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Rod photoreceptor activation alone defines the release of dopamine in the retina

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Abstract

Retinal dopamine is released by a specialised subset of amacrine cells in response to light and has a potent influence on how the retina responds to, and encodes, visual information. Here we address the critical question of which retinal photoreceptor is responsible for coordinating the release of this neuromodulator. While all three photoreceptor classes: rods, cones and melanopsin-containing retinal ganglion cells (mRGCs) have been shown to provide electrophysiological inputs to dopaminergic amacrine cells (DACs), we show here that the release of dopamine is defined only by rod photoreceptors. Remarkably, this rod signal coordinates both a suppressive signal at low intensities, and drives dopamine release at very bright light intensities. These data further reveal that dopamine release does not necessarily correlate with electrophysiological activity of dopaminergic amacrine cells and add to a growing body of evidence that rods define aspects of retinal function at very bright light levels.

Introduction

Dopamine is one of the most potent retinal neuromodulators, and its widespread functions define how retinal circuits encode visual information. Generally a signal for light-adaptation of the retinal circuitry, the effects of dopamine have been observed in growth and development, cell death, and myopia (reviewed [1, 2]). Therefore, there has been great interest in understanding the photoreceptor(s) and circuits responsible for coordinating its light-dependent release. Over recent years, the electrophysiological inputs to dopaminergic amacrine cells (DACs) have been defined in great detail, showing that all three photoreceptor classes: rods, cones and melanopsin-containing retinal ganglion cells (mRGCs), provide substantial input to these cells [3-7]. Conversely however, measurement of dopamine release in response to light does not correlate with electrophysiological input; while mRGC input alone can increase spiking of DACs this is not sufficient to drive dopamine release [8-10]. This finding matches recent discoveries in striatum-projecting midbrain dopaminergic neurons that display similar characteristics where dopamine release is not simply a passive read-out of midbrain cell firing [11]. It is proposed that dopamine can be locally released from axonal terminals in the striatum but the mechanism underlying this occurrence is unknown. The retina represents an ideal model to study the discontinuity between spiking and dopamine release given the well-defined and accessible nature of this tissue.

Retinal DACs reside in the middle of the retina where they give rise to an extensive dendritic/axonal plexus [12, 13]. Dopamine release is thought to occur by volume transmission that diffuses throughout the entire depth of the retina to activate dopamine receptors on all known classes of neuron [14, 15]. Here, we systematically investigate light-dependent release of dopamine in both wild-type and a variety of transgenic mice with altered photoreception and by using chemogenetics tools. Our data demonstrates that light-induced dopamine release is unexpectedly high threshold, not being released until bright photopic light intensities (> 400 lux), and, surprisingly, that this threshold is defined by the level of rod function. Previously rods had been thought to only govern dim light vision, but recent reports have suggested a novel function for them under very bright-light [16, 17]. Additionally, we reveal an inhibitory rod input that actively suppresses dopamine release under dim- to mesopic light intensities. We conclude that a balance of excitatory and inhibitory inputs, driven by rod photoreceptors, defines the release of dopamine in the retina over 9 log units of light stimulation. Furthermore, we examine the mechanisms governing dopamine release and propose that it can be segregated within individual DACs to specific axonal/dendritic varicosities.

Results

Light-induced dopamine release is high-threshold

Measurement of dopamine release in neuronal tissue has been approached in a variety of ways over the years. Here we use two complementary techniques to assess dopamine release in the mouse retina (Fig 1A): i) the ratio of one of the primary metabolites of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) to total DA (DOPAC:DA ratio) as an *in vivo* measure of dopamine turnover and ii) the release of dopamine from a dissected eye-cup (cornea, lens and iris removed) into an artificial media (artificial cerebrospinal fluid; aCSF) as an *ex vivo* measure. We were surprised to discover that a significant increase in light-induced dopamine

turnover (DOPAC:DA ratio) or DA release (*ex vivo*), only occurred under bright photopic illumination ($> 5 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$; $\sim 400 \text{ lux}$; Fig 1B&C). When these two measures are directly compared after normalisation (R/R_{max}) and corrected for differences in retinal illuminance (see methods) this threshold was the same irrespective of the mode of measurement and lies $> 6 \log$ units above the threshold for an electroretinogram (ERG) threshold response (Fig 1D). While such a high threshold for dopamine release was unexpected, the agreement between these two very different methods to assess dopamine release gives us confidence in the results. Moreover, the correlation of DOPAC:DA ratio to actual dopamine release from the retina further supports the utility of this indirect measure as a correlate of dopamine release.

Dim light actively suppresses dopamine release

The lowest irradiance point in Fig 1B was dim red light ($\sim 0.017 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) and was generally used as the “dark” condition in accordance with previous work [10, 18, 19], though rods will be activated by this stimulus. This point corresponds to $< 1 \text{ min}$ of light exposure during removal of the retinal tissue, as opposed to a 1hr white pulse at all other irradiances. However, when retinae were extracted under infrared light (IR; $-2.5 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) over a similar time-scale, an *increase* in dopamine turnover was observed (Fig 1E). These data indicate that the dim red light, presumably acting via rods at these intensities, is actively suppressing dopamine release.

Circadian rhythmicity of light-induced dopamine release is not observed

As light-induced dopamine turnover has been previously shown to be released in a circadian manner, with greater turnover at subjective midnight (CT18) as opposed to subjective midday (CT6) [10] we compared irradiance responses under these conditions (Fig 1F). Contrary to previous findings, no circadian differences were observed at any of the irradiances tested. This is likely due to the fact that animals were anaesthetised while light-pulsed, rather than free-moving in their home-cage. While we have previously shown that anaesthesia does not change the amplitude of the response, we find that it greatly reduces intra-individual variability [20]. We hypothesize that anaesthesia allows a more standardized light pulse to be applied to the animals rather than when they are free-moving in their home cage. All further experiments were performed within 2 hrs of CT6. No circadian difference in dark adapted dopamine release was observed in agreement with previous studies on C57BL/6 mice that lack melatonin [21].

Cone input is not required for light-induced dopamine release

While previous data suggests that rods predominately drive dopamine release [10], this is hard to rectify with the fact that the threshold of activation is at least 6 log units above that of rods making cones or mRGCs a more likely origin for this input. We first assessed cone input to light-induced dopamine release in a mouse model lacking cone phototransduction (*Gnat2*^{A518G}). These animals have morphologically normal cones and little cellular reorganisation at the ages used (2 – 3 months) but lack any measurable cone response [22]. In comparison to wild-type animals, *Gnat2*^{A518G} mice showed indistinguishable light-induced dopamine release that could be fit with the same curve (Fig 2A; extra-sum-of-squares F test, $P = 0.70$; also observed using the *ex vivo* protocol Fig S1B), suggesting cone function is not necessary to drive this response or set the threshold. Furthermore, dim red light produced the same level of suppression when compared to infrared meaning cones are not driving this

suppression in dim light (Fig 2B). In agreement with wild-types, *Gnat2*^{A518G} animals did not show any circadian differences in irradiance response (Fig 2C).

To determine the function of rods under the intensities that elicit dopamine release we recorded light-adapted flash electroretinograms from *Gnat2*^{A518G} and wild-type animals under two different adapting backgrounds. Michaelson contrast was constant for both conditions (0.99) and intensities of the background and flash (2 ms) were controlled by the addition of neutral density filters. Background A (Fig 2D) is below the dopamine release threshold ($3.3 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) and *Gnat2*^{A518G} animals display a measurable rod response, although it is smaller and slower than that of the wild-type (blue; Fig 2E). However, background B is just above the dopamine release threshold ($5.3 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) and *Gnat2*^{A518G} do not display any noticeable response, whereas the wild-type response is very similar to that recorded under background A (Fig 2F). While rods are not “silent” under these conditions, these data suggest that they do not contribute significantly to the transient response to light flashes in the presence of a background above the dopamine release threshold.

Given that some mouse models of cone inactivation have been shown to be incomplete, due to the second-messenger promiscuity of opsins [23], we assessed the impact of cone photoreception on light induced dopamine release in an alternative way utilising mice expressing the human L-cone opsin in place of the mouse M-cone opsin (*Opn1^{mwr}*) [24]. Activation of cones in these animals can be spectrally segregated from that of rods and mRGCs. We designed a red (624 nm) light stimulus that activates L-cones above the “threshold” for dopamine release, (see effective photon calculations in Fig S2) but is below threshold for rods and mRGCs, allowing us to ascertain if it is cones that are determining this threshold. We found that this red stimulus was not sufficient to drive light-induced dopamine release in the *Opn1^{mwr}* mouse (although a positive trend was observed; Fig 2G), indicating that cones are not setting the threshold for full amplitude light-induced dopamine release. We then applied a red stimulus that was matched for rod sensitivity with that of a blue pulse (434 nm) in wild-type animals and found that red light *can* drive significant dopamine release but only when it is bright enough to stimulate rods above the dopamine threshold (Fig 2H). Taken together these experiments indicate that a photoreceptor with a spectral sensitivity matching that of rod opsin determines the dopamine release threshold.

As cone input can be transient due to adaptation [25], and because previous data has shown that input to DACs can be enhanced by using a flickering light [26, 27], we compared dopamine turnover in response to a flickering or continuous white light stimulus just below the dopamine release threshold ($4.6 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) in wild-type animals. Flickering light did not enhance the response to white light at this intensity (Fig 2I) suggesting that the light input to DACs to elicit dopamine release is specific to the intensity of light and cannot be enhanced by summing many transient ON inputs.

mRGCs are neither necessary nor sufficient to drive light-induced dopamine release

We assessed mRGC input to light-induced dopamine release in a variety of ways. Firstly, using rodless coneless (*rd/rd*) mice we completed an irradiance response curve and found that dopamine turnover is not significantly altered at any light intensity (Fig 3A). Furthermore, mice lacking melanopsin (*Opn4^{cre/cre}*) show irradiance response curves that are

indistinguishable from wild-type animals (Fig 3B; extra-sum-of squares F-test, $p = 0.88$; also observed using the *ex vivo* protocol Fig S1). Additionally, while *Opn4^{cre/cre}* mice showed normal suppression of dopamine turnover by dim red light compared to infrared, no difference between the two was observed in the *rd/rd* animals (Fig 3C), further implicating rods in driving this suppression.

The retinae of the *rd/rd* animals undergo significant neural reorganisation following photoreceptor degeneration and although mRGC electrophysiological input to DACs is maintained in these animals [3] we investigated if dopamine could actually be released from DACs in *rd/rd* mice. Inclusion of high extracellular concentration potassium (15 mM as opposed to 3 mM) in the aCSF to elicit neuronal depolarisation caused significant dopamine release both under dark and light conditions (Fig 3D). These results show that while the *rd/rd* retina is capable of releasing dopamine, it cannot be triggered by mRGC input.

Finally, to assess mRGC input to DACs in a non-degenerate retina we turned to chemogenetic activation of mRGCs in the *Opn4^{cre/+}* retina. The clozapine-*N*-oxide (CNO) sensitive hM3D Gq-coupled receptor fused to mCherry was introduced via intravitreal injection of pAAV2-hSyn-DIO-hM3D(Gq)-mCherry. Expression was confirmed via immunohistochemistry as described previously [28]. mRGC activation by CNO was confirmed *in vivo* by pupil constriction (under IR > 950 nm) and *c-fos* activation of mCherry positive cells (Fig 3E), and *in vitro* by measuring mRGC spiking via patch-clamp recording of mCherry expressing cells (Fig S3). No significant increase in dopamine turnover occurred when hM3Dq expressing *Opn4^{cre/+}* animals were injected intraperitoneally (under dim red light) with 5 mg/kg CNO and maintained for 1 hr in the dark (Fig 3F). As *c-fos* activation of TH-expressing cells has been shown to correlate with dopamine release, it was assessed in tandem. While $68.8\% \pm 3.7$ (mean $\pm$ SEM) of TH⁺ amacrine cells in wild-type light-pulsed ($5.3 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$; 90 min) retinae expressed *c-fos*, only $4.7\% \pm 1.7$ of TH⁺ cells expressed *c-fos* in CNO activated hM3Dq expressing retinae (Fig 3G; no *c-fos* positive cells were found in dim red/control conditions). As we know that mRGC activity is upregulated in a consistent and prolonged way: *in vivo* (pupil remains constricted up to 2 hrs; Fig S3A) and *in vitro* (prolonged increase in spiking; Fig S3B-D), this indicates that even with persistent activation of mRGCs by hM3Dq receptors for 1hr, dopamine is not released from DACs.

Taken together with previous results, these data indicate that it is rod photoreceptor input that defines dopamine release in the retina at all light intensities, while cones and mRGCs are neither necessary nor sufficient to drive this response.

Pharmacological block of light-induced dopamine release

We next aimed to identify the major rod pathways that convey light information to DACs. To do this we utilised the *ex vivo* method that allows pre-treatment of retinae with pharmacological agents prior to light pulsing. We found that the glycine receptor blocker strychnine (10 μ M) reduced basal dopamine release under dim red light but did not inhibit light-induced dopamine release (Fig 4A). The GABA_{A/C} receptor blocker picrotoxin (50 μ M) caused significant dopamine release, to light levels, in dim red but did not augment the light response (Fig 4A). These data suggest that the bright light input may be relieving a constitutive GABAergic inhibition of DACs, as previously suggested by others [29, 30]. In agreement with previous reports, the mGluR6 receptor agonist L-AP4 (50 μ M; blocking ON bipolar input)

reduced dopamine release both in dim red and bright light conditions suggesting the origin of light-induced dopamine release occurs in the outer retina (Fig 4B). In contrast, while the AMPA/kainite receptor blocker CNQX (10 μ M) blocked light-induced dopamine release, it had no effect on basal levels (Fig 4B). Surprisingly, we found that the NMDA receptor blocker AP5 (10 μ M) significantly reduced, although did not entirely eliminate, light-induced dopamine release (Fig 4B). These data indicate that the light-input must travel through ON bipolar cells and be mediated by AMPA/kainite *and*, to a large part, NMDA receptor input at some point in the circuit.

Gap-junctions mediate input to dopaminergic amacrine cells

It is known that rod input to ON ganglion cells relies heavily on gap-junction connectivity between All amacrine cells and cone ON bipolar cells (primary rod pathway) and between rods and cone photoreceptors (secondary rod pathway)[31, 32]. Since light-induced dopamine release is blocked by L-AP4, our current understanding of the circuitry suggests the rod signal travels through one, or both, of these pathways. Using the pan-connexin blocker meclofenamic acid (MFA; 200 μ M) we see that the majority of light induced dopamine release is attenuated in both wild-type and *Gnat2*^{A518G} animals (Fig 4C). However, significant release is maintained in both genotypes suggesting the existence of a gap-junction independent pathway driven by rods. When comparing the magnitude of dopamine release between wild-types and *Gnat2*^{A518G} animals we see that light-induced absolute amplitude is reduced when cone function is eliminated. This is contrary to the *in vivo* turnover of dopamine (Fig 2A) and may reflect morphological changes in these animals that limits the diffusion of dopamine out of the retina. However, threshold for release when amplitudes were normalised was not significantly different between wild-types and *Gnat2*^{A518G} animals using the *ex vivo* protocol (Fig S1).

Spiking and synaptic inputs combine to cause light-induced dopamine release

To further investigate the mechanisms governing dopamine release we assessed the impact of synaptic input and/or spiking. DACs, unlike some amacrine cells, fire action potentials and dopamine release is correlated with spiking [33]. Addition of the voltage-gated sodium channel (Nav) blocker tetrodotoxin (10 μ M TTX) abolished light-induced dopamine release and significantly reduced basal levels under dim red light confirming that spiking is necessary for dopamine release (Fig 5A). Conversely, increasing neuronal depolarisation, and spiking, in the retina as a whole with 15 mM KCl did not increase dopamine release under dim red light (although a positive trend was observed), but significantly augmented light-induced release (Fig 5B). These data suggest that both spiking and light-induced synaptic input are important to promote dopamine release. Furthermore, addition of Mg²⁺-free solution to *rd/rd* retinæ to allow activation of NMDA receptors without coincident depolarisation caused a significant increase in dopamine release in both dim and bright-light conditions in *rd/rd* retinæ (Fig 5C). These data implicate a glutamate input to NMDA receptors that must be paired with spiking to produce significant dopamine release.

Duration response characteristics of dopamine release

To further characterise dopamine release dynamics using both *in vivo* and *ex vivo* protocols, we completed a duration-response curve for the brightest light intensity (6.8 log R* rod⁻¹ s⁻¹; Fig 6A). Animals were light pulsed *in vivo* with different duration pulses up to 1 hr and then maintained in the dark for 1-60 min until their retinæ were extracted. The DOPAC:DA ratio

showed a rapid increase within 30 sec and a peak at 1 min that reduced and recovered within the following 10 min in an oscillatory manner. By 15 min dopamine turnover plateaued and remained at this level for the remainder of the hour. After cessation of the light, the DOPAC:DA ratio showed a transient increase (although not significant) and then declined quickly and was significantly reduced within 10 min, and back to baseline levels after 1 hr. These data suggest that the DOPAC:DA ratio gives an “instantaneous” measure of dopamine release within the retina with an integration time of less than 2 min.

A duration-response curve was also completed for the *ex vivo* conditions over 15 min and these results are overlayed with *in vivo* DOPAC:DA ratio in Fig 6B. *Ex vivo* dopamine release showed a slower initial increase, likely reflecting the rate of DA diffusion out of the retina into the surrounding aCSF. Taken together, these data indicate that while *in vivo* and *ex vivo* measures of dopamine release can report similar intensity release profiles, they display quite different temporal release properties.

Mathematical model of dopamine turnover

Taking this duration-response curve into account, we aimed to construct a mathematical model to describe dopamine release in response to a step of bright light. Since the light input to DACs is affected by gap-junction contribution (Fig 4C) we must consider the fact that dopamine itself alters gap junction connectivity between many cell types in the retina [34-37]. Consequently, if dopamine itself is reducing the light input to DACs by closing gap junctions we might expect that dopamine release would exhibit an oscillatory release profile, i.e. more release when dopamine concentration is low and gap junctions are open, and less release when dopamine is high and gap junctions are closed. In fact, we do see a glimpse of this negative feedback in our time-course of the DOPAC:DA ratio (Fig 6A), with dopamine peaking at 1 min, reducing to a trough at 5 min and then returning to this peak at 15 min. However, the response to light off is somewhat different with a counterintuitive initial peak in dopamine release at ~60s and gradual exponential decline over time. While these occurrences could be caused by mechanisms intrinsic to DOPAC production itself (monoamine oxidase enzyme activity or dopamine uptake efficiency) the simplest mathematical model we found to elicit the observed response is illustrated in Fig 6C&D. A similar dopamine turnover profile (Fig 6C (ii)) can be replicated by applying a step light input (Fig 6C (i)) low-pass filtered ($\tau = 20$ s) minus a negative feedback component (Fig 6C (iii)) and a direct inhibitory component (Fig 6C (iii)). The negative feedback is the effect of dopamine release itself on the amplitude of the light input low-passed ($\tau = 100$ s) and delayed 60 s, which declines slowly over time ($\tau = 1000$ s; Fig 6D(iii)). The direct inhibitory component comes directly from the light input (Fig 6D(iv)), however, while this direct inhibition is included in both the light and dark responses its impact is only clearly observable as the transient increase in dopamine release following transition to dark (3600 s; 60 min). The model also shows that dopamine release exhibits hysteresis, whereby the transfer function in response to light is different to the dark response ($\tau = 1000$ s), suggesting that a reduction in retinal dopamine in the dark is slower than the increase in the light.

The model therefore fits well with the rest of our data that suggest rods both inhibit and stimulate dopamine release via separate circuits and suggests that dopamine itself provides an inhibitory feedback on its own release. It appears that the direct inhibition we observe under dim-light (Fig 1E) can be overcome at bright light intensities and we propose that a

balance of excitation and inhibition driven by rods alone defines the amplitude of dopamine release in the mouse retina.

Discussion

This study has identified the profile of dopamine release in the mouse retina over a large range of light intensities. This release appears to be exclusively defined by rod input and shows a U-shape, with more dopamine released under dark and bright conditions and less dopamine released under dim and mesopic conditions.

The high threshold of dopamine release ($>5 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$; equating to $\sim 400 \text{ lux}$) was unexpected given that it is driven by a rod input as we might expect rod photoreceptors to be already saturated at these light intensities. However, our data definitively show that dopamine release is not dependent on cone photoreceptors or mRGCs. In the last few years rods have been shown to function at intensities far above what was originally assumed [16, 17] so it is plausible that bleaching adaptation within rod photoreceptors preserves their function at such bright light intensities. The light intensities that elicit dopamine release, while bright, are normal physiological irradiances that would be experienced outdoors during the day-time (ranging from $\sim 400 - 200,000 \text{ lux}$). The threshold for release corresponds to the switch from mesopic (rod and cone shared) function of the retina, to purely photopic (cone only) [38]. Indeed, rod responses cannot be recorded via ERG in the *Gnat2*^{A518G} retina when a background above the dopamine threshold is applied ($5.3 \text{ R}^* \text{ rod}^{-1} \text{ s}^{-1}$; Fig 2F), although a significant ERG can be recorded with a background below this threshold ($3.3 \text{ R}^* \text{ rod}^{-1} \text{ s}^{-1}$; Fig 2E) suggesting that this is around the light intensity where rods are no longer useful to the system. Based on these data, it is likely that widespread dopamine release is causing removal of this “inoperable” rod input from the cone pathway to optimize the retina for vision under day-time light intensities.

Furthermore, our data imply that a dim light input to rods is actively suppressing dopamine release. However, this suppression must occur over a very short time scale as animals were only exposed to the red head torch for $< 1 \text{ min}$ before the retina was removed and snap frozen in the dark. Block with L-AP4 further decreases dopamine release in dim red light suggesting that suppression at these intensities does not occur via ON bipolar mediated pathways. It is possible that basal dopamine release under dim light intensities is mediated by a separate circuit to that of light-induced dopamine release, possibly acting via a direct rod to OFF bipolar cell synapse [39]. Dopamine has been shown to actually sensitize rod ON bipolar function at dark and very dim light intensities [40] implying that as light intensity moves from around rod threshold to $\sim 2\text{-}3 \text{ log units}$ above, dopamine release is reduced causing graded desensitization of rod ON bipolar cells.

One of the most noteworthy conclusions from our data is that dopamine release does not match electrophysiological input to TH labelled amacrine cells [3-6]. Specifically, and controversially, we comprehensively show that the input from mRGCs does not elicit dopamine release (Fig 3). This is further supported by our result that L-AP4 abolishes light-induced dopamine release in the wild-type retina (Fig 4B), as the electrophysiological input from mRGCs to TH amacrine cells persists in the presence of L-AP4 [3]. It is important to

distinguish the fact that neuronal activation (specifically spiking) does not necessarily equal neurotransmitter release, two distinct processes that are often used interchangeably in the literature. This is perhaps more specific to dopamine release as it has been suggested in striatum-projecting midbrain dopaminergic neurons that dopamine can be released via a local presynaptic mechanism that triggers dopamine release directly from axonal varicosities in the striatum, bypassing activity in dopaminergic somas [41]. We propose that this situation is mirrored in the retina where dopamine release can be triggered in axonal/dendritic varicosities by microcircuits comprising both presynaptic inputs and vesicle release in the same structure (Fig 7). Certainly, dopamine is thought to be mainly released from DAC processes [42], whereas all electrophysiological inputs have been described at the soma. Electrotonic isolation of distal neurites is likely given the lack of voltage-gated sodium channels (Na_v) found in DAC processes [43], and there is certainly precedent for this mechanism in the retinal A17 amacrine cells that are able to operate more than 100 independent microcircuits within the same cell [44]. In this scenario, while the basal firing rate of the neuron at the soma undoubtedly has an influence on the physiology of the cell, dopamine release could be triggered locally from each individual varicosity. This type of mechanism would be useful in a DAC to limit dopamine release to the vicinity of the light (synaptic) input. If this were not the case, activation of a dopaminergic cell soma would elicit dopamine release throughout the entire network of processes (up to 1.6 mm in diameter [13]). This hypothesis is supported by our data that simply increasing spiking of DACs by applying high KCl does not cause significant dopamine release, whereas high KCl + light gives an augmented response. Interestingly, *rd/rd* animals show the opposite effect where an increase in spiking (or NMDA channel disinhibition) does cause significant release. However, these animals show significant synaptic reorganisation [45] and spontaneous activity [46] that alter their functional circuitry. Finally, if changes in spiking of DACs are not directly correlated with dopamine release, what are the function of these inputs? Since TH-labelled cells have been shown to release both GABA and ATP [47, 48], electrophysiological inputs from mRGCs and cones may be more important to these neurotransmitter systems.

NMDA receptor activation appears necessary for the majority of light-induced dopamine release. Extra-synaptic NMDA receptors have been shown to augment AII and A17 amacrine cell light responses, specific to rod pathways [49]. Therefore, it may be at the rod ON bipolar to AII or A17 amacrine synapse where NMDA activation greatly increases the rod input at bright light intensities through glutamate spill over. The finding that both spiking and synaptic input is required to promote dopamine release suggests that glutamate activation of NMDA channels, only in combination with spiking, causes dopamine release. GluR1/GluR4 and NR1 are certainly found on the spines of TH-labelled cell varicosities [12] providing a possible mechanism to spatially segregate dopamine release to discrete parts of a single DAC.

The most parsimonious mathematical model we determined to explain dopamine release in response to rod input indicates that release is determined by a balance between excitatory and inhibitory pathways. In this model, “lights on” provides both excitation and direct inhibition of dopamine release. Interestingly, this direct inhibition, incorporated in the model to explain the transient increase in dopamine release at light offset, is of a similar magnitude to the reduction in dopamine release caused by dim red light (Fig 1E). The model suggests that this direct inhibition is still present during light-induced dopamine release, but that it is overwhelmed by the excitatory input. We must be careful here to point out that “excitation”

and “inhibition” in the model relates to increased or decreased dopamine release rather than the specific neurotransmitters involved in these processes e.g. an “excitatory input” could occur via a reduction in GABAergic input. Furthermore, the model predicts that the “lights on” excitatory input is reduced by the release of dopamine itself as a negative feedback. This likely relates to the effect of dopamine on retinal circuitry, possibly via a reduction in gap junction conductance. This negative feedback from dopamine attenuates significantly over time. While we do not know the mechanism governing this reduction, it may reflect desensitisation of dopamine receptors. However, since we are examining the dopamine turnover in the entire retina and we do not have spatial information, it may be that the initial large amplitude release of dopamine from the DACs reaches a high concentration in the INL/IPL that becomes diffused within the whole retina over time. Therefore, determining the spatial and temporal dynamics of dopamine diffusion is important to describe the mechanism of this negative feedback. Newly developed genetically encoded dopamine sensors would be ideal to analyse this process in more detail in future studies [50, 51].

Summary of dopamine release in the mouse retina

Taking this data together, we present a summary figure for the possible circuitry governing dopamine release in response to light stimulation (Fig 7). Under dark conditions, tonic spiking occurs that causes release of a basal level of dopamine; there is no inhibition or excitation at the level of dopaminergic varicosities as the light signal is absent. Under dim scotopic and mesopic light stimuli there is an increase in GABA inhibition to varicosities, likely driven through the OFF system. Glutamate release is also increased and stimulates synaptic AMPA/kainite receptors, but does not outweigh the inhibition causing an overall reduction in basal dopamine release. Under bright light conditions both inhibition and excitation are increased to the varicosity but due to glutamate spill over to extra-synaptic NMDA receptors the level of excitation tips the balance and allows a large release of dopamine. While it is likely that the precise circuitry mediating dopamine release is much more complex than that depicted above, all our data points toward the regulation of dopamine release in the retina relying on the balance between inhibition and excitation driven exclusively by rod photoreceptors.

The retina represents an ideal model tissue in which to study dopamine release mechanisms in the CNS given that the inputs causing release can be precisely defined and localised. Future work examining both the spatial and temporal aspects of light-induced dopamine release in the retina will likely be of great importance in elucidating the mechanisms governing release from dopaminergic cells in the rest of the brain.

STAR★Methods

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Donkey Anti-Mouse IgG (H+L) Fluorescein (FITC)	Jackson ImmunoResearch Laboratories, INC.	Cat#715-095-151, RRID:AB_2335588
Donkey Anti-Rabbit IgG (H+L) Rhodamine (TRITC)	Jackson ImmunoResearch Laboratories, INC.	Cat#711-025-152, RRID:AB_2340588
Goat Anti-Rabbit (H+L) Alexa Fluor 488	Life Technologies	Cat#A-11008, RRID:AB_143165
Rabbit Monoclonal Anti-c-Fos (9F6)	Cell Signalling Technology	Cat#2250, RRID:AB_2247211
Sheep Polyclonal Anti-Tyrosine Hydroxylase	Merck Millipore	Cat#657012, RRID:AB_696697
Bacterial and Virus Strains		
pAAV-hSyn-DIO-hM3D(Gq)-mCherry	Addgene	Cat#44361-AAV2
Chemicals, Peptides, and Recombinant Proteins		
(2R)-amino-5-phosphonovaleric acid (AP5)	Cayman Chemical	Cat#14539, CAS:79055-68-8
6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)	Cayman Chemical	Cat#14618, CAS:115066-14-3
Alexa Fluor 488 Hydrazide	Invitrogen	Cat#A10436
Biocytin	Life Technologies	Cat# B1592, CAS:576-19-2
Clozapine N-oxide (CNO)	Hellobio	Cat#HB6149, CAS:5786-21-0
DOPAC-d5	Cambridge Isotope Laboratories	Cat#DLM-2499-PK, CAS:60696-39-1
Dopamine-d4	CDN Isotopes	Cat#D-1540, CAS:203633-19-6
Hypromellose	Alcon	GenTeal
Ketamine	Troy, Illium	CAS:6740-88-1
L-2-amino-4-phosphonobutyric acid (L-AP4)	Abcam	Cat#ab120002, CAS:23052-81-5
Meclofenamic Acid (MFA)	Sigma-Aldrich	Cat#M4531-5G, CAS:6385-02-0
Phenylephrine	Bausch and Lomb	N/A
Picrotoxin	Sigma-Aldrich	Cat#P1675, CAS:124-87-8
Salts, acids and bases	Sigma-Aldrich	N/A
Streptavidin	Life Technologies	Cat#S11223, CAS:9013-20-1
Strychnine	Sigma-Aldrich	S0532, CAS:57-24-9
Tetrodotoxin (TTX)	TOCRIS	Cat#1078, CAS:4368-28-9

Tropicamide	Alcon	Mydracyl 1%
Xylazine	Troy, Illium	CAS:7361-61-7
Experimental Models: Organisms/Strains		
Mouse: C57BL/6J	ARC, Canning Vale, Australia	JAX: 000664
Mouse: <i>Gnat2</i> ^{A518G}	(Jobling, Vessey, Waugh, Mills, & Fletcher, 2013)	N/A
Mouse: <i>Opn4</i> ^{Cre/+} / <i>Opn4</i> ^{Cre/Cre}	(Ecker et al., 2010)	N/A
Mouse: <i>Opn1</i> ^{mwr}	(Smallwood et al., 2003)	N/A
Mouse: <i>rd/rd</i> (C57BL/6J-Pde6b ^{rd1-2J} /J)	The Jackson Laboratory	Stock no: 004766
Software and Algorithms		
GraphPad Prism 7	GraphPad Software	N/A
LabChart	ADInstruments	N/A
MassLynx 4.1	Waters MassLynx Software	N/A
MATLAB	MathWorks	N/A
pClamp 10	Molecular Devices	N/A
Metavue v7.8.4.0	Molecular Devices	N/A
OceanView 1.6.7	Ocean Optics	N/A
Other		
0.9 ND filters	LEE filters	Cat#211 0.9ND
Collimated LED	Thorlabs	Cat#MCWHLP1
Blue filter (434/17nm)	Semrock	Cat#FF01-434/17-25
Red filter (624/40nm)	Edmund Optics	Cat#67035
Head torch 8 LED	VARTA	N/A
Head torch red filter	LEE filters	Cat#787 Marius Red
IR Goggles	Night Vision Australia	Cat#D2MVSL
Photometer	Irradian	Cat#L202 Macam
SAD Lamp	Litebook	Cat#Edge SAD Lightbox
Spectroradiometer	Ocean Optics	Cat#USB2000+XR1-ES

Contact for Reagent and Resource Sharing

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Morven A. Cameron (m.cameron@westernsydney.edu.au).

Experimental Model and Subject Details

Mice

Animal care was in accordance with either the Australian Code for the Care and Use of Animals for Scientific Purposes or the UK Animals, Scientific Procedures, Act 1986 (c-fos DREADDs experiment). Protocols were approved and monitored by the Western Sydney University Animal Care and Ethic Committee, project numbers: A11900 and A12402. Wild-type C57BL/6J (ARC, Canning Vale, Australia), *Gnat2*^{A518G} (C56BL/6J backcrossed), *rd/rd* (C57BL/6J backcrossed), *Opn1*^{mwr}, *Opn4*^{cre/cre} and *Opn4*^{cre/+} (mixed C57BL/6 and 129sv) mice

were bred on site. Offspring (both male and female) 60 - 100 days were used. Animals were maintained under a 12hr light: dark cycle at ~300 lux illumination during the daytime.

Method Details

Light pulsing

For *in vivo* experiments, mice were handled under either dim red or infrared light, anaesthetized with an intraperitoneal injection of 70 mg/kg Ketamine + 7 mg/kg Xylazine (Troy Laboratories), and their eyes dilated with Tropicamide 1% (Alcon) before light pulsing. Control animals were not anaesthetised and were maintained in complete darkness, we have previously shown that anaesthesia does not affect dopamine release under dark conditions [20]. After light pulsing, or control dark conditions, mice were sacrificed by cervical dislocation and eyes enucleated (under the same lighting conditions as the pulse, or red/infrared light) and retinae extracted through a lateral incision along the *ora serrata* by adding pressure to both sides of the cut. Each retina was put into a 0.5 mL plastic tube and immediately frozen in liquid nitrogen. The tubes containing the retinae were stored at -80 °C until analysed. As the response to light stimulation was the same at 15 min as 1 hr (Figure 6A) pulsing was shortened to 15 min as described in the corresponding figure legends.

For *ex vivo* experiments, after overnight adaptation to the dark, mice were sacrificed by cervical dislocation under dim red light, eyes enucleated, and the cornea and lens removed. The remaining eyecup was placed, with the retina facing up, into a 0.5 mL plastic tube with 40 µL of oxygen-bubbled, HEPES-buffered aCSF (NaCl 140, HEPES 10, KCl 3, NaH₂PO₄ 1.25, CaCl₂ 2 and MgCl₂ 1, Glucose 10 mM) at room temperature, and either light-pulsed or kept in the dark. All pharmacological agents were dissolved in HEPES-buffered aCSF to a final concentration as stated. Tissue was incubated in each agent for > 10 min (30 min for meclofenamic acid) prior to light-pulsing or dark incubation. The supernatant aCSF was then removed and frozen in liquid nitrogen and stored at -80 °C until analysed. For *ex vivo* pharmacology experiments dark and light controls were completed in parallel as intra-experiment variability was much lower than inter-experiment variability.

Retinae from the *in vivo* experiment were homogenized using a pellet pestle cordless motor inside a 40 µL solution consisting of: 1% formic acid (Sigma-Aldrich), 0.5 mM ascorbic acid (Sigma-Aldrich) and 10 ng/mL the deuterated standards of dopamine-*d*4 (CDN Isotopes) and DOPAC-*d*5 (Cambridge Isotope Laboratories). Supernatant from the *ex vivo* experiments was made up to a solution of 1% formic acid, 0.5 mM ascorbic acid, 10 ng/mL deuterated standards. Then, for both *in vivo* and *ex vivo* preparations, samples were centrifuged at 14,000 r.p.m. for 10 min at 4 °C and the supernatant placed in clear glass total recovery vials (Waters) for chromatographic analysis.

UHPLC-MS/MS

Ultra-high performance liquid chromatography and tandem mass spectrometry was performed as described in [20]. Briefly, samples were analysed on a Waters Xevo TQ-MS triple quadrupole mass spectrometer, fitted with an electrospray ionization source. The desolvation gas flow (N₂) was 800 L/h, desolvation temperature was 450 °C, cone gas 0 L/h, and collision gas (Ar) flow of 0.15 mL/min, which gave a collision cell pressure of 2.6×10^{-3} mbar. In positive ion mode the capillary voltage was set at 1.2 kV and in negative mode 1.0 kV. Liquid chromatography was performed using a Waters Acquity UPLC, working at a flow rate of 0.20 mL/min. A Waters Acquity UPLC BEH C18 column of 1.7 µm particle size and dimensions 2.1

× 150 mm was used, operating at 40 °C. Solvent A consisted of 0.1% formic acid in ultrapure water and solvent B was 0.1% formic acid in methanol. A 20 min run was employed, commencing at 5% B for 1 min, increasing linearly to 100% B by 10 min, then returning immediately to 5% B at 15 min. The sample manager was kept at 4 °C and injections of 10 µL were made in full loop mode. Retention times for dopamine and dopamine-*d*₄ were 2.1 and 2.04 min respectively and were analysed in positive ion mode. DOPAC and DOPAC-*d*₅ were 5.09 and 5.01 min respectively and were analysed in negative ion mode. Dopamine only (*ex vivo*) or dopamine and DOPAC (*in vivo*) for each retina were quantified and dopamine per retina (*ex vivo*) or DOPAC: dopamine ratio (*in vivo*) is expressed as mean ± S.E.M.

Light calculations

Light stimuli for *in vivo* and *ex vivo* irradiance response curves were provided by an LED array (EDGE SAD Lightbox; Litebook) delivered from above the animal/eye preparation. Irradiance at the brightest setting (in µW cm⁻² nm⁻¹) was quantified between 200 – 1000 nm using a spectroradiometer fitted with a cosine diffuser (USB2000+XR1-ES; Ocean Optics) measured at eye level. Irradiance at each wavelength was then converted to photons cm⁻² s⁻¹ nm⁻¹. Total effective photons cm⁻² s⁻¹ for each photoreceptor was calculated taking into account the spectral sensitivity of each photoreceptor type using Govardovskii nomograms [52] for a vitamin A1 based opsin of $\lambda_{\max}$ as follows: rod – 498 nm, M-cone – 508 nm, S-cone – 360 nm, human L-cone – 556 nm, Opn4 – 480 nm. Photoisomerisations per rod, per second (R* rod⁻¹ s⁻¹) were calculated by converting effective rod photons µm⁻² s⁻¹, assuming a rod collecting area of 0.5 µm² [53]. *In vivo* retinal illuminance was estimated by correcting for lens transmission [54], whereas the lens was removed in the *ex vivo* preparation, so transmission was assumed to be 100% at all wavelengths. The eye aperture was estimated to be ~ 3.2 mm² (fully dilated pupil) *in vivo*, and ~ 8 mm² *ex vivo* (cornea and iris removed) giving a multiplication factor of 0.18 and 0.45 respectively (assuming a retinal area of 17.8 mm² [55]). Generally light is expressed as R* rod⁻¹ s⁻¹ (corrected for either *in vivo* or *ex vivo*) however, illuminance at the level of the cornea in lux was calculated by Ocean View software and independently confirmed via a photometer (L202 Macam; Irradian) and is used alongside R* rod⁻¹ s⁻¹ to provide context to readers. Neutral density film (0.9ND; LEE filters) was applied to the lamp to reduce intensity without changing spectral composition.

Electroretinogram (ERG) stimuli, the red and blue light-pulses (Fig 2E) and flicker stimuli were provided by a single high-power collimated LED (MCWHLP1; Thorlabs) and delivered either via ganzfeld illumination (ERG), or from above the animal/eye prep. Red and blue light-pulses employed a 624 nm and a 434 nm filter. Irradiance was precisely controlled by a combination of the voltage output for the LED and neutral density filters (Edmund Optics).

“Dim-red” for *in vivo* conditions involved exposing the animal to an LED headtorch filtered with red film (> 650 nm; Marius Red; LEE Filters) for < 1 min to isolate the retina, whereas “dim-red” for the *ex vivo* prep involved dissection of the tissue under a dissection scope illuminator filtered with the same red film that took ~ 3 mins. Infrared conditions were only employed *in vivo* using IR goggles (NVAD2MV; Night Vision Australia), the IR illuminator on these goggles was > 800 nm. Spectra of all stimuli were measured as described above, and R* rod⁻¹ s⁻¹ calculated. Experiments involving *Opn1^{mwR}* animals employed only IR goggles for “dark” conditions.

Chemogenetics

Intravitreal injections of AAV2-hSyn-DIO-hM3Dq-mCherry vector (2.3×10^{13} genomic particles/ml; The UNC Vector Core) were performed as previously reported [28, 56] and employed hyaluronan lyase and heparinase III (0.2U each) to maximise retinal penetration (total volume 2.5ul injected over 1 min). Control eyes underwent the same procedure, with injections including glycosylidic enzymes but with the virus replaced by vehicle (PBS). Mice were allowed at least 6 weeks to recover before being used in *in vivo* studies.

Dopamine release: *Opn4^{Cre/+}* mice expressing hM3Dq in the right eye only, were dark-adapted overnight. CNO (5mg/kg) was administered i.p. under dim-red light and kept for 1 hr in the dark. Both retinæ were removed under dim-red light and the left eye served as a control for the hM3Dq expressing eye. Pupil constriction in response to CNO was quantified 2 weeks prior using the same methods and visualised using an IR webcam (DCS-930L; D-Link) illuminated by a custom-made IR LED array (> 950 nm). Animals with pupil constriction of less than 80% in response to CNO injection were excluded.

c-fos: *Opn4^{Cre/+}* mice expressing hM3Dq were dark-adapted overnight, CNO (5mg/kg) was administered i.p. under dim red light and mice were kept for 90 min in the dark. After 90 minutes of treatment mice were deeply anaesthetized with i.p. injection of urethane (2.2g/kg; 30%w/v; Sigma-Aldrich, UK) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Wild-type animals underwent the same conditions, but without CNO injection and one group was light-pulsed, free-moving in their home cage with LED light ($5.3 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$).

Immunohistochemistry

Immunohistochemistry on retinal sections was performed as previously described [28, 56]. c-Fos staining employed rabbit anti-c-Fos primary (Cell Signaling 9F6; 1:250), and Alexa 488 conjugated goat anti-rabbit secondary (Life Technologies; 1:300) antibodies. TH staining employed sheep anti-TH primary (Merck AB1542; 1:300), and Alexa 647 conjugated donkey anti-sheep (Life Technologies, 1:300) antibodies. Endogenous mCherry fluorescence revealed expression of hM3Dq-mCherry in hM3Dq-expressing mice. Images were collected on an Olympus BX51 upright microscope using a [20x / 0.5 UPlan FLN] objective and captured using a [Coolsnap ES2 camera (Photometrics)] through Metavue v7.8.4.0 software (Molecular Devices). More than 15 retinal sections were analysed per each retina.

Electroretinogram

Animals were anaesthetised under IR illumination with i.p. injection of ketamine (70mg/kg) and xylazine (7mg/kg) and maintained with s.c. injection of ketamine (72mg/kg) and xylazine (5mg/kg). Mydriatics (tropicamide 1% & phenylephrine 2.5%) and hypromellose solution (0.5%) (Alcon, Belgium; Bausch and Lomb, UK) were applied to the recording eye prior to application of an active electrode consisting of a platinum wire (100 μm thickness) embedded in a contact lens made from Aclar transparent film (ProSciTech, Australia). A needle reference electrode was inserted approximately 5mm from the base of contralateral eye, distal enough to preclude signal contamination. A platinum wire bite bar was used to support the head and to act as ground. Electrode setup and injection of anaesthetic agents was conducted under IR light, the experimental setup was then covered with a light-tight faraday cage. Electrodes were connected to a PowerLab 26T where they were differentially amplified $\times 2000$, band-

pass filtered 0.5 to 300Hz (sampling rate 10kHz) and digitized to a Mac running LabChart Software (ADInstruments). Core body temperature was maintained by placing animals in a custom-made hose coil connected to a constant temperature water source. In all cases electrode stability was confirmed for 10 min prior to recording.

All mice were long-term dark adapted (>12hr) prior to recording ERGs. Flash and background stimuli were white light from a collimated LED (described above) reflected in a custom made Ganzfeld dome and attenuated with neutral density filters (Edmund Optics). For dark-adapted irradiance response measures a series of 10 ms flashes were delivered at 1Hz for the lowest intensity, up to 0.01 Hz at the brightest intensity with repetitions decreasing accordingly. An average trace was then generated for each irradiance. The b-wave amplitude was quantified by summing the absolute a-wave (unfiltered) amplitude and the b-wave amplitude when filtered (low pass 20 Hz) to remove the influence of oscillatory potentials. Simple sigmoidal curves were fitted to the irradiance response data for both amplitude and implicit time.

$$y = V_{min} + \frac{V_{max} - V_{min}}{1 + 10^{(\log EC_{50} - x)}}$$

In order to take account of the initial manifestation of the a-wave on the ERG waveform, the b-wave amplitude responses were fitted with separate simple sigmoidal functions at low and high irradiances [57]. Light-adapted ERGs were recorded (after 20 min adaptation to the background) in response to 20 repeats of a 2 ms flash of 0.99 Michaelson contrast (controlled via LED voltage). Light intensity of the background and flash were controlled by filtering with neutral density filters (Edmund Optics). ERGs were recorded through the middle of the subjective day (CT2-10). To express ERG responses in comparison to continuous light in the dopamine release experiment, irradiance was converted to $R^* \text{ rod}^{-1} \text{ s}^{-1}$ as described above. Therefore, in the dark-adapted irradiance response curve the maximum intensity flash ($6 \log R^* \text{ rod}^{-1} \text{ s}^{-1}$) elicits $4 \log R^* \text{ rod}^{-1}$ ($\sim 1.6 \log \text{ cd-s/m}^2$).

Modelling

To estimate the dynamics of dopamine release in response to light, we developed a simplified model in Simulink (Matlab, The Mathworks). While the number of empirical data points available to describe the light response curves are limited, they are sufficient to highlight some key features on the basis of which the model is created. Independent model elements are described as transfer functions in the Laplace domain. Indeed, as all transfer functions used were first order low pass filters, each function is a variant of

$$G(s) = \frac{\text{Output}}{\text{Input}} = \frac{1}{\tau s + 1}$$

where τ is the time constant and s is a complex number, $s = a + j\omega$. The Laplace transform $G = G(s)$ of any time function $f = f(t)$ are related by the equation

$$G(s) = \int_0^{\infty} f(t) e^{-st} dt$$

Gain factors, time delays, signal limits and simple arithmetic functions complete the model.

Electrophysiology

Animals were euthanized using cervical dislocation, eyes enucleated, cut along the ora serrata, and placed in artificial cerebrospinal fluid (ACSF) containing (mM): NaCl 125, NaHCO₃ 25, KCl 3, NaH₂PO₄ 1.25, CaCl₂ 2 and MgCl₂ 1, Glucose 10, at room temperature (RT) within 1 min of euthanasia under dim red light conditions. Whole-mount retinae were isolated and cut into 4 pieces. As needed, retinal pieces were then transferred to the recording chamber where they were infused with carbogen (5% CO₂/95% O₂) bubbled ACSF at a rate of 2-3 ml/min. A tissue anchor (harp) made of platinum with nylon threads (~500 µm apart) was used to keep the tissue in place. Whole-cell current clamp recordings were made from mCherry expressing retinal ganglion cells with patch electrodes of resistances 5.0–8.0 MΩ. Electrodes were filled with (mM): 120 KMeSO₄, 10 KCl, 0.008 CaCl₂, 0.5 EGTA, 1 MgCl₂, 10 HEPES, 4 ATP – Na₂, and 0.5 GTP – Na₃, adjusted to pH 7.4 with KOH. Alexa Fluor 488 Hydraz (70 µM, Invitrogen A10436) and biocytin hydrazide (0.3%, Life Technologies B1592) were also included in the pipette solution for morphological analysis. The series resistance (R_s) was monitored throughout the experiments and was in the range of 10-30 MΩ. Data was acquired with a Multiclamp 700B amplifier, low-pass filtered at 10 kHz at the amplifier output and digitized by a Digidata 1440A at 50 kHz and conveyed to a computer running pClamp 10 (Molecular Devices). CNO was dissolved in aCSF and applied either via whole-bath application or puffed from a ~3 MΩ patch pipette approximately 10 µm from the cell body.

Following recording, retinal pieces were fixed in 4% PFA for 1 hr, then washed thoroughly with Tris 0.1M pH 7.4. Alexa488 tagged streptavidin (Life Technologies, S11223) was applied in Tris-Triton (0.3%, Sigma X-100) at a dilution of 1:200 for 24 hrs before retinae were mounted on glass slides and coverslipped with Vectashield (Vectorlabs H-1000).

Quantification and Statistical Analysis

Chromatograms for dopamine, DOPAC and their respective deuterated standards were analysed with MassLynx and are presented as DOPAC: dopamine ratio as means ± SEM as noted in the text and figure legends. For *in vivo* experiments (DOPAC: dopamine) each *n* represents the mean between the ratios of both retinae from the same animal, whereas for *ex vivo* experiments (pg dopamine/retina) each *n* represents a single retina since once extracted can undergo different treatments or serve as dark controls. One-way analysis of variance (ANOVA) with Dunnett's or Tukey's multiple comparison test was applied when comparing different light or pharmacological conditions to dark or untreated control, and Student's test was applied when comparing between two light conditions within the same treatment. All graphs and statistical analysis were performed with GraphPad Prism 7. Statistical significance was defined as a *p* value smaller than 0.05.

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Author Contributions

M.A.C. conceived of the project, designed the experiments and wrote the manuscript. V.P.F. completed all of the *in vivo* and *ex vivo* experiments involving dopamine release and quantification by UHPLC-MS/MS. A.E.A. and N.M. designed and completed the chemogenetics experiments. P.B. designed and completed the mathematical modelling. K.A.V., A.I.J. and E.L.F. provided the *Gnat2*^{A518G} mouse and provided feedback on all experiments. J.W.M. provided equipment and reagents and was involved with the design and interpretation of all experiments. All authors provided feedback on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Figure legends

Figure 1. Light-induced dopamine release is high-threshold.

- A) Two complementary methods were used to measure dopamine release. *In vivo*: anaesthetised mice were light pulsed, retinae extracted and DOPAC:DA ratio quantified. *Ex vivo*: Eyes were removed from the animal, cornea, iris and lens removed and the eye submerged in artificial cerebrospinal fluid (aCSF). Eyes were light pulsed in plastic tubes and the supernatant aCSF was analysed for dopamine content (maximum duration of pulse was 15 min).
- B) Significant light-induced dopamine turnover *in vivo* occurs only above 5 Log R* rod⁻¹ s⁻¹ compared to dark-adapted mice dissected under dim red light (0.017 Log R* rod⁻¹ s⁻¹, >650 nm). One-way ANOVA with Dunnett's multiple comparison test ($n \geq 4$ each point; all white pulses 1hr).
- C) Light-induced dopamine release *ex vivo* showed the same threshold as *in vivo*. One-way ANOVA with Dunnett's multiple comparison test ($n \geq 5$ each point; all white pulses 15 min).
- D) After normalisation ($R/R_{\max}$) *in vivo* and *ex vivo* data can be fit with the same curve (extra sum-of-squares F test, $p = 0.53$, $F = 0.79$). ERG b-wave amplitude in response to the same light stimulus (but 10 ms flashes) is plotted for reference ($n = 6$ each point).
- E) Dopamine turnover is significantly greater in dark-adapted mice dissected under infrared light (-2.5 Log R* rod⁻¹ s⁻¹, >800 nm) compared to those dissected under dim red light (0.017 Log R* rod⁻¹ s⁻¹, >650 nm). Unpaired Student's t-test *** $p < 0.0001$ ($n \geq 6$; animals exposed to light for < 1 min).
- F) No statistical differences are observed between equivalent irradiance points at subjective midday (CT6; $n \geq 4$ each point) and subjective midnight (CT18; $n \geq 3$ each point), one curve fits all data sets (extra sum-of-squares F test, $p = 0.84$; $F = 0.34$; all white pulses 1hr). Mean $\pm$ SEM for all data points presented.

Figure 2. Cone input is not required for light-induced dopamine release.

- A) *Gnat2*^{A518G} ($n \geq 9$ each point) mice lacking cone function show an indistinguishable dopamine irradiance response to *wild-type* mice ($n \geq 4$ each point), one curve fits all data sets (extra sum-of-squares F test, $p = 0.7$; $F = 0.54$; all white pulses 1hr).
- B) Dopamine turnover is significantly greater in dark-adapted *Gnat2*^{A518G} mice dissected under infrared light (-2.5 Log R* rod⁻¹ s⁻¹, >800 nm) compared to those dissected under dim red light (0.017 Log R* rod⁻¹ s⁻¹, >650 nm). Unpaired Student's t-test ** $p < 0.01$ ($n \geq 6$; animals exposed to light for < 1 min).
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- D-F) Representative light-adapted electroretinograms (ERGs) recorded in *Gnat2*^{A518G} and *wild-type* mice in response to 0.99 Michaelson contrast at two different background luminances reveal that measurable rod-driven responses can be recorded in *Gnat2*^{A518G} with a background below (E) but not above (F) the dopamine release threshold. Animals were adapted to the background for 20 min prior to recording.
- G) *Opn1*^{mwr} mice do not exhibit significant light-induced dopamine release in response a red-light pulse (624 nm; 15 min) designed to activate L-cones above the dopamine release threshold but not rods or *Opn4*. Normal amplitude dopamine release still occurs under bright

white light ($6.8 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$; 15 min). One-way ANOVA Dunnett's multiple comparison test ($*p < 0.05$, $n \geq 5$).

H) When red (624 nm; 15 min) and blue (434 nm; 15 min) light-pulses are matched for rod sensitivity in wild-type animals both cause significant dopamine release compared to dim-red light. One-way ANOVA Dunnett's multiple comparison test ($***p < 0.001$, $n = 5$).

I) No light-induced dopamine release occurs in *wild-type* mice at $4.6 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$ under either continuous or flickering (0.5 sec ON, 1.5 sec OFF; total 15 min) light. One-way ANOVA ($p > 0.05$, $n \geq 4$ each). All data points mean $\pm$ SEM. See Figure S1 for more information on the red and blue pulses.

Figure 3. mRGCs are not necessary or sufficient to drive light-induced dopamine release.

A) *rd/rd* mice lacking rods and cones do not show light-induced dopamine release at any given light intensity. One-way ANOVA ($p > 0.05$, $n \geq 12$ each).

B) Mice lacking melanopsin (*Opn4^{Cre/Cre}*) show an indistinguishable dopamine irradiance response to *wild-types* ($n \geq 4$ each), one curve fits both data sets (extra sum-of-squares F test, $p = 0.88$; $F = 0.3$).

C) Dopamine turnover is significantly greater under infrared light ($-2.5 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$, $>800 \text{ nm}$) compared to dim red light ($0.017 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$, $>650 \text{ nm}$) in *Opn4^{Cre/Cre}*, but not *rd/rd* mice. Unpaired Student's t-test ($**p < 0.01$, mean $\pm$ SEM, $n \geq 4$ each).

D) *rd/rd* retinæ release dopamine in a high-potassium media (15 mM) in both dim red and light conditions. One-way ANOVA with Tukey's multiple comparison tests ($*p < 0.05$, mean $\pm$ SEM, $n \geq 5$ each).

E) Injection of CNO (5 mg/kg) caused activation of mRGCs in *Opn4^{Cre/+}* hM3Dq-infected mice. Top: representative image of infected eye before, and 10 min after, i.p. injection of CNO (contralateral uninfected eye showed similar constriction). Bottom: immunohistochemistry of a vertical retinal section showing co-localisation of c-fos (green, marker for neuronal activation) and mCherry (red, transfected mRGCs; scale bar 30 μm).

G) Activation of mRGCs by CNO does not cause increased dopamine turnover in the hM3Dq-infected eye of *Opn4^{Cre/+}* mice. Paired two-tailed t-test, left vs right eye ($p = 0.6$, mean $\pm$ SEM, $n = 5$ each). *Opn4^{+/+}* littermates show normal light-induced dopamine release, unpaired Student's t-test, $***p < 0.001$.

H) Immunohistochemistry reveals that dopaminergic amacrine cells (labelled with TH in red) express c-fos (green) in *wild-type* mice in response to a 90 min light-pulse, but not in the hM3Dq-infected eye of *Opn4^{Cre/+}* mice 90 min after i.p. CNO injection. No c-fos was observed in dim red/control conditions (scale bar 30 μm). See Figure S2 for more details on mRGC activation by CNO.

Figure 4. Effect of pharmacological blockers on dopamine release.

A) Glycine receptor antagonist strychnine significantly reduces dopamine release in dark and has no effect on light-pulsed retinæ when compared to control ($p < 0.05$). GABA_{A/C} receptor antagonist picrotoxin significantly increases dopamine turnover in the dark up to control light-pulsed levels, without additional effect to treated light-pulsed retinæ (one-way ANOVA with Tukey's multiple comparisons ($p < 0.001$; $n = 4$ each). # denotes significant difference to equivalent control conditions (dotted lines).

B) Light-induced dopamine release is abolished by the mGluR6 receptor agonist L-AP4, and dopamine release is significantly decreased in both dark-adapted and light-pulsed conditions compared to controls ($p < 0.05$). AMPA/kainite receptor antagonist CNQX significantly

prevents light-induced dopamine release ($p < 0.001$) without any effect on dark-adapted retinæ. NMDA receptor antagonist AP5 significantly reduces light-induced dopamine release ($p < 0.001$), but a difference between dim-red and light treated is maintained ($*p < 0.05$). One-way ANOVA with Tukey's multiple comparisons between treated and untreated, # denotes significant difference to equivalent control conditions (dotted lines). Unpaired Student's t-test within treatment groups ($n = 4$ each).

C) Gap-junction blocker meclofenamic acid (MFA) significantly reduces, but does not block, light induced dopamine release in both wild-type and cone-inactivated *Gnat2*^{A518G} mice. One-way ANOVA with Tukey's multiple comparisons between treated and untreated; unpaired Student's t-test within treatment groups ($**p < 0.01$, $***p < 0.001$; # denotes significant difference to equivalent control conditions; $n \geq 4$ each; mean $\pm$ SEM for all data points).

Figure 5: Dopamine release requires spiking and synaptic input.

A) Block of voltage-gated sodium channels by tetrodotoxin (TTX) significantly prevents light-induced dopamine release in *wild-type* mice. One-way ANOVA with Tukey's multiple comparisons between treated and untreated; unpaired Student's t-test within treatment groups ($*p < 0.05$, mean $\pm$ SEM, $n = 4$ each).

B) Media containing high concentration of potassium (15 mM KCl) significantly enhances light-induced dopamine release ($*p < 0.05$) but has no effect under dim red light conditions. One-way ANOVA with Tukey's multiple comparisons between treated and untreated; unpaired Student's t-test within treatment groups (mean $\pm$ SEM, $n = 4$ each).

C) Dopamine turnover is increased in *rd/rd* mice in Mg^{2+} -free media under both dim red and bright light conditions. One-way ANOVA with Tukey's multiple comparisons between treated and untreated ($**p < 0.01$, mean $\pm$ SEM, $n = 4$ each). All y-axes same units.

Figure 6: Duration response characteristics of dopamine release

A) Significant increase in light-induced dopamine turnover *in vivo* occurs after 30 sec of light exposure ($6.8 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$) compared to dark-adapted mice dissected under dim red light. This difference is maintained as long as tested (60 min). When lights are turned off from a light-adapted retina, a significant decrease in dopamine release results after 10 min. The decrease continues for 60 min until overnight dark-adapted levels are reached. One-way ANOVA with Dunnett's multiple comparison test ($p < 0.05$, $n \geq 4$ each point except $t=65, 90, 120\text{min } n=2$).

B) *Ex vivo* dopamine release displays a gradual increase over 15 min that can be fit with a sigmoidal curve (dotted line; $n = 2-6$ each point). *In vivo* data is repeated for comparison (black line).

C-D) Matlab Simulink model – (i) Light input to the system - LIGHT ON intensity is an arbitrary value (0.8) chosen to more closely replicate the empirical response, LIGHT OFF has a value of 0. The primary dopamine release response is switched between alternate pathways depending on the light condition. (ii) Dopamine release output of the model (iii) Negative feedback to light input - Light input to these pathways is reduced by negative only delayed feedback system which has dopamine release as an input. (iv) Light mediated direct inhibition of dopamine release.

Figure 7: Mechanism of dopamine release in the mouse retina [in discussion]

Suggested model to describe dopamine release in the mouse retina. Left: in the dark no light input to dopaminergic varicosities means dopamine release is not inhibited resulting in a high

basal release. Middle: scotopic/mesopic rod activation drives GABAergic inhibition, and a small excitatory glutamatergic input only to synaptic AMPA/kainite receptors causing reduced basal dopamine release. Right: activation by bright light also drives GABAergic inhibition but activates extra-synaptic NMDA receptors via glutamate spill over resulting in more excitation than inhibition causing a large release of dopamine.

Figure S1: In vivo and ex vivo dopamine release comparison for wild-type, *Gnat2*^{A518G} and *Opn4*^{Cre/Cre} animals. Related to Figures 1, 2 & 3.

A) *In vivo* DOPAC:DA ratio was normalised ($R/R_{\max}$) to reveal that one curve fits release profiles for wild-type, *Gnat2*^{A518G} and *Opn4*^{Cre/Cre} animals (extra sum-of-squares F test, $p = 0.99$; $F = 0.2$).

B) *Ex vivo* dopamine release (pg/retina) was normalised ($R/R_{\max}$) to reveal that one curve fits release profiles for wild-type, *Gnat2*^{A518G} and *Opn4*^{Cre/Cre} animals (extra sum-of-squares F test, $p = 0.3$; $F = 1.2$). EC_{50} of the fitted curves for each genotype was not significantly different between *in vivo* and *ex vivo* (paired Student's t-test $p > 0.05$; $n \geq 3$ each point).

Figure S2: Red and blue stimuli. Related to Figure 2.

A) Top: dotted red line - red light pulse (624 nm; 15 min) used in Figure 2G. Bottom: red solid and blue dotted lines - rod matched stimuli used in Figure 2H.

B-F) Theoretical dopamine release curves in response to white light plotted against effective log photons $\text{cm}^{-2} \text{s}^{-1}$ assuming this response is driven by B – rods (498 nm), C – M-cones (508 nm), D – L-cones (556 nm), E – UV-cones (360 nm) or F – melanopsin (480 nm $\lambda_{\max}$). Red and blue pulse effective log photons $\text{cm}^{-2} \text{s}^{-1}$ for each photoreceptor is plotted on each individual graph. Inserts: B, ERG response in a wild-type mouse to a dim version of the rod matched red and blue stimuli (10 rod effective log photons $\text{cm}^{-2} \text{s}^{-1}$; below cone threshold; scale bar 200 ms, 50 μV). D, ERG flicker (40 Hz) response of wild-type (green) and *Opn1*^{mWR} (red) mice to the red light pulse (dotted red; scale bar 100 ms, 10 μV).

Figure S3: Activation of mRGCs in *Opn4*^{Cre/+} hM3Dq-infected animals with clozapine N-oxide (CNO). Related to Figure 3.

A) Intraperitoneal injection (i.p.) of CNO causes prolonged pupil constriction under infrared illumination ($> 950 \text{ nm}$) that begins to subside 4 hrs after injection.

B) hM3Dq-expressing cells can be visualized by mCherry (red) expression. Yellow/green cell shows both mCherry expression and biocytin (green) following patch-clamp recording (scale bar 30 μm).

C) Current-clamp recording from yellow/green cell in B shows that a local puff of 10 μM CNO causes rapid depolarisation and spiking that decreases following the puff.

D) Slow bath application of 10 nM CNO initially causes neuronal silencing for $\sim 2.5 \text{ min}$, followed by a large increase in firing rate that was maintained for the recording ($> 60 \text{ min}$; trace truncated).

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B) Significant light-induced dopamine turnover *in vivo* occurs only above 5 Log R^* rod $^{-1}$ s $^{-1}$ compared to dark-adapted mice dissected under dim red light (0.017 Log R^* rod $^{-1}$ s $^{-1}$, >650 nm). One-way ANOVA with Dunnett's multiple comparison test ($n \geq 4$ each point; all white pulses 1hr).

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E) Dopamine turnover is significantly greater in dark-adapted mice dissected under infrared light (-2.5 Log R^* rod $^{-1}$ s $^{-1}$, >800 nm) compared to those dissected under dim red light (0.017 Log R^* rod $^{-1}$ s $^{-1}$, >650 nm). Unpaired Student's t-test *** $p < 0.0001$ ($n \geq 6$; animals exposed to light for < 1 min).

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G) Activation of mRGCs by CNO does not cause increased dopamine turnover in the hM3Dq-infected eye of *Opn4^{Cre/+}* mice. Paired two-tailed t-test, left vs right eye ($p = 0.6$, mean $\pm$ SEM, $n = 5$ each). *Opn4^{+/+}* littermates show normal light-induced dopamine release, unpaired Student's t-test, *** $p < 0.001$.

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Figure 5: Dopamine release requires spiking and synaptic input.

A) Block of voltage-gated sodium channels by tetrodotoxin (TTX) significantly prevents light-induced dopamine release in *wild-type* mice. One-way ANOVA with Tukey's multiple comparisons between treated and untreated; unpaired Student's t-test within treatment groups (* $p < 0.05$, mean $\pm$ SEM, $n = 4$ each).

B) Media containing high concentration of potassium (15 mM KCl) significantly enhances light-induced dopamine release (* $p < 0.05$) but has no effect under dim red light conditions. One-way ANOVA with Tukey's multiple comparisons between treated and untreated; unpaired Student's t-test within treatment groups (mean $\pm$ SEM, $n = 4$ each).

C) Dopamine turnover is increased in *rd/rd* mice in Mg^{2+} -free media under both dim red and bright light conditions. One-way ANOVA with Tukey's multiple comparisons between treated and untreated (** $p < 0.01$, mean $\pm$ SEM, $n = 4$ each). All y-axes same units.

Figure 6: Duration response characteristics of dopamine release

A) Significant increase in light-induced dopamine turnover *in vivo* occurs after 30 sec of light exposure ($6.8 \text{ Log R}^* \text{ rod}^{-1} \text{ s}^{-1}$) compared to dark-adapted mice dissected under dim red light. This difference is maintained as long as tested (60 min). When lights are turned off from a light-adapted retina, a significant decrease in dopamine release results after 10 min. The decrease continues for 60 min until overnight dark-adapted levels are reached. One-way ANOVA with Dunnett's multiple comparison test ($p < 0.05$, $n \geq 4$ each point except $t=65, 90, 120 \text{ min}$ $n=2$).

B) *Ex vivo* dopamine release displays a gradual increase over 15 min that can be fit with a sigmoidal curve (dotted line; $n = 2-6$ each point). *In vivo* data is repeated for comparison (black line).

C-D) Matlab Simulink model – (i) Light input to the system - LIGHT ON intensity is an arbitrary value (0.8) chosen to more closely replicate the empirical response, LIGHT OFF has a value of 0. The primary dopamine release response is switched between alternate pathways depending on the light condition. (ii) Dopamine release output of the model (iii) Negative feedback to light input - Light input to these pathways is reduced by negative only delayed feedback system which has dopamine release as an input. (iv) Light mediated direct inhibition of dopamine release.

Figure 7: Mechanism of dopamine release in the mouse retina [in discussion]

Suggested model to describe dopamine release in the mouse retina. Left: in the dark no light input to dopaminergic varicosities means dopamine release is not inhibited resulting in a high basal release. Middle: scotopic/mesopic rod activation drives GABAergic inhibition, and a small excitatory glutamatergic input only to synaptic AMPA/kainite receptors causing reduced basal dopamine release. Right: activation by bright light also drives GABAergic inhibition but activates extra-synaptic NMDA receptors via glutamate spill over resulting in more excitation than inhibition causing a large release of dopamine.

Figure S1: In vivo and ex vivo dopamine release comparison for wild-type, $Gnat2^{A518G}$ and $Opn4^{Cre/Cre}$ animals. Related to Figures 1, 2 & 3.

A) *In vivo* DOPAC:DA ratio was normalised (R/R_{max}) to reveal that one curve fits release profiles for wild-type, $Gnat2^{A518G}$ and $Opn4^{Cre/Cre}$ animals (extra sum-of-squares F test, $p = 0.99$; $F = 0.2$).

B) *Ex vivo* dopamine release (pg/retina) was normalised (R/R_{max}) to reveal that one curve fits release profiles for wild-type, $Gnat2^{A518G}$ and $Opn4^{Cre/Cre}$ animals (extra sum-of-squares F test, $p = 0.3$; $F = 1.2$). EC_{50} of the fitted curves for each genotype was not significantly different between *in vivo* and *ex vivo* (paired Student's t-test $p > 0.05$; $n \geq 3$ each point).

Figure S2: Red and blue stimuli. Related to Figure 2.

A) Top: dotted red line - red light pulse (624 nm; 15 min) used in Figure 2G. Bottom: red solid and blue dotted lines - rod matched stimuli used in Figure 2H.

B-F) Theoretical dopamine release curves in response to white light plotted against effective log photons $\text{cm}^{-2} \text{s}^{-1}$ assuming this response is driven by B – rods (498 nm), C – M-cones (508 nm), D – L-cones (556 nm), E – UV-cones (360 nm) or F – melanopsin (480 nm λ_{max}). Red and blue pulse effective log photons $\text{cm}^{-2} \text{s}^{-1}$ for each photoreceptor is plotted on each individual graph. Inserts: B, ERG response in a wild-type mouse to a dim version of the rod matched red and blue stimuli (10 rod effective log photons $\text{cm}^{-2} \text{s}^{-1}$; below cone threshold; scale bar 200 ms, 50 μV). D, ERG flicker (40 Hz) response of wild-type (green) and *Opn1^{mwR}* (red) mice to the red light pulse (dotted red; scale bar 100 ms, 10 μV).

Figure S3: Activation of mRGCs in *Opn4^{Cre/+}* hM3Dq-infected animals with clozapine N-oxide (CNO). Related to Figure 3.

A) Intraperitoneal injection (i.p.) of CNO causes prolonged pupil constriction under infrared illumination (> 950 nm) that begins to subside 4 hrs after injection.

B) hM3Dq-expressing cells can be visualized by mCherry (red) expression. Yellow/green cell shows both mCherry expression and biocytin (green) following patch-clamp recording (scale bar 30 μ m).

C) Current-clamp recording from yellow/green cell in B shows that a local puff of 10 μ M CNO causes rapid depolarisation and spiking that decreases following the puff.

D) Slow bath application of 10 nM CNO initially causes neuronal silencing for ~ 2.5 min, followed by a large increase in firing rate that was maintained for the recording (> 60 min; trace truncated).