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Consensual pupillary light response in the red-eared slider turtle (*Trachemys scripta elegans*)

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ABSTRACT

Purpose of this study was to determine if the turtle has a consensual pupillary light response (cPLR), and if so, to compare it to its direct pupillary light response (dPLR). One eye was illuminated with different intensities of light over a four log range while keeping the other eye in darkness. In the eye directly illuminated, pupil diameter was reduced by as much as ~31%. In the eye not stimulated by light, pupil diameter was also reduced but less to ~11%. When compared to the directly illuminated eye, this generated a ratio, cPLR–dPLR, equal to 0.35. Ratio of slopes for log/linear fits to plots of pupil changes versus retinal irradiance for non-illuminated (−1.27) to illuminated (−3.94) eyes closely matched at 0.32. cPLR had time constants ranging from 0.60 to 1.20 min; however, they were comparable and not statistically different from those of the dPLR, which ranged from 1.41 to 2.00 min. Application of mydriatic drugs to the directly illuminated eye also supported presence of a cPLR. Drugs reduced pupil constriction by ~9% for the dPLR and slowed its time constant to 9.58 min while simultaneous enhancing constriction by ~6% for the cPLR. Time constant for the cPLR at 1.75 min, however, was not changed. Results support that turtle possesses a cPLR although less strong than its dPLR.

1. Introduction

1.1. Consensual pupillary light response

The consensual pupillary light response (cPLR) is the reflexive constriction of the pupil in response to illumination of the contralateral eye (McIlwain, 1996) and is strongest in frontal-eyed mammals (Carpenter & Pierson, 1973; Clarke, Zhang, & Gamlin, 2003a, 2003b; Guth & Bailey, 1960; Pong & Fuchs, 2000; Smith, Ellis, & Smith, 1979) with significant binocular visual fields (Walls, 1942). In lateral-eyed mammals (Campbell & Lieberman, 1985; Clark & Ikeda, 1985; Inoue, 1980; Trejo, Rand, & Cicerone, 1989; Young & Lund, 1994), frog (von Campenhausen, 1963), and fish (Douglas, Harper, & Case, 1998), visual fields overlap less, and the cPLR is less strong. Although chicken also has a cPLR (Li & Howland, 1999), pupils of pigeon (Gamlin, Reiner, Erichsen, Karten, & Cohen, 1984), barn owl (Levine, 1955; Schaeffel & Wagner, 1992), urodeles (Henning, Henning, & Himstedt, 1991), and gecko (Denton, 1956) are thought to respond independently.

1.2. Aim of study

Since several turtle species possess partial overlap of their visual fields (Granda & Maxwell, 1978; Hergueta et al., 1992), which could make use of binocularly coordinated pupil responses, we hypothesized that the turtle possesses a cPLR but less strong as in lateral-eyed mammals, frog, and fish. To our knowledge, no one has systematically examined for a cPLR for only recently has a slow acting, direct pupillary light response (dPLR), been reported (Dearworth et al., 2009; Granda, Dearworth, Kittila, & Boyd, 1995). Although parasympathetic and sympathetic pathways contribute to efferent mechanisms controlling the dPLR (Dearworth et al., 2009; Dearworth & Cooper, 2008; Dearworth, Cooper, & McGee, 2007; Iske, 1929), the source generating its sluggish dynamic is still not known. To obtain better understanding of how afferent pathways carrying light signals from the retinas of the eyes combine and drive pupil responses in turtle, we tested for a cPLR functioning with the dPLR.

2. Methods

2.1. Animals

Seven *Trachemys scripta elegans* were bought from Kons Scientific Co. Inc., (Germantown, WI, USA). Animals weighed from 0.44 to 1.2 kg with carapace lengths ranging from 16 to 24 cm. Turtles were housed in a warm animal suite containing a 60-gallon tub equipped with a filtering system. The environment was maintained on a 14/10 h light/dark cycle with water temperature at 22 °C. Lights were turned on at 6:00 AM and turned off at 8:00 PM. Brick

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islands were placed in the tank for turtles to rest on and bask under 250 W infrared lamps. Using a radiometer (model DR-2000-LED, Gamma Scientific, San Diego, CA), radiant intensity from lights was measured at 3.86×10^{-2} W cm^{-2} sr^{-1}. Animals were fed Rise floating fish diet (Pro-Pet, L.L.C., St. Marys, OH) *ad libitum* every other day, and water in the tank was replaced weekly. All care and procedures of turtles were performed in accordance within the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Lafayette College.

2.2. Apparatus

After turtles were secured in a restraint that immobilized their heads, they were placed into a 15 cm light-integrating sphere, which was separated into left and right halves. Each turtle was form fitted with a black fabric hood that interfaced with the light-integrating sphere to allow independent illumination of each eye. Small holes were cut in the hood for full viewing of each pupil. Holes of 4.5 cm diameter also were cut into each side of the light-integrating sphere at an angle of 45° from the longitudinal axis of the head and below the animal's field of view so that infrared cameras could be inserted into the sphere for viewing each eye. Infrared light-emitting diodes were aligned with cameras to monitor pupil movements. Images of both pupils were captured from cameras and delivered to a computer controlling a commercial eye tracking system (ViewPoint EyeTracker® Arrington Research, Inc., Scottsdale, AZ), which allowed measurement of horizontal pupil diameters with a resolution of 0.03 mm.

Light adaptation came from a 150 W tungsten halogen light source which was shuttered controlled and mounted into the left half of the sphere behind the line of sight of the left eye. Spectral content of the source was checked by the radiometer and confirmed a broad band emission with greater contribution from longer wavelengths in the visible range (Fig. 1). Neutral density filters were used to attenuate the intensity of light. The right half of the sphere which enclosed the right eye was kept in darkness throughout all experimental trials. To test for trans-illumination, one of the turtles was euthanized and its right eye removed. Head of the animal was inserted into the apparatus, and the left side of the sphere illuminated. Radiant intensities in units of W cm^{-2} sr^{-1} were measured in each side of the sphere and converted to retinal irradiance using derivation by Dvorak, Granda, and Maxwell (1980) and the schematic of the turtle eye (Northmore & Granda, 1991). Intensities measured in the illuminated left half versus the dark right half of the light-integrating sphere are summarized in Table 1. Maximal retinal irradiance in the left illuminated half was equal to 6.91×10^{-6} J cm^{-2} s^{-1}. Levels of other light attenuations were relative to this maximum in units of optical density (OD).

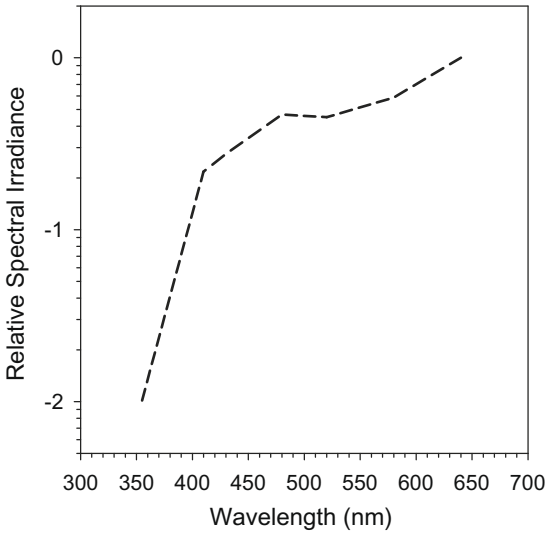

Fig. 1. Relative spectral irradiance for the tungsten halogen light source.

2.3. Protocol using different light intensities

Pupil responses from left eyes directly illuminated at different intensities were compared to their corresponding right eyes, which were kept in the dark. Animals were acclimated to the setup under darkness for 10 min before trials began. Once the trial began, measurements from both pupils were taken for 30 min in the dark and continued after turning on the light to the left side of the sphere. Because of the slow pupil responses, measurements were taken at 2-min intervals. All trials were completed at some time during their housed, 14-h light cycle, and turtles were given at least 24 h of rest between trials.

2.4. Mydriatic drug treatment

Mydriatic drugs were applied to the eye stimulated with light to test for enhancement of a cPLR (Theofilopoulos, Longmore, Kerr, Szabadi, & Bradshaw, 1988). After 10 min of light adaptation at 3.31×10^{-7} J cm^{-2} s^{-1} (1.3 OD) to the left eye, measurements of pupil size were taken to define a baseline cPLR. The light was then turned off, and topical application of agents to the corneas of eyes begun. Vecuronium bromide, a nicotinic cholinergic antagonist, was applied in combination with phenylephrine, a α_1 adrenergic agonist, to the cornea of the left eye; and the cornea of the right eye was treated with saline (0.9% NaCl). Applications were delivered in volumes of 0.04 ml, four times at 15 min intervals, using a schedule adapted from prior studies done in bird and turtle (Dearworth & Cooper, 2008; Dearworth et al., 2007; Loerzel, Smith, Howe, & Samuelson, 2002). At a concentration of 0.4% for vecuronium bromide, four applications of 0.04 ml delivered 0.64 mg of the drug. Four administrations of 2.5% phenylephrine accounted for a total instillation of 4 mg. At these amounts in turtles, this combination has been shown to be an effective mydriatic (Dearworth & Cooper, 2008) with peak drug effects occurring between 55 and 110 min. After 70 min in darkness and the start of drug application, during peak pharmacologically induced mydriasis, light adaptation was returned to the left side of the sphere for comparison of responses measured in both eyes prior to drug applications. Pupil sizes were tracked for an additional 40 min after turning the light back on.

2.5. Data analysis

Data collected by the eye tracking software were imported into Microsoft® Excel (Microsoft Corporation, Redmond, WA). To compare responses among animals of different sizes, data were normalized as percent difference from the maximum pupil diameter. Means ± standard error (SE) were plotted as a function of time. Analysis of variance (ANOVA) and *t*-tests were used for statistical comparison. Statistically significant differences were established at levels of $P < 0.05$.

To quantify the rates of pupillary changes, a time constant equation was used (Clarke, 2007; Dearworth et al., 20072009; Dearworth & Cooper, 2008; Granda et al., 1995). SigmaPlot (SPSS Inc., Chicago, IL) was used for curve fitting. Data were fit to equation, $y = y_0 + a(1 - e^{-t/\tau})$, where y is pupil size, y_0 is offset, a is the amplitude of pupil movement, t is time, and τ is the time constant.

Table 1
Retinal irradiances.

Light attenuation (OD)	Directly illuminated left eye (J cm^{-2} s^{-1})	Right eye in dark (J cm^{-2} s^{-1})
0.0	6.91×10^{-6}	4.97×10^{-12}
0.8	1.06×10^{-6}	1.02×10^{-12}
1.3	3.31×10^{-7}	1.31×10^{-12}
2.9	9.18×10^{-9}	7.00×10^{-13}
3.4	2.51×10^{-9}	5.10×10^{-13}

Radiometer measure in dark = 6.79×10^{-13} J cm^{-2} s^{-1}.

3. Results

3.1. Representative responses

An example of a single trial from turtle A is shown in Fig. 2. Pupils of both eyes dilated during 30 min of darkness to their largest diameters. Right eye (Fig. 2, black squares) dilated to maximum 2.97 mm at 28 min, and left eye (Fig. 2, white squares) dilated to its maximum 2.77 mm at 24 min. After left eye was illuminated at 30 min, both pupils slowly became smaller in size. Left eye responded more, reducing pupil diameter to a minimum of 2.03 mm and becoming 73.29% of the maximum measured in the dark. Right eye, which was in darkness, reduced its diameter to 2.64 mm, 88.89% of its maximum. Retinal irradiance evoking the direct pupillary response in left eye was 6.91×10^{-6} J cm^{-2} s^{-1}, 0.0 OD (Table 1). Response in the right eye was evoked under conditions approximating complete darkness. Quantity of light passing through the skull of a euthanized turtle into the right orbit was measured at an irradiance of 4.97×10^{-12} J cm^{-2} s^{-1}, a level of light approximately six log units less than what was measured for the light illuminating the left eye, and within one log unit of the radiometer measurement in the dark at 6.79×10^{-13} J cm^{-2} s^{-1}.

Mean responses ($N = 3$) to retinal irradiance of 1.06×10^{-6} J cm^{-2} s^{-1} (0.8 OD) from turtle B are shown in Fig. 3. Duration of light adaptation was extended to 40 min to show that pupil size in both eyes did not change significantly beyond 10 min. Quantity of light passing through the skull to the right eye of the euthanized turtle was 1.02×10^{-12} J cm^{-2} s^{-1}, again about six log units less than the intensity for the directly illuminated left eye and close to the level measured in complete darkness (Table 1). Left

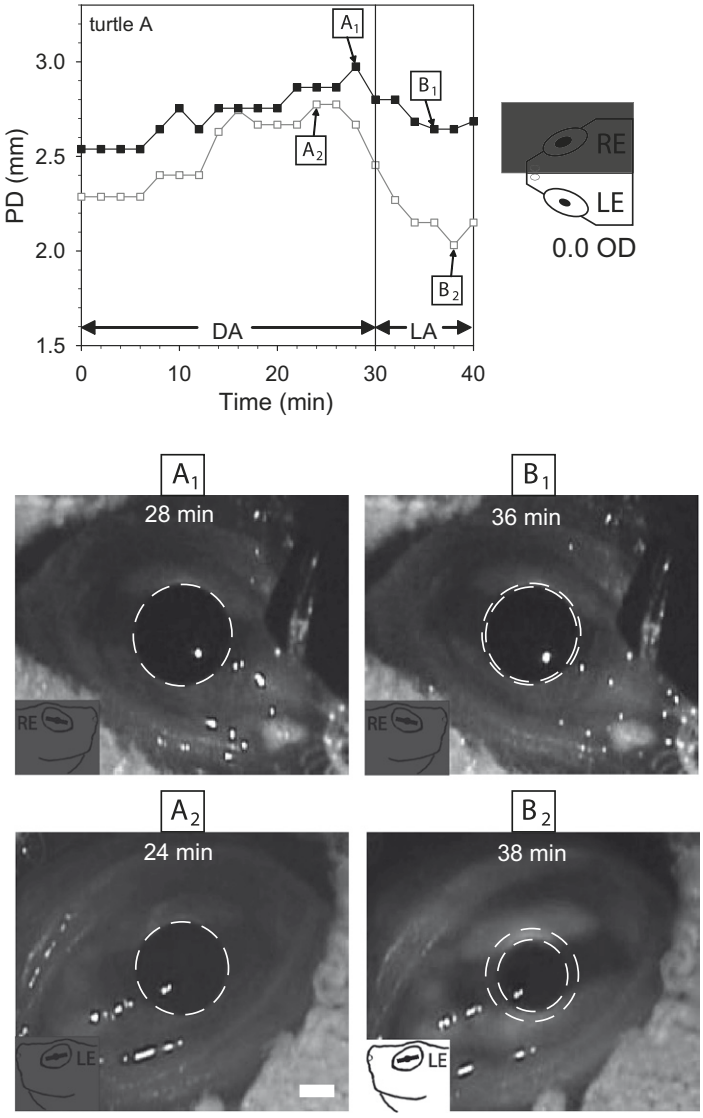

Fig. 2. dPLR by left eye (LE) and cPLR by right eye (RE). After both eyes were dark-adapted (DA) for 30 min, left eye (white squares) was light-adapted (LA) for 10 min at the light level coded as 0.0 optical density (OD). Right eye (RE) remained in the dark. Images at bottom show pupils at their maxima in dark (A_1 and A_2) and minima in light (B_1 and B_2). White scale bar in A_2 = 1 mm. Dashed white circles are fitted to maxima in A_1 and A_2 and superimposed on B_1 and B_2 for comparison. Sketches at lower left corners of images show orientation of the eye and iris line in the head (after Brown, 1969; Rodieck, 1998).

eye (Fig. 3, white squares) dilated to a maximum response of 3.00 mm ± 0.05 (SE) in dark adaptation at 30 min, and then to a minimum diameter of 1.83 mm ± 0.08 at 70 min during light adaptation. Right eye (Fig. 3, black squares) kept in the dark dilated to its greatest diameter 2.90 mm ± 0.11 at 26 min, then constricted to a minimum size of 2.36 ± 0.16 at 56 min. Reduction for left eye was 61.00% from maximum size in the dark, and for right eye was 81.38%.

3.2. Pooled responses for different light intensities

Data were pooled from five turtles (A, B, C, D, and E) to compare pupil responses evoked by different light intensities (Fig. 4). When left eye was exposed to light, pupil sizes for both eyes were significantly reduced. Greatest reduction in pupil sizes occurred when left eye was illuminated at 0.0 OD (Fig. 4, bottom). Left pupils were reduced from 100% ± 0.98 at 26 min to 69.27% ± 2.98 at 38 min (Table 2, *t*-test, $P < 0.001$); right eyes maintained in the dark had their pupils reduced from 100% ± 1.60 at 18 min to 89.02% ± 3.33 at 36 min ($P < 0.03$). Comparisons of maximum pupil diameters in the dark to minimum sizes during light also were done for the other intensities using *t*-tests, and likewise, all were statistically different ($P < 0.03$). ANOVAs for the changes measured in the eyes showed statistical differences ($P < 0.03$), except for one, the right eye in dark ($P = 0.10$), during illumination of the left eye at 3.4 OD.

3.3. Time constants equations fitted to pupil constrictions

Exponential curves generated from time constant equations are included in Fig. 4 to quantify response changes by directly illuminated left eyes and right eyes in the dark. For directly illuminated left eyes, amplitudes of constrictions ranged from a low of 17.36% ± 2.40 for 3.4 OD and increased to a high of 30.65% ± 1.46 at 0.0 OD (Table 3). Time constants, however, were not different and had overlapping SEs for different light levels. Range low was 1.41 min ± 0.42 at 0.8 OD, and high was 2.00 ± 0.74 at 3.4 OD. Results were similar for right eyes kept in the dark except the range of amplitudes for constriction was less than that observed for directly illuminated eyes. Lowest constriction was 5.86% ± 1.69 at 3.4 OD, and highest was 10.21% ± 1.17 at 0.0 OD. Low for time constants was 0.60 min ± 0.37 at 2.9 OD, and high was 1.20 ± 0.44 at 0.0 OD, but again with SEs that overlapped.

3.4. Mydriatic drugs with light adaptation

Results using mydriatic drugs (vecuronium bromide with phenylephrine) with light adaptation at 1.3 OD are shown in Fig. 5 (turtle F, $N = 4$). Pupil diameters of both eyes changed significantly versus time (Table 2, ANOVA, $P < 0.0001$). Ten min of light adaptation to left eye initially constricted both pupils. Before turning off the light illuminating the left eye, left pupil started at 70.23 ± 1.52 (Fig. 5, white triangle with dot), and the right at 92.66 ± 2.04 (Fig. 5, black triangle with dot). After applying drugs (vertical arrows in Fig. 5), both pupils reached 100% maxima at 60 min. When light adaptation was returned to the left eye at 70 min, both pupils were reduced in size from their maxima. Left pupil size in Fig. 5 was reduced from the maximum (*t*-test, $P < 0.0005$) after return to light adaptation at 70 min but was not reduced to the same size as was measured in light at the start prior to drugs. Minimal size reached at 110 min (Fig. 5, white triangle with cross) was 79.65% ± 1.62, and was significantly larger by ~9% ($P < 0.003$) than the size measured at the start (Fig. 5, white triangle with dot). The right pupil that was kept in the dark also was reduced significantly from its maxima ($P < 0.0003$) to 86.28 ± 1.03 at 110 min (Fig. 5, gray triangle with cross); however, it was affected oppositely with regard to the start (Fig. 5, black triangle with dot) and became significantly smaller by ~6% ($P < 0.05$).

As before, rates of constriction were quantified by fitting data to exponential curves. For the left pupil (Fig. 5, gray curve) rate of constriction by time constant was quantified as 9.58 min ± 3.03 ($r^2 = 0.92$), over four times greater than the other time constants fitted to constrictions occurring at the other light levels (0.0, 0.8, 2.9, and 3.4) in absence of drugs (cf. Table 3). Associated was an amplitude fit (17.78% ± 2.19) that was much lower than necessary to offset pupil size back to its original. Exponential fit (Fig. 5, black curve) for constriction in the right eye ($\tau = 1.75$ min ± 1.60, $a = 11.52\%$ ± 1.72, $r^2 = 0.88$); however, was still comparable to those measured at the other light levels.

3.5. Summary of effects by light intensities and drugs

Minimal pupil sizes reached for both eyes shown in Fig. 4 were plotted as a function of the values for retinal irradiances, which were presented to the left eye (Fig. 6). Log/linear fits to data for illuminated left eyes (gray line, $Y = -3.94 \log X + 48.16$, $r = 0.99$) and right eyes in dark (black line, $Y = -1.27 \log X + 82.07$, $r = 0.98$) are shown with ±95% confidence limits (CL). Minimum pupil sizes of both eyes reached during the different light adaptations of left eye before drugs (triangles with dots) and after drugs (triangles with crosses) also are plotted.

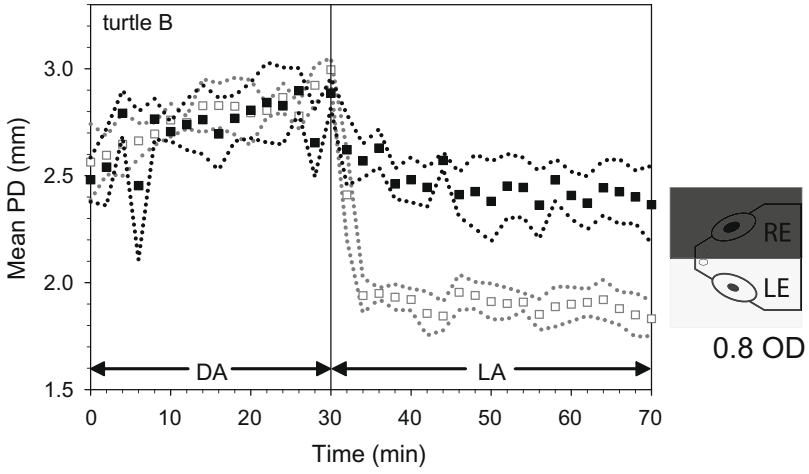

Fig. 3. Pupil responses during an extended light level of 0.8 OD to the left eye (white squares). Right eye (black squares) was kept in the dark. Mean pupil diameters (PD) ± standard errors (dotted lines) were derived from three trials.

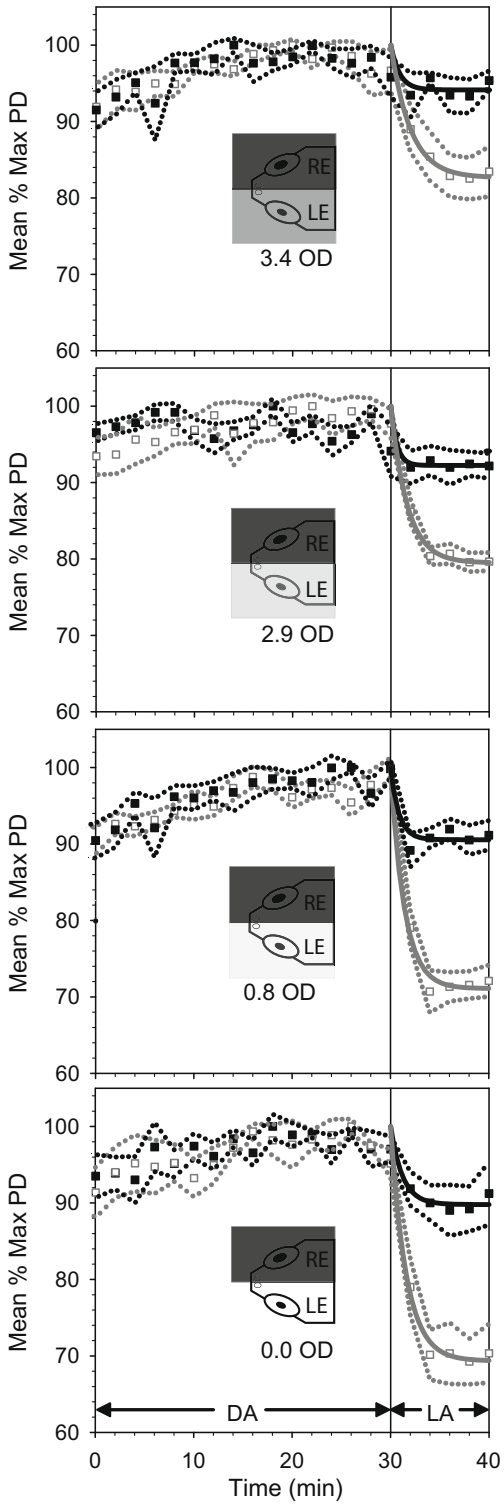

Fig. 4. Pooled responses from five different turtles at four light levels: 3.4 OD, $N = 6$; 2.9 OD, $N = 7$; 0.8 OD, $N = 9$; and 0.0 OD, $N = 4$. Pupil changes were normalized to maximal pupil diameters. Time constant (τ) equations were computed for both direct (gray curve) and consensual (black curve) responses.

4. Discussion

4.1. Range of the cPLR

The red-eared slider possesses a cPLR, which is weaker than its dPLR. Greatest intensity of light presented to the ipsilateral eye constricts the contralateral pupil ~11% (from 100% in dark to ~89%). Compared to constriction of the directly illuminated pupil, ~31% (100% to ~69%), the ratio of cPLR to the dPLR is 0.35 (Fig. 4, bottom). Strength of the response is weaker than observed in other lateral- and frontal-eyed vertebrates. For example, in rat, the ratio is 0.78 (Trejo et al., 1989), and in fish, for the midshipman species, a ratio as high as ~0.60 has been shown (Douglas et al., 1998, Fig. 9). Studies in monkey and humans range from essentially no difference between direct and consensual responses (Carpenter & Pierson, 1973; Clarke et al., 2003a; Loewenfeld, 1993) to showing values which come close to rat at 0.79 (Pong & Fuchs, 2000). Amplitude of the cPLR functioning with the dPLR for turtle also increases with light intensity over a four log range (Fig. 6) just as in monkey (Clarke et al., 2003a; Pong & Fuchs, 2000). Log/linear fits to data possessed slopes of −1.27 for the right eyes kept in darkness and −3.94 for directly illuminated left eyes, and when compared to each other generate a ratio of 0.32. This correlates well with the ratio of cPLR to the dPLR at maximum intensity (0.35). Similar correlation is observed in monkey (Pong & Fuchs, 2000) where ratio for the slopes of log/linear fits is 0.83, also close to its cPLR to dPLR ratio (0.79).

Since red-eared sliders have heavily pigmented skin (Brown, 1969) and a substantial interorbital septum, which develops with its chondrocranium (Tulenko & Sheil, 2007), a cPLR in turtle due to trans-illumination is unlikely. In the frame still of Fig. 2 B_1, the pupil is dark, and no light coming through the other side can be seen. As measured by radiometer, light intensities at the right orbit in the dark (Table 1) were essentially zero, within a log unit of the probe measurement in total darkness. But even as small as the trans-illumination irradiance measurements seem to be, they are likely to overestimate the amount of light reaching the retina of the non-illuminated eye. A more precise measurement could have been acquired by only removing the anterior part of the right eye (cornea, iris, lens, and vitreous) instead of the whole eye. Nonetheless, based on Granda et al. (1995), this low level of light is two or more log units below the retinal irradiance required to drive any measurable dPLR in alert behaving turtles.

4.2. Mydriatic drugs enhance the cPLR

Presence of a cPLR in turtle is also supported by enhancement of the cPLR during reduction and slowing ($\tau = 9.58$ min) of the dPLR using mydriatics (Figs. 5 and 6). As the illuminated eye is dilated with drugs, more photons are permitted to enter the eye and strike the retina, which increases light signals carried by pathways going to the contralateral eye, thus causing an enhanced consensual constriction. Studies done in human (Theofilopoulos et al., 1988) and turtle (Dearworth & Cooper, 2008) using mydriatics support this observed compensatory miosis. For example, in the study in turtle, when phenylephrine was used to dilate the pupil, the contralateral eye, which was treated with saline, constricted. The result, however, was not conclusive enough to claim that a cPLR exists in turtle since the reduction observed during treatments of combination of vecuronium bromide with phenylephrine in the same study was not significant. Possible reason for the lack of statistical power was that both eyes were illuminated by the same intensities of light during drug application, which could have masked statistical significance. In contrast, the current work independently stimulated the drug treated eye with light while shielding the control eye treated with saline in the dark.

4.3. Function of the slow dPLR and cPLR

The cPLR possess time constants ranging from 0.60 to 1.20 min (Fig. 4 and Table 3) and are similar to those observed for the dPLR in turtle (1.41–2.00 min) (cf. Dearworth et al., 2009; Granda et al.,

Table 2
Summary statistics.

Light attenuation (OD)	Directly illuminated left eye				Right eye in dark			
	% Max. PD vs. time ANOVA *P* value	$DA_{Max.}$ (100%) ±SE	$LA_{Min.}$ % ± SE	$DA_{Max.}$ vs. $LA_{Min.}$ *t*-test *P* value	% Max. PD vs. time ANOVA *P* value	$DA_{Max.}$ (100%) ±SE	$LA_{Min.}$ % ± SE	$DA_{Max.}$ vs. $LA_{Min.}$ *t*-test *P* value
0.0	<0.0001	±0.98	69.27 ± 2.98	<0.001	<0.03	±1.60	89.02 ± 3.33	<0.03
0.8	<0.0001	±1.25	70.71 ± 2.76	<0.0001	<0.0001	±0.57	89.17 ± 2.21	<0.001
2.9	<0.0001	±1.53	79.58 ± 1.24	<0.0001	<0.0005	±1.02	91.99 ± 2.26	<0.03
3.4	<0.0001	±0.78	82.53 ± 2.72	<0.001	0.10	±0.88	93.32 ± 2.22	<0.03
1.3 and after mydriatics	<0.0001	±1.41	79.65 ± 1.62	<0.0003	<0.0001	±0.49	86.28 ± 1.03	<0.0003

$DA_{Max.}$ (100%) = mean maximum pupil diameter during dark adaptation.
$LA_{Min.}$ = mean minimum pupil diameter during light adaptation of directly illuminated left eye.

Table 3
Amplitude changes and time constants for pupillary constrictions.

Light attenuation (OD)	Directly illuminated left eye			Right eye in dark		
	a ± SE (%)	τ ± SE (min)	r^2	*a* ± SE (%)	τ ± SE (min)	r^2
0.0	30.65 ± 1.46	1.60 ± 0.21	0.99	10.21 ± 1.17	1.20 ± 0.44	0.96
0.8	28.91 ± 1.67	1.41 ± 0.24	0.99	9.44 ± 1.79	0.85 ± 0.73	0.90
2.9	20.50 ± 0.86	1.68 ± 0.19	0.99	7.76 ± 0.57	0.60 ± 0.37	0.98
3.4	17.36 ± 2.40	2.00 ± 0.74	0.92	5.86 ± 1.69	0.90 ± 1.09	0.80
1.3 and after mydriatics	17.78 ± 2.19	9.58 ± 3.03	0.92	11.52 ± 1.72	1.75 ± 1.60	0.88

a = amplitude.
τ = time constant.

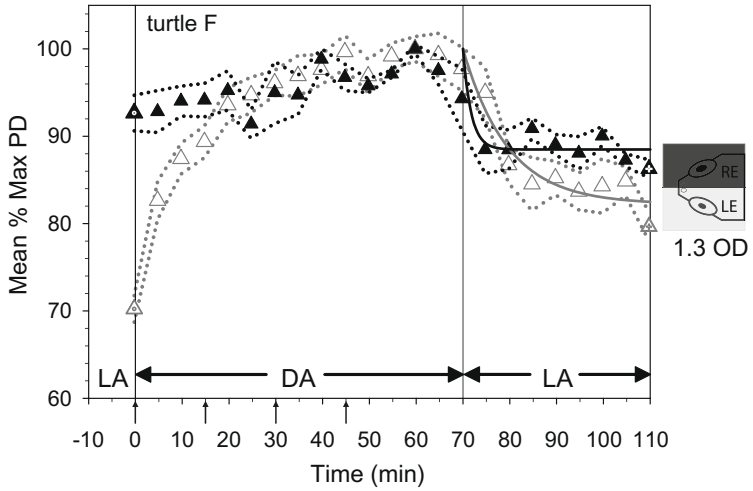

Fig. 5. Mydriatic drugs reduced the dPLR and enhanced the cPLR. Left eye (LE) was light-adapted (LA) at 1.3 OD for 10 min; right eye (RE) was maintained in darkness throughout the trial. To obtain baseline pupil sizes (triangles with dots), pupils were measured 15 s prior to dark-adapting (DA) the left eye. At time 0, drugs (vertical black arrows on time axis) were applied to left eye (white triangles), and saline was applied to right eye (black triangles). At 70 min, left eye was light-adapted (LA) again at 1.3 OD. Both pupils reached new minimal sizes at 110 min (triangle with crosses). Time constant equations were fitted to data to quantify rates of pupil constrictions for dPLR (gray curve) and for cPLR (black curve).

1995). They are also comparable to the dynamics reported for amphibian and fish (Bailes, Trezise, & Collin, 2007; Cornell & Hailman, 1984; Douglas, Collin, & Corrigan, 2002; Douglas et al., 1998; Henning et al., 1991; Kuchnow, 1971). Although these pupil changes are relatively modest in comparison to most vertebrates, which questions their functional significance, responses are clearly present. Perhaps turtle is similar to frog, where reduced amplitude and slowness has been suggested to be an evolutionary adaptation, resulting from interaction among other compensating factors (circadian rhythm, phototactic behaviors, and photoreceptive adaptations occurring with retinomotor responses) working together to provide optimal vision for the animal within its environment (Cornell & Hailman, 1984).

4.4. Possible mechanism and circuitry

Neural processes underlying these factors are poorly understood. In turtle, complex psychophysical visual mechanisms (Granda & Sisson, 1989; Sisson & Granda, 1989) and rapid adaptations by photoreceptors (Granda, Maxwell, & Zwick, 1972) reflect possible sources for compensation. Turtle too possesses retinomotor responses (Ali, 1971; Drenckhahn & Wagner, 1985). Another source could involve intrinsically photosensitive retinal ganglion cells (ipRGC) containing the photopigment melanopsin (Berson, Dunn, & Takao, 2002; Provencio, Jiang, De Grip, Hayes, & Rollag, 1998; Provencio et al., 2000; Warren, Allen, Brown, & Robinson, 2003), which in other vertebrates are involved with slow sustained pupil

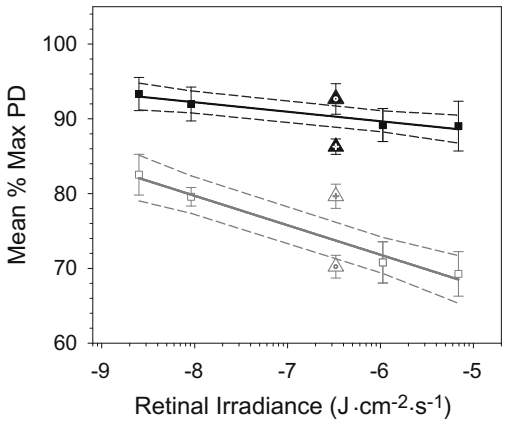

Fig. 6. Minimum pupil sizes for dPLR (white squares) and cPLR (black squares) during different levels of light adaptation to left eyes plotted versus retinal irradiance on logarithmic scale. Data are fit with log/linear regression lines with 95% confidence limits (dashed lines). Data points in response to illumination of the left eye at 1.3 OD before drugs (triangles with dots) fall within confidence limits of fit, but data points from after drugs (triangle with crosses) fall outside limits further indicating that the dPLR is reduced in the left eye (mydriasis) while simultaneously increasing the cPLR in the right eye (compensatory miosis).

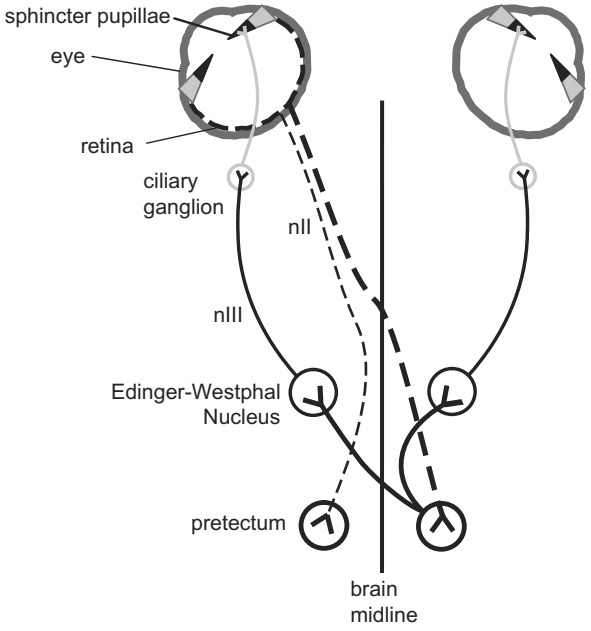

Fig. 7. Diagram of neural pathways in turtle, which could carry signals controlling its slow dPLR and cPLR.

responses (Gamlin et al., 2007; Lucas, Douglas, & Foster, 2001; Young & Kimura, 2008). It is not yet known if ipRGCs are expressed in turtle, but if they are, the afferent pathways carrying their signals could also contribute to the retinal circuitry and photoreceptive mechanisms driving slow pupil responses.

Ipsilateral pathways, although less than the contralateral (Fig. 7), have been reported to diffusely project from the retina to the pretectum in *T. scripta elegans* as well as in other related turtle species (Bass & Northcutt, 1981; Hergueta, Lemire, Ward, Rio, & Reperant, 1992 for review; Reiner, Zhang, & Eldred, 1996) and could support cPLR, which is weaker than a dPLR. The pretectum in turtle also makes connections with the accessory optic system to coordinate visuomotor responses involved in stabilizing retinal images, such as optokinetic reflex behaviors (Fan, Weber, Pickard, Faber, & Ariel, 1995; Weber, Martin, & Ariel, 2003), and pupil constriction is likely another function given that connections by the pretectum to other brain regions in turtle are extensive and similar to birds and mammals (Kenigfest et al., 2000, 2004). In support of this, in other vertebrates, both lateral- and frontal-eyed, pathways from the pretectum project bilaterally via the posterior commissure to Edinger-Westphal nuclei and are shown to mediate pupil constriction (Clarke et al., 2003b; Henning & Himstedt, 1994; Itoh, 1977; Young & Lund, 1994). In contrast, chicken also possess a cPLR, but instead of using bilateral projections to the pretectum, bilateral retinal projections going to the suprachiasmatic nuclei then to Edinger-Westphal are suggested to be responsible (Fitzgerald, Gamlin, Zagvazdin, & Reiner, 1996; Li & Howland, 1999). This suggests that there are several connections possible within the visual system of turtle, which could share information between the two sides of the brain and process a cPLR.

Although a common photoreceptive mechanism with central processing, which is bilaterally unequal, is one cause for a cPLR weaker than the dPLR, another reason could be due to a photointrinsic iris. Several vertebrates, mammalian and non-mammalian, including turtle, possess light-sensitive irises (von Studnitz, 1933; for review see Barr, 1989). Preliminary results from our laboratory have confirmed this for the red-eared slider after testing for light responses in the pupils of enucleated eyes (Dearworth, Cooper, & Littlefield, 2006; Sipe, Dearworth, Blaum, & McDougal, 2009). Alternatively then, central processing may be equal, and the greater magnitude of the dPLR relative to the weaker cPLR could be instead from summation of central processing and the photointrinsic response, where the dPLR is augmented by a photointrinsic response. In either case, our results suggest that turtle possesses a cPLR although less strong than its dPLR.

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