Dopamine D1 and D4 receptors contribute to light adaptation in ON-sustained retinal ganglion cells

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Abstract:

Adaptation of ganglion cells to increasing light levels is a crucial property of the retina. The retina must respond to light intensities that vary by 10-12 orders of magnitude, but the dynamic range of ganglion cell responses only covers ~1000 orders of magnitude. Dopamine is a crucial neuromodulator for light adaptation and activates receptors in the D1 family – D1Rs that are expressed on horizontal cells and some bipolar and ganglion cells- and the D2 family – D2Rs that are expressed on dopaminergic amacrine cells and D4Rs that are primarily expressed on photoreceptors. However, how these receptors change the synaptic properties of the inputs to ganglion cells is not yet clear. Here we used single cell retinal patch-clamp recordings from the mouse retina to determine how activating D1Rs and D4Rs changed the light-evoked and spontaneous excitatory inputs to ON-sustained (ON-s) ganglion cells. We found that both D1R and D4R activation decrease the light-evoked excitatory inputs to ON-s ganglion cells, but that only the sum of activating the two receptors was similar to the effect of light adaptation to a rod-saturating background. The largest effects on spontaneous excitatory activity of both D1R and D4R agonists was on the frequency of events, suggesting that D1Rs and D4Rs are acting upstream of the ganglion cells.

Keywords: retina, ganglion cell, dopamine, light adaptation

Introduction

During the course of a normal day, our eyes are exposed to lighting conditions that may vary in intensity by 10-12 orders of magnitude (Shapley and Enroth-Cugell 1984). In contrast, retinal ganglion cells can only modulate their firing rates by a factor of ~100-1000 (Sakmann et al. 1969). In order to maintain a proper signaling response to light, the retina must constantly tune its sensitivity to ambient luminance conditions. The multiple processes involved in this retinal tuning are collectively referred to as light adaptation.

Studies on light adaptation have found that typically ganglion cell light sensitivity and receptive field size are reduced by increased background illumination (Barlow et al. 1957; Enroth-Cugell and Shapley 1973; Gouras 1967; Ke et al. 2014; Sakmann et al. 1969; Wiesel 1959). However, light adaptation can have variable impacts depending on the ganglion cell type (Barlow and Levick 1969; Yoon 1972) and can induce changes in ganglion cell integration dynamics (Grimes et al. 2014) or even response polarity (Kuffler 1953; Rivlin-Etzion et al. 2018; Tikidji-Hamburyan et al. 2015; Wienbar and Schwartz 2018) depending on luminance intensity. Because the retina consists of at least 40 populations of ganglion cells (Baden et al. 2016; Bae et al. 2018; Masland 2012; Sanes and Masland 2015), each with a particular set of conductances and connectivity, it is possible that light adaptation imparts changes to retinal encoding that are unique to each of these individual populations.

For more than half a century visual neuroscientists have known that dopamine is integral to retinal light adaptation, but the full scope of its sites of action and neuromodulatory effects remain inadequately understood and quantified (Roy and Field 2019; Witkovsky 2004). In the retina, dopamine is produced by a specific population of dopaminergic amacrine cells that are located within the inner nuclear layer (Jackson et al. 2012; Witkovsky 2004). When the retina is exposed to increased light levels, dopamine is released by dopaminergic amacrine cells into the retinal space (Mills et al. 2007; Perez-Fernandez et al. 2019). Dopamine mediates its effects primarily in a paracrine manner by binding to dopamine type-D1, -D2, and -D4 receptors located on retinal neuronal subtypes (Cohen et al. 1992; Derouiche and Asan 1999; Farshi et al. 2016; Klitten et al. 2008; Li et al. 2013; Veruki and Wassle 1996). Dopamine type D1 receptors (D1Rs) are expressed on horizontal cells and select populations of bipolar, amacrine and ganglion cells (Farshi et al. 2016; Veruki and Wassle 1996). Dopamine type D4 receptors (D4Rs) are primarily located on photoreceptors (both rods and cones), but there is also evidence

for their expression in some inner nuclear and ganglion cell populations (Cohen et al. 1992; Li et al. 2013; Van Hook et al. 2012; Yin et al. 2020). Dopamine type D2 receptors are mainly expressed by dopaminergic amacrine cells, where they act as autoreceptors to limit dopamine release (Derouiche and Asan 1999; Li et al. 2013; Veruki 1997). Although the effects of dopamine and its agonists on ganglion cells have been partially characterized (Chen and Yang 2007; Hayashida et al. 2009; Jensen and Daw 1986; Li et al. 2016; Maguire and Hamasaki 1994; Ogata et al. 2012; Van Hook et al. 2012; Vaquero et al. 2001), most studies to date have focused on spike rate modulation in eyecup preps or in dissociated ganglion cells. However, this approach cannot differentiate the complex circuit dynamics that occur upstream of ganglion cells.

In this study, we isolated excitatory currents to ON sustained (ON-s) ganglion cells, which reflect glutamate release by ON bipolar cells. By targeting this synapse, the differential effects of D1R and D4R signaling pathways on light adaptation-induced changes to ganglion cell excitatory inputs could be determined. In addition, the site(s) of action presynaptic or postsynaptic to ganglion cells could be identified. By isolating this single site within intact retinal tissue, we quantified the impact of dopaminergic network modulation upon signal output from ON pathways of the inner retina. Through studying the effects of adaptation on circuit elements upstream of ganglion cells, where the added complexity of distinct inhibitory inputs onto ganglion cells is removed, it should be possible to glean more consistent rules for how all channels of retinal processing are ultimately modulated.

Methods

Retinal slice and whole mount preparation.

Animal protocols conformed with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were approved by the University of Arizona Institutional Animal Care and Use Committee. As previously described (Eggers et al. 2013), C57BL/6J male mice (Jackson Laboratories, Bar Harbor, ME) 35–60 days of age were euthanized using carbon dioxide. The eyes were enucleated, the cornea and lens removed, and the eyecup was incubated in cold extracellular solution (see Solutions and drugs) with 800 U/ml of hyaluronidase for 20 min. The eyecup was washed with cold extracellular solution, and the retina was removed. For

slice preparation, the retina was trimmed into an approximate rectangle and mounted onto 0.45-µm nitrocellulose filter paper (Millipore, Billerica, MA). The filter paper containing the retina was then transferred to a hand chopper, sliced into 250-µm-thick slices, rotated 90°, and mounted onto glass coverslips using vacuum grease. Retinal whole mounts were prepared according to the methods outlined by Ivanova et al. (2013). Briefly, a hydrophilized cell culture filter insert (Millipore Sigma, Burlington MA) was trimmed down with a Dremel tool to a height of ~1mm. The retina was cut into 4 equal quadrants and mounted onto our trimmed cell culture insert as needed, one quadrant per ganglion cell experiment. Quadrants were mounted photoreceptor side down by applying slight negative pressure with a custom-trimmed 1 mL syringe. All dissections and light response recording procedures were performed under infrared illumination to preserve the light sensitivity of our preparations.

Solutions and drugs.

Extracellular solution used as a control bath for dissection and whole cell recordings was bubbled with a mixture of 95% O2-5% CO2, which set the pH to ~7.4 and contained the following (in mM): 125.00 NaCl, 2.50 KCl, 1.00 MgCl2, 1.25 NaH2PO4, 20.00 glucose, 26.00 NaHCO3, and 2.00 CaCl2. The intracellular solution in the recording pipette used for monitoring excitatory ganglion cell currents contained the following (in mM): 120.00 CsOH, 120.00 gluconic acid, 1.00 MgCl2, 10.00 HEPES, 10.00 EGTA, 10.00 tetraethylammonium-Cl, 10.00 phosphocreatine-Na2, 4.00 Mg-ATP, 0.50 Na-GTP, and 0.1% sulforhodamine-B dissolved in water and was adjusted to pH 7.2 with CsOH. With these concentrations, the driving force for Cl− was calculated as 60 mV in all solutions.

The partial D1R agonist SKF-38393 (SKF, 20 µM; Tocris, Bristol, United Kingdom) was used to selectively activate D1Rs, and the D4R agonist PD-168077-maleate (PD, 500 nM; Sigma) was used to selectively activate D4Rs. Each was diluted in extracellular solution to the given concentration and applied to the bath during the recordings by a gravity-driven superfusion system (Cell Microcontrols, Norfolk, VA) at a rate of ~1 ml/min. For D4R agonist experiments, as DMSO was initially used to solubilize the drug, our perfusate had a final working DMSO concentration of less than 0.0025% by mass. Chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise indicated.

Whole cell recordings

All light response recordings were first performed on retinal slices or whole-mounts in a dark-adapted state, followed by recordings performed under drug-added or light-adapted conditions (see Light Stimuli). Retinal slices on glass coverslips or whole-mount preps were placed in a custom chamber and heated to 32° by a TC-324 temperature controller coupled to an SH-27B inline heater (Warner, Hamden CT). For D1R/D4R agonist experiments, dark-adapted light responses were measured first, followed by a 5-min incubation period with the agonist, after which light responses were again measured in the presence of continuous agonist perfusion. For light-adaptation experiments, dark-adapted light response measurements were followed by 5 min of light adaptation, before again recording light responses (with background illumination maintained.) Whole cell voltage-clamp recordings were made from ON-s ganglion cells in retinal slices or whole-mount preperations. Light-evoked (L-) and spontaneous (s) excitatory post-synaptic currents (EPSCs) were recorded from ON ganglion cells voltage clamped at -60 mV, the reversal potential calculated for Cl^- currents. For all recordings, series resistance was uncompensated. Electrodes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) using a P97 Flaming/Brown puller (Sutter Instruments, Novato, CA) and had resistances of 3–7 MΩ. Liquid junction potentials of 20 mV, calculated with Clampex software (Molecular Devices, Sunnyvale, CA), were corrected for before recording. Light responses and spontaneous events were sampled at 10 kHz and filtered at 6 kHz with the four-pole 165-Bessel filter on a Multi-Clamp 700B patch-clamp amplifier (Molecular Devices) before being digitized with a Digidata 1140 data acquisition system (Molecular Devices) and Clampex software.

Confirmation of ganglion cell morphology and presence of an axon was done at the end of each recording using an Intensilight fluorescence lamp to visualize sulforhodamine-B

fluorescence and images were captured with a Digitalsight camera controlled by Elements software (Nikon Instruments, Tokyo, Japan).

Light stimuli

Full-field light stimuli were evoked with a light-emitting diode (LED; HLMP-3950, λpeak = 525 nm; Agilent, Palo Alto, CA) that was calibrated with an S471 optometer (Gamma Scientific, San Diego, CA) and projected through the camera port of the microscope and onto the stage via a 4x objective. The stimulus intensities were chosen to cover both rod-dominant (9.5, 95.0, 950.0 photons$\cdot\mu m^{-2}\cdot s^{-1}$) and cone-dominant ($9.5\cdot10^3$, $9.5\cdot10^4$, and $9.5\cdot10^5$ photons$\cdot\mu m^{-2}\cdot s^{-1}$) response ranges. These intensities were calculated to be equivalent to 4.75, 47.50, 475.00, $4.75\cdot10^3$, $4.75\cdot10^4$, and $4.75\cdot10^5$ R*$\cdot$rod$^{-1}\cdot s^{-1}$, respectively (Field and Rieke 2002). Sequential light responses were recorded with a stimulating interval of 30 s. Stimulus intensities, background rod-saturating light (950 photons$\cdot\mu m^{-2}\cdot s^{-1}$), and duration (30 ms) were controlled with Clampex software by varying the current through the LED. The background intensity was chosen, as it was shown to maximally activate rods (Wang and Kefalov 2009). A rod-saturating background light was applied for 5 min to light adapt the retina slice or whole-mount prep and was sustained throughout all light-adapted recordings.

Data analysis and statistics

Between two and four traces of L-EPSCs for each condition were averaged using Clampfit (Molecular Devices). The peak amplitude, charge transfer (Q), time to peak, and decay to 37% of the peak (D37) were determined. The bounds for integration used to calculate Q were marked by the times at which the longest-duration response began and when it returned to baseline, typically 1–2 s. These integration bounds were kept constant for all light responses recorded from the same cell. The time to peak was calculated as the temporal difference between stimulus onset and the response peak amplitude. Because the decay time could not be easily fitted with either a single or double exponential curve, the D37 was determined by computing the time it took for the EPSC to decline from its peak amplitude to 37% of its peak amplitude.

For L-EPSCs, data from each cell were normalized to the maximum response recorded; typically this maximum occurred under dark-adapted conditions at the highest intensity ($9.5\cdot10^5$

photons·$\mu m^{-2} \cdot s^{-1}$). If there was no discernable response for a given light intensity after filtering and averaging, the peak amplitude was recorded as 0, and it was excluded from our analysis of response kinetics. Comparisons between experimental conditions and luminance intensities were made with two-way repeated-measures (RM) ANOVA tests using the Student-Newman-Keuls (SNK) method for pairwise comparisons in SigmaPlot (Systat Software, San Jose, CA). If any data were shown to have a non-normal distribution or unequal variance, tests were repeated on the log10 values (or square root values for peak amplitudes) of data.

For spontaneous currents, events were included in the analysis if they occurred after a light response had returned to baseline but before the 1 s baseline preceding the subsequent light stimulus. Frequency, amplitude, interevent interval (IEI), and decay constants were calculated using MiniAnalysis software (Synaptosoft, Fort Lee, NJ). Decay constants were fit with a single exponent. Effects of treatments on sEPSCs were analyzed at the single cell level with Kolmogorov-Smirnov (K-S) tests in Clampfit. Amplitude, decay τ, and IEI histogram distributions were normalized to the number of events. Average effects of a D1R agonist, a D4R agonist, or light adaptation on sEPSCs in the same cells were analyzed with paired t-tests, and comparisons between different groups of cells were analyzed with unpaired t-tests, after normalizing each cell to its dark-adapted state. Individual cells were only included in the analysis if they had 10 or more spontaneous events per treatment condition. For all studies, differences were considered significant when $P \leq 0.05$. All data are reported as means ± 95% confidence interval.

Results

Light adaptation reduces L-EPSCs and sEPSCs in ON-s ganglion cells

To assess the contributions of D1R- and D4R-dependent signaling to light adaptation we first had to quantify the effects of a fixed background luminance on ON-s ganglion cell EPSCs, since retinal conditions can vary significantly between different experimental paradigms. For our light adaptation protocol we chose a background intensity equal to 950 photons·$\mu m^{-2} \cdot s$, since this background has been shown to saturate the rod response (Wang and Kefalov 2009). The light adaptation protocol significantly reduced both the peak amplitude (Fig. 1A) and Q (Fig. 1B) of L-EPSCs in ON-s ganglion cells (2-way ANOVA, peak amplitude $p<0.001$; Q $p<0.001$) at all three intensities tested (SNK, $p<0.001$). Additionally, light adaptation significantly increased

time to peak values (2-way ANOVA, p=0.001, Fig. 1C) but decreased 37% decay values (2-way ANOVA, D37 p<0.001, Fig. 1D) at all intensities tested (SNK, p<0.001).

To determine whether these declines in ON-s ganglion cell L-EPSCs are attributable to pre- or post-synaptic effects, concurrent changes in sEPSC frequency or amplitude were measured (Fig. 2). Analysis of cumulative histograms showed that light adaptation caused significant shifts in sEPSCs towards smaller amplitudes (Fig. 2B) and longer inter-event intervals (Fig. 2C). On average, light adaptation caused a large decline in sEPSC frequency (-61.4% ± 7.4%, paired t-test p<0.00001) as well as a slight decline in amplitude (-19.5 ± 4.3%, paired t-test p=0.00044). This suggests that light adaptation modulates the activity of circuits upstream of ganglion cells, and may induce post-synaptic changes to ganglion cell receptors.

D1R activation reduces the magnitude of L-EPSCs in ON-s *ganglion cells*

Given that light adaptation significantly modulates ON-s ganglion cell L-EPSCs, the contributions of D1R and D4R receptors were then determined. First, the effect of a D1R agonist on ON-s L-EPSCs was measured (Fig. 3A). L-EPSC peak amplitudes (Fig. 3B) and Q (Fig. 3C) were significantly diminished (2-way ANOVA, peak amplitude p<0.001; Q p=0.009) with significant pairwise differences at the top 4 intensities for peak amplitudes (SNK, p<0.05) and at $9.5 \cdot 10^4$ photons$\cdot \mu m^{-2} \cdot s^{-1}$ for Q (SNK, p=0.04). D1R agonist application significantly increased time to peak values (2-way ANOVA, p=0.015, Fig. 3D) with a significant pairwise difference at 950 photons$\cdot um^{-2} \cdot s^{-1}$ (SNK, p=0.013), but did not have a significant impact on D37 values (2-way ANOVA, p=0.079, Fig. 3E). However, light adaptation caused significantly greater reductions to peak amplitude and Q values (2-way ANOVA, peak p=0.002, Q p<0.001) than D1R activation (Fig. 3B, C). Light adapted L-EPSCs also had significantly longer time to peak values and significantly shorter D37 values (2-way ANOVA, T2P p<0.001, D37 p=0.03, Fig. 3D, E) than D1R L-EPSCs. These results suggest that while D1R application has a significant impact on L-EPSCs, it does not fully recapitulate the effects of light adaptation. In analyzing our data, we also took note of significant variability in the response of individual ganglion cells to our D1R agonist, as shown by the significant overlap in shaded 95% confidence intervals between groups. The overall significance of this observation is unclear.

D1R activation reduces frequency and amplitude of sEPSCs in some but not all ON-s *ganglion cells*

We next examined the effects of D1R activation on sEPSCs in the cells that light responses were recorded from (Fig. 4A). Analysis of cumulative histograms showed that D1R activation significantly shifted sEPSC amplitudes (Fig. 4B) and inter-event intervals (Fig. 4C) towards smaller and longer values, respectively. Although the sEPSC distributions were shifted significantly, average sEPSC amplitude and frequency values after D1R treatment were smaller, (Fig. 4D,E), but not to a significant degree (paired t-test, amplitude $p=0.46$; frequency $p=0.125$, n = 5 cells from 5 animals). The magnitude of D1R-mediated effects on average sEPSC amplitude and frequency varied, and some cells even exhibited increased amplitude or frequency after D1R application. When we compared amplitude and inter-event interval distributions on a single cell level, we found that D1R activation caused significant shifts towards smaller amplitudes and longer inter-event intervals in 3/5 cells (K-S, $p<0.05$). We found no correlation between the degree of changes in sEPSC amplitudes or frequencies and changes in L-EPSCs. When we compared the normalized changes induced by D1R activation and light adaptation to average sEPSC amplitude and frequency, we found no significant differences. Owing to variability in this data it is unclear whether light adaptation mediates its effects on sEPSCs via D1Rs.

D4R activation decreases L-EPSCs in ON-s *ganglion cells*

Similar to our analysis of the effects of a D1R agonist, we examined the effects of a D4R agonist on light-evoked currents in ON-s ganglion cells (Fig. 5A). Both peak amplitude (Fig. 5B) and Q (Fig. 5C) were significantly decreased by D4R activation (2-way ANOVA, $p<0.001$, n=7 cells from 7 animals), with significant pairwise differences at 950, $9.5\cdot10^4$ and $9.5\cdot10^5$ photons$\cdot\mu m^{-2}\cdot s^{-1}$ for peak amplitudes and at $9.5\cdot10^3$, $9.5\cdot10^4$, $9.5\cdot10^5$ photons$\cdot\mu m^{-2}\cdot s^{-1}$ photons for Q (SNK, $p<0.05$). No significant difference in time to peak (Fig. 5D) or D37 (Fig. 5E) between dark-adapted and D4-agonist treated responses (2-way ANOVA, time to peak $p=0.904$; D37 $p=0.719$, n=6 cells from 6 animals) were identified. However, similar to the data on D1R activation, light adaptation caused significantly greater reductions to both peak amplitude and Q than D4R activation (2-way ANOVA, peak amplitude $p<0.001$, Q $p<0.001$, Fig. 5B,C). Light-adapted L-EPSCs also had significantly longer times to peak and shorter D37 values (2-way ANOVA, T2P $p<0.001$, D37 $p=0.026$, Fig. 5D,E) than L-EPSCs after activating D4R. This data

suggests that while D4R application has significant impacts on L-EPSCs, it cannot fully account for changes that occur during light adaptation. In analyzing our data, we again took note of significant variability in the response of individual ganglion cells to our D4R agonist, as shown by the significant overlap in shaded 95% confidence intervals between groups.

D4R activation decreases the frequency of sEPSCs and slightly decreases sEPSC amplitudes in ON-s *ganglion cells*

We next analyzed the impact of D4R activation upon ON-s ganglion cell sEPSCs (Fig. 6A). Cumulative histograms showed that D4R activation significantly shifted sEPSCs towards smaller amplitudes (Fig. 6B) and longer inter-event intervals (Fig. 6C). Treatment with a D4R agonist caused significant reductions in the average amplitude (Fig. 6D) and frequency (Fig. 6E) of sEPSCs in ON-s ganglion cells (paired t-test, frequency $p=0.00369$; amplitude $p=0.00353$). On the single cell level, D4R activation caused significant shifts in amplitudes towards smaller values, and inter-event intervals towards longer values, in 4/5 cells (K-S, $p<0.01$). This suggests pre-synaptic changes in tonic bipolar cell release, and potential post-synaptic modifications to receptors on ganglion cells. Again, we found no correlation between the magnitude of D4R activation's impact on sEPSC and L-EPSC parameters. There were no significant differences between the normalized changes induced by D4R activation and light adaptation to average sEPSC amplitudes and frequencies. Collectively, this data suggests that light adaptation mediates its effects on sEPSCs via D4Rs.

Combined effects of D1R/D4R activation approximate some but not all changes to L-EPSCs and sEPSCs that are induced by light adaptation

To see whether the effects of light adaptation on ON-s GC light responses could be fully explained by dopamine receptor activation we created a simple additive model for our L-EPSC peak amplitude and Q values (Fig. 7A,B). For peak amplitude values (Fig. 7A) the hypothetical combination of D1R/D4R agonist treatment (dashed gold line) fairly closely approximated the measured response reduction caused by light adaptation (grey line). However, this was not the case for our Q data (Fig. 7B) where our additive model did not cause nearly the same reduction as light adaptation. We also created an additive model for our sEPSC amplitude and frequency values (Fig. 7C,D) showing the hypothetical effects of D1R/D4R agonist co-activation. In this

case, the model produced similar declines to average sEPSC peak amplitudes (Fig. 7C), but decreased average sEPSC frequency to a greater degree than light adaptation (Fig. 7D). These models suggests that D1R and D4R activation may have synergistic effects on these parameters, or perhaps that there are additional changes induced by light adaptation that act through dopamine-independent mechanisms.

Discussion

In this study we examined the effects of light adaptation and dopamine receptor agonists on excitatory inputs to ON-s ganglion cells, in order to gauge the relative contribution of dopaminergic pathways to luminance adaptation. Light adaptation reduced the magnitude of L-EPSCs while delaying their times to peak and speeding up their decays and decreased the frequency and amplitude of sEPSCs. These measured effects of light adaptation on excitatory inputs to ON-s ganglion cells largely fit with prior data, as previous reports have demonstrated reduced light sensitivity (Barlow et al. 1957; Enroth-Cugell and Shapley 1973; Gouras 1967; Sakmann and Creutzfeldt 1969; Wiesel 1959), as well as faster response kinetics (Ogawa et al. 1966; Tikidji-Hamburyan et al. 2015) of ganglion cell spike rates in the light-adapted retina. There is also good evidence to suggest that light adaptation induces significant changes in resting ganglion cell spike rates (Barlow and Levick 1969; Freeman et al. 2008), which could be mediated by the changes in spontaneous currents measured here**.** Because dopamine is released during light adaptation and modulates retinal activity via activation of both D1Rs and D4Rs (Roy and Field 2019; Witkovsky 2004), it makes sense that treatment with either a D1R or D4R agonist seems to mediate a portion of light adaptation's effects. but fails to completely recapitulate them.

Activation of D1Rs can modulate retinal output at multiple synaptic sites

Activating D1Rs decreased L-EPSCs magnitude and slightly increased time to peak (Fig. 3). Our analysis of D1R activation's effects on sEPSCs suggested that most of these changes are attributable to decreased ON bipolar cell output rather than direct effects upon ON-s ganglion cells. Upsteam of ON-s ganglion cells there are multiple sites where D1R activation should modify retinal activity. In the outer plexiform layer, photoreceptor activity should not be directly

modified as they do not express D1Rs (Farshi et al. 2016; Nguyen-Legros et al. 1999; Veruki and Wassle 1996). However, the synaptic output of photoreceptors is regulated by horizontal cells – when horizontal cells are depolarized they inhibit voltage-gated calcium currents in photoreceptors (Babai and Thoreson 2009; Verweij et al. 1996), thus reducing glutamate release onto themselves and bipolar cells (Liu et al. 2013). A D1R specific agonist has been shown to reduce voltage-gated calcium currents in murine horizontal cells (Liu et al. 2016), which should reduce negative feedback onto photoreceptors and result in enhanced glutamate release. However, a study performed in rabbit retina demonstrated a significant depolarization of horizontal cell resting membrane potential upon selective D1R activation (Pflug et al. 2008), which should inhibit photoreceptor release of glutamate. The authors attributed this depolarization to enhanced horizontal cell glutamate sensitivity, noting the absence of this dopamine induced depolarization under blockade of photoreceptor transmission (Hankins 1991). It is difficult to say which of these mechanisms would be larger. In the same paper, horizontal cell hyperpolarizing light responses after D1R activation were enhanced in size and duration, which could suggest larger declines in photoreceptor release. However, these changes could be attributable to the diverse set of horizontal cell conductances (Blanco and de la Villa 1999; Sun et al. 2017) and their modulation by dopamine (Knapp and Dowling 1987; Liu et al. 2016; Pfeiffer-Linn and Lasater 1993), or as a side effect of the well-established decoupling of horizontal cell gap junctions upon D1R activation (Dong and McReynolds 1991; Ribelayga and Mangel 2003; Zhang et al. 2011). The complexity of this synapse renders an overly simplistic interpretation of such observation as speculative at best (see Chapot et al. 2017), barring further experimental evidence.

In addition to glutamatergic signals from photoreceptors, the response and output of ON cone bipolar cells are shaped by multiple sources of inhibitory and excitatory signaling. In addition to their effects on photoreceptor release, there is some evidence that horizontal cells can act via feed-forward $GABA_A$ synapses to modify ON cone bipolar cell output (Du and Yang 2000; Hare and Owen 1996; Haverkamp et al. 2000; Herrmann et al. 2011; Shields et al. 2000; Thoreson and Mangel 2012). A recent study in rabbits found evidence for a D1R-mediated increase in $GABA_A$ receptor expression in the dendrites of ON cone bipolar cells (Chaffiol et al. 2017), though in this case the authors suggested a mechanism involving tonic GABA-induced depolarization. Interestingly, the same study found that, in the absence of dopamine, cone bipolar

cell light-evoked voltage responses increased with stimulus size, while the selective activation of D1Rs (but not D2/D4Rs) caused responses to decline with increased stimulus size, presumably through modulation of surround strength. Here a full-field light stimuli was used that should maximally activate the surrounds of upstream ON cone bipolar cells. Thus, if D1R activation increases the strength of ON cone bipolar cell surrounds, application of a D1R agonist should result in decreased ON cone bipolar cell responses to light, which would help to explain our result of reduced excitatory input to ON-s ganglion cells.

Although horizontal cells undoubtedly play a part in shaping the inhibitory surrounds of bipolar cells, there is a growing literature concerning the role of amacrine cells in generating bipolar cell receptive fields under both dark- and light-adapted conditions (Bloomfield et al. 1997; Chavez et al. 2010; Dedek et al. 2008; Diamond 2017; Euler and Masland 2000; Flood et al. 2018; Lukasiewicz 2005; Mazade and Eggers 2020; 2016; Mazade et al. 2019; Molnar and Werblin 2007; Oesch and Diamond 2019; Zhao et al. 2017). Rod bipolar and ON cone bipolar cells both receive mixed $GABA_AR/GABA_CR$-mediated inhibition (Chavez et al. 2010; Eggers and Lukasiewicz 2006; Euler and Wassle 1998; Shields et al. 2000), while rod bipolar cells also receive glycinergic input (Chavez and Diamond 2008; Eggers and Lukasiewicz 2011; Ivanova et al. 2006). The amount of evoked inhibition these cells receive is dependent upon the size of a light stimulus; initially inhibitory inputs will increase with growing stimulus size as the surround is activated, but beyond a certain stimulus size the amount of evoked inhibition will decline as recruitment of serial inhibitory synapses between amacrine cells serve to limit their release (Eggers and Lukasiewicz 2010; Hsueh et al. 2008; Zhang et al. 1997). There is some evidence that D1R activation can modulate the strength of these inhibitory synapses between amacrine cells (Smith et al. 2015b; Travis et al. 2018), possibly through the potentiation of $GABA_A$ currents that are thought to underlie them (Feigenspan and Bormann 1994a). Additionally, we have previously shown that D1R activation can reduce evoked inhibitory inputs to rod bipolar cells independent of this serial inhibitory network (Flood et al. 2018), possibly by inhibiting calcium currents as it does in horizontal cells (Liu et al. 2016).

D1R activation reduced light-evoked inhibitory currents onto rod bipolar cells (Flood et al. 2018), which presumably should increase their excitatory drive to the AII amacrine cells → ON cone bipolar cell network, resulting in increased excitatory currents in ON ganglion cells. However, the results presented here showed a decrease in ON-s ganglion cell L-EPSCs when

D1Rs were activated. Growing evidence for an amacrine cell population that receives primarily ON cone bipolar cell excitatory input and inhibits AII amacrine cells (Lauritzen et al. 2019; Park et al. 2020) could explain our findings, as this network would selectively reduce excitatory contributions from the rod pathway to cone bipolar cells. It is also possible that D1R activation could decrease inhibitory inputs to ON cone bipolar cells, as has been previously demonstrated for OFF cone bipolar cells (Mazade and Eggers 2020; Mazade et al. 2019) although we would instead expect an enhancement of inhibitory currents to explain our current results. Lastly, outside of changes to ON cone bipolar cell inputs, it is likely that some populations of ON cone bipolar cells are directly affected by D1R activation. Farshi et al. (2016) demonstrated D1R expression in ON type 5-2, 6 and 7 bipolar cells, all of which likely form synapses onto ON-s ganglion cells (Dunn and Wong 2014; Helmstaedter et al. 2013) with ON type 6 bipolar cells forming the majority (Schwartz et al. 2012). Previous research has demonstrated a role for D1Rs in reducing voltage-gated currents in ON cone bipolar cells (Hellmer et al. 2020; Ichinose et al. 2005; Smith et al. 2015a), depolarizing their resting membrane potential (Chaffiol et al. 2017)and modulating GABAergic conductance (Dong and Werblin 1994; Feigenspan and Bormann 1994b; Wellis and Werblin 1995), all of which could have significant impacts on excitatory output to ON ganglion cells.

Activation of dopamine type-4 receptors likely reduces glutamate release by photoreceptors

After D4R activation, we found that L-EPSCs were significantly reduced in magnitude (Fig. 5), with a concurrent decrease in sEPSC frequency (Fig. 6C,E). These changes suggest D4R-induced modulation of both phasic and tonic release of glutamate by ON cone bipolar cells. We also found that D4R activation reduced sEPSC amplitude (Fig. 6D), suggesting a post-synaptic change in ionotropic glutamate receptors that could help explain our findings. However, it is possible that this difference in average sEPSC amplitude may simply arise from reductions in coordinated vesicle release by ON cone bipolar cells. Overall, our results show that D4R activation reduces excitatory inputs to ON-s ganglion cells.

Previous studies have explored the effects of D4R-mediated signaling on ganglion cell responses. Jensen and Daw (1986) found that inhibiting dopamine type-2 family receptors in light-adapted rabbit retina increased spontaneous spiking activity in ON-s ganglion cells, suggesting that activation of these receptors should decrease spontaneous spiking. Our results

showing diminished sEPSC frequency and amplitude upon D4R activation could help to explain this change in spontaneous spiking. Another study showed that antagonizing D4Rs in rat retina decreases light sensitivity and maximum firing rate in ON ganglion cells, suggesting that D4R activation should increase both of these parameters (Jensen 2015). This finding is thus contrary to our current results. However, it is difficult to directly compare our findings with those from these previous two studies as they attempted to block D4Rs under light-adapted conditions (when D1R- and light-induced changes should be active), whereas we examined the effects of selectively agonizing D4Rs under dark-adapted conditions. Additionally, these studies also used extracellular spikes as their metric, which cannot differentiate between pre- and post-synaptic effects. The seemingly contradictory results between our study and Jensen's (2015) are thus likely attributable to the involvement of D4R-modulatable voltage-gated currents in ON-s ganglion cells.

There is evidence for D4R expression in certain ganglion cell populations (Hayashida et al. 2009; Koulen et al. 1999; Ogata et al. 2012; Van Hook et al. 2012; Veruki 1997). A recent study performed on isolated rat ganglion cells found that activation of dopamine type 2 family receptors enhances ganglion cell excitability by modifying voltage-gated channels (Yin et al. 2020), but this would have little effect in the voltage-clamp experiments performed here. Most dopamine receptor localization studies agree that the predominant location for D4Rs in the retina, is on photoreceptors (Cohen et al. 1992; Deming et al. 2016; Derouiche and Asan 1999; Klitten et al. 2008; Patel et al. 2003; Tran and Dickman 1992), suggesting that they are the main site of D4R agonist action. Studies suggest that dopamine diminishes photoreceptor light sensitivity (Nikolaeva et al. 2019) and modifies calcium currents (Ivanova et al. 2008; Stella and Thoreson 2000; Thoreson et al. 2002), with the overall effect of decreasing glutamate release (Firsov and Astakhova 2015). If these changes result in decreased tonic glutamate release from photoreceptors, we would expect a sustained depolarization of ON bipolar cells, which could lead to a diminished capacity for both spontaneous and light-evoked glutamate release onto ON ganglion cells via activity-dependent adaptation mechanisms (Nawy 2000; Snellman et al. 2008).

The full effects of light adapting mechanisms likely require light

We found that neither a D1R nor D4R agonist were able to completely recapitulate the effects of our light adaptation protocol on excitatory ganglion cell currents. Our simple additive

model for the proposed effects of D1R/D4R co-activation also failed to do so (Fig. 7), although the true effects of such a treatment may be synergistic rather than additive. However, it is very likely that light adaptation also involves some mechanisms that are dopamine-independent. Several studies have identified activity-dependent effects on vesicle supply (Jarsky et al. 2011; Oesch and Diamond 2011; Singer and Diamond 2006), calcium influx (Nawy 2000; Snellman et al. 2008), glutamate receptor cycling (Jones et al. 2012; Xia et al. 2007) and release probability (Grimes et al. 2014) that modify the behavior of retinal circuits. In addition, light-dependent release of nitric oxide (Jacoby et al. 2018; Tooker and Vigh 2015) has been implicated as another source of retinal adaptation that can modify the activity of multiple retinal populations (Agurto et al. 2017; Maddox and Gleason 2017; Tooker et al. 2013). In our study, we utilized brief light stimuli that were separated by significant periods of darkness to reduce the impact of these mechanisms, but many of these changes were probably induced to some degree by our light-adapting background.

Conclusion

Light adaptation, although a straightforward concept from the perspective of signal processing, is a phenomenon of intricate complexity when considering the diverse molecular, cellular and circuit mechanisms that are involved. In this study, we assessed the contributions of specific dopaminergic signaling pathways to light adaptation of a relatively well-characterized retinal circuit. We found that activation of either dopamine pathway contributed to light-induced changes via modulation of retinal activity upstream of ON-s ganglion cells, and perhaps via modulation of post-synaptic receptors on ON-s ganglion cells. Although the main likely sites of dopaminergic action of these two pathways are located at different retinal populations, their effects on full-field light-evoked excitatory currents at the ganglion cell level may be similarly brought about through modulation of photoreceptor release dynamics. Future work will need to be done to characterize the differential contribution of dopaminergic signaling to the modulation of retinal interneuron receptive fields, and to isolate the actions of these pathways on inner vs. outer retinal activity.

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Figure legends

Figure 1. Light adaptation decreases the magnitude and kinetics of L-EPSCs in ON-s GCs. **A**. Example L-EPSC traces from the same cell before (black, dark-adapted, DA) and after (gray, light-adapted, LA) light adaptation at three different light intensities**.** 30-ms light stimuli are represented by small golden bars. Stimulus intensities in total rod photoisomerizations are given above each set of traces. **B-E**. Average peak amplitude (B), Q (C), time to peak (D) and D37 (E) of normalized L-EPSCs before (black) and after (gray) light adaptation. 2-way ANOVA main effects of adaptation p values are reported at the top of each graph. * = significant main effects difference between dark and light adapted states. # = significant pairwise difference between dark and light adapted states at individual light intensities. Error bars = +/- 95% confidence interval. For all of thes experiments, n = 17 cells from 12 animals.

Figure 2. Light adaptation decreases the frequency and amplitude of sEPSCs in ON-s GCs. **A**. Example sEPSC traces from the same cell before (black,left) and after (gray,right) light adaptation**. B-C**. Average cumulative histogram of sEPSC amplitudes (B) and inter-event intervals (C) before and after light adaptation. Shaded regions represent 95% confidence intervals. Bin frequencies were normalized to total number of events recorded under each condition, and amplitude/IEI values were normalized to maximum values recorded on a cell-by-cell basis. 2-way ANOVA main effects p-values for DA vs LA are reported above each graph. **D-E.** Average changes in sEPSC amplitude (D) and frequency (E) after light adaptation. Values were normalized on a cell-by-cell basis to that recorded under dark-adapted conditions. Individual data points are marked by empty white circles. Error bars = +/- standard error. For all of these experiments, n = 15 cells from 10 animals.

Figure 3. D1R activation decreases the magnitude and times to peak of L-EPSCs in ON-s GCs **A**. Example L-EPSC traces from the same cell at increasing light intensities before (black) and after (light blue) application of the D1R agonist SKF-38393 (20 μM). The gold bars represent 30 ms light stimuli. Stimulus intensities in total rod photoisomerizations are given above each set of traces. **B-E.** Comparison of average peak amplitude (B), Q (C), time to peak (D) and D37 (E) between dark-adapted (DA) and D1R-agonized (DA+D1) conditions. All values are normalized on a cell-by-cell basis to the maximum values of each parameter recorded for each cell. Average

normalized values of light-adapted ganglion cells from figure 1 are included for reference. Main effects of agonist treatment p-values for two-way ANOVAs are shown above each graph. * = significantly different main effect between dark adapted and agonist-treated states at each light intensity. # = significantly different pairwise comparison between dark-adapted and D1R activated states. † = significantly different pairwise comparison between D1R activated and light-adapted states. Error bars = +/- 95% confidence interval. For all of our D1R L-EPSC experiments, n = 9 cells from 8 animals.

Figure 4. D1R activation decreases the frequency of sEPSCs in some but not all ON-s GCs. **A**. Example sEPSC traces from the same cell before (black,left) and after (blue,right) application of the D1 agonist SKF 38393 (20 µM)**. B-C**. Average cumulative histogram of sEPSC amplitudes (B) and inter-event intervals (C) before and after D1R activation. Shaded regions represent 95% confidence intervals. Bin frequencies were normalized to total number of events recorded under each condition, and amplitude/IEI values were normalized to maximum values recorded on a cell-by-cell basis. 2-way ANOVA main effects p-values for DA vs D1 are reported above each graph. **D-E.** Average changes in sEPSC amplitude (D) and frequency (E) after D1 agonist application or light adaptation. Average values for each treatment were normalized on a cell-by-cell basis to that recorded under dark-adapted conditions. Individual data points are marked by empty white circles. Error bars = +/- standard error. # = significant difference between dark-adapted and D1 agonist conditions. * = significant difference between D1 agonist and light-adapted conditions. For all of these experiments, n = 5 cells from 5 animals.

Figure 5. D4R activation decreases the magnitude but not kinetics of L-EPSCs in ON-s GCs **A**. Example L-EPSC traces from the same cell at increasing light intensities before (black) and after (red) application of the D4R agonist PD-168077 maleate (500 nm). The gold bars represent 30 ms light stimuli. Stimulus intensities in total rod photoisomerizations are given above each set of traces. **B-E.** Comparison of Q (B) peak amplitude (C), time to peak (D) and D37 (E) between dark-adapted (DA) and D4R-agonized (DA+D4) conditions. Light adapted (LA) values from figure 1 are included for comparison. Main effects of agonist treatment p-values for two-way ANOVAs are shown above each graph. * = significantly different main effect between dark adapted and agonist-treated states at each light intensity. # = significantly different pairwise

comparison between dark-adapted and D4R activated states. † = significantly different pairwise comparison between D4R activated and light-adapted states. Error bars = +/- 95% confidence interval. For our D4R L-EPSC experiments, n = 7 cells from 6 animals.

Figure 6. D4R activation decreases the frequency of sEPSCs and slightly diminishes sEPSC amplitudes in ONs GCs . **A**. Example sEPSC traces from the same cell before (black,left) and after (red,right) application of the D4 agonist PD-168077 maleate (500 nM)**. B-C**. Average cumulative histogram of sEPSC amplitudes (B) and inter-event intervals (C) before and after D4R activation. Shaded regions represent 95% confidence intervals. Bin frequencies were normalized to total number of events recorded under each condition, and amplitude/IEI values were normalized to maximum values recorded on a cell-by-cell basis. 2-way ANOVA main effects p-values for DA vs D4 are reported above each graph. **D-E.** Average changes in sEPSC amplitude (D) and frequency (E) after D4 agonist application or light adaptation. Average values for each treatment were normalized on a cell-by-cell basis to that recorded under dark-adapted conditions. Individual data points are marked by empty white circles. Error bars = +/- standard error. # = significant difference between dark-adapted and D1 agonist conditions. * = significant difference between D1 agonist and light-adapted conditions. For these experiments, n = 5 cells from 4 animals.

Figure 7. Additive effects of dopaminergic signaling do not fully explain light-adaptation. **A-B.** Average percent of response remaining (as compared to under dark-adapted conditions) after light adaptation (LA, gray), application of a D1R agonist (D1, blue), or D4R agonist (D4, red), as well as the theoretical reduction one would witness from the additive effects of D1R-D4R agonist co-application (D1+D4, dashed line). The additive effects of D1R/D4R agonist co-application seem to approximate the effects of light adaptation in diminishing peak amplitude (A, but do not match the decline in charge transfer witnessed upon light adaptation (B). **C-D**. Average % of sEPSC amplitude (C) and frequency (D) compared to dark-adapted conditions after light adaptation (LA, grey), D1R activation (D1, blue), and D4R activation (D4, red). The hypothetical impact of combined D1R/D4R activation are also shown (D1+D4, gold). The combined effects of dopamine agonists seems to approximate light-adaptations effects upon average sEPSC amplitude (C), but not average sEPSC frequency (D).

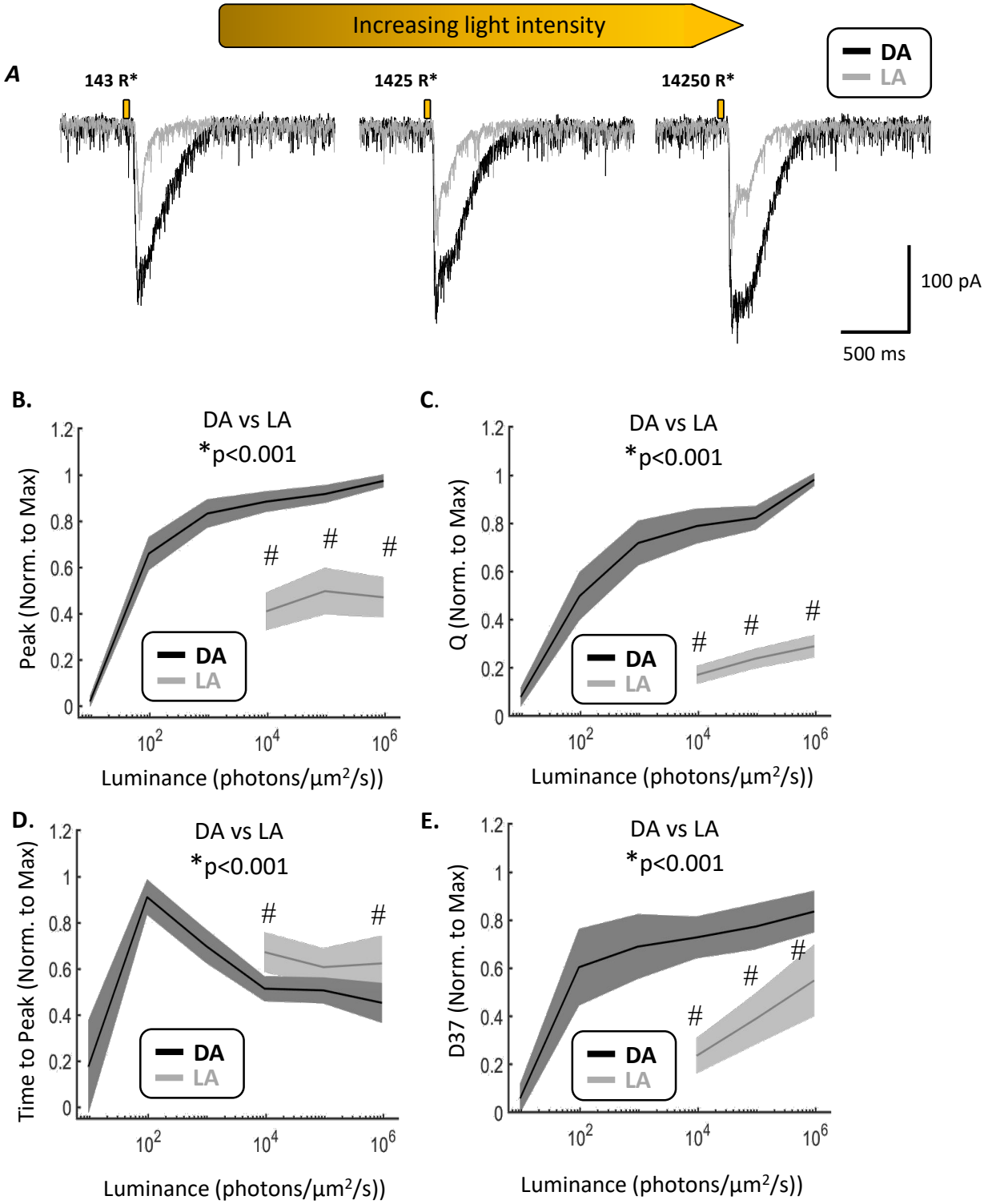
Increasing light intensity
A
143 R*
1425 R*
14250 R*
DA
LA
100 pA
500 ms
B.
DA vs LA
*p<0.001
Peak (Norm. to Max)
Luminance (photons/µm²/s))
C.
DA vs LA
*p<0.001
Q (Norm. to Max)
Luminance (photons/µm²/s))
D.
DA vs LA
*p<0.001
Time to Peak (Norm. to Max)
Luminance (photons/µm²/s))
E.
DA vs LA
*p<0.001
D37 (Norm. to Max)
Luminance (photons/µm²/s))

Figure 1

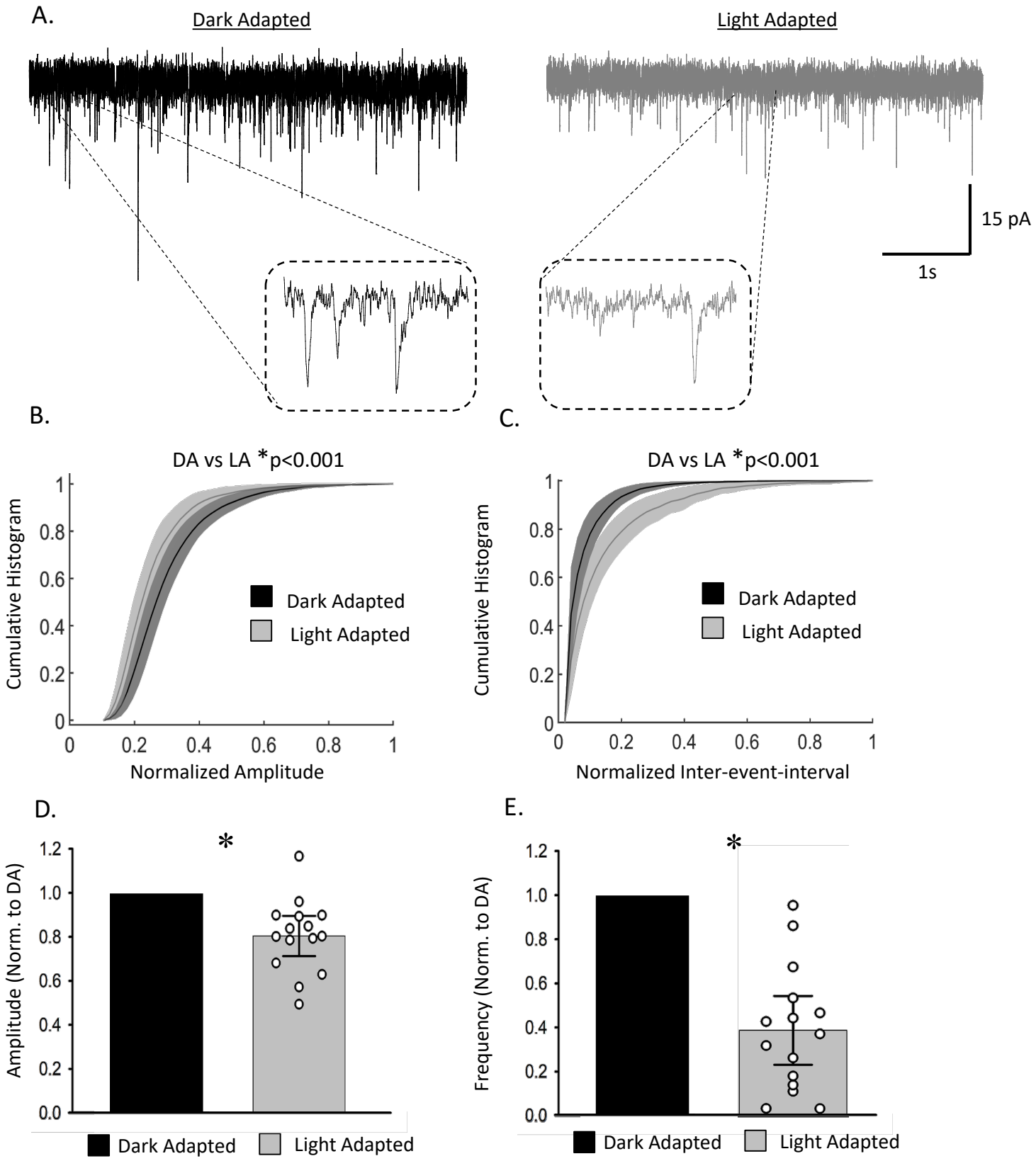
A.
Dark Adapted
Light Adapted
15 pA
1s
B.
DA vs LA *p<0.001
Cumulative Histogram
Normalized Amplitude
Dark Adapted
Light Adapted
C.
DA vs LA *p<0.001
Cumulative Histogram
Normalized Inter-event-interval
Dark Adapted
Light Adapted
D.
Amplitude (Norm. to DA)
*
Dark Adapted
Light Adapted
E.
Frequency (Norm. to DA)
*
Dark Adapted
Light Adapted

Figure 2

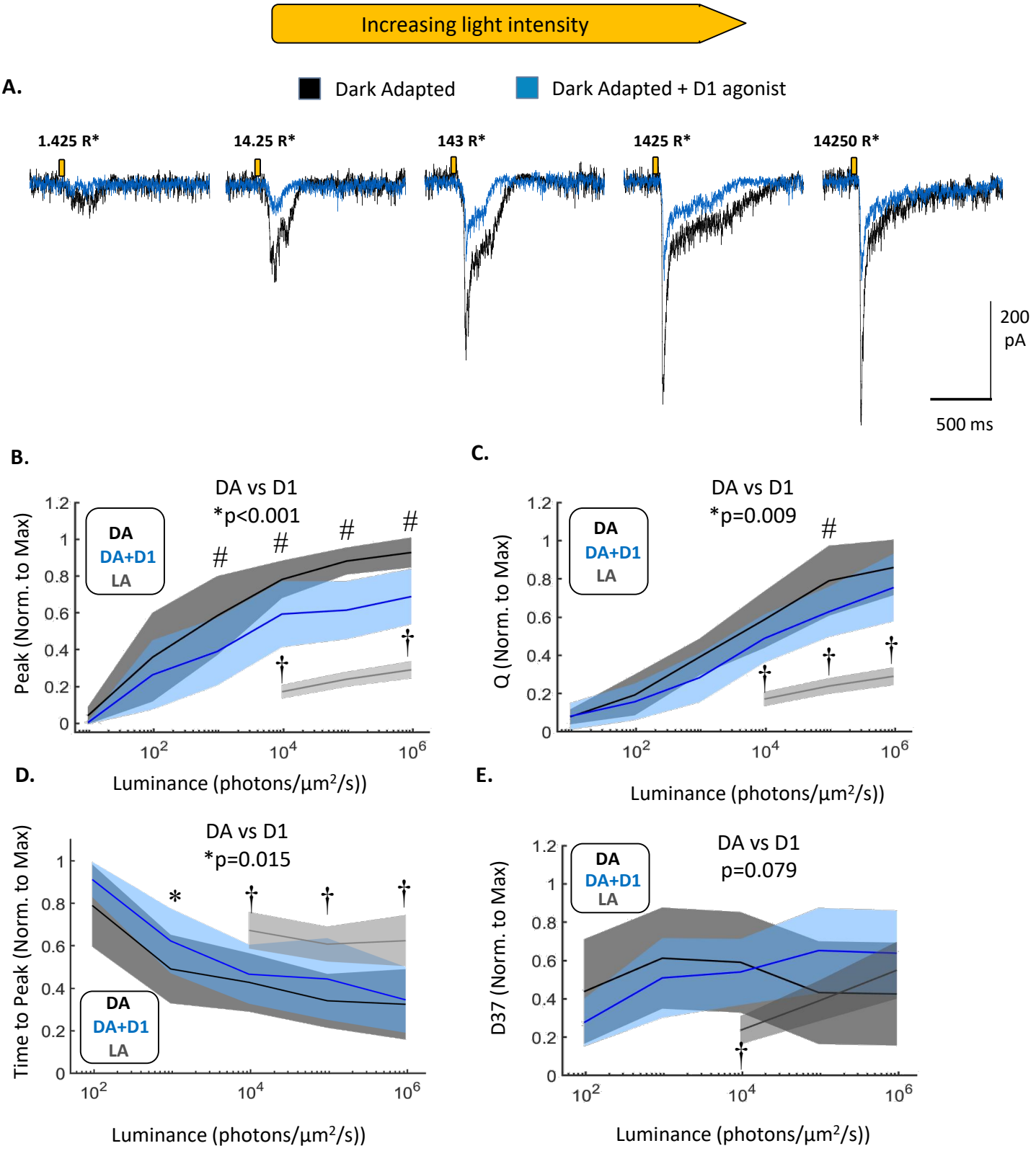
Increasing light intensity
A.
Dark Adapted
Dark Adapted + D1 agonist
1.425 R*
14.25 R*
143 R*
1425 R*
14250 R*
200 pA
500 ms
B.
DA vs D1
*p<0.001
DA
DA+D1
LA
Peak (Norm. to Max)
Luminance (photons/µm²/s))
C.
DA vs D1
*p=0.009
Q (Norm. to Max)
Luminance (photons/µm²/s))
D.
DA vs D1
*p=0.015
Time to Peak (Norm. to Max)
Luminance (photons/µm²/s))
E.
DA vs D1
p=0.079
D37 (Norm. to Max)
Luminance (photons/µm²/s))

Figure 3

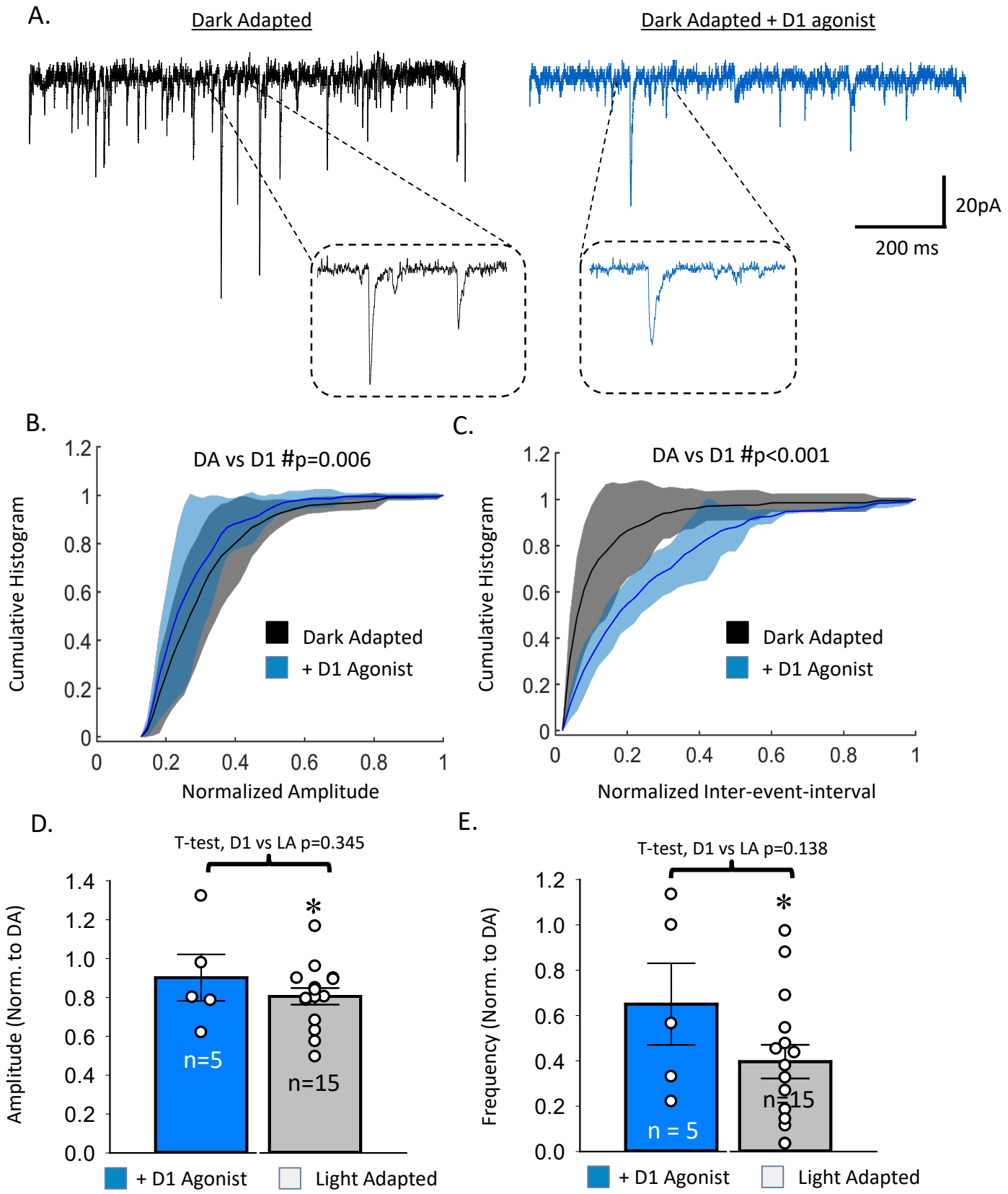
A.
Dark Adapted
Dark Adapted + D1 agonist
20pA
200 ms
B.
DA vs D1 #p=0.006
Cumulative Histogram
Normalized Amplitude
Dark Adapted
+ D1 Agonist
C.
DA vs D1 #p<0.001
Cumulative Histogram
Normalized Inter-event-interval
Dark Adapted
+ D1 Agonist
D.
T-test, D1 vs LA p=0.345
Amplitude (Norm. to DA)
n=5
n=15
+ D1 Agonist
Light Adapted
E.
T-test, D1 vs LA p=0.138
Frequency (Norm. to DA)
n = 5
n=15
+ D1 Agonist
Light Adapted

Figure 4

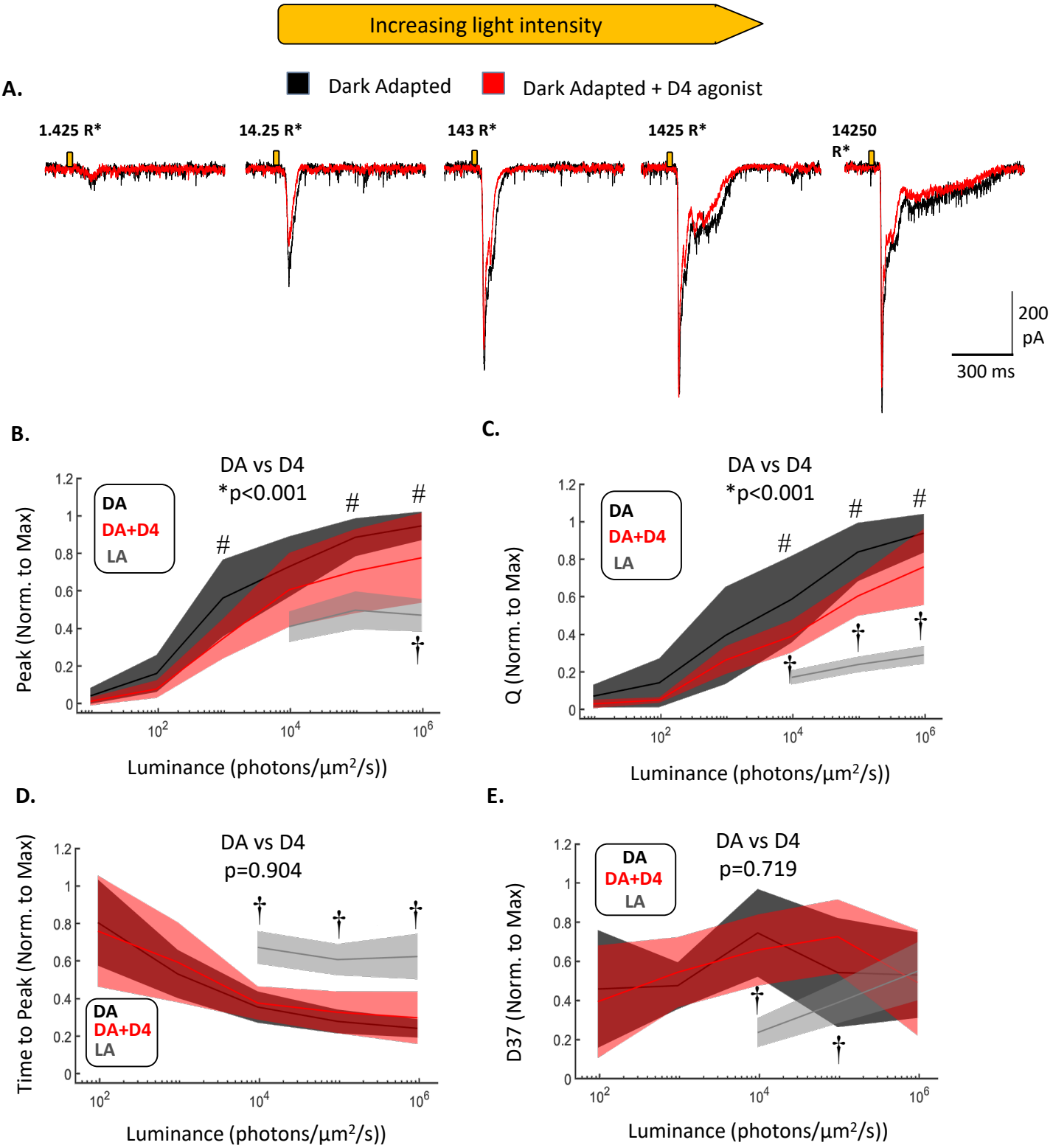
Increasing light intensity
A.
Dark Adapted
Dark Adapted + D4 agonist
1.425 R*
14.25 R*
143 R*
1425 R*
14250 R*
200 pA
300 ms
B.
DA vs D4
*p<0.001
DA
DA+D4
LA
Peak (Norm. to Max)
Luminance (photons/µm²/s))
C.
DA vs D4
*p<0.001
Q (Norm. to Max)
Luminance (photons/µm²/s))
D.
DA vs D4
p=0.904
Time to Peak (Norm. to Max)
Luminance (photons/µm²/s))
E.
DA vs D4
p=0.719
D37 (Norm. to Max)
Luminance (photons/µm²/s))

Figure 5

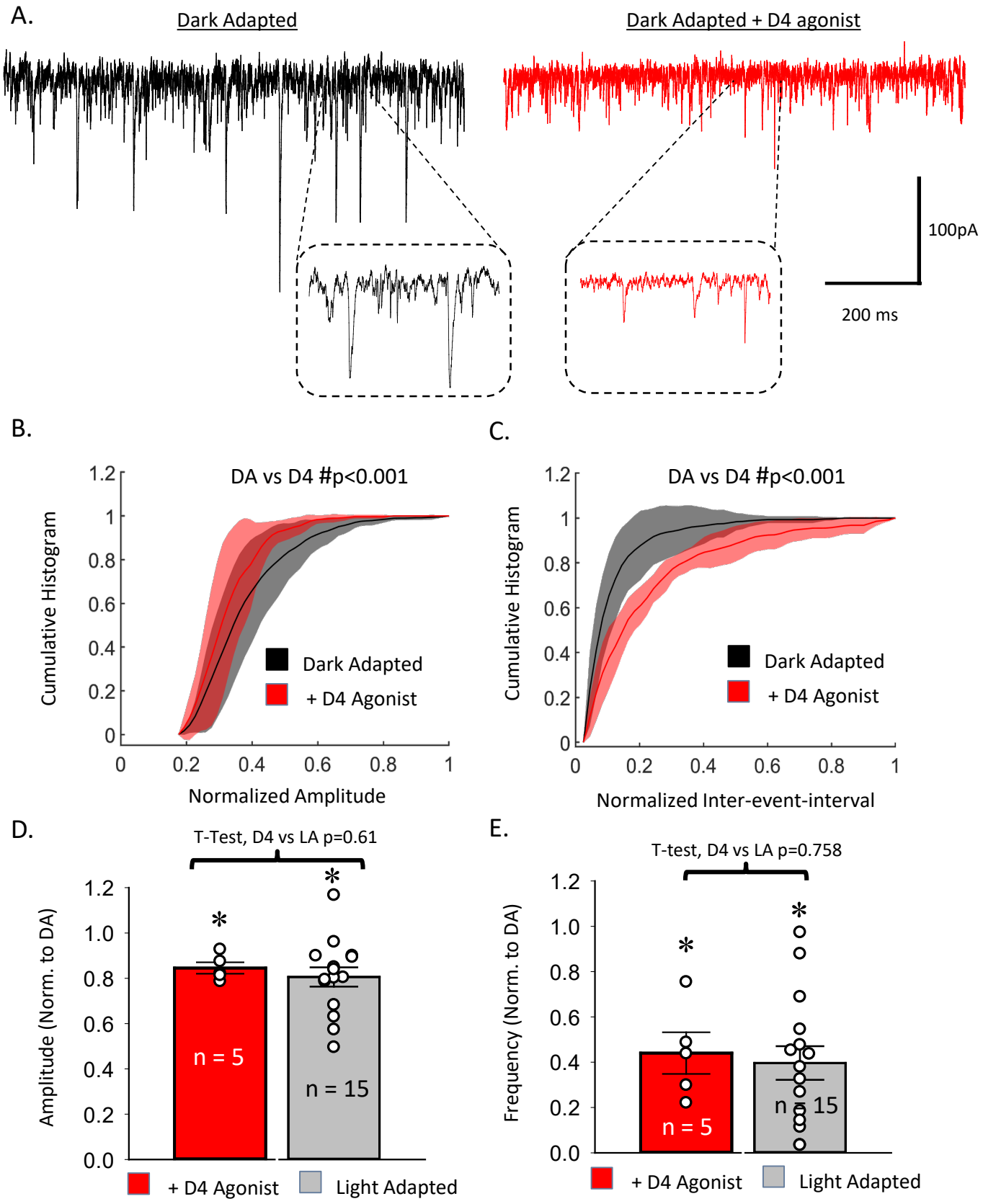
A.
Dark Adapted
Dark Adapted + D4 agonist
100pA
200 ms
B.
DA vs D4 #p<0.001
Cumulative Histogram
Normalized Amplitude
Dark Adapted
+ D4 Agonist
C.
DA vs D4 #p<0.001
Cumulative Histogram
Normalized Inter-event-interval
Dark Adapted
+ D4 Agonist
D.
T-Test, D4 vs LA p=0.61
Amplitude (Norm. to DA)
n = 5
n = 15
+ D4 Agonist
Light Adapted
E.
T-test, D4 vs LA p=0.758
Frequency (Norm. to DA)
n = 5
n = 15
+ D4 Agonist
Light Adapted

Figure 6

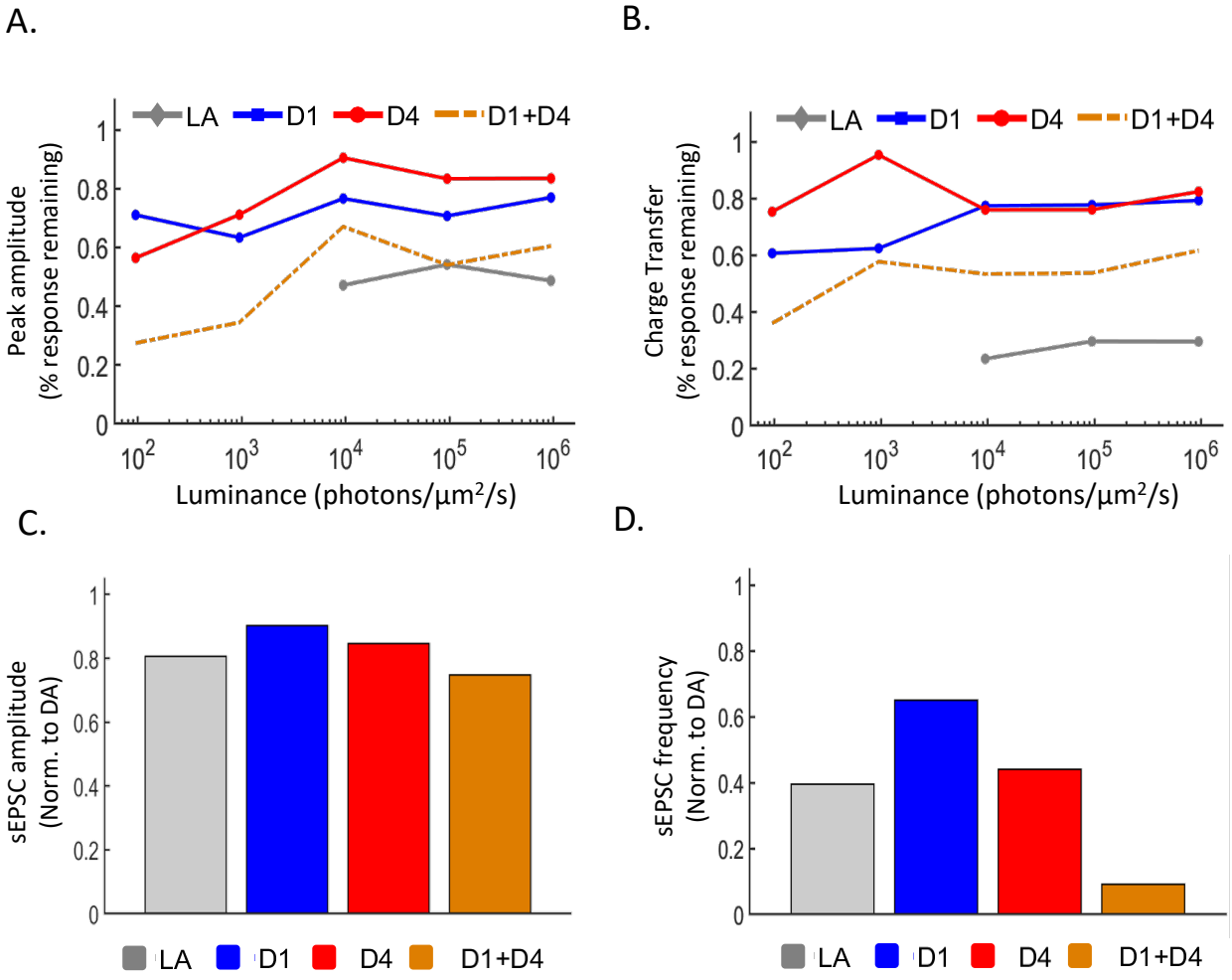
A.
B.
C.
D.
LA
D1
D4
D1+D4
Peak amplitude
(% response remaining)
Charge Transfer
(% response remaining)
Luminance (photons/μm²/s)
sEPSC amplitude
(Norm. to DA)
sEPSC frequency
(Norm. to DA)

Figure 7