Project for the Workshop

Title: "The role of the retinal bipolar cells in the sequential processing of the visual information"

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- **The retina as a model of the Central Nervous System:**

Since Cajal's works were published the retina has been considered a model of the Central Nervous System (CNS) in reason of two facts. The first one is that the retina is the only part of the CNS accessible to the simple clinical exploration, and it is also very to perform experimental manipulations. The second reason is that it has a very well organised nature, so it has been quite easy to find some of the circuits that underlay the processing of the visual information. The understanding of the retinal information processing can help to improve the electronic-computational devices inspired in the CNS.

The retina transforms the energy, from luminous to electrical and this to chemical. So, the visual information travels through the CNS and allows the animal to perceive the visible world. However, the retina is much more than a transductor of the light into electrical energy. Nowadays, it is classical the explanation of the "Parallel Processing" of the information performed by the retina (for review see Boycott and Wässle, 1999; Wässle and Boycott, 1991; Kolb, 1994). The retina performs a sequential as well as a parallel processing of the information. In this workshop we propose a short review to have a look to the first steps in the processing of the visual information. We will focus our explanation and examples in the scotopic or nocturnal pathway.

- **The cellular components of the retinal circuits:**

The neuronal circuits are composed by different neurons and some glial cells. Each cellular type has her own characteristics and perform a kind of response. There is some tendency to consider the neuron as a mere part of this "biological" circuit, but to date is more and more evident that the cells are overall dynamics.

The cellular response cause radical changes in the cellular membrane proprieties as well as in the intracellular space composition. Such cellular changes perturb the closest extracellular environment. So, each cellular response changes the physiochemical characteristics at each step of the circuit. The membrane changes and reacts to the stimulation by the modification of the ionic channels and co-transporters and other membrane components, activating not only the enzymatic cascades but also modifying the intracellular ionic concentrations.

Another important point in the central nervous system is that the extracellular space is quite"virtual". Most of the "interneuronal" spaces are occupied by the intracelular space of any glial cell. So, the intracellular space composition is crucial to the cellular response and so to the circuit and to the system response. In fact, the cellular prolongations of the Müller glial cells are virtually around every little space in the retina.

It is necessary to consider each cell, neuronal and glial, as a very active component of the circuit and to consider the environment of the cells as a potential modulator of the transmitted signal.

Functionally all the retinal circuits can be grouped in two main pathways, the vertical pathway (from photoreceptors to ganglion cells) and the transversal pathway (mediated by horizontal and amacrine cells). The first one mediated by **glutamate** is considered the "transmission pathway" and the last one mediated mainly by **GABA**, the "modulation pathway". This structure give an idea of a sequential processing of the information, one step, from photoreceptors to bipolar cells modified by the horizontal cells activity would determine the next step, from bipolar to ganglion cells modified by the amacrine cells activity. In this basic organisation the bipolar cells have a central role: they are the *"key"* between the two main steps of retinal processing.

- **The rod bipolar cells as integrative units:**

The bipolar cells are second order interneurons in the pathways of the retina; they receive the inputs from the photoreceptors and send the outputs to the ganglion cells. They participate of the first synapse of the retina, where they make contact the photoreceptors, the horizontal and the bipolar cells in the called *"triads"*. In the inner plexiform layer participate of the second synapse of the retina linking with at least two types of amacrine cells. In fact, the retina is structured in the way that any spot of light that fall on any part of the retina affects to at least one cells of each type of bipolar cells. The visual information pass across these cells, thus any modification of their state will affect the transmitted information.

Most of the models of the retina make their point in the modulation at the synaptic level, considering the horizontal and the amacrine cells activity as the main factor of modulation of the information processing. The division in the "vertical-transmission pathway" and the "transversal-modulation pathway" allows this type of interpretation. However, we should not forget that the whole visual information passes across the bipolar cell population.

There are described between eight and ten types of bipolar cells, from them, in the mammalian retina, only one is considered to contact to the rod photoreceptors; the rod bipolar cells (RBC). These cells have a clear heterogeneous distribution of different membrane components. In fact, they have two different ionotropic GABA receptors, the $GABA_A$ and the $GABA_C$, that shows a different kinetic and a selective subcellular localisation (Lukasiewicz *et al.*, 1994; Qian and Dowling, 1995; Lukasiewicz, 1996; Lukasiewicz and Wong, 1997; Fletcher *et al.*, 1998; for review see Feingenspan and Bormann, 1998). The $GABA_A$ receptors are located mainly in the dendrites of the rod bipolar cells (Vaquero *et al.*, 1995). They have a quicker kinetic and they show a clear desensibilisation (Ishida, 1992, Feingenspan *et al.*, 1993). The $GABA_C$ receptors are located in the axon terminals of the rod bipolar cells (Vaquero *et al*., 1995). They have a slower kinetic and they show no evident desensibilisation (Feingenspan *et al.*, 1993; Feingenspan and Bormann, 1994; Bormann and Feingenspan, 1995; for review see Lucasiewicz, 1996). They also have a specific distribution of the glycine receptors, thus the concentration of these receptors is higher in the dendrites than in the axon terminals. The RBCs are ON-type cells (the light excites them), and so they have the metabotropic

glutamate receptor mGluR6. These receptors are located almost exclusively in the very tip of the dendrites of these cells.

These cells have a heterogeneous distribution not only of the ligand-dependent receptors but also of the membrane ionic co-transporters. They have two types of chloride co-transporters the KCC2 and the NKCC (Vardi and Sterling, 1994; Vardi *et al.*, 2000). The KCC2 is located in the dendrites and it is known to introduce chloride into the cell. The NKCC is located in the axon terminals of the RBC and it is known to extrude the chloride from the cell (Russel, 2000). It has been described a gradient in the intracelular chloride concentration in the RBC that allows an opposite response to GABA in the dendrites and in the axon terminals of these cells (Varela *et al.*, 2004). The KCC2 has been implicated in the type of GABAergic response, excitatory or inhibitory, during the developing of the Lateral Superior Olive Neurons (Kakazu et al., 1999). To date it has been shown that the control of the intracellular chloride concentration is also crucial in the GABAergic response of the Hippocampal cells (Stanley *et al.*, 1999; Wardle and Poo, 2003; Woodin *et al.*, 20003), in the circuits of the Substancia Nigra of the rat (Gulacsi *et al.*, 2003) and in the rod bipolar cells of the retina (Varela *et al.*, 2004).

The bipolar cells are not only a new step in the parallel processing of the visual information but also a sequential unit of integration of the message transmitted in this moment by this circuit of the retina. It have one pole that can be called "input" component (Figure 1), where the information arrive from photoreceptors and horizontal cells, an intermediate component of transmission, and an "output" pole where the message is still modified by the amacrine cells and then sent to the ganglion cells. These modifications of the cellular activity to the final "output-message" are dependent not only of the "synaptic modulation" but also to the "cellular-state modulation".

So in the "input" component of the RBC, the dendrites, there are the glutamate receptors mGluR6 that generated the cellular response, activation when the light arrives to the retina. The GABA receptors can modify the glutamatergic response, and they are extremely dependent of the intra and extracellular chloride concentrations. In the dendrites is located also the chloride co-transporter KCC2 that is known to allow a high intracellular chloride concentration. The activity of this chloride co-transporter is dependent of different intracellular factors as the calcium concentration and the pH of the cell and it is also dependent of extracellular factors as the extracellular chloride concentration or the HCO_3^- or the BDNF (Figure 1).

If we accept this idea of the bipolar cell as a unit of integration of the information we should consider the influence of the Müller glial cells in this integration. It has been already explained the influence of the intracellular ionic concentrations in the postsynaptic effect of a neurotransmitter as the GABA. Such intracellular ionic concentrations are in some extreme dependent to the ionic co-transporters and so by the extracellular ionic concentrations, the Müller Glial cells determine and control this extracellular space (Fig. 1).

- **The "functional units" in the circuit:**

As it has been already said the bipolar cells are interneurons localised between the outer plexiform layer and the inner plexiform layer. In the first one they make contacts with the photoreceptors as well as with the horizontal cells. It has been proposed that horizontal cells mediate the surround inhibition to the bipolar cells. For that assumption it is necessary that ON BC (rod- or cone-driven) depolarises with GABA (Vardi and Sterling, 1994). This

"excitatory behaviour" by GABA has been already described in the immature CNS and in certain mature cells.

The horizontal cells have a centre-periphery antagonism so they are depolarised when no light (dark condition) strikes on the centre of their receptive field. In these conditions they free the GABA to the first synapse of the retina. When light affects the periphery of the horizontal cell's receptive field the GABA from them inhibits the OFF bipolar cells that are already inhibited. If GABA would inhibit the ON bipolar cells, which have ionotropic GABA receptor in their dendrites, then HC can not mediated the surround inhibition of these cells: they will activate the bipolar cells with surround illumination and inactivated them with the centre illumination. So, at the dendrites an inhibitory effect of the GABA released by the HC would act against the activation of the ON rod bipolar cells when they must be activated. On the other hand, an excitatory effect of GABA in these cells will enhance the contrast detection at the borders.

As we have already said the action of a neurotransmitter in a postsynatic cell depend not as much of his nature but of the nature of the postsynatic receptors. Moreover, the response of an ionotropic receptor depends of the intracellular ionic concentration in this postsynatic cell. Then an effect "excitatory" or "inhibitory" in response to the activation of ionotropic GABA receptors will depend of the intracellular chloride concentration. The intracellular chloride concentration depends not only of the glial elements but also of the bipolar cell itself.

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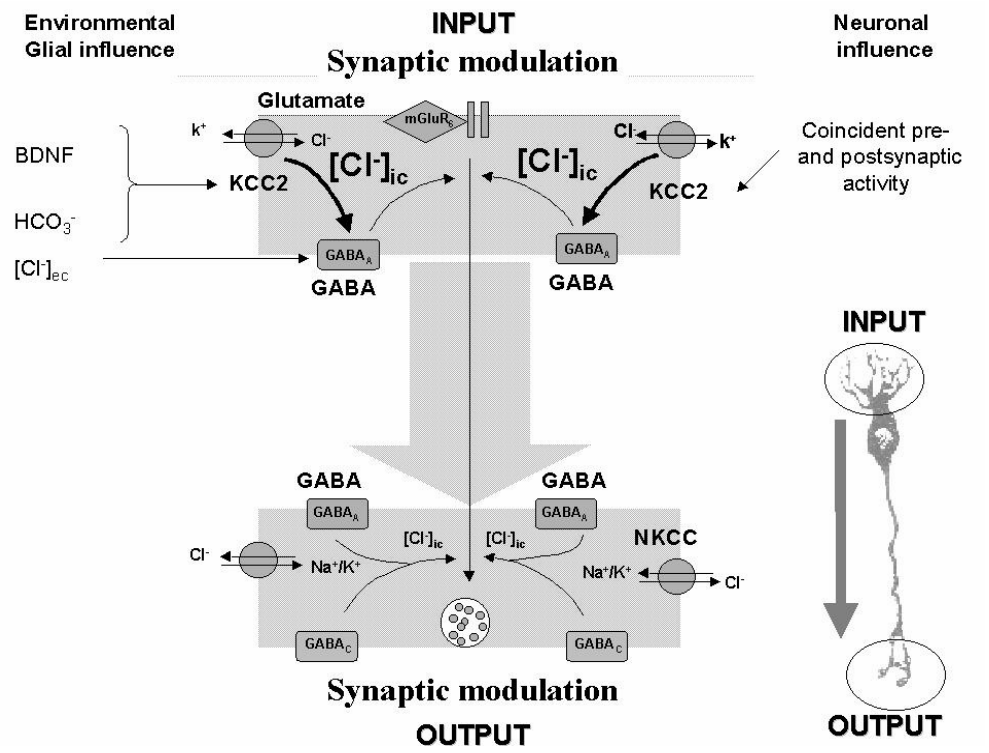

Figure 1: *The bipolar cell as a sequential unit of integration of the information*. The RBC has a clear polarisation in her morphology with a "input pole", the dendrites and a "output pole", in the axon terminals. There is also a polarisation of the membrane components, as the receptors of Glutamate and GABA or the ionic co-transporters. On other hand, the activity of some membrane components, different to the classical neurotransmitter receptors, as the KCC2 can be modified by intra and extracellular elements and they can also modify the response to the neurotransmitters, and to modify the cellular response. So, the intracellular state each moment of the cell determines the response to stimulus at this moment. The bipolar cell in the right bottom is a picture modified from Cajal.