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## Modulation of horizontal cell function by dopaminergic ligands in mammalian retina <sup>★</sup>

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### Abstract

Light responses of rabbit horizontal cell somata (HC) to flickering light stimuli recorded with sharp electrodes consist of a distinctive flicker component superimposed on a sustained hyperpolarisation. Activation of dopamine D1/D5 receptors depolarises HC dark membrane potential and suppresses the flicker component of responses to photopic stimuli without affecting the sustained hyperpolarising response component. Waveforms of responses to scotopic stimuli are preserved. Similar response modulation was observed in depolarising cells of the inner retina, suggesting that activation of D1/D5 receptors of HC causes modification of cone signal transmission to higher order neurons. The impact of dopamine D1/D5 receptor activation on the function of HC in the light stimulated retina is discussed.

### Keywords

Rabbit; Intracellular recording; Flicker responses; Dopamine

## 1. Introduction

Retinal dopamine release is subject to diurnal variation with an increase at subjective day and a decrease at subjective night (Ribelayga, Wang, & Mangel, 2004), but the most effective stimulation of dopamine release results from flickering light (Boelen, Boelen, & Marshak, 1998; Gustincich, Feigenspan, Wu, Koopman, & Raviola, 1997; Kramer, 1971; Wang, Harsanyi, & Mangel, 1997). Cell bodies of dopamine releasing cells lie among amacrine cells and have processes that extend to the outer plexiform layer, but for the most part dopamine reaches its target cells by volume transmission (Bjelke et al., 1996; Kolb, Cuenca, Wang, & Dekorver, 1990). Receptors for dopamine are found all over the vertebrate retina (Nguyen-Legros, Versaux-Botteri, & Vernier, 1999). In the outer retina D1/D5 receptors, a receptor type whose activation increases intracellular cAMP levels (Deary et

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al., 1990), are restricted to horizontal cells (Veruki & Wassle, 1996), whereas photoreceptors carry dopamine receptors that belong to the D2/D3/D4 type. Activation of this receptor type decreases intracellular cAMP levels (Cohen & Blazynski, 1990). D5 receptors have also been described on pigment epithelial cells (Versaux-Botteri, Gibert, Nguyen-Legros, & Vernier, 1997). Dopamine D2/D3/D4 receptors in the inner retina are found on amacrine cells and ganglion cells (Derouiche & Asan, 1999; Wagner, Luo, Ariano, Sibley, & Stell, 1993). On dopaminergic amacrine cells they may function as autoreceptors limiting dopamine release (Schorderet & Nowak, 1990; Veruki, 1997).

Many studies in lower vertebrates support the role of dopamine as a mediator of light adaptation. In recordings obtained from fish retina during subjective night, dopamine acts as a circadian clock effector for the day, as it increases cone input into HC (Wang et al., 1997). In *Xenopus* retina, where rods and cones provide parallel input into the same HC, dopamine enhances cone components of HC light responses and suppresses rod components (Witkovsky, Stone, & Besharse, 1988). The glutamate sensitivity of AMPA-kainate receptors, that mediate cone input to HC, is increased by dopamine (Knapp & Dowling, 1987; Schmidt, Kruse, & Hatt, 1994), probably compensating for reduced glutamate release by light-adapted, hyperpolarised cones. In addition dopamine contributes to the decrease of receptive field size of ganglion cells by uncoupling homologous gap junctions between HC (Mangel & Miller, 1987; McMahon, Packer, & Dacey, 2004).

In mammalian retina dopamine modulation of HC coupling can be seen using gap-junction-permeant tracer molecules (Baldrige, Vaney, & Weiler, 1998; Hampson, Weiler, & Vaney, 1994). Recently dopaminergic effects on receptive field size of the rod-driven, B-type HC axon terminals in rabbit has been investigated (Reitsamer, Pflug, Franz, & Huber, 2006). Only one study exists documenting the effects of dopamine on physiological light responses of the cone-driven HC cell bodies in mammals. This study was performed in rat (Hankins & Ikeda, 1991), a species deficient in mammalian A-type horizontal cells (Peichl & Gonzalez-Soriano, 1994).

In the present paper effects of several dopamine agonists and one dopamine antagonist on intracellular light responses of rabbit HC are investigated. Rabbit retina has two types of HC, A-type and B-type. From B-type cells arises a long axon with a wide-spread terminal solely connected to rods (Kolb, 1974; Raviola & Dacheux, 1990). HC somata of both types are connected to cones but not rods. Nevertheless HC somata exhibit mixed rod and cone input; rod input reaches them through gap junctions between rods and cones (Nelson, 1977), since the long and thin axon isolates the rod-connected terminal from the soma. Recordings of axon terminals, on the other hand exhibit only rod responses. By analogy to lower vertebrates mammalian HC somata are thought to direct their main output back to cones (Burkhardt, 1993). Changes in HC membrane potential shift the voltage dependence of the synaptic  $Ca^{2+}$  current of cones by one or more mechanisms (Hirasawa & Kaneko, 2003; Verweij, Kamermans, & Spekreijse, 1996).

One impact of a change in HC membrane potential has been observed by Nelson et al. and Pflug et al. (Nelson, Pflug, & Baer, 1990; Pflug, Nelson, & Ahnelt, 1990), recording responses to photopic flickering light stimuli from cat HC. These responses consisted of a

steady hyperpolarisation and a superimposed flickering response component. Maintained HC hyperpolarisation by a rod-saturating background light enhanced the photopic flickering component.

In this paper we report that dopamine agonists depolarise rabbit HC and reduce or eliminate the ability of HC to follow photopic flicker, while leaving scotopic flicker responses unaltered or somewhat enhanced. We suggest that the depolarisation induced by dopamine agonists arises through modulation of the conductivity of AMPA-kainate receptors (Knapp & Dowling, 1987), and we propose that reduction in photopic flicker responses arises through feedback-modulation of the cone synapse by depolarised HC. While supporting a role for HC membrane potential in modulating the gain of cone synapses, at a first glance these results seem to disagree with the commonly held view that during light adaptation, dopamine increases cone responses and decreases rod responses (Witkovsky et al., 1988). Under most physiological conditions the depolarising effects of dopamine would be offset by the hyperpolarising effects of steady light. In our study direct application of dopamine agonists unbalances this relation and accentuates the effect of dopamine, allowing us, in effect, to assess dopaminergic actions as relatively uncoupled from background illumination. When background light is added to treatments with dopamine agonists, HC photopic flicker responses return. Results suggest that for mammals, there is a more complex interaction between dopamine and light adaptation than previously supposed.

Parts of these results have been published in abstract form (Nelson & Pflug, 1989; Pflug & Nelson, 1994).

## 2. Methods

### 2.1. Preparation of rabbit retinas

Intracellular recordings of rabbit HC were obtained from isolated retinas. Eyes of deeply anaesthetised rabbits (Urethane, 1.7 g/kg) were enucleated under dim red light. The anterior part of the eye was removed and the retina gently peeled off the pigment epithelium. The retina was mounted in a chamber between two stretched nylon grids and perfused with tissue culture medium composed of three parts newborn calf serum and five parts MEM, the same as used in cat eyecup studies (Pflug et al., 1990). Retina and medium were maintained between 32.5 and 33.5 °C. Following enucleation animals were euthanised by an anesthetic overdose.

All animal experiments were carried out according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### 2.2. Drug application

Concentrated (5–10 mM) stock-solutions of drugs were injected into the perfusion line by means of a syringe pump and were diluted to pharmacological dosage by mixing with normal perfusate. Final concentrations of the unspecific dopamine agonist apomorphine (Sigma–Aldrich) dissolved in ascorbic acid (8 mM) to prevent oxidation, of the dopamine D1/D5-specific agonist SKF38393 and of the dopamine D2/D3/D4-specific agonist quinpirole (LY171555), all from Sigma–Aldrich, ranged between 20 and 350 µM. Injection

of a control solution of ascorbic acid only had no detectable effect on HC responses. Final concentrations of the dopamine D1-specific antagonist SCH23390 (Research Biochemicals) ranged between 80 and 300  $\mu\text{M}$ .

### 2.3. Electrodes and recording setup

Sharp microelectrodes were pulled from glass capillaries on a microelectrode puller (Model P-87, Sutter Instrument Company, Novato, CA, USA) and filled with 4% Neurobiotin and 1% Lucifer Yellow in 0.1 M Tris buffer (pH 7.6). The retinal layers were visualised by a CCD camera under infrared illumination to keep the retina in the dark. Tips of sharp electrodes were guided to the HC layer under visual control with a computer controlled micromanipulator (Reitsamer, Groiss, Franz, & Pflug, 2000).

### 2.4. Light stimulation

Full field light stimuli were generated on an optical bench by a 100 Watt Xenon lamp with electronically-driven shutters (Vincent Associates), and projected onto the retina through the condenser of an inverted microscope (Nikon Diaphot, Optoteam Präzisionsinstrumente GmbH, Vienna, Austria). Two optical outlets provided for test and background beams. Colour and intensity of stimuli were adjusted by means of interference filters and a set of neutral density filters.

### 2.5. Identification of horizontal cells

Penetrated HC somata and axon terminals were identified by the depth of retinal impalement and on the basis of their physiological response to bright flickering stimuli (Bloomfield & Miller, 1982; Reitsamer et al., 2006); axon terminals were discarded for the purposes of this study, except one example is illustrated for comparison to the soma recordings.

To differentiate horizontal cell types Neurobiotin was injected after recordings for 10 min (+1nA). After injection, retinal patches were equilibrated in oxygenated superfusion medium for 40 min at 4 °C and then fixed in paraformaldehyde (4%). Neurobiotin was visualised by using the Elite ABC-Kit (Vector), incubation with diaminobenzidine (0.03%) and reaction with  $\text{H}_2\text{O}_2$  (0.01%). Data resulting from 64 rabbit HC somata and 1 axon terminal were analysed in this study; 3 A-type and 5 B-type cells could be identified by staining.

### 2.6. Recording protocols

After successful impalement of HC somata, receptive field and intensity response protocols were performed. In general, recordings were made from cells of the mid periphery and the periphery of the dorsal and ventral retina. The drug pump was started after initial control measurements; then, after an additional 15 min drug equilibration time, the measurement protocols were repeated. In many, but not all recordings recovery from drug effects could be observed 15–20 min after switching off the drug pump.

### 2.7. Analysis

All recordings were carried out with an institutionally developed LabView (National Instruments Austria, Salzburg, Austria) based data acquisition system. Graphic and statistical analysis was performed with Sigma Plot and Sigma Stat (SPSS Inc., Chicago, IL,

USA), a paired *t*-test was used to assess drug effects, a MANOVA test followed by post hoc tests with Bonferoni correction was used for pointwise comparison of control and drug intensity—response series. *P*-values less than 0.05 (Bonferoni corrected 0.001) were considered significant. Statistical results are expressed as the means  $\pm$  standard error.

### 3. Results

#### 3.1. Identification of rod and cone components in HC responses

We have used full field flicker stimuli with frequencies between 7 and 10 Hz to facilitate the analysis of signal components within the intracellular responses of A- and B-type HC in rabbit retina. Cells were stained after recording for morphological identification. Fields of Neurobiotin—stained A-type and B-type HC are illustrated in Fig. 1. Their physiological responses and reaction to drugs were similar. Comparison of responses of A- and B-type HC somata with responses recorded from an axon terminal of a B-type HC allowed us to distinguish rod and cone mediated components. Fig. 2a shows the time course of responses of an A-type HC to increasing intensities of red (642 nm) flickering light stimuli. The soma responses follow the temporal modulation of the light stimulus at all intensities. Fig. 2b shows responses of a rod-driven axon terminal. These signals lack a flicker component for responses to bright ( $>5.5 \log \text{ quanta } \mu\text{m}^{-2} \text{ s}^{-1}$ ) stimuli. In this stimulus range they consist only of a sustained hyperpolarisation with a slow return to the baseline. Flicker responses to dim stimuli are visible in soma and in axon terminal recordings alike and are therefore considered as arising from rods, whereas flicker responses to bright stimuli are lacking in the solely rod-connected axon terminals, but present in the cone-connected soma responses, and therefore considered as arising from cones (Reitsamer et al., 2000, 2006). Fig. 2c compares the time course of two cycles of the flicker component of an A-type HC soma response to a dim stimulus ( $5.0 \log \text{ quanta } \mu\text{m}^{-2} \text{ s}^{-1}$ , upper trace) with the response to a bright stimulus ( $7.0 \log \text{ quanta } \mu\text{m}^{-2} \text{ s}^{-1}$ , lower trace). The flicker response to the bright stimulus is advanced in phase as compared to the flicker response to the dim stimulus. This further confirms that the dim flicker response is from rods, while the bright flicker response is from cones. In the following these stimuli and responses are therefore called photopic and scotopic, respectively. The transition from scotopic to photopic flicker appears to take place at about  $5.5 \log \text{ quanta } \mu\text{m}^{-2} \text{ s}^{-1}$ . It is indicated by a reduced flicker amplitude resulting from superposition of slow and fast signals at this intermediate intensity that leads to destructive interference of the two waveforms (Fig. 2a).

#### 3.2. Effects of dopamine agonists on HC light responses

Since both, D1/D5 and D2/D3/D4 dopamine receptors have been identified in the outer retina we have studied the influence of D1/D5 and D2/D3/D4 agonists on light responses of a total of 21 HC. Three of them could be identified by intracellular staining as A-type and 5 as B-type HC. Since drug effects were similar in stained A- and B-type cells the data from both types are pooled in the following.

We applied the specific dopamine agonist SKF38393 for the D1/D5 group ( $n = 16$ , Fig. 3a) and quinpirole for the D2/D3/D4 group ( $n = 5$ , Fig. 3b). Concentrations ranged from 20 to 350  $\mu\text{M}$ . Fig. 3a shows the influence of 200  $\mu\text{M}$  SKF38393. The D1 agonist strongly

influences the response kinetics of HC: both the overall and the flicker amplitudes for scotopic stimuli exceed the control response; initial transients clearly visible in the control response are cancelled; small transients at light offset are induced. Overall amplitudes of responses to photopic stimuli approximately match amplitudes of control responses, but flicker components of these responses are almost completely suppressed. Similar effects were observed in all recorded cells ( $n = 8$ ) at SKF38393 concentrations higher than  $70 \mu\text{M}$ . The full effects have never been observed at concentrations lower than  $40 \mu\text{M}$ , although increase of flicker amplitude in the scotopic intensity range and less obvious changes such as enhanced transients at the onset and the offset of the responses sometimes occurred at these lower concentrations. About 10 min after the start of drug application, drug effects were fully developed; original waveforms recovered almost completely after rinsing for up to 20 min with drug free medium (not shown). A slight enhancement of responses to dim stimuli by the D2 agonist quinpirole could occasionally be observed; otherwise the D2 agonist lacked the dramatic actions of the D1 agonist (Fig. 3b).

In addition to the suppression of flickering response components SKF 38393 depolarised the membrane potential ( $8.5 \pm 1.3 \text{ mV}$  at  $200 \mu\text{M}$ ,  $n = 4$ ,  $p < .001$ ). No significant change in membrane potential was observed with quinpirole.

Combined application of the D1/D5 and the D2/D3/D4-specific agonist (not shown), or superfusion with the nonspecific dopamine agonist, apomorphine (Fig. 4) had similar effects as the D1 agonist; depolarisation of cells by apomorphine was even greater than by SKF 38393 ( $14.2 \pm 3.3 \text{ mV}$  at  $200 \mu\text{M}$ ,  $n = 11$ ,  $p < .001$ ).

### 3.3. Effects of D1/D5 antagonist on HC light responses

The effects of D1/D5 agonists could not be blocked by the D1 antagonist SCH23390 (tested between 80 and  $300 \mu\text{M}$ ) as seen in Fig. 3c. Neither did application of SCH23390 alone produce effects contrary to SKF38393 (not shown), although coupling studies on horizontal cell axon terminals (Reitsamer et al., 2006) suggest that endogenous dopamine release occurs under these recording conditions.

### 3.4. Effects of dopamine agonists on rms flicker responses

The modifications in HC flicker amplitude induced by dopamine agonists are summarised in Fig. 5. In each group of drug and control comparisons amplitudes of drug and control responses were normalised to the highest rms (root-mean-square) amplitude of the control intensity series before averaging. The difference between control amplitudes and amplitudes during SKF38393 (Fig. 5b) and apomorphine (Fig. 5a), but not quinpirole (Fig. 5c) application is highly significant at photopic light intensities. ( $p < .05$ , MANOVA followed by post hoc tests with Bonferoni correction ( $p < .001$ )).

### 3.5. Responses of neurons of the inner retina

During the collection of the data we occasionally penetrated cells of the inner retina and recorded responses to flickering stimuli. In all depolarising cells of the inner retina recorded, application of the nonspecific dopamine agonist apomorphine induced the same suppression of photopic flickering response components as in HC. Fig. 6 shows three examples of

different inner retinal waveforms (an A8 amacrine cell, an unstained depolarising cell and an AII amacrine cell). For the morphological identification the nomenclature of MacNeil et al. (MacNeil, Heussy, Dacheux, Raviola, & Masland, 1999) has been used. In each case flicker suppression at high stimulus intensities can clearly be seen. We were able to apply the dopamine D1 antagonist SCH23390 (200  $\mu\text{M}$ ) on the amacrine cell in Fig. 6b. As observed in the HCs, SCH23390 did not block the effect of apomorphine (not shown).

### 3.6. Effect of light adaptation

The similarity of dopamine D1/D5 agonist actions on HC and on depolarising cells of the proximal retina raised the possibility that it is the depolarisation of HC that, through feedback-modulation of cone synapses, alters cone synaptic transmission, causing these modulations to be forwarded to all second and third order retinal cells. To answer the question whether flicker suppression is indeed related to HC depolarisation we exposed a HC, whose photopic flicker response was completely suppressed by apomorphine (100  $\mu\text{M}$ ), to a bright stimulus of white light that hyperpolarised the HC maximally. One second after this stimulus the response to the test stimulus was abolished, 12 s later this response had recovered and exhibited the flicker component demonstrated in the lower trace of Fig. 7. During further recovery from light adaptation, flicker was again lost and a response similar to the one before the white flash returned. A similar observation was made when recording intensity response series of HC during recovery from SKF: light adaptation with blue light (2.1 log quanta  $\mu\text{m}^{-2} \text{s}^{-1}$ ) almost doubled the flicker component of the response, although the overall response amplitude was reduced (not shown). Thus it appears that photopic flicker suppression by D1 agonists can be reversed by light stimuli that hyperpolarise HC in maintained fashion.

## 4. Discussion

### 4.1. Dopaminergic actions on HC light responses and membrane potential

This study reports the influence of dopaminergic agents on the soma responses of rabbit retinal horizontal cells to flickering light stimuli. Unlike axon terminals soma responses of A- and B-type HC are characterised by their ability to follow a flickering stimulus from dimmest up to the highest light intensities (Reitsamer et al., 2006). The main findings of this paper, valid for both A- and B-type HC soma recordings, are: (1) The dopamine D1 agonist SKF38393 and the unspecific dopamine agonist apomorphine depolarise HC somata; (2) both agonists suppress the flickering component of responses to temporally modulated photopic stimuli, but preserve the maintained, hyperpolarised, photopic component; (3) although effects of dopamine agonists show that flicker suppression in HC responses is mediated by D1/D5 receptors, effects of both, SKF38393 and apomorphine cannot be blocked by a D1/D5 antagonist; (4) hyperpolarisation by light acts oppositely to depolarisation induced by dopamine agonists, and at least partially restores the suppressed photopic flicker response components; (5) other less pronounced changes in HC response kinetics were noted: these include increase of both overall amplitudes and flickering components of responses to scotopic stimuli by D1/D5 and (to a lesser extent) D2/D3/D4 agonists, a block of initial transients, and enhancement of transients at response offset.

## 4.2. Depolarisation of HC by dopamine D1/D5 agonists

Dopamine induced depolarisation of HC has previously been reported in lower vertebrate, and in mammalian retinas. Experiments in teleost retina, with dopamine concentrations similar to this study, (Knapp & Dowling, 1987; Schmidt et al., 1994) attributed dopaminergic depolarisation to an increased sensitivity of HC AMPA-kainate-type glutamate receptors. These receptors continuously respond to photoreceptor-released glutamate. The increase in sensitivity of AMPA-kainate receptors in HC is mediated by D1/D5 receptors or by drugs increasing intracellular cAMP concentration (Raymond, Blackstone, & Haganir, 1993). Hankins and Ikeda (Hankins & Ikeda, 1991) report dopamine induced depolarisation of HC in the rod dominated rat retina. Rat retina contains an axon bearing HC analogous to cat and rabbit B-type HC, but has no analogue of A-type HC (Peichl & Gonzalez-Soriano, 1994).

The requirement of glutamate stimulation in order to reveal dopaminergic action on HC has been found in several studies: Knapp and Dowling (1987) demonstrate that dopamine does not increase currents of isolated teleost HC in the absence of glutamate. Hankins and Ikeda (1991) recorded from an isolated rat retina and reported that application of dopamine does not depolarise HC if synaptic transmission from photoreceptors is blocked by  $\text{Co}^{2+}$ . The glutamate receptor subunits GluR6/7, that appear subject to dopaminergic modulation (Raymond et al., 1993), are similarly distributed in the retina of a number of species including rat and rabbit (Peng, Blackstone, Haganir, & Yau, 1995), and have also been identified in cat A- and B-type HC (Qin & Pourcho, 2001). It is very likely that in rabbit retina these kainate-type receptor subunits are the basis for the substantial depolarisation induced by activation of dopamine D1/D5 receptors.

## 4.3. Effects of the D1/D5 antagonist SCH23390

Dopamine D1/D5 agonist-induced depolarisation could not be blocked by the D1 antagonist SCH23390, although block of the D1/D5 receptor would be expected to block intracellular cAMP production responsible for the modification of the glutamate gated channel. A similar inconsistency of agonist and antagonist effects is reported in other studies. Asare et al. (Asare, Nelson, & Connaughton, 2005) observed enhancement of glutamate induced depolarisation in zebrafish horizontal and bipolar cells by dopamine agonists. The depolarisation was potentiated, not blocked, by application of the dopamine antagonist SCH23390. Huppe-Gourgues et al. (Huppe-Gourgues, Coude, Lachapelle, & Casanova, 2005) report that D1 and D2 dopamine agonists as well as antagonists decreased the b-wave of the rabbit. The sometimes inconsistent actions of dopamine antagonists in retina merit further study. Whether such effects point to specialised retinal receptors with different pharmacological profiles, to circuitry interactions, or to complex intracellular mechanisms is beyond the scope of this study. The actions of D1/D5 agonists, however, are clear and consistent.

## 4.4. Effects mediated by D2/D3/D4 receptors

Though of similar type, effects exerted by the D2/D3/D4 agonist quinpirole were less pronounced and more variable than the effects exerted by D1/D5 agonists. D2/D3/D4 receptors are localised on photoreceptors and on the single type of dopamine releasing

neuron, the dopaminergic amacrine cell (Kolb et al., 1990); reviewed by Witkovsky (Witkovsky, 2004). The latter function as autoreceptors and decrease endogenous dopamine release. But the actions of the D2/D3/D4 agonist on HC appear similar to a dopamine increase, so this inner plexiform layer pathway is an unlikely cause of the effects seen in HC. Therefore it is possible that D2/D3/D4 receptors localised on photoreceptors (Wagner et al., 1993) could be responsible for some of the small response modulations observed. In *Xenopus* retina, Witkovsky and Besharse (Witkovsky et al., 1988) also found that both, D1/D5 and D2/D3/D4 agonists, altered HC cone responses in like manner, though in that species, both receptor groups were about equally effective. Activation of D2/D3/D4 receptors localised on photoreceptors, and known to modify photoreceptor cAMP content (Nir, Haque, & Iuvone, 2001), may be implicated. One potential site of action is the cone presynaptic Ca<sup>2+</sup> channel itself.

#### 4.5. Effects mediated by D1/D5 receptors

Activation of D1/D5 receptors either by the D1-specific agonist SKF38393, or by the unspecific agonist apomorphine, depolarise HC and profoundly change their response kinetics. D1/D5 receptors have been found on many neurons of the inner retina and on HC, but not on photoreceptors (Nguyen-Legros et al., 1999; Veruki & Wässle, 1996). Since photoreceptors provide the only known synaptic input into HC, effects of dopamine D1/D5 agonists observed in HC are very likely mediated by increase of intracellular cAMP concentration brought about by HC D1/D5 receptors.

#### 4.6. Modifications of HC voltage sensitive channels by dopamine

HC light responses are triggered by a reduction of photoreceptor glutamate release, and are additionally shaped by a number of voltage gated channels (Blanco & de la Villa, 1999). Depolarisation-activated Na<sup>+</sup> channels, depolarisation- and hyperpolarisation-activated K<sup>+</sup> channels and Ca<sup>2+</sup> channels modify the light response. In white-bass retina it has been demonstrated that L- and T-type Ca<sup>2+</sup> currents (Pfeiffer-Linn & Lasater, 1993) are altered by dopamine. These channel types exist in rabbit retina and their modulation might contribute to the observed enhancement of off-transients.

Dopaminergic modulation of potassium currents in retinal HC has as yet not been demonstrated. In striatal neurons activation of inwardly rectifying potassium currents has been shown to repress action potential burst firing (Pacheco-Cano, Bargas, Hernandez-Lopez, Tapia, & Galarraga, 1996). Since inwardly rectifying potassium currents exist in HC, dopaminergic increase of these currents could contribute to suppression of flickering response components by slowing the depolarising phase of photopic flicker cycles.

#### 4.7. Modifications of HC feedback by dopamine

We demonstrate in this study that dopamine D1/D5 agonists depolarise HC somata of rabbit retina, probably by increasing glutamate gated currents. In fish retina it has been demonstrated that changes of membrane polarisation of HC shift the voltage dependence of the synaptic Ca<sup>2+</sup> current of photoreceptors (Verweij et al., 1996) establishing a negative feedback action of horizontal cells on cone synaptic release. The mechanism of this feedback is still under discussion; an ephaptic interaction in the small invaginating synaptic

cleft where HC dendrites contact cones has been proposed (Kamermans et al., 2001), but several studies (Hirasawa & Kaneko, 2003), (Dmitriev & Mangel, 2006) argue in favour of other mechanisms. Hirasawa and Kaneko favour a modulation of cone  $\text{Ca}^{2+}$  currents by pH changes in the synaptic cleft. The pH changes are induced by polarisation changes of the HC membrane. Additionally modulation by GABA has been proposed (Tatsukawa, Hirasawa, Kaneko, & Kaneda, 2005). Independent of the mechanism, all of these models result in a shift of cone  $I_{\text{Ca}}$  activation to more negative values by hyperpolarisation of HC. A depolarisation of HC would therefore be expected to shift the working range of the synapse to more depolarised values. If dopamine application, by depolarising the HC membrane, shifted the  $\text{Ca}^{2+}$  activation curve to more positive values while the cone is hyperpolarised by light, transmission of the most negative part, i.e. the flickering part of the light response would be reduced or completely truncated, regardless of its origin in rods or cones. Predictions from this consideration are: (1) If the depolarisation of HC has an impact on photoreceptor  $\text{Ca}^{2+}$  currents and therefore transmitter release, cones would forward a modified response that should be recorded from all second and third order neurons that receive direct or indirect input from cones. (2) Hyperpolarisation of HC membrane potential should shift the working range of the cone synapse to more hyperpolarised values; this might result in a reversal of dopamine actions.

We favour the idea that the HC feedback pathway plays a major role in shaping HC and inner retinal light responses to photopic flicker stimuli, because (1) suppression of high amplitude flicker components of HC responses by D1/D5 ligands has indeed been observed in all photopically active neurons of the inner retina (Fig. 6); (2) flickering response components initially suppressed by apomorphine could be partially restored by light induced hyperpolarisation (Fig. 7). Further support comes from results of Nelson and Pflug (Nelson et al., 1990; Pflug et al., 1990) who recorded from HC of cat retina and demonstrated that photopic flicker components of HC responses were enhanced during hyperpolarisation of HC by background light.

In this study dopamine effects on outer retinal circuitry have been assessed by continuous application of dopamine agonists over a period of several minutes. Under these experimental conditions D1/D5 agonists had a severe impact on response waveforms of HC and inner retinal neurons. Endogenous dopamine release in a physiologically functioning retina varies and is controlled by time course and intensity of light stimulation. The control of the operating range of HC by light dependent dopamine release could be an essential contribution to the retinal adaptation to a bright ambience.

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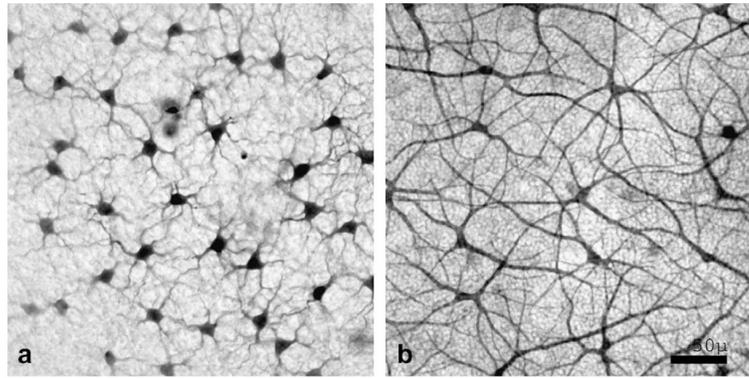
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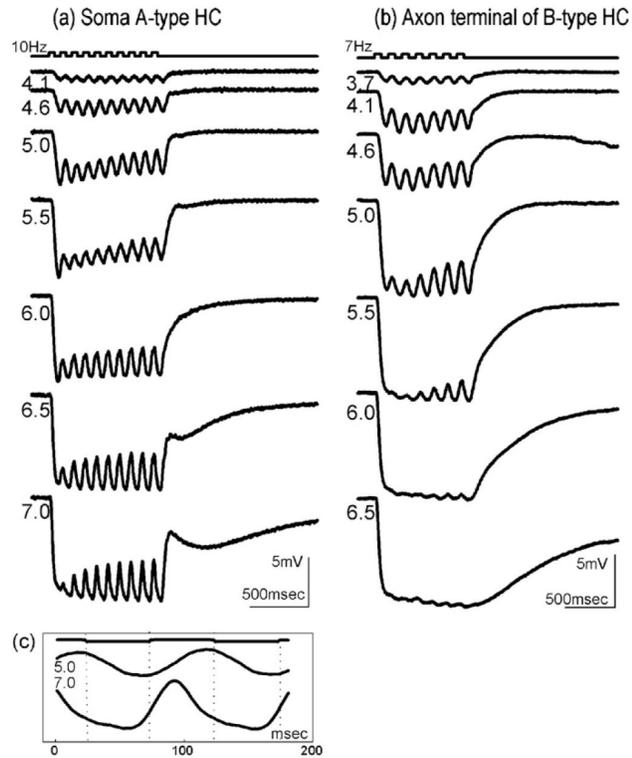
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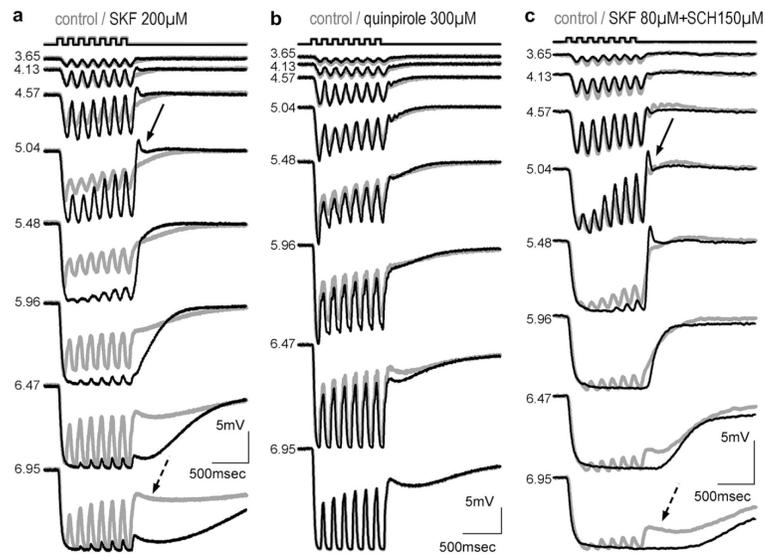


**Fig. 1.**  
Neurobiotin stained fields of B-type (a) and A-type (b) horizontal cells.

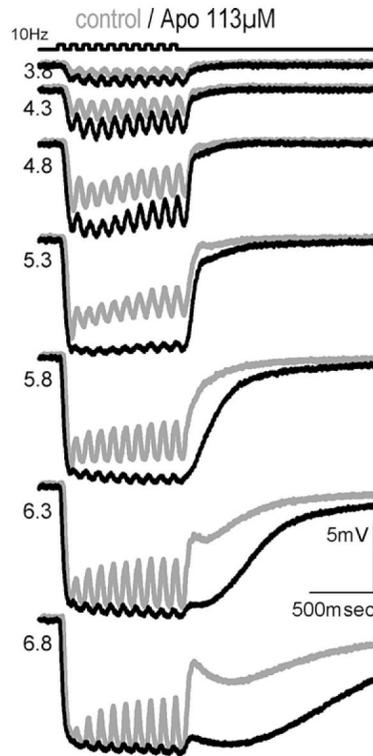


**Fig. 2.**

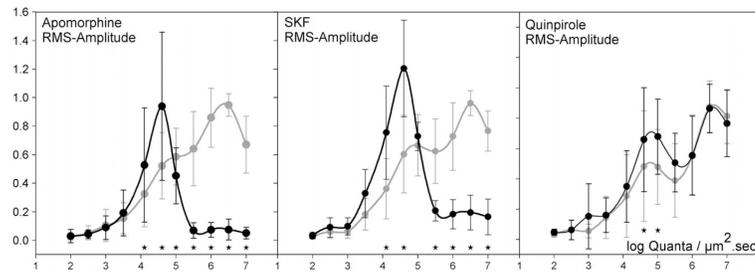
(a) Intracellular responses of an A-type HC and (b) an axon terminal of a B-type HC. (c) Flicker cycles of the A-type HC at two intensities: rod-driven (middle trace) and cone-driven (lower trace). Flicker cycles exhibit a clearly visible rod-cone phase shift. Top trace: light stimulus; intensity of flickering light stimuli are indicated next to the responses in log quanta  $\mu^{-2} s^{-1}$ .



**Fig. 3.** Intracellular responses of HC somata to flickering light of increasing intensity (indicated in log quanta  $\mu\text{m}^{-2} \text{s}^{-1}$ ; stimulus pattern on top); effects of drug application were fully developed after 5–10 min (drug indicated on top; grey: control; black: drug); (a) A-type HC: the D1 agonist SKF38393 enhances responses to dim stimuli and induces transients at response offset (solid arrow) but suppresses flicker components in responses to bright stimuli (dotted arrow); (b) A-type HC; the D2 agonist quinpirole changes responses only slightly; (c) B-type HC; effects of SKF38393 are not suppressed by the D1 antagonist SCH23390.



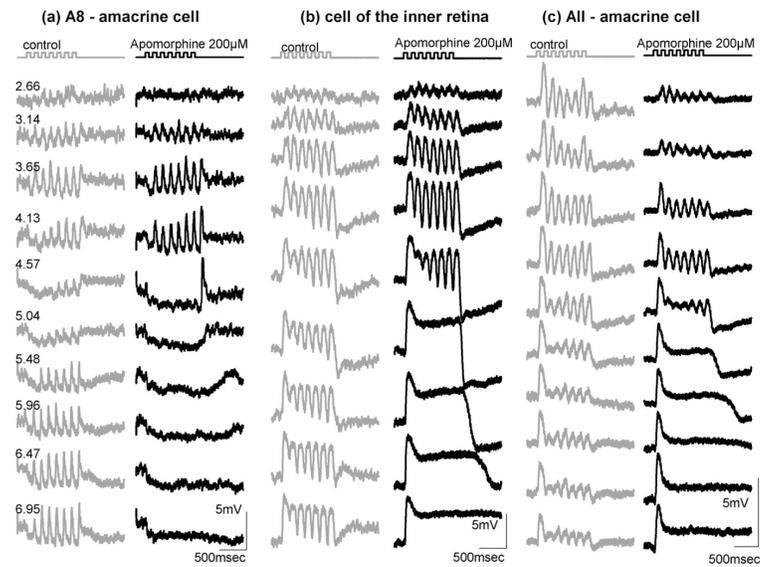
**Fig. 4.** A-type HC, effect of apomorphine on intracellular responses to a flickering light stimulus (top) of increasing intensity (indicated in log quanta  $\mu\text{m}^{-2} \text{s}^{-1}$ ).



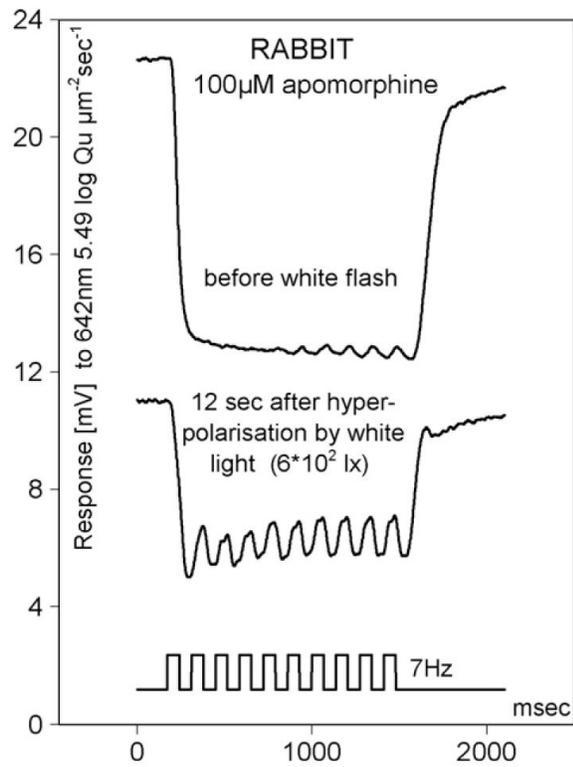
**Fig. 5.**

Mean normalised rms (root-mean-square) amplitude and SD of the flickering components of responses to a light stimulus (intensity indicated in the abscissa in  $\log \text{quanta } \mu\text{m}^{-2} \text{s}^{-1}$ ).

Apomorphine, 200  $\mu\text{M}$ ,  $n = 7$ ; SKF, 200  $\mu\text{M}$ ,  $n = 5$ ; Quinpirole, 300  $\mu\text{M}$ ,  $n = 5$ ; grey: control; black: drug. Responses indicated by a star are significant in a MANOVA post hoc—comparison with Bonferoni correction of control and drug-influenced amplitudes.



**Fig. 6.** Responses of cells of the inner retina to a flickering light stimulus (top) of increasing intensity indicated in log quanta  $\mu\text{m}^{-2} \text{s}^{-1}$  on the left. (grey: control; black: apomorphine, 200  $\mu\text{M}$ ).



**Fig. 7.** Rabbit HC responses during application of apomorphine. Light stimulus (bottom trace): 642 nm, 5.49 log quanta  $\mu\text{m}^{-2} \text{s}^{-1}$  before (top) and after (middle) a white flash of adapting light.