the same scotomata as in normals, evidence that the regenerated projection resembled the original. More recently, others have used slightly different behavioral methods to assess visual function in fish<sup>4,5</sup>, but both of these required that the animal be restrained, whereas the 'automated behavioral perimetry' is carried out on freely-swimming animals.

The aim is to have the fish indicate that it sees a target, the location of which is set by the experimenter. The fish swims in a cylindrical arena with equally spaced light-emitting-diodes on the walls. The fish is trained to orient toward a light when it turns on. With the use of a minicomputer to control the presentation of the stimuli and the collection of data, a large number of trials was possible. The computer turned on the stimulus, very briefly, and then analysed a video record of the fish's activity. This record was obtained from a video camera which viewed the fish from below, in silhouette. The analysis involved recognizing which end of the fish was the front, and then fitting a straight arrow through the fish's profile, thus establishing where it pointed. Fig. 1 shows some results, and two conclusions are evident. First, the method works impressively well, as it produces results with very small variability on a completely unrestrained animal. Secondly, it shows that the fish has an absolute sense of spatial position, since the stimulus was illuminated so briefly that the fish had not even begun to orient before the light went out. Therefore the fish could not have servoed in on the target, but instead made a ballistic movement.

The method was validated further by crushing one optic nerve, which resulted in the expected lack of an orientation response to that side, and by removing the caudal half of the tectum on one side. This abolished responses to stimuli in the posterior field on the contralateral side, as Sperry's results on frogs predicted. But since 1970, it has been known that when the caudal hemitectum is removed and the optic nerve severed, the projection from the entire retina compresses into the tectal remnant<sup>6</sup>. Northmore<sup>7</sup> reproduced this result behaviorally, as Fig. 2 illustrates, confirming an earlier behavioral study<sup>5</sup>. Moreover, since the response depended on accurate motor behavior,

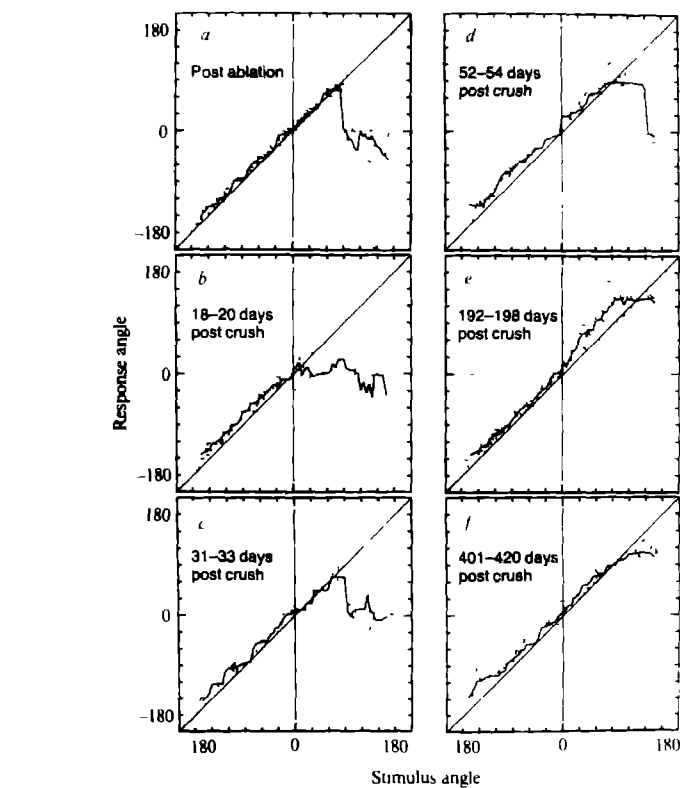


Fig. 2. Similar to the right column in Fig. 1. The caudal half of the left tectum was ablated, and the data in panel A obtained. Thirteen days later, the right optic nerve was crushed, and panels B-F were obtained thereafter. Note that the caudal right field was initially neglected, but gradually filled in to approximate the normal case. (Reproduced, with permission, from Ref. 7.)

the result demonstrated that the tectal 'motor map' was not fixed anatomically, but must also be modifiable as the sensory map was known to be.

Most recently, visuomotor perimetry has been supported by parallel automated electrophysiological maps.<sup>2</sup> As usual, the fish is immobilized, and the experimenter inserts a microelectrode into a tectal location. But in contrast to the usual, the experimenter then turns the decision-making over to a computer. The computer moves visual stimuli around the visual field, records the electrical responses to each stimulus location, and then decides where the receptive field is, on the basis of which locations evoked the biggest response. Of course, this is the same procedure that is ordinarily carried out by the experimenter, using his or her own hands and sensory apparatus, but the objective aspect of the decision making removes any suspicion of bias from the final result. There were no surprises in this experiment, the objectively derived map was similar to those acquired by more conventional means. One supposes that when the technique is applied to

experimentally altered projections, there will be less reason to doubt the results, given that the computer is free of prejudice.

In recent years, the field of retinotectology has shed its earlier reputation as a contentious 'random noise generator' (a description once offered at a meeting). The introduction of modern anatomical techniques and now, automated behavioral and electrophysiological ones, should further assist this metamorphosis.

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## The primate social environment, brain neurochemical changes and psychopathology

*Changes in cerebrospinal fluid (CSF) neurochemical measures associated with both depression and schizophrenia have been reported and replicated in several studies. Nevertheless, the causes of these changes remain uncertain. Neurochemical differences may be secondary effects of behavioral changes, altered diet, daily routine, and perhaps drug use. Alternatively, if neurochemical changes produce a behavioral disorder, then a further question arises as to what caused these changes in the first place. Recent animal studies suggest that disruption of social development or ongoing social relationships could cause some of these changes.*

There are a considerable number of neurochemical studies of human patients which focus on the catecholamine (CA) and indoleamine neurotransmitter biosynthetic and degradative pathways. In the CA pathway, the amino acid precursor, tyrosine, is converted to dopamine (DA). DA is then converted to norepinephrine (NE). In primates the major metabolite of DA is homovanillic acid (HVA), and the major metabolite of NE is 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). The precursor of the indoleamine neurotransmitter, serotonin (5-hydroxytryptamine, [5-HT]) is tryptophan. Its major metabolite is 5-hydroxyindoleacetic acid (5-HIAA). Measures of NE, HVA and 5-HIAA in CSF samples obtained from the cerebral ventricular system, or from the cisterna magna, are correlated with activity in periventricular brain neuronal mechanisms in nonhuman primates. Similar but less robust relationships can be demonstrated in CSF obtained at the lumbar region of the spinal cord in humans.<sup>1,2</sup>

The consistency of the relationship between changes in lumbar CSF neurochemicals and changes in behavior appears to be most potent in humans with higher CSF NE or lower CSF 5-HIAA concentrations than controls. Elevated CSF NE is associated with mania, anxious depression, and paranoid schizophrenia.<sup>2,3</sup> Reduced CSF 5-HIAA is associated with interpersonal violence and suicide. Studies of men with histories of violent aggression have related increased aggression to low concentrations of CSF 5-HIAA.<sup>4,5</sup> Suicide attempts by patients with low 5-HIAA are more frequent and more violent.<sup>6</sup> In a follow-up study, Traskman-Benz *et al.*<sup>7</sup> found that 6 out of 30 patients with CSF 5-HIAA values below the median of a 119 patient sample had committed suicide within one year of discharge from the hospital. The cause of

increased CSF NE or reduced 5-HIAA in humans remains obscure. However, five major factors that affect neurotransmitter and metabolite concentrations in CSF have been identified in human and nonhuman primates. They are:

(1) *Time of day* Several neurotransmitters and metabolites have diurnal rhythms in nonhuman primates. Among these are NE, DA, 5-HT, and HVA. CSF CA rhythm parallels wakefulness, activity, and body temperature, and is out of phase with 5-HT, cortisol, prolactin and testosterone.<sup>8,9</sup>

(2) *Precursor availability* Serotonin systems are affected by intake of precursor amino acids. CSF 5-HIAA increases with increased dietary tryptophan in humans.<sup>10</sup> CSF 5-HIAA is correlated with plasma tryptophan in vervet monkeys (*Cercopithecus aethiops*) and correlations are higher in males than females.<sup>11</sup> Dietary amines do not affect CSF NE concentrations in humans.<sup>12</sup>

(3) *Gender* CSF 5-HIAA, HVA and MHPG concentrations are lower in male adult (feral reared) vervets.<sup>11</sup> In humans, gender differences have been reported in some studies<sup>13</sup> but not others.<sup>14</sup> Of the variety of factors that can effect CSF neurochemical measures, most of them appear to have a greater impact on males.<sup>11,13,14</sup>

(4) *Development* HVA is higher in human infants and juveniles than in

adults. Developmental curves are most prominent in males. CSF HVA declines to adult levels by approximately 25 years of age.<sup>14</sup>

(5) *Social environment* Over and above the aforementioned factors, social interactions can markedly and persistently alter measures of neurotransmitter metabolism in nonhuman primates.

The quality of early social rearing experience produces differences in brain NE system function that lasts into adulthood in rhesus monkeys (*Macaca mulatta*).<sup>15</sup> Increased NE system activity has been related to anxious behavior<sup>16</sup>, and CSF NE concentrations may predict individual behavioral responses to social stressors. Increased CSF NE is associated with active or aggressive responses to social stressors.<sup>15</sup>

Several studies suggest that 5-HT mechanisms influence social interactions in nonhuman primates, and reciprocally, that social interactions affect 5-HT mechanisms. Early social separation and isolation in both human and nonhuman primates causes reductions in platelet 5-HT which return to normal when social interactions are restored.<sup>17</sup> Blood and CSF measures are positively correlated, especially in males.<sup>11</sup> Social dominance in male vervets, i.e., receiving deference from other males, is a primary social regulatory mechanism for aggression. Dominance is positively correlated with CSF 5-HIAA and whole blood 5-HT (WBS) concentrations.<sup>18</sup> Changes in male social dominance, whether naturally occurring, or experimentally induced, have a reciprocal effect on 5-HT mechanisms. Dominant vervets have WBS levels twice as high as subordinates, and these levels drop if dominance is lost.<sup>19</sup>

Social environment may affect CSF NE, HVA and 5-HIAA concentrations in humans as well. Repeated episodes

TABLE I. Factors currently associated with changes in CSF norepinephrine and 5-hydroxyindoleacetic acid concentration in human and nonhuman primates

CSF neurochemical	Association and direction of concentration change	
	Human	Nonhuman
Norepinephrine	mania ↑ paranoid schizophrenia ↑ anxious depression ↑ duration of hospitalization ↑	social stressors ↑ irritable aggression ↑ agitation ↑ circadian rhythm (day ↑, night ↓)
5-hydroxyindole-acetic acid (5-HIAA)	suicide? violence ↓ (when paired with NE ↑) repeated affective illness ↓	male social dominance ↑ subordination ↓