

SEROTONERGIC MODULATION OF EXCITATORY SYNAPTIC TRANSMISSION IN THE
MOUSE INNER RETINA: DISSECTING THE POTENTIAL MECHANISMS AND PLAYERS

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LIST OF ABBREVIATIONS

5-HIAA = 5-hydroxy-indoleacetic acid

5-HT = 5-hydroxytryptamine, serotonin

5HTR = 5-HT receptor

8-OH-DPAT (8-DPAT) = 8-hydroxy-7-Dipropylamino-5,6,7,8-tetrahydronaphthalen-1-ol

AC = amacrine cell

ACSF = artificial cerebrospinal fluid

AHP = afterhyperpolarization

AMPA = alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPA = AMPA receptor

BC = bipolar cell

BSA = bovine serum albumin

CNS = central nervous system

CPPG = alpha-cyclopropyl-4-phosphonophenylglycine

CTL = citalopram

DAPI = 4',6-diamidino-2-phenylindole dihydrochloride

DREADD = designer receptor exclusively activated by designer drugs

ERG = electroretinogram

EPSC = excitatory postsynaptic current

GABA = gamma-aminobutyric acid

GIRK = G protein-coupled inwardly-rectifying potassium channel

Glu = glutamate

GPCR = G protein-coupled receptor

HPLC = high-performance liquid chromatography

INL = inner nuclear layer

IPL = inner plexiform layer

IPSC = inhibitory postsynaptic current

KA = kainic acid

KAR = kainate receptor

KO = knock-out

MAO = monoamine oxidase

MDMA = methylenedioxymethamphetamine

mGluR = metabotropic glutamate receptor

mPFC = medial prefrontal cortex

NMDA = N-methyl-D-aspartate

NMDAR = NMDA receptor

ONL = outer nuclear layer

OPL = outer plexiform layer

PBS = phosphate buffered saline

PFA = paraformaldehyde

PKA = protein kinase A

PR = photoreceptor

RGC = retinal ganglion cell

SERT = serotonin transporter

SSRI = selective serotonin reuptake inhibitor

TPH = tryptophan hydroxylase

Trp = tryptophan

TRPM1 = transient receptor potential cation channel subfamily M member 1

V1 = primary visual cortex

VMAT2 = vesicular monoamine transporter

WT = wild type

Serotonin (5-HT) is a ubiquitous modulator of synaptic transmission in the brain regulating both cognitive and sensory function. In the mammalian retina, the first station for visual sensory processing, the serotonergic system has been shown to be expressed in all five classes of retinal neurons in several species, including rodents and humans. Previous evidence suggests that pharmacological manipulation of the 5-HT system can modify both spontaneous activity and light-evoked responses of retinal ganglion cells (RGCs), suggesting that 5-HT might shape the retinal output to the brain by regulating RGC function. This could be achieved either by a direct effect of 5-HT onto RGC excitability or by a modulation of the synaptic inputs they receive. Moreover, the transporter responsible for 5-HT reuptake (SERT) might also play a role in the modulation of visual processing and RGCs function by regulating the extracellular levels of 5-HT in the inner retina. However, how exactly 5-HT modulates retinal synapses in the inner retina and its impact on visual function remain poorly understood. Thus, in this thesis, we focused on elucidating the complex modulatory effects of the serotonergic system in the inner retinal circuitry with particular attention to the role of endogenous 5-HT, the receptors subtypes involved and the regulation by SERT.

To this end, we performed electrophysiological recordings from different RGCs, classified based on their response to light in ON, OFF and ON-OFF, to study 5-HT-mediated regulation of both spontaneous and evoked synaptic responses in acute mouse retinal slices. Our results demonstrated that exogenous 5-HT application produces a cell-type specific reduction of spontaneous excitatory postsynaptic currents (sEPSCs) and light-evoked EPSCs in ON-OFF RGCs, but not ON RGCs. Such depression is likely mediated by a presynaptic mechanism affecting glutamate release from bipolar cells. Likewise, we found that increasing the extracellular availability of endogenous 5-HT by using the selective serotonin reuptake inhibitor (SSRI) citalopram (CTL) also reduces in a cell-type specific manner both spontaneous and light-evoked EPSCs in ON-OFF RGCs, but not ON RGCs. Moreover, pharmacological manipulations suggested that both the 5HT1 and 5HT2 receptor families are involved in the modulation of light-evoked EPSCs in RGCs. Altogether these findings not only indicate that 5-HT modulates excitatory synapses onto RGCs in a cell-type specific manner, but also provide evidence for a tonic endogenous release of 5-HT in the mouse retina and the involvement of two 5-HT receptor families in the serotonergic modulation of excitatory transmission in the inner retina.

Given the important role of 5-HT in the physiological regulation of synaptic efficacy in ON-OFF RGCs, we finally asked if a chronic disruption in the serotonergic system could affect retinal function. To answer this question, we took advantage of a well-studied model of 5-HT disruption, the SERT knock-out (KO) mouse, in which 5-HT levels in brain circuits are altered due to the impairment of its reuptake from the extracellular space. Consistently, we found that 5-HT levels are reduced in the retina of both SERT heterozygous (HET) and KO mice compared to WT littermates. Multielectrode array recordings in whole mount retinas showed that the global spontaneous firing of all types of RGCs was significantly reduced in KO mice compared to WT littermates. This reduction in RGCs activity was not associated with a change in RGC intrinsic excitability, but likely involves an alteration of synaptic function in the inner retina. Importantly, ON-OFF RGCs were particularly affected in KO mice, supporting the possibility that this class of cell is a major target of serotonergic regulation in the mouse retina.

Altogether our results support the physiological role of 5-HT in the modulation of mouse inner retinal synapses, by showing not only that the endogenous serotonergic system modulates the excitatory inputs onto RGCs in the WT retina, but also that a disruption in the levels of retinal 5-HT results in an alteration in synaptic transmission onto RGCs, that ultimately impacts RGC function, the retinal output to the brain, potentially affecting visual function.