Response anisocoria in the pupillary light and darkness reflex

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Abstract
The pupil constricts or dilates in response to a luminance increase or decrease, and these transient pupillary responses are controlled by the parasympathetic and sympathetic pathways. Although pupillary responses of the two eyes are highly correlated, they are not always identical (referred to as anisocoria). For example, there are unequal direct and consensual pupillary constriction responses after an increase in luminance to one eye. While contraction anisocoria (i.e., constriction) has been demonstrated in the pupillary light reflex, it is not yet known if there is also dilation anisocoria in the pupillary darkness reflex. Unlike previous studies that focused on the pupillary light reflex, we examined response anisocoria in both pupillary light and darkness reflexes. While requiring participants to maintain central fixation, we presented a light or dark stimulus to either the right or left visual field to induce transient pupillary constriction or dilation. Both the pupillary light and darkness reflexes had significantly larger ipsilateral responses compared to the contralateral responses relative to the stimulated visual field. The observed ipsilateral effects occurred significantly faster in the light than darkness reflex, suggesting that larger ipsilateral pupillary dilation after a luminance decrease cannot be only attributed to the inhibition of the parasympathetic system, but is also mediated by the excitation of the sympathetic system. Together, our results demonstrated a larger ipsilateral pupil response in both the pupillary light and darkness reflex, indicating an asymmetry in ipsilateral and contralateral neural circuitry of the pupillary darkness reflex.

KEYWORDS
consensual response, parasympathetic, pupil constriction and dilation, sympathetic

1 | INTRODUCTION
Pupil size constantly changes to regulate the amount of light entering the retina, with pupillary constriction in response to

an increase in global luminance and pupillary dilation in response to a luminance decrease (Loewenfeld, 1999; referred to as the pupillary light and darkness reflex, respectively). Although the pupillary responses are highly correlated between the two eyes (Jones, 1949; Loewenfeld, 1954), they are not identical, referring to as anisocoria. When a single eye is stimulated by light, larger pupillary constriction is usually produced for the direct response of the stimulated eye compared to consensual response of the non-stimulated eye (referred to as contraction anisocoria) (Carle, Maddess, & James, 2011; Fan, Miles, Takahashi, & Yao, 2009; Schmid,

Contraction anisocoria can be explained by anatomical evidence in the pupillary light reflex pathway (Figure 1a). An increase in luminance results in the activation of the parasympathetic system to stimulate the pupillae sphincter muscles of the iris to decrease the pupil diameter (Gamlin & Clarke, 1995; Loewenfeld, 1999). Retinal ganglion cells that mediate luminance signals from the temporal retina project to the ipsilateral pretectal olivary nucleus (PON), whereas ganglion cells from the nasal retina project to the contralateral PON (Gamlin, 2006; McDougal & Gamlin, 2015). Neurons in the PON project bilaterally to the preganglionic Edinger-Westphal (EW) nuclei (Kozicz et al., 2011). Preganglionic parasympathetic neurons in the EW nuclei project ipsilaterally to the ciliary ganglion and continue to the pupillae sphincter muscle of the iris.

**FIGURE 1** Schematic of the pupillary light (a) and darkness (b) pathway. See text for details. (c) Each trial began with the appearance of a central FP (0.5° diameter; isoluminant of background) on a gray background (12 cd/m²). After 1,000–1,200 ms of fixation, the background luminance on the right or left visual field relative to the central fixation point either increased (20 cd/m²), decreased (4 cd/m²), or stayed the same (12 cd/m²) for 500 ms, and the participants were required to maintain steady fixation for an additional 2,000–2,500 ms. Note that the outline of the box here is only for illustration of the paradigm. Bkgd, background; CG, ciliary ganglion; EW, Edinger–Westphal nucleus; FP, fixation point; Hypoth, hypothalamus; PON, pretectal olivary nucleus; SCG, superior cervical ganglion. [Colour figure can be viewed at wileyonlinelibrary.com]
FIGURE 2  Pupillary light and dark responses after background luminance changes. (a) Pupil response following the change of background luminance (light reflex: increase 8 cd/m²; darkness reflex: decrease 8 cd/m²; CTRL: no changes). (b) Normalized pupil light and darkness response (light/darkness responses minus CTRL responses). (c,d) Normalized pupil diameter and velocity in the pupillary light reflex between the right and left pupil. (e,f) Normalized pupil diameter and velocity in the pupillary darkness reflex between the right and left pupil. The dark-gray horizontal bar on X-axis indicates the timeline of background luminance change. The shaded colored regions surrounding the pupillary response represent ± standard error range for different conditions. In a, c-f, the light and darkness epochs for pupil size and velocity are shaded in yellow and dark-orange, respectively. CTRL: control condition (no background luminance change), Bkgd: background, n: number of participants. [Colour figure can be viewed at wileyonlinelibrary.com]

There are denser projections from the nasal retina to the contralateral PON compared to those transmitted from the temporal retina to the ipsilateral PON (Hutchins & Weber, 1985; Kupfer, Chumbley, & Downer, 1967), and the projection from the PON to the EW nucleus is contralaterally predominant (Clarke, Blanks, & Giolli, 2003; Gamlin, Zhang, & Clarke, 1995; Pierson & Carpenter, 1974; Steiger & Büttner-Ennever, 1979). Therefore, there should be greater activity to stimulate the pupillae sphincter in the direct compared to the consensual eye.

The pathway for the darkness reflex is less well understood than the pupillary light reflex pathway. A decrease in pupil size (see Figure 1b). Although the retinotopic relationship between the retina and suprachiasmatic nucleus (SCN) is unclear, the visual fibers from the retina provide direct visual input to the SCN, located in the anterior hypothalamus (Abrahamson & Moore, 2001; Fernandez, Chang, Hattar, & Chen, 2016; Moga & Moore, 1997). The hypothalamus has projections through the brainstem to the ciliospinal center located in the intermediolateral cell column of the spinal cord at the level of C8–T2 (Holstege, 1987; Loewenfeld, 1999; Swanson & McKellar, 1979). However, how the luminaire signals actually travel from the SCN to the intermediolateral cell column of the spinal cord is not understood. Axons of the preganglionic neurons travel from the spinal cord to the superior cervical ganglia (SCG) (Kardon, 2005; Loewenfeld, 1999), and the postganglionic neurons of the SCG terminate on the dilatory pupilles muscle of the iris via the long and short ciliary nerves (Beatty & Lucero-Wagoner, 2000; Loewenfeld, 1999; McDougal & Gamlin, 2015).

Although response anisocoria has been investigated in the pupillary light reflex, it remains poorly understood in the pupillary darkness reflex. The goal of the current study is to investigate whether there is dilatation anisocoria in the pupillary darkness reflex. Constriction anisocoria is also examined to compare pupillary dilation with constriction responses. Unlike previous research presenting luminance change in one of the eyes, we presented luminance change in one visual field. Specifically, background luminance in a single visual field was increased or decreased to induce the pupillary light or darkness reflex (Figure 1c), and we compared the pupillary responses that are contralateral or ipsilateral to the stimulated visual field to examine response anisocoria.

2 | MATERIALS AND METHODS

2.1 | Participants

All experimental procedures were reviewed and approved by the Ethics Board of Queen’s University and were in accordance with the principles of the Canadian Tri-Council Policy Statetement (TCPS-2 2014) on Ethical Conduct for Research Involving Humans, and the Declaration of Helsinki (World Medical Association, 2001). Fifteen participants (seven males, eight females) were recruited for this study (mean age 22.1 ± 3.2 years). All participants had normal or corrected to normal vision, were naive to the purpose of the experiment, provided informed written consent, and were compensated for their participation.

2.2 | Recording and apparatus

Eye position and pupil size were measured by a video-based eye tracker (Eyelink-1000 binocular arm, SR Research,
Osgoode, ON, Canada) at a rate of 500 Hz with binocular recording. Eyelink Experimental Builder and Eyelink software controlled stimulus presentation and data acquisition. The visual stimuli were presented on a 17-inch LCD monitor at a screen resolution of 1,280 × 1,024 pixels (60 Hz refresh rate), subtending a viewing angle of 32° × 26°, and the distance from the eyes to the monitor was set at 58 cm. Pupil area values recorded from the eye tracker were transformed to actual pupil sizes in diameter following previously described methods (Steiner & Barry, 2011; Wang & Munoz, 2014). To maintain an accurate measurement of pupil size before, during and after visual stimulation, and to avoid contamination by saccadic eye movements, participants were required to maintain visual fixation on a point at the center of the screen throughout the trial.

2.3 | Experiment

Participants were seated in a dark room and the experiment consisted of 185 trials (Figure 1c). Each trial began with the appearance of a central fixation point (FP) (0.5° diameter; isoluminant of background) on a gray background (12 cd/m²). After 1,000–1,200 ms of central fixation, background luminance within the right or left visual field either increased to 20 cd/m², or decreased to 4 cd/m² for 500 ms (both with 66.67% contrast relative to the gray background), or stayed the same (12 cd/m²). It has been argued that the area of retina near the vertical meridian contains cells that can project either ipsilaterally or contralaterally (e.g., Gamlin, 2003). To compensate for this possibility, the region of luminance change was shifted 3° of visual angle off the vertical meridian in the direction of the hemifield being tested. With this arrangement, only one PON would be modulated at a time. The participants were required to maintain steady fixation for an additional 2,000–2,500 ms to provide a long duration to measure pupil responses. Visual field locations (right and left) and luminance changes (light, dark, and control) were randomly interleaved. Note that in this study, the pupil ipsilateral or contralateral to the stimulated visual field is referred to as the ipsilateral and contralateral pupil.

2.4 | Data analysis

Trials with blinks or an eye position deviation more than 2° from the central FP were excluded from analysis (<5%). There were at least 20 trials for each condition remaining for analysis. For each trial, pupil diameter values were subtracted from the baseline pupil diameter value determined by averaging pupil size from 100 ms before to background luminance change onset (Wang, Blohm, Huang, Boehnke, & Munoz, 2017; Wang, Brien, & Munoz, 2015). Because pupil size was constantly changing even when there was no stimulus presented, and to simplify data presentation and quantification, we normalized pupil diameter values by contrasting the background change versus no-background-change conditions directly. Specifically, pupil values from each background change trial were contrasted to the average pupil value from all control trials. Moreover, the two pupil measures were normalized separately to appropriately compare the difference between the right and left pupil response. We also computed instantaneous pupil velocity to further examine moment-to-moment pupillary changes. Two time windows were arbitrarily selected to separately capture transient pupil light and darkness peak responses: (a) an epoch spanning from 600 to 750 ms after the background change onset was selected for the pupillary light reflex because the time to peak constriction was ~726 ms; and (b) an epoch spanning from 650 to 800 ms was selected for the pupillary darkness reflex because the time to peak dilation was ~776 ms. Two velocity epochs were also selected accordingly to measure the peak response of light and darkness reflexes separately: (a) an epoch spanning from 300 to 450 ms for the light reflex was used because the time to peak velocity was ~357 ms; and (b) an epoch spanning from 450 to 600 ms for the darkness reflex was used to capture the sustained peak period from 375 to 659 ms after the background change onset. The pupil response onset latency (PROL) was defined as the earliest point in which pupil size in the background-change condition statistically (p < 0.05) exceeded the control condition or the baseline time window epoch (usually 0–100 ms after background luminance changes) and remained so for at least 100 ms. A two-sided t test (student t test) was used to inform the statistical differences.

3 | RESULTS

3.1 | Pupillary light and darkness reflex evoked by luminance changes in a single visual field

To first demonstrate the effect of pupillary light and darkness reflexes with changing background luminance in a single visual field, the right and left visual field condition and the right and left pupil data were collapsed. Change in background luminance resulted in transient pupillary responses, with pupillary constriction and dilation in response to luminance increase and decrease, respectively (Figure 2a). As seen in Figure 2a, after transient responses, the pupil dilated and constricted in the light and darkness reflex condition, respectively, partly responding to luminance decrease (light condition) and increase (darkness condition) due to the luminance change back to the background level. Notably, the magnitude for the darkness reflex was substantially smaller than that for the light reflex, and this could be due to a short presentation of the background luminance change. Because of the slower response for pupillary dilation than pupillary constriction, the
dilation force evoked by a luminance decrease was overridden by constriction force evoked by luminance increase back to the background level, therefore resulting in a smaller dilation. Nonetheless, these results were consistent with the literature and transient pupil response effects we previously observed with whole background luminance change (e.g., Barbur, Harlow, & Sahraie, 1992; Wang & Munoz, 2014). The pupil significantly constricted in the light condition (light epoch 600–750 ms, \( t(14) = 11.5, p = 1.7e-08 \)) and dilated significantly in the dark condition (darkness epoch 650–800 ms, \( t(14) = 2.48, p = 0.026 \)), relative to the control condition (no change in background). Consistent with the literature, the PROL (pupil response onset latency) was significantly faster in the light compared to the dark condition (calculated individually: \( t(14) = 4.76, p = 3.025e-04 \)), with 295 and 635 ms (calculated by the average curve) for the light and darkness reflex, respectively. These results are consistent with the idea that the transient response of pupillary light and darkness reflexes are primarily mediated by the parasympathetic and sympathetic pathways, respectively, thus producing shorter light and darkness response latencies (Bitsios, Prettyman, & Szabadi, 1996; Loewenfeld, 1999). To normalize pupil size, we contrasted the light and dark condition to the control condition, as illustrated in Figure 2b (see Materials and Methods for details). Note that although the darkness reflex has sometimes been described as the whole pupil response evoked by transient luminance decrease (Loewenfeld, 1999), the current study focused on the transient component of pupil dynamics to compare pupil constriction and dilation responses after background luminance changes.

3.2 | Similar pupillary light and darkness responses between the right and left pupil

To validate the accuracy of both pupil measurements, we separated the right and left pupil responses. As illustrated in Figure 2c–f, both pupils showed clear light and darkness reflexes, and responses in the right and left pupil were approximately the same. In the light condition, pupil size of the two eyes did not differ during the light response epoch (Figure 2c: epoch 600–750 ms, \( t(14) = 0.617, p = 0.55 \)), and the PROLs were not different between the right and left pupil, with mean latency being 273 and 273 ms for the right and left pupil, respectively (\( t(14) = 0.17, p = 0.87 \)). Similarly, pupil

**FIGURE 3** Pupil laterality effect after background luminance increase. (a,b) Normalized pupillary light responses between the right and left pupil following background luminance increase in the right and left visual field, respectively (\( n = 15 \)). (c) Pupil laterality effect relative to the stimulated visual field (ipsilateral minus contralateral pupil size) on the normalized pupil diameter (600–750 ms epoch post-luminance-change, \( n = 30 \)) when the background change was appeared in the left (◄: blue – red trace) or right (►: red – blue trace) field. (d,e) Normalized pupillary light velocity responses between the right and left pupil following background luminance increase in the right and left visual field, respectively (\( n = 15 \)). (f) Pupil laterality effect relative to the stimulated visual field (ipsilateral minus contralateral pupil velocity) on the normalized pupil velocity (300–450 ms epoch post-luminance-change, \( n = 30 \)). In a,b,d,e, the dark-gray horizontal bar on X-axis indicates the time line of background luminance change. The shaded colored regions surrounding the pupillary response represent ± standard error range for different conditions. The light epochs for pupil size and velocity are shaded in yellow. The cyan bar on X-axis indicates the timeline at which differences between the right and left pupils were statistically significant (\( p < 0.05 \)). In c,f, each triangle indicates one subject and filled-triangles indicate subjects with statistically significant differences (\( p < 0.05 \)). Bkgd: background. [Colour figure can be viewed at wileyonlinelibrary.com]
velocity was similar between the right and left pupil response (Figure 2d: epoch 300–450 ms, \( t(14) = 0.555, p = 0.59 \)). In the dark condition, pupil size of the two eyes was not different during the darkness response epoch (Figure 2e: epoch 650–800 ms, \( t(14) = 1.54, p = 0.15 \)). The PROLs were similar between the right and left pupil, with mean latency being 526 and 572 ms for the right and left pupil, respectively (\( t(14) = 1.08, p = 0.3 \)). Pupil velocity was also not different between the right and left pupil (Figure 2f: epoch 450–600 ms, \( t(14) = 0.0083, p = 0.94 \)). Together, these results suggest the accuracy of and similarity between the right and left pupil size measurement. To focus on the transient response evoked by background luminance changes, the timeline of the remaining figures was changed to 1,000 ms after background luminance changes.

3.3 | Larger pupillary constriction in the ipsilateral pupil after a luminance increase

Consistent with the literature (Carle et al., 2011; Fan et al., 2009; Schmid et al., 2000; Smith, Ellis, & Smith, 1979; Smith & Smith, 1980; Wyatt & Musselman, 1981), pupillary constriction evoked by a sudden luminance increase in the right or left visual field was not identical between the right and left pupils (Figure 3a,b, right and left visual field change). As predicted (Figure 1a), when the left visual field was brightened (Figure 3a), the left pupil had greater constriction (blue trace below red trace). When the right visual field was brightened (Figure 3b), the right pupil had greater constriction (red trace below blue trace). Figure 3c illustrates pupil size differences between the two conditions (ipsilateral minus contralateral pupil size during the epoch of 600–750 ms) when the background change was appeared in the left (◄: blue–red trace) or right (►: red–blue trace) field separately, showing that the pupil ipsilateral to the stimulated visual field had significantly larger constriction, compared to the contralateral pupil (\( t(29) = 4.55, p = 8.7e-05 \)). When background was presented on the right and left visual field, there were nine and eight (out of 15) participants with significantly larger pupil constriction, respectively. Moreover, the PROL was significantly faster for the ipsilateral compared to the contralateral condition, with mean latencies being 270 and 275 ms for the ipsilateral and contralateral condition, respectively (\( t(14) = 3.7, p = 0.0024 \), calculated by combining the right and left field conditions). Time to peak response (referred to as peak latency) was not different between the two conditions, with mean peak latencies being 744 and 749 ms for the ipsilateral and contralateral condition, respectively (\( t(14) = 1.37, p = 0.19 \), calculated by combining the right and left field conditions).

Pupil velocity dynamics revealed the same pattern of results (Figure 3d,e). Higher pupillary constriction velocity was observed in the pupil ipsilateral to the stimulated visual field in comparison to the contralateral pupil (Figure 3f: ipsilateral minus contralateral pupil velocity during the epoch of 300–450 ms: \( t(29) = 4.48, p = 0.0001 \)). There were nine (out of 15) participants with significantly larger pupil constriction velocity in the right and left visual field conditions. As mentioned, the current study was focused on the transient component of pupillary responses, and the whole dynamics of pupil responses are provided in Supporting information Figure S1.

3.4 | Larger pupillary dilation in the ipsilateral pupil after a luminance decrease

Although slower in response than the light reflex, the pupil darkness reflex also showed laterality effects. Transient pupil dilation after a background luminance decrease in the right and left visual fields was not identical between the right and left pupil. When the left visual field was darkened (Figure 4a), the left pupil had greater dilation (blue trace above red trace). When the right visual field was darkened (Figure 4b), the right pupil had greater dilation (red trace above blue trace; see Supporting information Figure S2 for the whole dynamics of pupil responses). Figure 4c illustrates pupil size differences (ipsilateral minus contralateral pupil size during the epoch of 650–800 ms) when the background change was appeared in the left (◄: blue–red trace) or right (►: red–blue trace) field separately, showing that the pupil ipsilateral to the stimulated visual field had significantly larger dilation, compared to the contralateral pupil (\( t(29) = 3, p = 0.0055 \)). There were six and eight participants with significantly larger pupil dilation in the right and left visual field, respectively. Moreover, the PROL and peak latency of pupil darkness responses were similar between the ipsilateral and contralateral conditions, with mean PROLs being 539 and 548 ms for the ipsilateral and contralateral condition, respectively (\( t(14) = 0.23, p = 0.82 \), calculated by combining the right and left field conditions) and mean peak latencies being 969 and 928 ms for the ipsilateral and contralateral condition, respectively (\( t(14) = 0.99, p = 0.34 \), calculated by combining the right and left field conditions). Pupil velocity dynamics revealed the same pattern of results (Figure 4d–f), higher pupillary dilation velocity was observed in the pupil ipsilateral to the stimulated visual field in comparison to the contralateral pupil (Figure 4f: ipsilateral minus contralateral pupil velocity during the epoch of 450–600 ms: \( t(29) = 3.59, p = 0.0012 \); four and six participants had significantly larger pupil dilation velocity in the right and left visual field, respectively).

3.5 | Laterality effects occurred earlier for pupillary light than darkness reflex

Because pupillary dilation could be caused by exciting the sympathetic system or inhibiting (or reduced activating) the
parasympathetic system, to understand the neural pathway mediating the laterality effect between the light and darkness reflex, we contrasted pupillary responses between the ipsilateral and contralateral pupil in both reflexes. Figure 5 illustrates differences between the ipsilateral and contralateral pupil responses (ipsilateral minus contralateral pupil size), showing initial larger pupillary constriction in the ipsilateral pupil in the pupillary light reflex, and larger pupillary dilation in the darkness reflex. Importantly, the ipsilateral effects occurred significantly earlier in the light than the darkness reflex ($t(14) = -4.54, p = 0.00046$), with 251 and 445 ms response latencies (calculated by the mean curve) for the light and darkness reflex, respectively.

4 | DISCUSSION

In response to an increase or decrease in luminance, the pupillary light and darkness reflexes, respectively, alter the diameter of the pupils by modulating the pupillae sphincter and dilator muscle tone to control the intensity of light that falls on the retinal ganglion cells. While contraction anisocoria has been demonstrated in the pupillary light reflex (Carle et al., 2011; Schmid et al., 2000; Smith & Smith, 1980; Smith et al., 1979; Wyatt & Musselman, 1981), it was not clear if these differences between two pupils were also present in the pupillary darkness reflex. Our results showed that in addition to the pupillary light reflex, the pupillary darkness reflex also displays robust response anisocoria, such that the ipsilateral pupil relative to the stimulated visual field had a larger dilation change compared to the contralateral pupil after background luminance decrease.

4.1 | Response anisocoria in the pupillary light and darkness reflex

The pupillary light reflex pathway that gives rise to contraction anisocoria has been described (Carle et al., 2011; Fan et al., 2009; Schmid et al., 2000; Smith & Smith, 1980; Wyatt & Musselman, 1981). As depicted in Figure 1a, the optic fibers from the nasal
retina, which cross over (at the optic chiasm), are more numerous than those from the temporal retina, which remain uncrossed (Kupfer et al., 1967). Moreover, there are more contralateral PON projections to the EW nucleus compared to the ipsilateral projections (Clarke et al., 2003; Gamlin et al., 1995; Hutchins & Weber, 1985; Pierson & Carpenter, 1974; Steiger & Büttner-Ennever, 1979). Due to the fact that our stimulus was configured to just activate one PON, our results showing larger pupillary constriction than those from the temporal retina, which remain uncrossed (Kupfer et al., 1967). Moreover, there are more contralateral PON projections to the EW nucleus compared to the ipsilateral projections (Clarke et al., 2003; Gamlin et al., 1995; Hutchins & Weber, 1985; Pierson & Carpenter, 1974; Steiger & Büttner-Ennever, 1979). Additionally, the ipsilateral bias was also observed in the darkness reflex (Figure 4), with larger dilation in the ipsilateral pupil than the contralateral pupil relative to the stimulated visual field after luminance decrease. Previous research has identified a sex difference in constriction anisocoria (Fan et al., 2009), which raises an interesting question whether similar effects can be found in dilatation anisocoria. Future research is required to investigate this question.

4.2 Potential neural substrates underlying the observed asymmetry in the pupillary darkness reflex

Two integrated processes take place during pupillary dilation: relaxation of the iris sphincter muscle, and contraction of the dilator pupillae muscle. A sudden decrease in luminance would reduce the activity in the parasympathetic pathway, in turn relaxing the iris sphincter muscle, therefore resulting in pupillary dilation. Moreover, neurons in the PON and EW nucleus in behaving monkeys have exhibited the firing rate of cells proportionally to luminance levels (Gamlin, 2000; Gamlin et al., 1995), suggesting that the parasympathetic pathway can continually monitor luminance changes and reduce its activity while detecting luminance decrease to cause pupillary dilation. Therefore, the observed bias in the darkness reflex could be mediated by this pathway (Figure 4). However, if the observed ipsilateral bias in pupillary dilation was mediated by the reduced activation on the parasympathetic pathway, then pupillary response latencies between the light and darkness reflex should be similar, as they would be mediated through the same pathway. In contrast, the ipsilateral pupil bias occurred ~200 ms earlier after background luminance changes in the light compared to darkness reflex (Figure 5). Differences in biased latency cannot merely be attributed to mechanics of sphincter and dilator muscles that cause pupillary constriction or dilation because studies in cats have shown similar response latencies ~200 ms on electrically stimulating both muscles (Schaeppi & Koella, 1964a,b). Although it is still possible that the relaxation time of the sphincter muscle is radically slower than its contraction time, it may suggest that the observed asymmetry between ipsilateral and contralateral pupil responses relative to the stimulated visual field in the darkness reflex could be partly mediated by the sympathetic pathway, as suggested by a previous study that the sympathetic system plays an active role in the darkness reflex (Bailey & Guth, 1959).

In contrast to most species, which have bilateral retinal efferents along the retinohypothalamic tract to the SCN with a slight to moderate contralateral predominance (Abrahamson & Moore, 2001; Fernandez et al., 2016; Hattar et al., 2006; Johnson, Morin, & Moore, 1988; Morin, Shivers, Blanchard, & Muscat, 2006; Muscat, Huberman, Jordan, & Morin, 2003; Pickard, 1982; Smale, Blanchard, Moore, & Morin, 1991), the retinohypothalamic projections in primates have been described as predominantly ipsilateral (Gooley & Saper, 2005; Magnin, Cooper, & Mick, 1989). Axons involved in the pupillary darkness reflex descend from the hypothalamus. A few fibers cross the midline in the dorsal commissure to reach the contralateral ciliospinal center, and continue from the ciliospinal center in an ipsilateral dominant fashion to the SCG, although significant contralateral activation was also observed from stimulating these pathways (Kardon, 2005; Loewy, Araujo, & Kerr, 1973). In our experiment, after presenting a dark background in one visual field, luminance change signals are received by the nasal retina of the ipsilateral eye (relative to the stimulated visual field) and the temporal retina of the contralateral eye. These
signals from the ipsilateral nasal retina and the contralateral temporal retina project primarily ipsilaterally through the darkness reflex pathway (Reeves & Posner, 1969). Although the retinotopic projections to the SCN have yet to be systematically studied, our results show larger dilation of the ipsilateral pupil, suggesting a stronger connection between the nasal retina in comparison to the temporal retina and the SCN in humans. Future research is required to test this prediction.

5 | CONCLUSION

Characterizing the neural pathways mediating pupillary reflexes is important because pupil responses are now being used to assess numerous neurologic and visual pathologies (e.g., Bremner, 2009; Daluwatte et al., 2013; Frost et al., 2013; Kankipati, Girkin, & Gamlin, 2011; Wang, McInnis, Brien, Pari, & Munoz, 2016). Our results demonstrated asymmetric pupillary responses during not only pupillary constriction but also pupillary dilation, and possibly also suggest the presence of unbalanced neural projections from the nasal and temporal retina to the SCN. Further research is required to fully understand the details of the neural pathway in the pupillary darkness reflex.

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CONFLICT OF INTEREST

The authors report no potential conflict of interest.

DATA ACCESSIBILITY

Data are available from C-A.W. upon reasonable request.

AUTHORS CONTRIBUTION

C.-A.W., J.H. and D.P.M. designed research; L.T. performed research; C.-A.W. analyzed data and wrote the manuscript; all co-authors contributed to editing the manuscript.

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