



# **La Sensibilidad Espectral Visual de la Araña de Rincón (Araña Reclusa Chilena) *Loxosceles laeta***

**Felipe Alejandro Tapia Pérez**

**Tesis para optar al grado de  
Magíster en Ciencias Biológicas Mención Neurociencia**

**Director de Tesis:  
Dr. Oliver Schmachtenberg**

**Valparaíso, Chile  
2020**

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# La Sensibilidad Espectral Visual de la Araña de Rincón (Araña Reclusa Chilena) *Loxosceles laeta*

La presente tesis se encuentra publicada en forma de un artículo de investigación en la revista "Journal of Experimental Biology" (<https://jeb.biologists.org/>) bajo el título "The visual spectral sensitivity of the Chilean recluse spider *Loxosceles laeta*", es posible acceder a la publicación en línea del artículo mediante el vínculo <https://jeb.biologists.org/content/223/2/jeb217133>.

## 1 ABSTRACT

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Las arañas son un gran grupo de artrópodos casi omnipresentes en climas cálidos y templados. Dependen de información táctil y visual para cazar y reproducirse, pero en comparación con sus sentidos mecánicos, poco se sabe acerca de sus sistemas visuales. En este estudio analizamos la sensibilidad espectral visual de la araña de rincón o araña reclusa chilena *Loxosceles laeta*, una especie sinantrópica que representa una amenaza importante a los humanos, utilizando registros electroretinográficos de sus tres tipos de ojos y pruebas tipo *open-field* con iluminación monocromática localizada para análisis de comportamiento. El electroretinograma mostró dos máximos de sensibilidad en el rango ultravioleta y verde, pero no se observaron diferencias entre los tres tipos de ojos o entre especímenes machos y hembras. La adaptación cromática selectiva redujo la sensibilidad general a la luz, pero no apoyó la posible presencia de más de un tipo de rodopsina en los fotorreceptores. La prueba *open-field* reveló preferencia por las esquinas por sobre los lados, y un aumento en el tiempo de exploración de las zonas abiertas iluminadas con luz de longitudes de onda corta (violeta a verde) comparado a zonas no iluminadas, no se observaron respuestas de comportamiento a luz roja o infrarroja. Esta información sugiere que *L. laeta* tiene visión monocromática sin especializaciones espectrales en sus tres pares de ojos secundarios.

## 2 INTRODUCCIÓN

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### 2.1 LA ARAÑA DE RINCÓN

La araña de rincón o araña reclusa chilena, *Loxosceles laeta* (Nicolet, 1849) es una especie de araña nativa de Sudamérica, común en Chile, además de Perú, Ecuador, Argentina, Uruguay y el sur de Brasil (Canals et al., 2016). Se trata de una araña con un tamaño promedio de entre 10-12 mm (desde el cefalotórax al abdomen) o 30 mm considerando las patas, de color marrón. En la cara dorsal del cefalotórax poseen una depresión más oscura en forma de violín, lo que les da además el nombre común de "araña violinista" (Cabrerizo et al., 2009). El número y distribución de los ojos es característico, y permite la identificación del género: poseen tres pares de ojos en forma de "V" (Cabrerizo et al., 2009).

*L. laeta* es una araña solitaria sinantrópica, es decir, de hábitats domésticos, por lo que se encuentra dentro de casas, usualmente en agujeros en paredes, espacios entre muebles, detrás de cuadros (Gonçalves-de-Andrade et al., 2007) y en esquinas oscuras (Canals et al., 2015) lo que da origen a su nombre común. Esta relación estrecha con el ser humano hace que el loxoscelismo, cuadro clínico resultante de la mordedura de las arañas del género *Loxosceles*, constituya un importante problema de salud pública en varios países sudamericanos, especialmente en Chile, para el cual no existe suficiente evidencia que permita orientar las decisiones sobre su manejo (Manríquez M and Silva V, 2009).

El veneno de esta araña es dermo-necrótico y víscero-tóxico. Es rico en enzimas de bajo peso molecular, de las cuales el principal componente tóxico es la esfingomielinasa D, que interactúa con las membranas celulares y otros elementos tisulares desencadenando alteraciones que involucran el sistema del complemento y activación de neutrófilos (Da Silva et al., 2004). Asociado a la mordedura de esta araña, se han descrito dos cuadros clínicos: El primero y más frecuente (83,3% del total) (Schenone, 2003), denominado loxoscelismo cutáneo, es un cuadro limitado, generalmente benigno, caracterizado por dolor, edema y eritema dentro de las primeras 6 a 8 horas de la mordedura, que progresa a un halo de vasoconstricción e isquemia que se extiende alrededor de la lesión. La mordedura tiende a curar por segunda intención en 6 a 8 semanas (Schenone, 2003). El segundo cuadro, el loxoscelismo visceral, se caracteriza por la presencia de compromiso sistémico, eventualmente letal, caracterizado por hemólisis, hematuria, hemoglobinuria, ictericia, fiebre e insuficiencia renal, que ocurre generalmente transcurridas las primeras 24 horas de la mordedura (Schenone, 2003).

A pesar de su importancia epidemiológica, la biología de esta especie y sus depredadores no es bien conocida (Canals et al., 2016). En lo referente a sus sistemas sensoriales, existe evidencia de la capacidad de estas arañas de detectar conoespecíficos mediante claves olfatorias (Calbiague et al., 2017), pero en cuanto a su visión, no existen estudios que exploren el tema.

## 2.2 VISIÓN EN ARAÑAS

Existen en el mundo más de 48 mil especies de arañas (World Spider Catalog 20.5, <https://wsc.nmbe.ch/>, accedido el 24-12-2019). Existe una enorme variabilidad en cuanto a las características de los ojos de estos animales, siendo por ejemplo la disposición de éstos un criterio para determinar la familia a la cual pertenece un espécimen.

La mayor parte de las especies de arañas posee ocho ojos dispuestos en dos filas en la región anterior del prosoma, sobre los quelíceros, siendo los más anteriores clasificados como anterolaterales (AL) y antero-mediales (AM), mientras que los más caudales corresponden las los postero-laterales (PL) y postero-mediales (PM). Sin embargo, existen grupos de arañas con menos ojos, como las especies de las familias *Dysderidae*, *Scytodidae*, *Pholcidae* y *Sicariidae* que poseen seis ojos, las del género *Tetrablemma* (*Theriidae*) que poseen cuatro o las de la familia *Caponiidae* que sólo poseen dos.

Los lentes corresponden a engrosamientos de la cutícula detrás de los cuales se ubican las células columnares vítreas (DeVoe et al., 1969). Los ojos AM, llamados también ojos principales, se diferencian de los demás (ojos secundarios) de 4 formas: (1) Los ojos principales se desarrollan de un engrosamiento del prosoma que produce tres capas, mientras que los secundarios se forman de

sólo una invaginación; (2) Los núcleos de los receptores se ubican en el centro de la célula, con los rabdómeros distales a ellos; (3) Carecen de un *tapetum*; (4) La retina es móvil (Barth, 1985). Los ojos principales son los más relevantes para la agudez visual, presentando la capacidad de formar imágenes de baja resolución (Land and Nilsson, 2012).

Como se mencionó previamente, las arañas del género *Loxosceles*, al cual pertenece la araña de rincón, poseen seis ojos distribuidos en tres díadas. Los ojos AM se encuentran ausentes y los PM han migrado a una posición más anterior (Barth, 1985; DeVoe et al., 1969; Gertsch and Ennik, 1983). Por ello, los ojos de *L. laeta* corresponden en su totalidad a ojos del tipo secundario, los que se asocian más con la navegación y la detección de pistas celestes como el sol u otras estrellas durante la noche (Land and Nilsson, 2012).

Una forma de caracterizar la visión en animales es mediante el estudio de la sensibilidad espectral, que corresponde al recíproco de la intensidad de luz necesaria por cada longitud de onda para obtener una respuesta con una cierta amplitud criterio (DeVoe et al., 1997). Ya que la sensibilidad espectral de los fotorreceptores depende a su vez del espectro de absorción de los fotorpigmentos que expresa (DeVoe et al., 1997), este método no sólo entrega información respecto al rango absoluto de sensibilidad sino también del número y características de los distintos tipos de pigmentos que pueda presentar la retina.

Respecto a la sensibilidad espectral en arañas, existen estudios iniciales como los de George y Peckham en el siglo XIX, en el que se observó que la araña lobo *Hogna frondicola* (Emerton, 1885) descansaba preferencialmente bajo un vidrio rojo comparado con vidrios azules, amarillos o verdes (George and Peckham, 1887). Una de las arañas mejor estudiadas desde el punto de vista de su sistema nervioso es *Cupiennius salei* (Keyserling, 1877). En el estudio de Barth 1993, se determina la sensibilidad espectral utilizando la técnica de electroretinograma (ERG). Las curvas de sensibilidad tanto en los ojos principales como secundarios mostraban un máximo verde prominente entre 520-540 nm con un hombro en el ultravioleta entre 340-380 nm. La adaptación selectiva de los ojos no evidenció cambios en la sensibilidad relativa, indicando la no presencia de más de un tipo espectral de receptor (Barth et al., 1993). Un estudio posterior por Walla, evidenció, utilizando registros intracelulares, la presencia de 3 grupos de células con sensibilidades máximas en el azul (480 nm), verde (520 nm) y UV (340 nm), las células azules y verdes además mostraban máximos secundarios en el UV a 340-380nm (Walla et al., 1996). Esto contrasta con lo observado en un grupo cercano taxonómicamente a *Ctenidae* (la familia a la que *C. salei* pertenece), las arañas lobo (Lycosidae), las cuales corresponden a arañas cazadoras al igual que *C. salei* (Walla et al., 1996), pero poseen sólo un tipo de receptor con una absorción máxima entre 505-510nm en los ojos secundarios (DeVoe et al., 1969) y dos tipos en los ojos principales, el ya mencionado y un receptor con una absorción máxima en el ultravioleta entre 360-370nm (DeVoe, 1972).

Finalmente, para ejemplificar la amplia variabilidad de las características visuales de los ojos de las arañas cabe citar el ejemplo de uno de los grupos con tal vez los ojos principales más complejos, *Salticidae*. Estas arañas poseen no menos de 4 tipos de receptores, con sensibilidades máximas en el UV (360 nm), azul (480-500 nm), verde (520-540 nm) y amarillo (580 nm), estando además estos receptores ubicados en 4 capas distintas en la retina (Yamashita and Tateda, 1976). Cabe destacar además que los ojos secundarios de estas arañas poseen sólo un pigmento con sensibilidad máxima entre 535-540 nm (Yamashita and Tateda, 1976).

Cómo se puede observar, los ojos de las arañas están muy lejos de ser simples o tener características uniformes entre los distintos grupos del taxón (Barth et al., 1993), por lo tanto, es imposible generalizar los resultados de estos estudios funcionales a otras familias, especialmente en el caso de *L. laeta*, ya que no existen datos respecto a las sensibilidades espectrales de ningún miembro de Sicariidae o de alguna familia cercana.

Con el objetivo de profundizar el conocimiento respecto a la biología y ecología de esta especie de gran importancia epidemiológica en Chile, decidimos estudiar la sensibilidad espectral de sus ojos. Dado sus hábitos nocturnos y al hecho de que sólo poseen ojos de tipo secundario, hipotetizamos que su sensibilidad espectral sería similar al de otras especies nocturnas como *C. salei* (Barth et al., 1993) y que prefieren lugares oscuros.

### 3 DESCRIPCIÓN GENERAL DEL ESTUDIO

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Con el objetivo de determinar la sensibilidad espectral de *Loxosceles laeta* se utilizaron dos aproximaciones metodológicas:

#### 3.1 ELECTRORETINOGRAMA

Se realizaron registros de la respuesta eléctrica de la retina frente a la estimulación con destellos de luz monocromáticos entre 370 y 760 nm, utilizando un conjunto de LEDs (19 en total). La sensibilidad espectral se obtiene al determinar el inverso de la intensidad lumínica necesaria para obtener una respuesta de amplitud criterio (20  $\mu$ V) para cada uno de los LEDs disponibles. Se repitió el protocolo para cada tipo de ojo (PM, AL, PL) y se comparó también entre machos y hembras. Finalmente se analizó más en detalle la posible presencia de múltiples pigmentos visuales adaptando selectivamente la retina utilizando luces monocromáticas.

#### 3.2 OPEN-FIELD TEST

Se permitió a las arañas explorar una caja de vidrio cuadrada por un período de 10 minutos, mientras se registraba la actividad en video. La caja estaba cubierta con cartulina negra, excepto por pequeñas ventanas cuadradas la base de cada uno de los lados. Antes de cada experimento se elegía un lado al azar y se posicionaba una luz monocromática (violeta, azul, verde, roja e infrarroja). Se midió el tiempo relativo de exploración para determinar preferencia.

### 4 DISCUSIÓN

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Los registros de ERG evidenciaron un rango espectral absoluto entre los 370 y 650 nm, con la posibilidad de que este se extienda más profundamente en el UV. La curva además presenta dos máximos de sensibilidad, en el verde y UV. Estas características fueron iguales entre los distintos pares de ojos y entre machos y hembras, sugiriendo que no existen especializaciones a nivel retiniano. Los ensayos conductuales mostraron un rango de respuesta similar, con un aumento

del tiempo de exploración de los sectores iluminados con luces entre el violeta y verde, sin respuesta en el rango del rojo al infrarrojo.

Los análisis utilizando adaptación selectiva apoyan la presencia de un solo tipo de fotopigmento, compatible con la rodopsina 1 (Rh1) descrita en otras arañas (Nagata et al., 2012; Varma et al., 2019), que sería responsable del máximo verde. En cuanto al máximo UV, los datos de los que se dispone en este estudio no permiten esclarecer su naturaleza, pero dos posibles explicaciones existen, la presencia de un pigmento accesorio sensibilizante, como el descrito en insectos (Kirschfeld and Vogt, 1986; Stavenga et al., 2017) o una banda  $\beta$  aumentada de Rh1.

Es de interés además el hecho de que las arañas, a pesar de mostrar en general una preferencia por las esquinas durante la prueba conductual (haciendo honor a su nombre común de “araña de rincón”), al ser expuestas a la estimulación lumínica estas aumentaron su tiempo de exploración en lugar de mostrar conductas aversivas, como podría pensarse inicialmente para un animal nocturno. Sin embargo, el significado exacto de esta conducta no se puede determinar en base sólo a este estudio.

Finalmente, este estudio revela pistas iniciales que pueden ser de gran ayuda para esclarecer qué factores determinan los hábitats que esta especie elige para vivir, y por ende qué medidas pueden desarrollarse para evitar la exposición humana a este peligroso animal.

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# The Visual Spectral Sensitivity of the Chilean Recluse Spider *Loxosceles laeta*

Felipe Tapia<sup>1,2</sup>, Jesús Olivares<sup>1</sup> and Oliver Schmachtenberg<sup>1</sup>

<sup>1</sup>Centro Interdisciplinario de Neurociencia de Valparaíso (CINV), Facultad de Ciencias, Universidad de Valparaíso, Valparaíso, Chile.

<sup>2</sup>PhD Program in Neuroscience, Universidad de Valparaíso, Valparaíso, Chile

## Corresponding author:

Oliver Schmachtenberg

e-mail: [oliver.schmachtenberg@uv.cl](mailto:oliver.schmachtenberg@uv.cl)

Tel: +56-32-2995505

**Keywords:** Arthropods, Eye, Retina, Ultraviolet vision, Electroretinogram, Open field test

## Summary statement

*Loxosceles laeta* has monochromatic vision with a spectral range between the ultraviolet and green and no eye type- or sex-specific spectral specializations, evidenced using electroretinogram and behavioral assays.

## Article Information

<b>DOI</b>	<a href="https://doi.org/10.1242/jeb.217133">https://doi.org/10.1242/jeb.217133</a>
<b>PubMed</b>	31852757
<b>Published By</b>	The Company of Biologists Ltd.
<b>Print ISSN</b>	0022-0949
<b>Online ISSN</b>	1477-9145
<b>History</b>	Received October 22, 2019 Accepted December 10, 2019 Posted online December 18, 2019.
<b>Copyright &amp; Usage</b>	© 2019. Published by The Company of Biologists Ltd <a href="http://www.biologists.com/user-licence-1-1/">http://www.biologists.com/user-licence-1-1/</a>

## 1 ABSTRACT

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Spiders are a large group of arthropods and nearly omnipresent in warm and temperate climates. They rely on tactile and visual information to hunt and breed, but compared with their mechanical senses, little is known about their visual systems. In this study, we analyzed the visual spectral sensitivity of the Chilean recluse spider *Loxosceles laeta*, a synanthropic species posing a significant threat to humans, using electroretinogram recordings of its three eye types and open field tests with localized chromatic illumination for behavioral analysis. The electroretinogram displayed two sensitivity peaks in the ultraviolet and green ranges, and no differences were observed between the three eye types and between male and female specimens. Selective chromatic adaptation reduced overall light sensitivity, but did not support the expression of more than one type of rhodopsin in photoreceptors. The open field tests revealed a preference for corners over side areas, and an increased exploration of open field areas illuminated by shorter wavelength (violet to green) light compared with non-illuminated areas, while no behavioral responses to red and near-infrared light were observed. These data suggest that *L. laeta* has monochromatic vision without spectral specializations in its three secondary eye pairs.

## 2 INTRODUCTION

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There are over 48 thousand spider species on Earth (World Spider Catalog 20.5, <https://wsc.nmbe.ch/>, accessed on 9 January 2020), but comparatively little is known regarding their visual capabilities, although these aroused an early scientific interest. In the nineteenth century, it was shown that some spiders have the ability to distinguish colors, besides being able to recognize objects such as their egg cocoons and their conspecifics at close range (George and Peckham, 1887). Unlike the compound eyes of insects, spider eyes are simple lens eyes (Land and Nilsson, 2012). Most spider species have eight eyes arranged in two rows in the anterior region of their prosoma above the chelicerae, with those nearest to the clypeus usually classified as anterior lateral (AL) and anterior median (AM) eyes, while the more caudal eyes are termed posterior lateral (PL) and posterior median (PM) eyes. However, there are spider species that have fewer eyes. For instance, species of the families *Dysderidae*, *Scytodidae*, *Pholcidae* and *Sicariidae* have six eyes, while other species have only four eyes, such as representatives of the genus *Tetrablemma* (family *Theridiidae*), or two eyes, such as spiders of the family *Caponiidae*. Some cavern-dwelling species lack eyes completely (Foelix, 2011). The eyes of spiders can be classified as principal and secondary depending on their embryological origin, which has implications for their morphology and function.

The AM eyes, generally referred to as principal eyes (Barth, 2002b; Yamashita, 1985), express photopigments in microvillous photoreceptor segments near the lens, unlike the secondary eyes, whose photoreceptors point in the opposite direction, with cell bodies closer to the lens and the photopigment-containing segment forming the first portion of the axon (Blest, 1985). Principal eyes are relevant for visual acuity, forming low-resolution images, while secondary eyes are thought to

be related to navigation and may serve to detect celestial cues such as the sun or other stars at night (Land and Nilsson, 2012).

Certain spiders have very well-developed visual systems, such as species of the families *Salticidae*, *Lycosidae* and *Thomisidae* of mostly diurnal habits (Defrize et al., 2011; Nuboer, 1986). Examples of spiders with complex visual systems include the lycosids, which have the ability to detect polarized light, mainly used for spatial orientation (Dacke et al., 2001). Both principal and secondary eye types have a spectral sensitivity ranging from 380 to 580 nm, with photoreceptors that absorb maximally between 505 and 510 nm, and a second absorption peak in the AM eyes at 380 nm (DeVoe et al., 1969). Furthermore, thomisids, voracious hunters that ambush their prey, have the ability to choose the plants they use for hiding based not only on their shape but also on their spectral reflectance patterns, and can also change their body color to adapt to the environment. Thomisid spiders of the species *Misumena vatia* were reported to have two classes of photoreceptor cells, one with a sensitivity peak at 340 nm and another with maximum sensitivity around 520 nm (Defrize et al., 2011).

In contrast, salticids have up to four classes of photoreceptors in their principal eyes, based on their cellular spectral sensitivities, and are thought to possess color vision (Foelix, 2011; Nuboer, 1986). They are also able to judge distance precisely based on visual cues, ostensibly through an elaborate combination of chromatic aberration and image defocus, detected by four tiered photoreceptor layers in the eye (Nagata et al., 2012). The vision of some salticid species is necessary and sufficient to induce courtship behavior (Cross et al., 2008). Another exceptional case is the jumping spider *Evarcha culicivora*, which is able to detect whether a mosquito has recently consumed human blood, based on both olfactory and visual cues (Jackson et al., 2005; Nentwig, 2013). More recently, processing of visual information has been demonstrated in the visual brain centers of *Phidippus audax* (Menda et al., 2014). Among the species with nocturnal habits, probably the most studied is the wandering spider *Cupiennius salei*, a Central American species of the family *Ctenidae*, which has served as a model in many studies of spider biology since the 1960s (Barth, 2002a).

As mentioned above, spiders of the genus *Loxosceles* (*Sicariidae*) have six eyes arranged in three dyads (Fig. 1). The AM eyes are absent and the PM eyes have migrated to a more anterior position, remaining in a recurved disposition in relation to the lateral dyads (Gertsch and Ennik, 1983). These animals occupy a wide variety of habitats, both domestic and natural. As a species of synanthropic habits, they are usually found in fissures, spaces between furniture, and behind photographs and paintings (Gonçalves-de-Andrade et al., 2007), while in natural environments, several species of the genus live in cracks of trees, rock walls and caves (Canals et al., 2015a; Elliott et al., 2017; Tacaure-Ríos, 2011). Being a group of highly venomous species common in Latin America, with closely related albeit slightly less poisonous species inhabiting the southern USA, the Mediterranean and other temperate parts of the world, it seems relevant to better understand what sensory stimuli attract or repel species of the genus *Loxosceles*, and by what criteria they choose their habitat. However, to the best of our knowledge, there are no data available regarding the visual capabilities of any of the species of the genus *Loxosceles*, and only a handful of reports have shed light on its biology (Calbiague et al., 2017; Canals and Solís, 2014; Canals et al., 2015a,b,c).

The Chilean recluse spider *Loxosceles laeta* is considered the most dangerous species of this genus (Binford and Wells, 2003), as a result of its mostly synanthropic habits and highly necrotoxic venom, which may cause serious disfiguring tissue damage and potentially lethal systemic reactions in a minor percentage of the bites (Chaves-Moreira et al., 2017). However, despite its public health relevance, next to nothing is known about the sensory physiology of this species. Here, we analyzed the visual response spectrum of live *L. laeta* specimens by complementary electrophysiological and behavioral experiments. Given their mostly nocturnal habits, we hypothesized that they present a visual spectral range similar to the secondary eyes of other nocturnal spiders (Barth et al., 1993), and prefer darker versus brighter places to dwell.

Accordingly, we observed electroretinogram (ERG) and behavioral responses to light in the range from ultraviolet (UV) to green wavelengths. Surprisingly, an increased exploratory behavior of illuminated versus dark areas was evident in the open field test. These findings provide data on a public health-relevant species that will allow a better understanding of its behavior and possibly the development of strategies to reduce human exposure to its bites.

## 3 MATERIALS AND METHODS

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### 3.1 ANIMALS

*Loxosceles laeta* (Nicolet 1849) (Fig. 1A) individuals were supplied by students of the Faculty of Science of the University of Valparaíso, as they are present in the majority of homes in central Chile. The spiders were kept in 100 ml plastic containers adapted for adequate ventilation under a natural photoperiod at 20–24°C and 60–80% relative humidity. Specimens were staged by size according to the description by Canals and Solís (2014), and further classified by sex based on visual inspection of the pedipalps. Twelve animals (6 females and 6 males) were used for ERG recordings and 8 animals (6 females and 2 males) for behavioral tests. The mass (mean±s.d.) of the spiders was 69.65±39.7 mg for females and 75.77±34.57 mg for males, and the average leg length was 2±0.37 cm for females and 2.45±0.42 cm for males. Before the experiments, the specimens were anesthetized with CO<sub>2</sub> in a closed plastic container for a period of 3 min at room temperature. This allowed safe manipulation of the animals during the procedures.

### 3.2 HISTOLOGY

The legs and abdomen were removed from the cephalothorax, which was fixed in 4% paraformaldehyde solution for 24 h at 4°C. For cryosections, the tissue was cryoprotected in 30% sucrose in PBS for 48 h, embedded in tissue freezing medium (Tissue Tek, Sakura, Mountain View, CA, USA) and cut in a cryostat (Leica CM 1900, Berlin, Germany) at –20°C into 18 µm sections, which were stained in 1% Toluidine Blue (Sigma-Aldrich, MO, USA) for 1 min. Alternatively, fixed tissue was dehydrated in a graded series of ethanol and acetone and embedded in Eponate (Pelco, Fresno, CA, USA). Semi-thin sections (1 µm) were cut in an ultramicrotome (Reichert, Austria) and stained with 1% Toluidine Blue for 2 min.

### 3.3 ERG RECORDINGS

Anesthetized animals were placed inside a Petri dish and the legs were kept in position using two paper strips fastened with masking tape (Fig. 2A). The dish was covered with 5% type VII-A agarose (Sigma-Aldrich), leaving the prosoma exposed. Under this configuration, animals remained immobile for the duration of the experiments, avoiding the necessity of further anesthesia. The animal was then placed on a rotatory platform under a stereomicroscope, which allowed us to position different pairs of eyes towards the stimulation source. A fiberoptic light guide (model 77526, Newport, Irvine, CA, USA) was placed 2 cm away from the recorded eye, and the correct positioning of the light source was verified using the reflection of the light on the tapetum (Fig. 2B). The light guide was connected to one of the LEDs in a custom array with emission spectra spanning from UV to infrared (IR) wavelengths. The spectral characteristics for each LED were measured with a calibrated fiberoptic spectrometer (Ocean Optics USB4000, Largo, FL, USA), and are detailed in Table S1. The LED array was controlled using custom software, and the irradiance of each individual LED was calibrated using three repeated measurements with an optical power meter (1918-R, Newport). ERG recordings were obtained using tungsten electrodes with an impedance of about 13 M $\Omega$  (FHC, Bowdoin, ME, USA) connected to a differential amplifier (Model 3000, A-M Systems, Carlsborg, WA, USA) used in differential and AC mode (band-pass filtered between 1 and 100 Hz, gain 10k). Despite the unstable baseline, several ERG recordings in DC mode were also obtained (Fig. 3A), which confirmed the proportionality of AC versus DC amplitude measurements (Fig. S1). Recordings were digitized using an analog-to-digital converter card (PCI-6221 with chassis BNC2090, National Instruments, Austin, TX, USA) controlled by WinWCP 4.9.4 software (University of Strathclyde, Glasgow, UK). A recording electrode was inserted through the junction between the lens and the chitin covering the cephalothorax (Fig. 2C); a reference electrode was placed in the anterior trochanter of the first leg ipsilateral to the recorded eye (Fig. 2D), and a ground electrode was inserted into the agarose. The three right eyes were recorded, one for each eye pair. To establish the amount of time necessary for dark adaptation prior to the ERG recordings, a series of 500 ms green (533 nm) light flashes were presented at increasing time intervals until the amplitude of the response reached a plateau. The data were fitted to an exponential function using the least square method to obtain the maximum steady-state response amplitude (Fig. 3B). The calculated amount of time to reach 99% of the maximum steady state response amplitude value was  $8.79 \pm 0.13$  min (N=3); therefore, the animals were dark adapted for a period of 15 min before the experiments.

To construct the spectral sensitivity curves, monochromatic 500 ms test flashes were presented to the eye, starting from the minimum intensity available and increasing until a criterion response was achieved. This criterion response was set to 20  $\mu$ V above the baseline noise (average noise  $22.6 \pm 1.4$   $\mu$ V) and was determined using the on-line analysis tool of WinWCP 4.9.4. This protocol was then repeated two more times, for a total of three recordings for each LED available in the array. Each test flash was separated by a period of 10 s. The spectral sensitivity was obtained as the inverse of the quantal intensity necessary to produce the criterion response. The same protocol was repeated to test selective monochromatic adaptation. To this end, the animals were first dark adapted for 15 min, and then adapted to UV (380 nm), green (533 nm) or long wavelength light (approximately 490–790 nm; white LED with long wavelength filter) using LEDs connected to the eyepiece of the

stereomicroscope for another 15 min. The adapting light was kept on for the duration of the experiments, except for a period of 500 ms prior to and after each test flash. At the end of the ERG recordings, the animals were anesthetized again, removed from the agarose and returned to their containers.

### 3.4 BEHAVIORAL ASSAYS

The apparatus used for open field tests was a custom-built 30×30 cm glass box, the walls of which were covered on the outside using black card stock and on the inside with polytetrafluoroethylene strips to prevent the animals from climbing out (Fig. 2E). Each of the four sides of the box had a 1×2 cm rectangular hole in the bottom center of the cardboard. At the start of each test, one of the four sides of the box was chosen using a random number generator (<https://www.random.org/>), and a stimulator containing a LED and a variable neutral density filter (ND 2-400 filter, Neewer, Shenzhen, PRC) was placed facing the hole in the wall from the outside, at a distance of 1 cm and an angle of 30 deg downwards. Seen from the inside of the box, this produced a discrete square area of illumination (Fig. 2F). The LED was turned on and the animals (under light-adapted conditions) were released in the middle of the box and allowed to freely explore the inside for 10 min. The procedure was repeated for the following wavelengths: violet, 422 nm; blue, 457 nm; green, 517 nm; red, 635 nm; and IR, 855 nm. Negative controls were performed without light stimulation. The intensity of the different LEDs was equated to the LED with the least brightness – in this case, the violet LED – using the variable density filter and calibrated with an optical power meter (1918-R, Newport). The target intensity in radiant flux was  $1.14 \times 10^{16}$  photons  $s^{-1} cm^{-2}$ . The behavior of the animal was recorded immediately after its release using a webcam (C920 HD Pro, Logitech) located above the apparatus at a constant frame rate of 2 frames  $s^{-1}$ , using a dim white background illumination (44 lx in the center,  $28 \pm 5$  lx at the edges). Between trials, the inside of the box was thoroughly cleaned with 70% ethanol. Each wavelength was tested on separate days. For analysis, the open area was divided into nine sectors, with four sectors comprising the corners of the field (sectors 1, 3, 7 and 9), four sectors comprising the open sides of the walls (sectors 2, 4, 6 and 8) and one central sector (sector 5) (Fig. 4A).

### 3.5 DATA ANALYSIS

ERG recordings were stored and analyzed offline using WinWCP 4.9.4 software (University of Strathclyde, Glasgow, UK). Sensitivity curves were first normalized per individual and then averaged between individuals using custom software written in Python 3.7.1. The visual pigment template described by Stavenga (2010) was fitted to the green sensitivity peak using the least squares method via the `curve_fit` function from the open source SciPy 1.3.1 package. The video recordings were analyzed offline with a modified version of a custom-programmed tracking algorithm previously used by the laboratory (Calbiague et al., 2017), which is available online at <https://sourceforge.net/projects/spider-tracking/>. Statistical analysis was performed using Stata 13.0 software (StataCorp, College Station, TX, USA). The data were first analyzed for normal distribution using the Shapiro–Wilk test. In all cases, at least one variable was found not to conform

to a normal distribution, so significant differences were established with the Kruskal–Wallis test followed by Dunn’s multiple comparisons test. For all tests, the  $\alpha$  value was set to 0.05. Unless otherwise stated, data are presented as means $\pm$ s.e.m. Graphics were constructed with custom software written in Python 3.7.1, using the open source Matplotlib 3.0.3 package.

## 4 RESULTS

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### 4.1 HISTOLOGICAL ANALYSIS

Transverse semi-thin sections and cryosections through the median eyes of *L. laeta* revealed a simple structure with a one-tiered retina and photoreceptor rhabdoms oriented towards the reflective tapetum (Fig. 1C,D), consistent with the secondary spider eye type (Foelix, 2011). This is in line with the notion that all eyes of sicariids are secondary eyes, the primary eyes being absent (Barth, 2002b; Schmidt-Rhaesa et al., 2015), and the central eyes corresponding to displaced PM eyes (Gertsch and Ennik, 1983). We estimate that the eyes of *L. laeta* each contain about 100 photoreceptors.

### 4.2 ERG RECORDINGS

The ERG responses obtained corresponded to monophasic cornea negative potentials (Fig. 3A), similar to the ones already described in other spider species (Barth et al., 1993; Defrize et al., 2011), but with comparatively low amplitudes in the microvolt range. All three eye types displayed a main sensitivity peak around 500 nm and another one extending into the UV range, past the limit of our equipment (Fig. 3C). When fitted using a rhodopsin template as described by Stavenga (2010), the obtained peaks were 534, 530 and 525 nm for the PM, AL and PL eyes, respectively ( $R^2$  values: PM, 0.97; AL, 0.97; PL, 0.98). The values for the green sensitivity peak are similar to that measured for spider rhodopsin-1 (Rh1), recently cloned and described in jumping spiders (Koyanagi et al., 2008; Nagata et al., 2012). The spectral sensitivity plots showed similar characteristics for all eye types, and no statistically significant differences were found between the three eye pairs. These results suggest that no spectral specialization exists between the three secondary eye pairs. As no differences were found between eyes of the same animals, possible spectral sensitivity specializations of male or female specimens were analyzed. To this end, averaged sensitivity curves from the PM eyes of both sexes were compared, but neither the PM eyes (Fig. 3D) nor the PL and AL eyes displayed any statistically significant differences (Fig. S2). These results argue against the presence of sex-specific visual specializations at the retinal level.

Finally, to test whether the observed spectral sensitivity peaks correspond to two or more different visual pigments, spectral sensitivity curves were constructed under chromatic adaptation to UV, green and long wavelength light. While the overall sensitivity decreased under each type of adaptation by up to two log units compared with dark-adapted conditions, no significant difference was observed between the three adaptation curves in their overall sensitivity level (Fig. 3E). When comparing the ratio between the green and UV sensitivity peaks, a significant difference was found

between the dark-adapted condition and the different chromatic adaptations, but no differences were found between each chromatic adaptation (dark,  $1.02 \pm 0.17$ ; UV,  $6.92 \pm 1.84$ ; green,  $7.27 \pm 0.6$ ; long,  $5.18 \pm 2.07$ ; dark-adapted included,  $P=0.03$ ; dark-adapted excluded  $P=0.74$ ), arguing against the possibility of more than one functionally independent light-sensitive pigment in the eyes of *L. laeta*.

### 4.3 BEHAVIORAL TESTS

To further characterize the visual system of *L. laeta* and to obtain a behavioral correlate to our electrophysiological data, we tested the preference for different wavelengths using an open field test. Open field tests are widely used to evaluate exploratory behavior, and have been previously used with spiders (Baatrup and Bayley, 1993; Carducci and Jakob, 2000). When exposed to our open field, the specimens showed a highly thigmotactic behavior, exploring the borders of the field and avoiding the central sector (Fig. 4). In negative controls (without light stimulus), the spiders preferred the corner sectors over the open side sectors (corners,  $87.1 \pm 2.9\%$ ; sides,  $12.2 \pm 2.5\%$ ; center,  $0.7 \pm 0.5\%$ ;  $P=0.0001$ ). No preference was observed for any of the open side sectors (side 2,  $33.2 \pm 5.8\%$ ; side 6,  $21.3 \pm 6.1\%$ ; side 8,  $25.4 \pm 6.3\%$ ; side 4,  $20.2 \pm 6.5\%$ ;  $P=0.3$ ) (Fig. 4A). For this reason, the remainder of the tests were analyzed comparing the open side sectors only. The overall activity level of the spiders was assessed using the number of sector boundary crossings. There were no significant differences between conditions (violet,  $39.8 \pm 4.4$ ; blue,  $42 \pm 7.6$ ; green,  $31.9 \pm 5.9$ ; red,  $31.5 \pm 6$ ; IR,  $45.6 \pm 9$ ; control,  $18 \pm 2.4$ ;  $P=0.39$ ).

To test the behavioral spectral preference, we added a light source to one of the sides of the open field, producing a discrete illuminated zone in one of the open side sectors. For analysis, we defined a stimulation sector (Stim), and numbered the rest of the open side sectors clockwise. Surprisingly, the presence of the light source caused the spiders to increase the time exploring the illuminated sectors. This effect was clearly observed when using the violet (Stim,  $45.9 \pm 6.2\%$ ; side 1,  $21 \pm 5.2\%$ ; side 2,  $16.6 \pm 2.5\%$ ; side 3,  $16.4 \pm 2.6\%$ ;  $P=0.0014$ ) (Fig. 4B), blue (Stim,  $42.6 \pm 5.4\%$ ; side 1,  $23.7 \pm 3.8\%$ ; side 2,  $17.6 \pm 2.3\%$ ; side 3,  $16.1 \pm 4.4\%$ ;  $P=0.0075$ ) (Fig. 4C) and green lights (Stim,  $45.3 \pm 5.7\%$ ; side 1,  $22.4 \pm 4.1\%$ ; side 2,  $19.8 \pm 5.9\%$ ; side 3,  $12.4 \pm 2.9\%$ ;  $P=0.0025$ ) (Fig. 4D), with an increase of the time spent in the illuminated side sector compared with the other sectors. The effect was abolished when using the red (Stim,  $24.3 \pm 4.4\%$ ; side 1,  $26 \pm 7.2\%$ ; side 2,  $19.8 \pm 3.9\%$ ; side 3,  $30 \pm 7.3\%$ ;  $P=0.88$ ) (Fig. 4E) and IR lights (Stim,  $16.1 \pm 3.2\%$ ; side 1,  $26.1 \pm 6.1\%$ ; side 2,  $30 \pm 5.5\%$ ; side 3,  $27.8 \pm 6.8\%$ ;  $P=0.3$ ) (Fig. 4F). These results are in line with the electrophysiological data, showing that *L. laeta* has a spectral sensitivity spanning from the UV to the green spectrum that decreases towards longer wavelengths, without any apparent IR sensitivity.

## 5 DISCUSSION

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We used the flash ERG technique to measure the spectral sensitivity of the eyes of *L. laeta*. The data obtained show responses between the UV (370 nm) and red (650 nm) parts of the spectrum, which is consistent with previous studies in different genera, including *Cupiennius* (Barth et al., 1993; Walla et al., 1996), *Lycosa* (DeVoe, 1972; DeVoe et al., 1969), *Deinopis* (Laughlin et al., 1980), *Leucorchestris* (Norgaard et al., 2008) and *Misumena* (Defrize et al., 2011). The ERG amplitudes obtained, generally in the 25– 100  $\mu$ V range under dark-adapted conditions, were rather low compared with those of the aforementioned studies, which may be due to the small eye size of *L. laeta* or to a low shunting resistance of the ERG circuit that reduced the response amplitude while preserving the signal-to-noise ratio. Based on the shape of the spectral sensitivity plots obtained, the sensitivity range probably extends deeper into the UV range, but this was not testable with our equipment. Importantly, there were no significant differences between the sensitivity spectra of the three eye pairs, suggesting that there is no spectral specialization between eye types in this spider species, as opposed to others (Defrize et al., 2011; DeVoe et al., 1969). However, different kinds of eye type-specific specializations, like motion or polarization detection, as described for other species (Mueller and Labhart, 2010; Neuhofer et al., 2009), cannot be excluded.

The sensitivity spectra observed in *L. laeta* are consistent with the expression of the bistable spider Rh1 (Nagata et al., 2012; Varma et al., 2019), responsible for the peak in the green range, but the marked sensitivity peak in the UV range remains to be explained. One potential mechanism is the presence of an accessory sensitizing pigment absorbing in the UV range. The presence and function of a sensitizing pigment, presumably 3-hydroxyretinol, has been reported for several insect species (Kirschfeld and Vogt, 1986; Stavenga et al., 2017). A related vitamin A derivative, 11-cisretinol, was detected by HPLC in the eyes of *C. salei* (Barth et al., 1993), but the presence of 3-hydroxyretinol was not reported and, to our knowledge, no other studies have investigated the possible role of UV-absorbing sensitizing pigments in spider photoreceptors. A more likely possibility is that the UV peak of *L. laeta* corresponds to an enhanced  $\beta$ -band of Rh1. Indeed, the absorption spectra of invertebrate rhodopsins were recently shown to depend strongly on the counter-ions provided by amino acids close to the chromophore (Nagata et al., 2019); thus, a variant of Rh1 might be responsible for the observed spectrum.

After chromatic preadaptation with UV, green and long wavelength light, the overall sensitivity was significantly reduced when compared with the dark-adapted condition. We did not observe significant differences between the preadapted conditions in terms of overall sensitivity and relative sensitivity between the green and UV peaks, which is not compatible with the expression of more than one type of rhodopsin in the same or different photoreceptors, although we cannot fully exclude the possibility of the expression of two rhodopsin pigments with similar peak absorption in the green range, as reported in *C. salei* (Walla et al., 1996). Therefore, despite its two spectral sensitivity peaks, *L. laeta* is likely to have only monochromatic vision. Prior studies in spiders have found evidence for differential rhodopsin expression in photoreceptors, and two or three sensitivity peaks in ERG recordings in a few model species, but evidence for dichromatic or trichromatic color

vision in arachnids remains scarce (Barth, 2002a; Foelix, 2011; Morehouse et al., 2017; Walla et al., 1996; Zurek et al., 2015). For example, the wandering spider *Leucorchestris arenicola* displayed only one peak in the green spectrum in all eye types (Norgaard et al., 2008). Spectral sensitivity analysis using ERG recordings revealed two peaks, in the UV and green range, in all eye types of the crab spider *M. vatia* (Defrize et al., 2011), similar to our results. Different wolf spider (*Lycosa*) species showed two similar peaks in the AM eyes, while their secondary eyes only had one green sensitivity peak (DeVoe et al., 1969). In contrast, differential expression of UV- and green-sensitive opsins in photoreceptors of principal and secondary eyes was found in specimens of the genera *Cupiennius*, *Phidippus*, *Lycosa* and *Thomisus*, using intracellular recording of photoreceptors (Defrize et al., 2011; DeVoe, 1972, 1975; Walla et al., 1996). In *C. salei*, initial ERG studies revealed the presence of a main green peak (520–540 nm) and a UV shoulder (340–380 nm), but selective chromatic adaptation failed to indicate the existence of more than one type of photoreceptor (Barth et al., 1993). Later studies using intracellular recordings suggested the presence of three different photoreceptor types, with sensitivity maxima in the blue (480 nm), green (520 nm) and UV (340 nm) for all eyes (Walla et al., 1996). The same authors also noted the possibility of more than one visual pigment per photoreceptor, as seen in other arthropods (Sakamoto et al., 1996) and proposed for *Lycosa* (DeVoe, 1972), or the existence of electrical coupling between different photoreceptor cells (Walla et al., 1996). Yet, a newer study showed that, when tested using moving color stripes over backgrounds of varying gray levels, *C. salei* individuals were unable to discriminate the blue-, green- and red-colored stimuli from certain gray levels (Orlando and Schmid, 2011). The authors concluded that even though *C. salei* has three types of photoreceptors, the secondary eyes, the ones in charge of movement detection, are apparently color blind (Orlando and Schmid, 2011).

In order to further characterize the effect of chromatic stimulation on the behavior of *L. laeta*, it was necessary to develop a specific paradigm, as assays involving the movement of the retina, such as those described by Orlando and Schmid (2011), were not possible because of the lack of retinal muscles, and other behavioral paradigms, such as the one used by Zopf et al. (2013) involving the search for a black target, did not elicit any directed response in *L. laeta*. Thus, the physiological sensitivity spectrum obtained here by ERG recordings was complemented by behavioral data obtained with a modified open field test, in which the animals were exposed to discrete illuminated areas in specific sections of their path. The observation of a significantly longer exploration time of areas illuminated by light in the violet to green range, without reaction to red and near-IR light, might be explained by a higher overall sensitivity of *L. laeta* to short wavelength as opposed to longer wavelength light, which is in line with our electrophysiological findings, and does not necessarily indicate a preference for short wavelength light.

Interestingly, the available evidence classifies this species as a nocturnal animal (Canals et al., 2015a), but our behavioral assays showed no avoidance of illuminated areas, and instead an increased exploration time of these compared with unilluminated side areas, although the exact significance of the observed exploratory behavior remains to be studied. It is of note, however, that the (darker) corners of the maze remained most attractive to the animals, in accordance with their local name of araña de rincón ('corner spider').

Efforts are underway to assemble the genome of *Loxosceles reclusa* (Brown Recluse Spider Genome Project, <https://www.hgsc.bcm.edu/arthropods/brown-recluse-spider-genome-project>). This will reveal whether a variant of jumping spider Rh1 is indeed present in the *Loxosceles* genome, and whether other types of opsins are also present. Further studies are clearly needed to better understand the visual systems, and visually driven behaviors, of this and other spider species.

## 6 ACKNOWLEDGEMENTS

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We wish to thank Dr Alex Vielma for a critical revision of the manuscript and advice on ERG recordings, Dr Adrián Palacios for access to the ERG stimulation apparatus and optical power meter, and Dr Nicolás Palanca for helpful advice.

## 7 COMPETING INTERESTS

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The authors declare no competing or financial interests.

## 8 AUTHOR CONTRIBUTIONS

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Conceptualization: O.S., F.T., J.O.; Methodology: F.T.; Software: F.T.; Validation: F.T.; Formal analysis: F.T.; Investigation: O.S., F.T., J.O.; Resources: O.S.; Data curation: F.T.; Writing - original draft: O.S., F.T., J.O.; Writing - review & editing: O.S., F.T., J.O.; Supervision: O.S.; Project administration: O.S.; Funding acquisition: O.S.

## 9 FUNDING

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This study was supported by FONDECYT (grant no. 1171228), CONICYT PhD scholarship (F.T.) and the Millennium Institute Interdisciplinary Center of Neuroscience of Valparaiso (CINV: grant no. ICM P09-022-F).

## 10 SUPPLEMENTARY INFORMATION

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Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.217133.supplemental>

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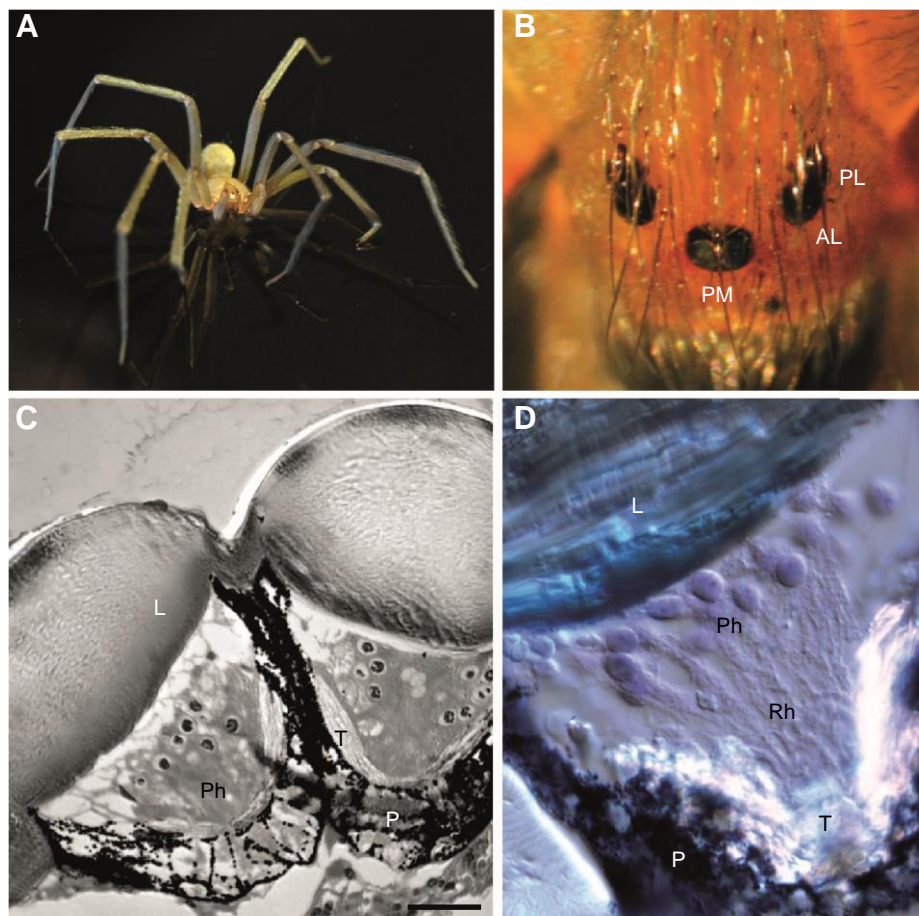
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## 12 FIGURES

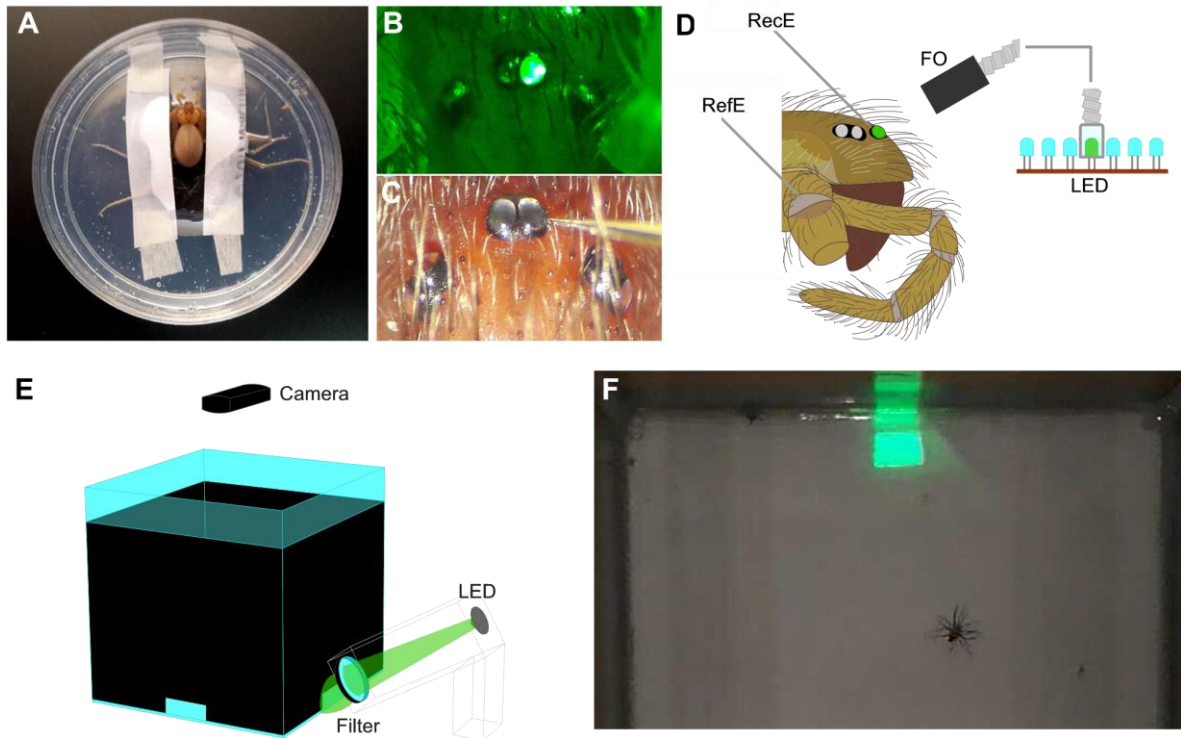
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### 12.1 FIGURE 1



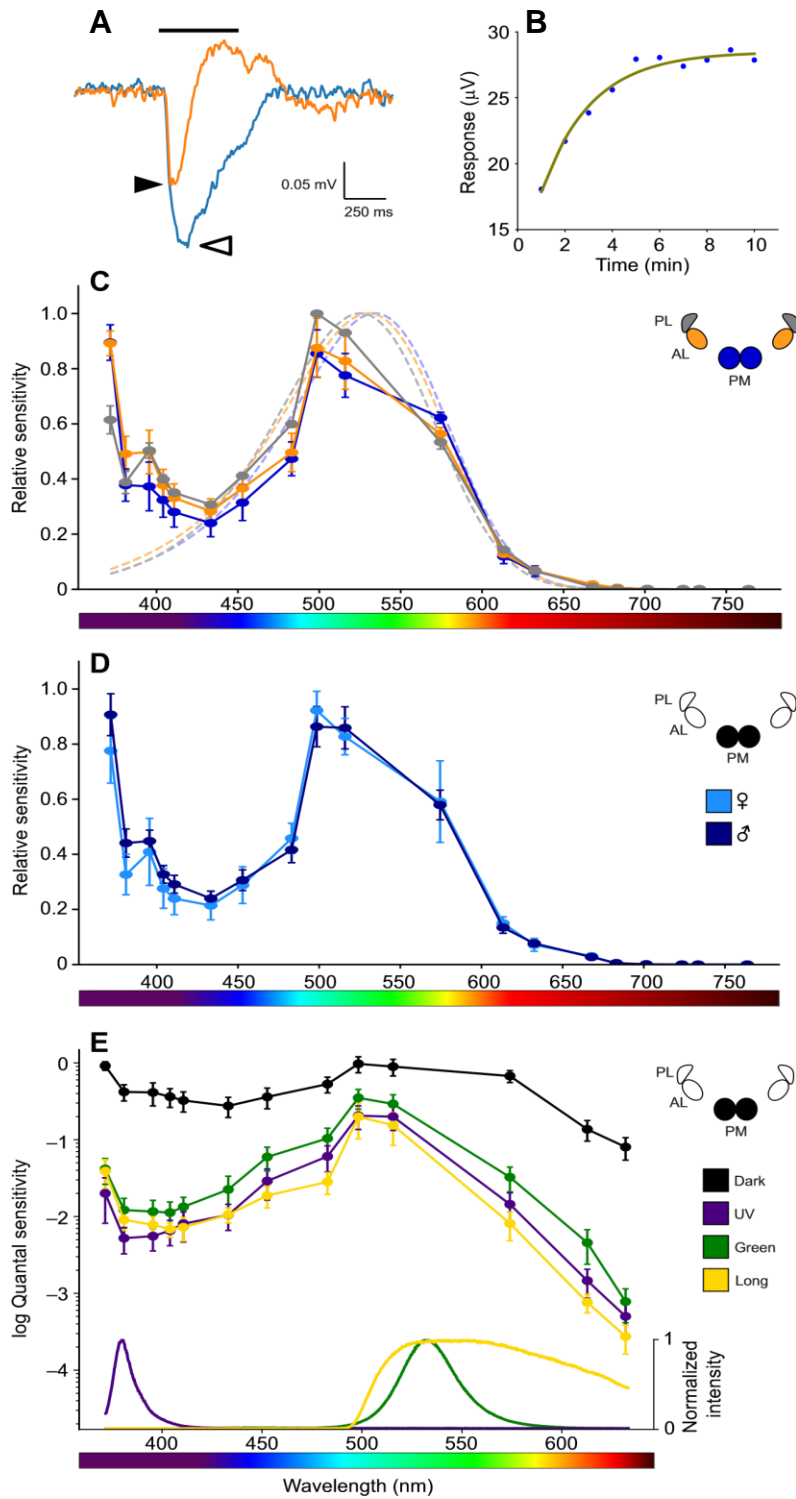
**Figure 1. The eyes of *L. laeta*.** (A) Male specimen of *L. laeta*. (B) Overview of the position of the three eye pairs of *L. laeta*. PM, posterior median, AL, anterior lateral, PL, posterior lateral. Note missing AM eyes. (C) Toluidine blue-stained semithin cross-section of *L. laeta* PM eyes. (D) Detail of a PM eye, showing the primitive-type secondary eye structure with photoreceptor somata close to the lens and rhabdome towards the tapetum. L, lens, Ph, photoreceptors, Rh, rhabdome, T, tapetum; P, pigment layer. Scale bars: (A): 1 cm; (B): 1 mm; (C, D): 100  $\mu$ m.

## 12.2 FIGURE 2



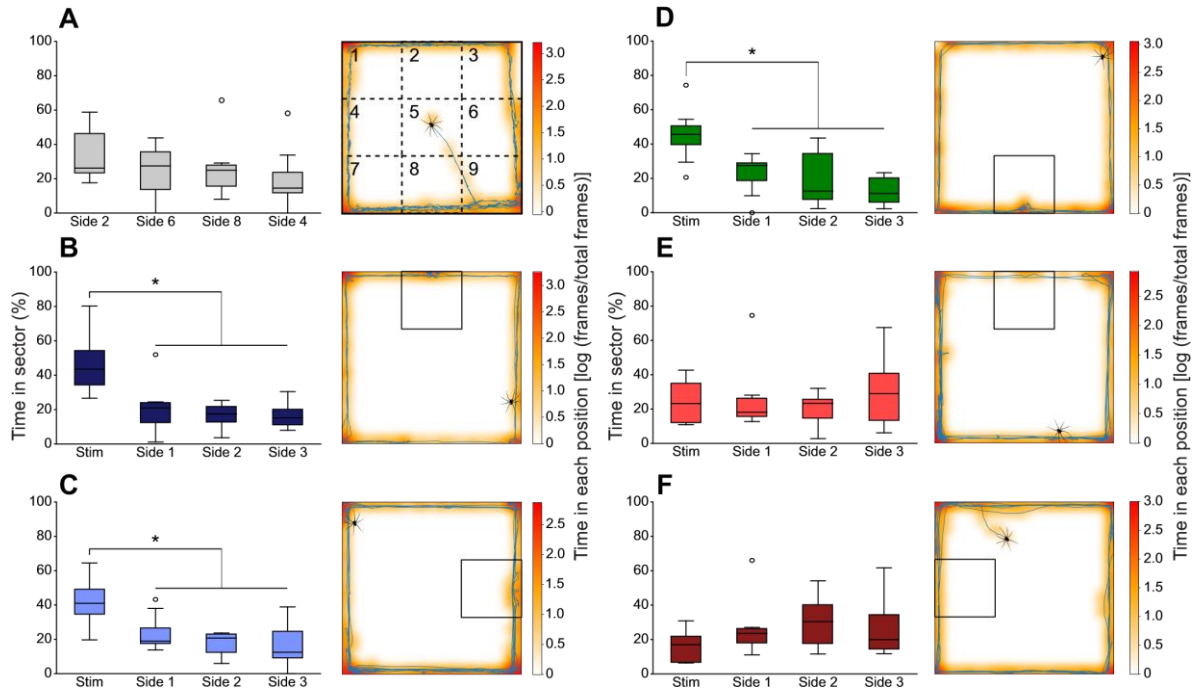
**Figure 2. Methodological overview.** (A) For immobilization, the legs of the animal were kept in place using paper strips fastened with masking tape and covered with agarose, leaving the body exposed. (B) The reflection in the tapetum was used to determine a correct alignment of the fiberoptic light guide before electrode placement (C). (D) Schematic representation of the ERG setup. The recording electrode (RecE) is placed in the cornea, the reference electrode (RefE) in the trochanter of the first ipsilateral leg and a fiberoptic light guide (FO) is positioned in front of the eye and connected to one of the LEDs of the custom array (LED). (E) Schematic representation of the behavioral setup. A box covered in black cardboard was used for open field tests. A small window was opened on each side of the box and a stimulator containing a LED and a variable neutral density filter to control the intensity of the light was placed adjacent to one of the openings. Above the box, a camera (Cam) was placed to record the behavior of the animal. (F) From the inside of the box, the setup produced a discrete square area of illumination. The image corresponds to a frame from a representative video recording.

12.3 FIGURE 3



**Figure 3. Spectral sensitivity of the three eye pairs of *L. laeta*.** (A) Representative ERG recording showing a cornea-negative potential as a response to a 499 nm light stimulus. Analysis were performed using the peak amplitude of the response. Bar, stimulation period; filled arrowhead, peak response amplitude in AC mode; empty arrowhead, peak response amplitude in DC mode. (B) Representative plot of response amplitudes during dark adaptation, fitted to an exponential curve. (C) Normalized average spectral sensitivities for the three eye pairs of *L. laeta* (mean  $\pm$  s.e.m, n = 6). The colors correspond to each eye pair as shown in the inset (blue, posterior median; orange, anterior lateral; gray, posterior lateral). Dotted lines represent the pigment template curves fitted to the data as described by Stavenga (Stavenga, 2010). (D) Comparison of the normalized average spectral sensitivity for male (dark blue) and female (light blue) individuals for the posterior median eyes (mean  $\pm$  s.e.m, n = 6 per sex, n = 12). No significant differences between the sexes were observed. (E) Sensitivity curves for chromatic adaptation experiments, expressed as the log of the quantal sensitivity (top). Quantal sensitivity is defined as the inverse of the quanta requires to elicit a criterion response. Each adaptation light is shown in a different color, as defined in the legend, with the dark-adapted condition shown in black (mean  $\pm$  s.e.m, n = 6 per condition). The normalized spectral characteristics of each adaptation light source are shown at the bottom using the same colors.

## 12.4 FIGURE 4



**Figure 4. Open field test.** (A-F) Left, box plots showing the time spent in each of the side sectors as a percentage of total time, with the illuminated sector marked as Stim, and the other three side sectors counted clockwise. Right, heat map showing a representative path of a single animal (blue line); the colors represent the amount of time spent in each position (as the logarithm of the number of video frames over total video frames). The test area was divided into 9 sectors, which were then grouped into corner sectors (1, 3, 7 and 9), side sectors (2, 4, 6 and 8), and central sector (5) as shown in (A). The stimulation sector is marked with a black square (B-F). Data are shown for the control condition (no test light; A), and using the violet (422 nm; B), blue (457 nm; C), green (517 nm; D), red (635 nm; E) and infrared (855 nm; F) LEDs ( $n = 8$  per condition, Kruskal-Wallis test followed by Dunn's multiple comparisons test,  $* p < 0.05$ ). For box plots, bars represent the medians, boxes the interquartile range and whiskers the data range; circles are outliers.

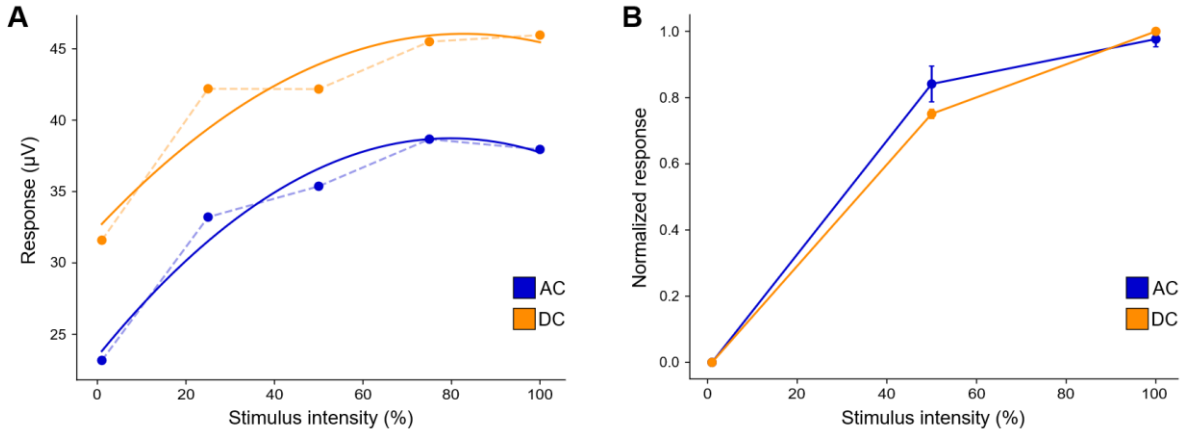
## 13 SUPPLEMENTARY MATERIAL

### 13.1 TABLE S1

LED array			LEDs for behavioral experiments		
LED	Peak value (nm)	Half width (nm)	LED	Peak value (nm)	Half width (nm)
1	371.57	12.92	Violet	421.53	21.9
2	380.99	9.21	Blue	456.82	24.28
3	395.51	11.43	Green	517.48	34.67
4	404.06	14.05	Red	635.11	17.59
5	410.78	12.6	IR	854.55	40.84
6	433.27	16.16	LEDs for monochromatic adaptation		
7	452.81	20.29	LED	Peak value (nm)	Half width (nm)
8	483.01	23.68	UV	380.18	11.67
9	498.51	30.9	Green	532.82	32.16
10	515.91	32.11			
11	574.49	14.51			
12	613.28	16.02			
13	632.47	16.85			
14	667.8	20.18			
15	683.05	22.62			
16	701.52	23.37			
17	723.48	24.05			
18	733.47	24.28			
19	763.71	25.74			

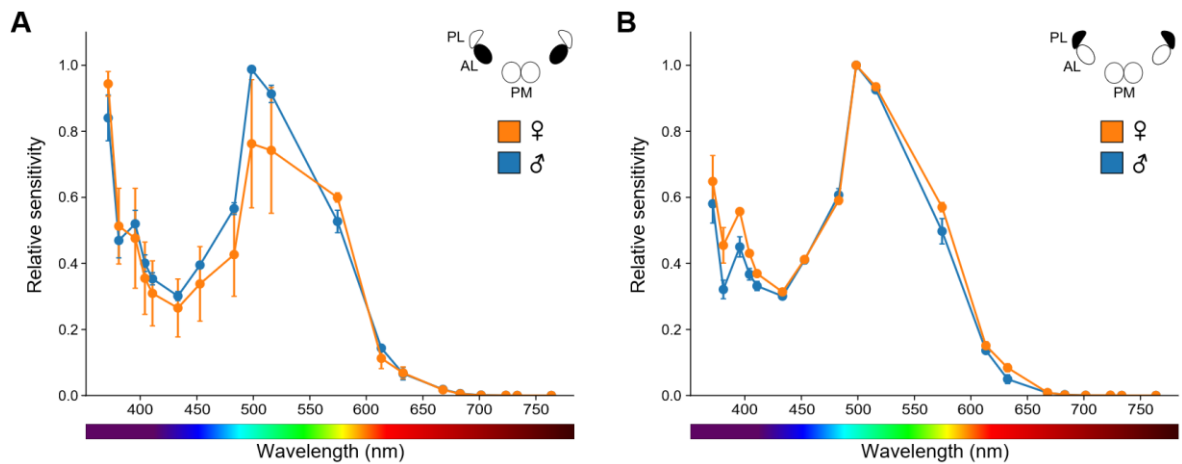
**Table S1. Wavelengths of the LEDs used for the different experiments.** The table details the spectral characteristics (peak wavelength and spectral half-width) for all the LEDs used across the experiments (ERG recordings, chromatic adaptation and behavioral experiments).

### 13.2 FIGURE S1



**Figure S1. Relationship between response peaks in AC and DC mode.** (A) Representative trace of the peak response values to a 516 nm light pulse in AC (blue) and DC (orange) modes at different intensities, showing the correlation between the peaks in both modes. Data are fit using a linear regression (AC,  $R^2 = 0.98$ ; DC,  $R^2 = 0.98$ ). (B) Average of three normalized response/intensity plots in both AC and DC modes, again showing the correlation between them (mean  $\pm$  s.e.m,  $n = 3$ ).

### 13.3 FIGURE S2



**Figure S2. Spectral sensitivity of the AL and PL eyes by sex.** (A-B) Comparison of the normalized average spectral sensitivity for male (dark blue) and female (light blue) individuals for the anterior lateral (A) and posterior lateral (B) eyes (mean  $\pm$  s.e.m,  $n = 3$  per sex,  $N = 6$ ). No significant differences between the sexes were observed.