● PERSPECTIVE

Serotonin controls axon and neuronal regeneration in the nervous system: lessons from regenerating animal models

Traumatic brain injury (TBI) is a mechanical injury to brain tissue that leads to an impairment of function and a broad spectrum of symptoms and disabilities; often, it is followed by diffuse axonal injury, which causes denaturation of the white matter and axon retraction, leaving patients with severe brain damage or even in a persistent vegetative state. Spinal cord injury (SCI) is defined as a lesion within the spinal cord that results in the disruption of nerve fibre bundles that convey ascending sensory and descending motor information. In mammals, including humans, SCI can lead to permanent disability and an irreversible loss of function below the site of injury due to the disruption of motor, sensory and autonomic systems. The inability of axons to regrow within the injured central nervous system (CNS) of adult mammals is a fundamental feature that explains the poor regenerative capacity observed after TBI or SCI. In addition, TBI and SCI cause a loss of cells (neurons and glia) due to the primary physical injury. Also, after the primary injury, a cascade of secondary injury events expands the zona of neural tissue injury causing further cell death.

In contrast to mammals, invertebrates and fishes are able to recover spontaneously from traumatic nervous system injuries. The process of recovery in these animals involves regenerative events, including the production of new neurons and glial cells to replace those lost after the injury and the regeneration of axotomized axons to reconnect to their appropriate targets. Studies in these animal models can help to find molecules that control the spontaneous regeneration of cells and axons in the CNS. The aim is to use the information obtained in these models to propose new therapies in pre-clinical studies using non-regenerating models of TBI or SCI. One of the strategies to find molecules involved in CNS regeneration is to use the information provided by developmental studies. Regeneration is not a complete recapitulation of developmental processes, but a number of studies have shown that a large proportion of the molecules involved in CNS development also play a crucial role in CNS regeneration.

More and more studies are revealing the role of classic neurotransmitters in CNS development. However, fewer studies have looked at the role of neurotransmitters in regeneration following a traumatic nervous system injury; even when these point to a key role of neurotransmitters in axon or cell regeneration. One of the main neurotransmitter candidates to be a mediator in regenerative processes in the CNS is serotonin (5-HT). 5-HT plays an important role during the development of the nervous system; for example, in dendrite and axon growth (reviewed by Trakhtenberg and Goldberg, 2012). Here, we review the current knowledge on the role of this neurotransmitter in cell and axon regeneration based on *in vitro*, *ex vivo* and *in vivo* studies in regenerating species (*e.g.*, invertebrates and fishes). This review shows that 5-HT plays an important role both in the modulation of axon re-growth and in the generation of new neurons after nervous system injury in regenerating species. We also give our own perspective on how the study of the role of 5-HT in nervous system regeneration should move forward. Manipulating neurotransmitter systems like 5-HT could be an innovative way to promote regeneration after CNS injuries.

The role of 5-HT in axon re-growth and regeneration: In **Additional Table 1**, we present a summary of the different pharmacological and genetic manipulations that have revealed a role of the serotonergic system in nervous system regeneration. Three studies have looked at the role of 5-HT in axonal regeneration in invertebrate species (Murrain et al., 1990; Koert et al., 2001; Alam et al., 2016). In *Caenorhabditis elegans*, 5-HT promotes axon regeneration (Alam et al., 2016). Non-serotonergic neurons of *C. elegans* temporarily express tryptophan hydroxylase (*tph*; the rate-limiting enzyme in 5-HT synthesis) in response to axotomy promoting their own regeneration. Mutations in the tph gene caused deficiencies in axon regeneration in *C. elegans* and *tph* upregulation after injury appears to be induced by hypoxia inducible factor-1 (HIF-1; Alam et al., 2016). However, Koert et al. (2001) reported a different role of 5-HT in axon regeneration by studying the regenerative properties of serotonergic cerebral giant cells (CGCs) of the snail *Lymnaea stagnalis*. CGCs of this snail are able to regenerate and reform functional synapses after a nerve crush. This work showed that autoreleased 5-HT is inhibitory for neurite outgrowth in cultured CGCs and suggested that, *in vivo*, 5-HT acts as a regulator of adequate guidance and branching after nerve crush by the induction of growth cone collapse (Koert et al., 2001). An *ex vivo* study in the pond snail, *Helisoma trivolis*, also showed that the spontaneous regeneration of B19 and C1 neurons was inhibited by the application of 5-HT (Murrain et al., 1990). In this model, 5-HT seemed to cause its inhibitory effect by increasing intracellular calcium levels (Murrain et al., 1990). In this sense, are also the results obtained from studies that used the *ex vivo* goldfish, *Carassius auratus*, retina as a model. Neurite regeneration of neuronal explants of the goldfish retina, after a crush injury of the optic nerve, was decreased by 5-HT (Lima et al., 1994). This was confirmed in the *ex vivo* cultured post-crush retina of goldfish after the application of 5-hydroxytryptophan (the precursor of 5-HT synthesis), imipramine (a monoamine reuptake inhibitor) or citalopram (a 5-HT reuptake inhibitor), which also inhibited neurite outgrowth (Lima et al., 1994, 1996). After an optic nerve lesion in goldfish, 5-HT levels drop initially and then they recover to control levels 10 to 15 days later, which led to hypothesize that 5-HT may be acting as an outgrowth inhibitor to contribute to proper optic nerve regeneration in goldfish (Lima et al., 1994). These studies indicate [with the exception of the *C. elegans* work by Alam et al. (2016)] that 5-HT exerts mainly a negative effect on axon re-growth.

Taken together, the results from the studies in *C. elegans vs*. snails and goldfish might seem to be contradictory; but, we should consider that the extracellular 5-HT signal is transduced to the intracellular milieu by a variety of 5-HT receptors. 5-HT receptors are a large and diverse family of receptor proteins with different signal transduction mechanisms, which could explain the different effects of 5-HT in different neurons, species and injury models. In the following section, we present the current knowledge on the role of 5-HT receptors in axonal regeneration.

The role of 5-HT receptors in axon regeneration: 5-HT receptors are divided in seven distinct classes (5-HT1 to 7), which mostly are G-protein coupled metabotropic receptors, with one class being a ligand-gated ion channel (5-HT3 receptor). As far as we are aware, studies in regenerating species have only looked at the role of 5-HT1A and 5-HT7 receptors in axonal regeneration.

5-HT1A receptor: Studies by the Lima group have shown that treatments with 5-HT1A agonists [8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and buspirone] reduce neurite outgrowth from retinal explants of goldfish retinas with a prior crush of the optic nerve (Lima et al., 1994; Schmeer et al., 2001). Further analyses revealed that the negative effect of 8-OH-DPAT in neurite outgrowth was correlated with an increase in cAMP concentration (Urbina et al., 1996; Schmeer et al., 2001).

5-HT1A is coupled negatively to adenylate cyclase by Gi/Go to inhibit cyclic adenosine monophosphate (cAMP) production; although, some studies have also reported that it can stimulate adenylate cyclase under some conditions. In addition, 8-OH-DPAT acts as an agonist of the 5-HT1A receptor, but it can also act as an agonist of the 5-HT7 receptor, which is positively coupled to adenylate cyclase, and as a 5-HT reuptake inhibitor. So, the increase of cAMP concentration observed by Schmeer et al. (2001) in goldfish retinal explants after the 8-OH-DPAT treatment could due to the activation

of 5-HT7 receptors or because in the goldfish retina 5-HT1A receptors are positively coupled to adenylate cyclase (as the authors suggested in their study). In fact, a treatment with the cAMP analogue 8-Br-cAMP significantly reduced neurite length in goldfish post-crush retinal explants (Schmeer et al., 2001). These studies by the Lima group seem to contradict the current assumption on the role of cAMP as a second messenger that promotes axonal growth and regeneration. These might be explained due to the use of an *ex vivo* system and the non-specificity of the drugs. It is necessary to perform new *in vivo* studies in vertebrate models using genetic knockdowns of the 5-HT1A receptor or more specific drugs to clarify the role of this receptor in axonal regeneration.

5-HT7 receptor: The 5-HT7 receptor is positively coupled to adenylate cyclase. The study using the nematode *C. elegans* revealed a positive role of this receptor in axonal regeneration. Mutations in the 5-HT7 receptor gene caused defects in axon regeneration after axotomy (Alam et al., 2016). The effect of the 5-HT7 receptor promoting the regeneration of *C. elegans* axons occurs through the activation of two G proteins; the $G_{\alpha 12}$ protein, which is involved in Rho family GTPase signalling, and the activation of $G_{\alpha s}$, which stimulates the membrane-associated enzyme adenylate cyclase to produce cAMP (Alam et al., 2016). Now, it would of interest to study the role of this receptor in axon regeneration in vertebrate models, especially because some of the effects observed in the pharmacological studies by the Lima group could be related to a non-specific activation of 5-HT7 (see above).

The role of 5-HT in neuronal regeneration: Recent work has shown that 5-HT also plays a role in motor neuron regeneration following a complete spinal cord injury in adult zebrafish (Barreiro-Iglesias et al., 2015). 5-HT treatments promoted the proliferation of spinal cord motor neuron progenitor cells that become activate after a SCI; these progenitor cells are Olig2 expressing radial glial cells located in a ventrolateral position respect to the central canal (Barreiro-Iglesias et al., 2015). The increased proliferation of these progenitor cells, due to the 5-HT treatment, led to an increase in the number of newly generated motor neurons. Conversely, a treatment with the toxin 5,7-dihydroxytryptamine, which specifically ablates serotonergic axons, caused a decrease in the production of new motor neurons after SCI. These results indicate that endogenous 5-HT is a signal that promotes the production of new motor neurons in the spinal cord of adult zebrafish after a complete SCI. Now, it would be of interest to determine the receptor/s that mediate the pro-regenerative 5-HT in signal in motor neuron progenitor cells. Pilot experiments showed that 5-HT1A receptors are highly expressed in these progenitor cells of the adult zebrafish spinal cord (Barreiro-Iglesias et al., 2015). Interestingly, 5-HT1A receptor expression is increased around the central canal of the spinal cord following a complete SCI in lampreys (Cornide-Petronio et al., 2014) and 5-HT1A receptors mediate, at least in part, the positive effect of 5-HT in the production of spinal cord motor neurons during the embryonic development in zebrafish (Barreiro-Iglesias et al., 2015). Now, it would be of interest to study the role of this receptor in motor neuron regeneration and also the downstream pathways activated by 5-HT that lead to an increased proliferation of intrinsic spinal cord stem cells.

Conclusions: Studies in regenerating models like invertebrates and fishes are revealing a crucial role of 5-HT in the modulation of regenerative processes (either neurite or neuronal regeneration) after traumatic CNS injuries. However, more work is needed to fully understand the role that different neurotransmitter receptors play in this process, which appears to be species and neuron type dependant. The use of genetic tools or more specific drugs is needed to decipher the contribution of each 5-HT receptor to axon or neuronal regeneration. Further work should also aim to translate the findings in regenerating species to non-regenerating and more clinically relevant models of TBI or SCI. Once we have a clear picture of the specific role of 5-HT and each of its receptors in regeneration, the possible translation of this knowledge to the clinic will be facilitated by the existence of serotonergic drugs that are already in use in human patients with other conditions.

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***Additional file:** Additional Table 1: Studies looking at the role of 5-HT in nervous system regeneration in regenerating species.*

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