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• Basic Research •

Pharmacological and light-driven pupillary accommodation in guinea pigs: comparative effects of muscarinic modulators and illumination across developmental stages

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Abstract
● **AIM:** To investigate the effects of different light intensities and various mydriatic and miotic drugs on pupil accommodation in guinea pigs.
● **METHODS:** Forty-two-week-old guinea pigs were randomly divided into four groups to assess pupillary responses under varying light intensities (100, 250, 500 lx) and pharmacological interventions (1% atropine, 1% cyclopentolate, 1% tropicamide, or 2% pilocarpine). Baseline pupil size and eccentricity were recorded using a non-contact Python-based imaging system integrating edge detection and pixel-to-distance conversion. Direct illumination effects were measured at sequential time points, followed by drug administration and longitudinal tracking of pupillary changes. The protocol was repeated at 12wk of age for developmental comparisons. Post-experiment, enucleated eyes were analyzed to evaluate *in vitro vs in vivo* differences.
● **RESULTS:** Significant age-dependent differences in pupil dynamics were observed. Both 2- and 12-week-old guinea pigs exhibited marked pupil constriction under direct illumination (P<0.001), with decreased eccentricity post-constriction (P<0.001). Indirect illumination caused inconsistent pupil size changes (2-week: P=0.68; 12-week: P=0.49). Pharmacologically, atropine, cyclopentolate, and tropicamide induced pupil dilation (P<0.001), whereas pilocarpine caused constriction (P<0.001). All drug groups showed reduced eccentricity (P<0.001). *In vivo/in vitro* comparisons revealed significant structural differences.
● **CONCLUSION:** This study investigates pupillary responses in developing guinea pigs, revealing a direct pupillary light reflex (PLR) with light intensity-dependent responses, while indirect PLR was undetectable. The differential effects of muscarinic modulators on pupillary responses underscore the critical role of cholinergic signaling in ocular accommodation, with age-related variations in sensitivity. Additionally, a novel non-contact measurement methodology achieved a precision of 0.01 mm for pupillary quantification, enhancing accuracy in ocular studies.
● **KEYWORDS:** pupil; guinea pig; muscarinic receptor modulator; light reflex
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INTRODUCTION
It has been confirmed that many animal species exhibit complex accommodative responses in their eyes, with most mammals, including humans, demonstrating ocular adaptability[1]. However, not all mammalian species show accommodative responses. For example, the gray squirrel does not exhibit a notable accommodative response to muscarinic receptor agonists[1]. Some mice, commonly used in myopia and related genetic studies, also appear to lack this accommodative phenomenon[2]. Guinea pigs, belonging to the order Rodentia within the class Mammalia, are chosen in this study to further investigate the accommodative ability of mammalian eyes.

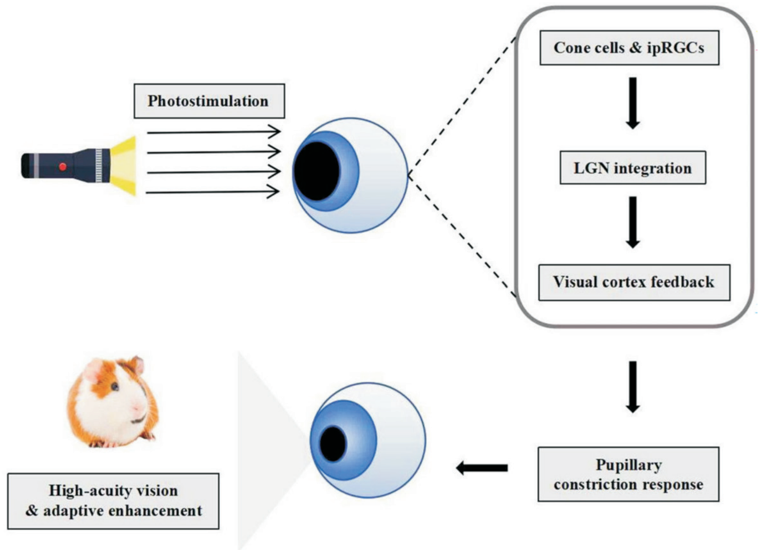

Figure 1 The neural pathway underlying the PLR and visual adaptation Photosimulation activates retinal pathways, where signals are transmitted to the LGN for initial integration. Subsequently, the visual cortex processes these signals and provides modulatory feedback to refine sensory encoding, enhancing high-acuity vision and environmental adaptability. PLR: Pupillary light reflex; LGN: Lateral geniculate nucleus.

The aim is to better understand the functional characteristics of rodent species as models for myopia research.

The pupillary light reflex (PLR) is a neurophysiological process involving the perception, transmission, and response to light stimuli, primarily manifested as pupil constriction[3] (Figure 1). In mammals, the PLR begins when photoreceptors in the retina detect changes in light intensity, which are then transmitted *via* the optic nerve to the central nervous system (CNS). Ultimately, the autonomic nervous system controls the contraction of the pupillary sphincter muscle. This reflex not only reflects the functional status of the optic nerve and CNS but is also widely used in clinical settings for evaluating neurological function[4].

Light intensity and age are pivotal factors modulating PLR. In mammals, pupillary responses mature postnatally, reflecting CNS development[5-6]. For instance, rodents exhibit delayed CNS maturation at birth, with accelerated neural refinement in early weeks[7-9]. This developmental trajectory likely impacts pupillary reflex efficiency, yet comparative data across ages are sparse. Similarly, light intensity directly governs pupil constriction amplitude and latency, but species-specific thresholds and age-related adaptations are underexplored. No studies have concurrently assessed light- and pharmacologically-driven pupillary changes in guinea pigs across developmental stages.

Previous studies have shown that M1-M5 subtypes of muscarinic receptors are present in the retina, choroid, sclera, and iris-ciliary body of guinea pigs[10], with the M3 receptor being the most predominant subtype[11]. Atropine, cyclopentolate, and tropicamide are muscarinic receptor antagonists, commonly used in clinical practice as fast-acting mydriatic agents. Although their pharmacological effects differ slightly, all three drugs achieve pupillary dilation and inhibition of accommodative responses by blocking muscarinic receptors on the iris sphincter and ciliary body. Pilocarpine, a muscarinic receptor agonist, induces ciliary body contraction, resulting in pupil constriction (Figure 2), and is widely used in various ophthalmic studies[12-13].

The research group has previously demonstrated that by applying edge detection, curve fitting, and pixel conversion principles using Python software to build a mathematical model[14-15], high-quality biological measurements of the guinea pig eye were successfully obtained. This study investigated the effects of local administration of 1% atropine, 1% tropicamide, 1% cyclopentolate, 2% pilocarpine, and light exposure on the pupillary response and accommodation in guinea pigs of different ages. Measured parameters included the rate and amplitude of pupillary accommodation, as well as eccentricity. Additionally, we analyzed changes in pupil parameters *in vivo* and *in vitro*. These experiments aim to further explore the ocular accommodation mechanisms and characteristics in guinea pigs, to better utilize them as an experimental animal model.

MATERIALS AND METHODS

Ethical Approval This research was conducted in full compliance with the relevant provisions of the Regulations on the Management of Laboratory Animals issued by the China Science and Technology Commission, and was approved by the Laboratory Animal Management and Ethics Committee of the Shanghai Clinical Center for Public Health (No.2022-A009-01).

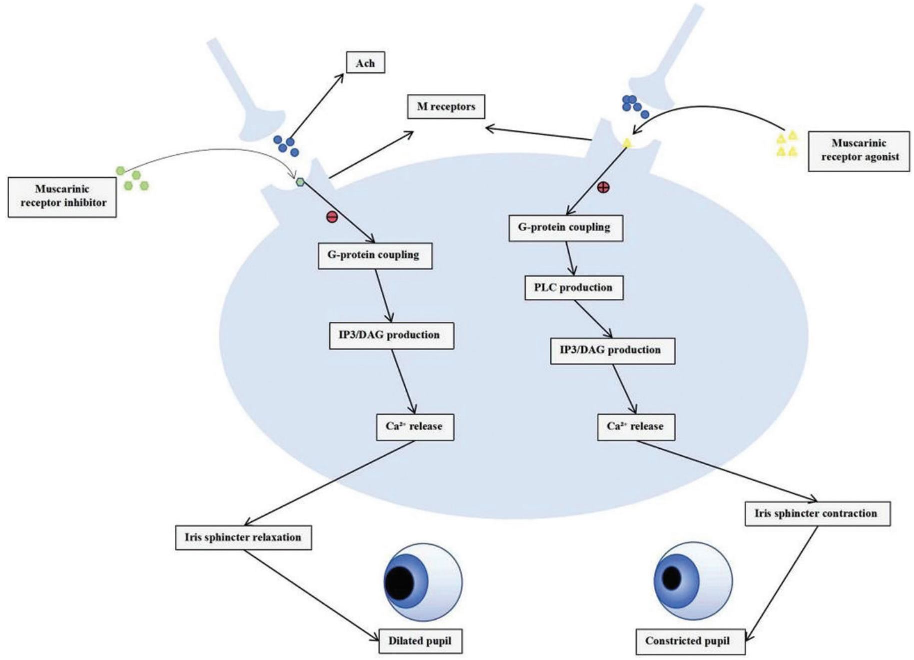

Figure 2 The pharmacological modulation of pupillary responses *via* muscarinic acetylcholine receptors Antagonists bind to mAChRs, competitively inhibiting acetylcholine binding and blocking G-protein coupling. This suppression reduces IP3/DAG synthesis, leading to diminished intracellular Ca^{2+} release. The resultant relaxation of the iris sphincter muscle induces pupil dilation, often accompanied by ciliary muscle paralysis. Conversely, agonists like pilocarpine selectively activate M3 receptors, stimulating Gq-protein-dependent PLC activation, which elevates IP3/DAG levels and mobilizes Ca^{2+}. Increased Ca^{2+} triggers iris sphincter contraction, producing pupil constriction. IP3: Inositol triphosphate; DAG: Diacyl glycerol; PLC: Phospholipase C; M3: Muscarinic M3 receptor.

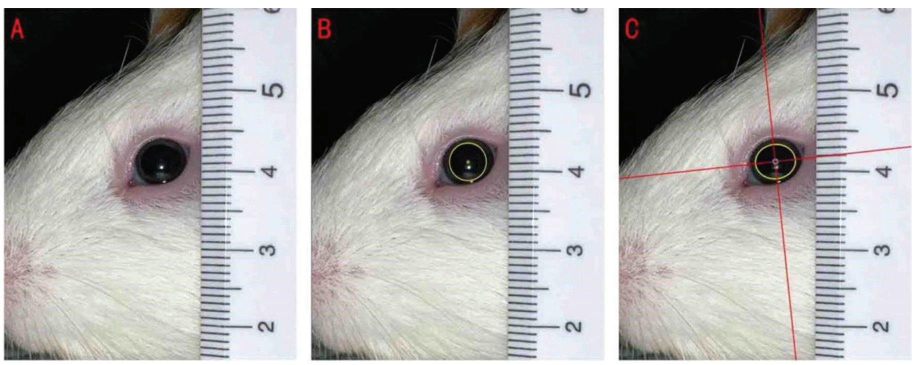

Figure 3 Non-contact measurement of guinea pig pupil parameters using Python-based image analysis A: Photograph of the pupil of the left eye of a guinea pig, with pixel-actual distance conversion *via* a ruler scale; B: Calculation of guinea pig pupil diameter by circular fitting of the guinea pig pupil; C: Calculation of eccentricity by ellipse fitting to the guinea pig pupil.

Experimental Animals Forty British shorthair guinea pigs (2-week-old) were housed at (25±1)°C and 60%±5% humidity under a 12-hour light/dark cycle. The average light intensity during the light period was 20 lx. No abnormalities in the pupil, cornea, or other ocular surfaces were observed under slit-lamp examination.

Data Measurement In a calm state, clear and stable images of the guinea pig's eyes were captured from both the left and right sides at a distance of 33 cm using two cameras. The guinea pig was induced to fixate on colorful, attention-grabbing objects and sounds, and images were taken when the most significant eye movement occurred. All measurements were taken in an environment with a luminance of 50 lx to minimize the influence of lighting on pupil size. An illuminance meter was used to measure the light levels near the guinea pig's eyes. The images were imported into Python 3.9 programming software[14] (a specialized software designed for non-contact measurement of experimental animal data; Figure 3) to measure baseline values of pupil size and eccentricity. Pupil images were analyzed using a custom Python system that combines edge detection, ellipse fitting, and calibrated distance conversion[15]. This approach provided non-contact measurements with 0.01 mm resolution, minimizing observer bias (The complete code is available from the corresponding author upon reasonable request).

Pupillary Light Reflex A 500 lx illumination was used to irradiate the left eye of two-week-old and twelve-week-old guinea pigs. The light source was about 40 cm away from the guinea pig's eyeball for 2s. Baseline pupil size and measurements at different time points (0.5s, 1s, 1.5s, 2s) following light exposure were recorded. Observe whether the direct PLR is present in the left eye and whether the indirect PLR is present in the contralateral (right) eye. And differences between guinea pigs of different ages were compared. Each PLR experiment was conducted prior to the drug administration to avoid any potential confounding effects caused by the drugs.

After the PLR experiment, the left pupil of twelve-week-old guinea pigs was exposed to light intensities of 100, 250, and 500 lx. The effect of different light intensities on pupillary accommodation was observed. Each measurement was performed after the pupil size had returned to baseline levels following the previous measurement.

Pupillary Accommodation Drug Sensitivity Forty-two-week-old guinea pigs were randomly divided into four groups. Using a 1 mL syringe, 0.5 mL of 1% atropine, 1% cyclopentolate, 1% tropicamide, or 2% pilocarpine was administered to each guinea pig's eye (Figure 4). The time points at which the pupil diameter reached maximum, minimum, and returned to baseline values were recorded for each group. The entire process of pupillary changes following drug administration was documented, and the same procedure was repeated when the guinea pigs reached 12wk of age.

Pupillary Parameters *in vitro* After completing the above procedures, the guinea pigs were euthanized with an overdose of sodium pentobarbital. The eyeballs were then excised, and the surrounding fascial and muscular tissues were carefully removed to prevent any morphological changes in the eyeballs caused by the dissection. Eighty eyeballs were placed against a white background at a distance of at least 33 cm and photographed. The photographs were then imported into Python 3.9 software, where they were converted to pixel-to-real distance to obtain the conversion coefficients. The pupil diameter and eccentricity of the guinea pigs were subsequently calculated using the curve-fitting function (Figure 5).

Statistical Analysis Pupil size was compared across different intensities of light using analysis of variance (ANOVA), and paired samples t-tests were used to compare results between baseline values and later time points. All data in this study are mean±standard deviation unless otherwise stated. Differences between any two parameters were defined as significant at $P<0.05$ and highly significant at $P<0.001$. The resultant data was imported into Python 3.9 for statistical analysis and charting.

RESULTS

Changes in Pupil Parameters Before and After Illumination

With 500 lx illumination, the left pupil size of two-week-old guinea pigs decreased from a baseline of 3.65±0.13 to 1.85±0.11 mm at 2s. The most significant reduction occurred within 1.5s ($P<0.001$; Figure 6A). For twelve-week-old guinea pigs, the left pupil size decreased from a baseline of 5.54±0.22 to 2.13±0.12 mm at 2s, with the majority of the reduction occurring within 1s ($P<0.001$, Figure 6B). No significant differences were found in the right pupil size of both two-week-old and twelve-week-old guinea pigs before and after light stimulation ($P>0.05$). With varying light intensities, significant differences were observed in the left pupil diameter of twelve-week-old guinea pigs ($P<0.001$), while no significant differences were found in the right pupil ($P>0.05$; Table 1). In terms of eccentricity, the left pupil of twelve-week-old guinea pigs decreased from 0.35±0.11 before illumination to 0.22±0.03 after illumination.

Changes in Pupillary Parameters Before and After Drug Administration

Atropine group The pupil size of the left eye following 1% atropine drops in 2-week-old guinea pigs (Figure 7A) gradually increased from a baseline of 3.61±0.10 mm, peaked at 4.16±0.11 mm at approximately 20min, and remained at the peak until the third day before gradually decreasing. It returned to baseline on day 5 (t=0.35, P=0.717). The left pupil diameter of 12-week-old guinea pigs (Figure 7B) reached a maximum of 6.73±0.17 mm at 10min from a baseline of 5.57±0.23 mm, which persisted until day 4 before gradually decreasing and returning to baseline on day 6 (t=-0.43, P=0.960). No statistically significant difference was observed between the pre- and post-drug measurements of the right pupil in both 2-week-old and 12-week-old guinea pigs ($P>0.05$, repeated measures ANOVA). The pupil eccentricity of the left eye (Figure 8A) decreased from 0.32±0.11 to 0.27±0.01 in 12-week-old guinea pigs following dosing.

Cyclopentolate group After 1% cyclopentolate eye drops were applied to the left eye of two-week-old guinea pigs, the pupil size (Figure 7C) increased from a baseline value of 3.66±0.13 mm, reaching a maximum of 4.15±0.15 mm at 20min. After 4h, the pupil gradually began to shrink and returned to baseline values at 8h (t=-0.50, P=0.630). For twelve-week-old guinea pigs, the left pupil diameter (Figure 7D) increased from a baseline value of 5.60±0.24 mm to a maximum of 6.73±0.10 mm at 9min. The pupil started to decrease after 6h and returned to baseline values at 10h (t=0.77, P=0.463). No significant changes in the right pupil diameter of either two-week-old or twelve-week-old guinea pigs were observed before and after drug application ($P>0.05$, repeated measures ANOVA). After drug application in twelve-week-old guinea pigs, the left pupil eccentricity (Figure 8B) decreased from 0.35±0.12 to 0.27±0.03.

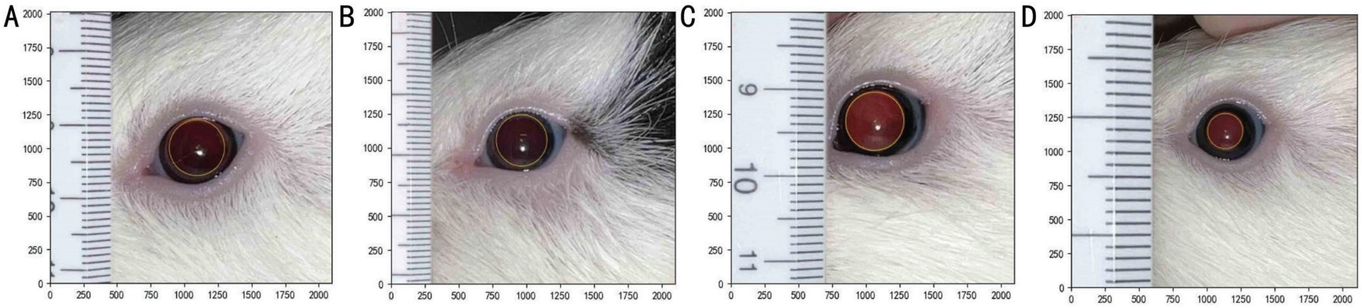

Figure 4 Photograph of the pupil of a 12-week-old guinea pig after a drug drop in the eye A: Pictures taken at 10min after 1% atropine eye drops in guinea pigs. Mean maximum pupil size of guinea pig pupil in 1% atropine group: 6.73±0.17 mm. B: Pictures of guinea pigs taken at 9min after 1% cyclopentolate eye drops. Mean maximum pupil size of guinea pig pupil in 1% cyclopentolate group: 6.73±0.10 mm. C: Pictures taken at 5min after 1% tropicamide eye drops in guinea pigs. Mean maximum pupil size of guinea pig pupil in 1% tropicamide group: 6.91±0.11 mm. D: Pictures taken at 18min after 2% pilocarpine eye drops in guinea pigs. Mean minimum pupil size of guinea pig pupils in 2% pilocarpine group: 2.64±0.13 mm.

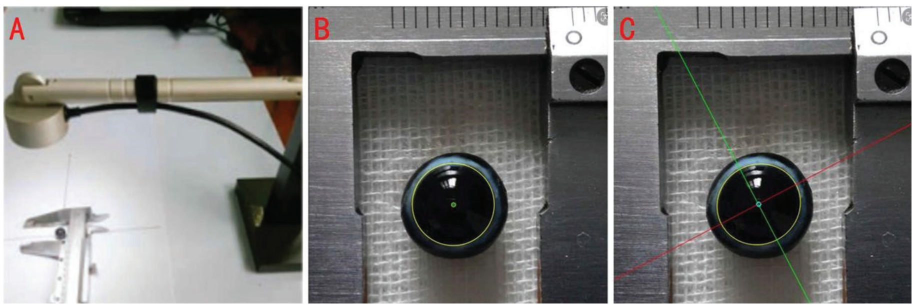

Figure 5 *In vitro* eye pupil data measurement A: A specialised overhead camera unit for photographing eye structures; B: Pupil circle fitting in guinea pig isolated eyes; C: Ellipse fitting of the pupil of the isolated eye of the guinea pig.

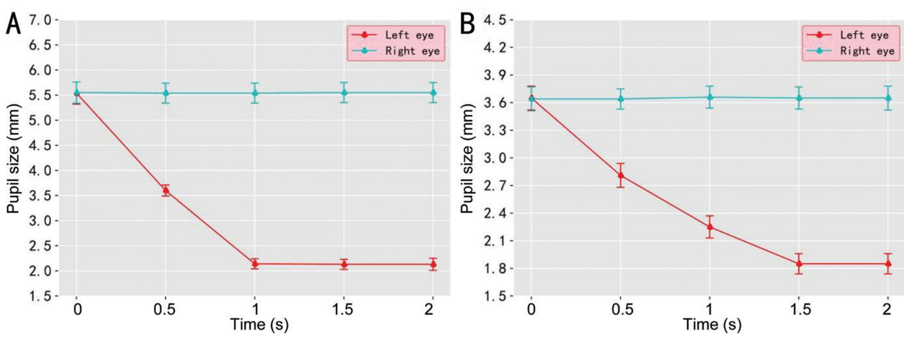

Figure 6 Changes in pupil size of guinea pig eyes on both sides before and after illumination The pupil size of the left eye (light-exposing eyes) of 12-week-old guinea pigs reached its minimum before that of 2-week-old guinea pigs. Identically, the pupil size of the right eye (contralateral eyes) was not significantly altered in both (P>0.05). A: The pupil of the left eye of 12-week-old guinea pigs began to shrink after light exposure, reached a minimum after 1s, and continued until the end of light exposure; B: The pupil of the left eye of 2-week-old guinea pigs reached a minimum about 1.5s after the start of light exposure and then continued until the end of light exposure.

Table 1 Pupil diameter of 12-week-old guinea pigs under three light intensities mean±SD (range)

Eyes	100 lx	250 lx	500 lx	*F*	*P*
Left eyes	5.25±0.12 (4.99-5.47)	4.05±0.14 (3.83-4.29)	2.13±0.12 (1.96-2.34)	6291.48	<0.001
Right eyes	5.55±0.21 (5.20-5.89)	5.54±0.21 (5.21-5.90)	5.55±0.20 (5.24-5.90)	0.0005	0.10

Lx: Lumen per square meter (SI unit of illuminance); SD: Standard deviation.

Tropicamide group The pupil size of the left eye of 2-week-old guinea pigs after 1% tropicamide eye drops (Figure 7E) increased rapidly from a baseline of 3.62±0.14 mm, peaking at 4.22±0.10 mm at 12min, and then gradually constricted after two hours, returning to baseline at the eighth hour (t=0.09, P=0.931). The pupil size of the right eye of 12-week-old guinea pigs (Figure 7F) had a baseline of 5.50±0.24 mm, peaked at 6.91±0.11 mm at 5min, persisted until the 4th hour,

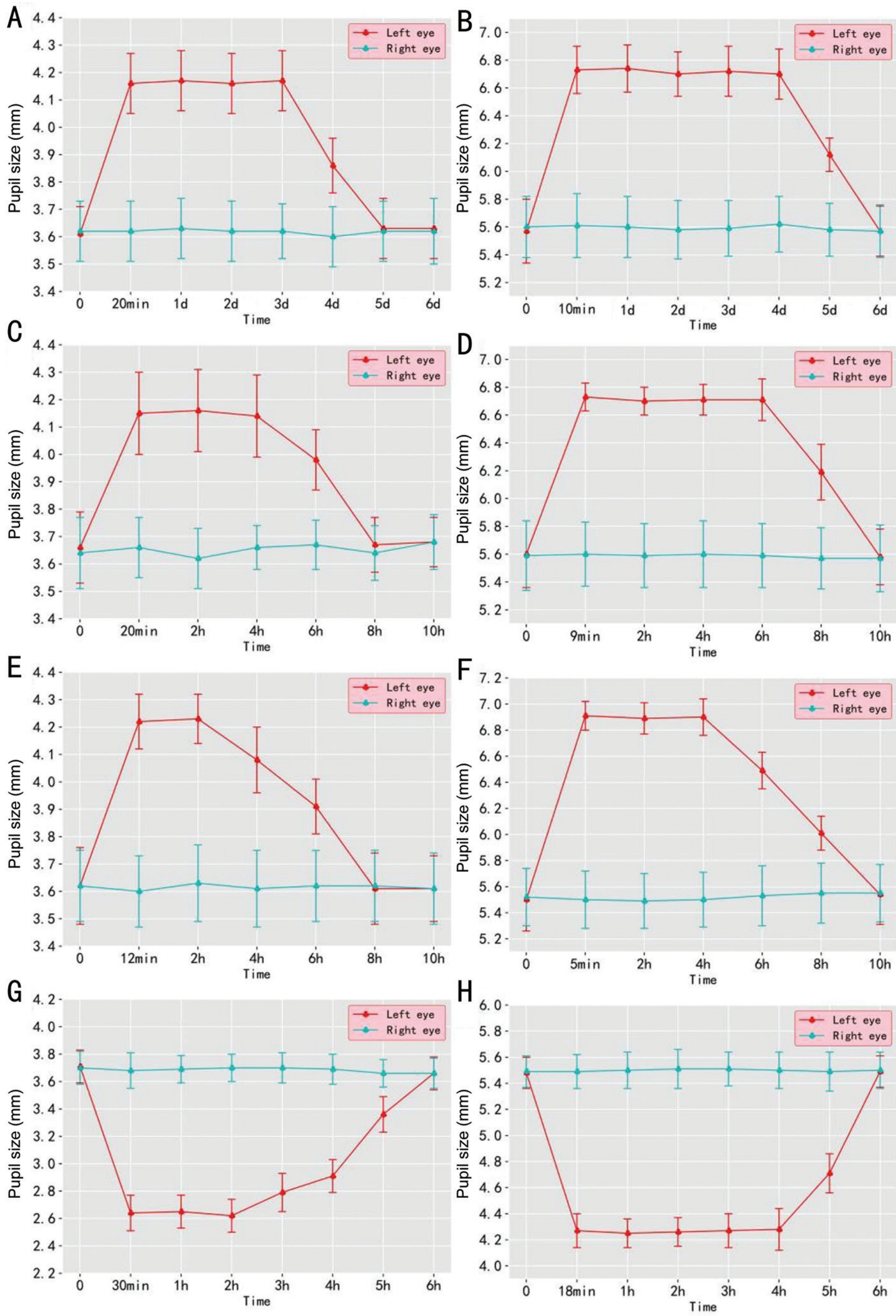

Figure 7 Changes in pupil size of two-week-old and twelve-week-old guinea pigs after eye drops of different drugs A: Changes in pupil size of guinea pigs in the 2-week-old atropine group; B: Changes in pupil size of guinea pigs in the 12-week-old atropine group; C: Changes in pupil size of guinea pigs in the 2-week-old cyclopentolate group; D: Changes in pupil size of guinea pigs in the 12-week-old cyclopentolate group; E: Changes in pupil size of guinea pigs in the 2-week-old tropicamide group; F: Changes in pupil size of guinea pigs in the 12-week-old tropicamide group; G: Changes in pupil size of guinea pigs in the 2-week-old pilocarpine group; H: Changes in pupil size of guinea pigs in the 12-week-old pilocarpine group.

and then gradually constricted, returning to baseline at the 10^{th} hour (t=-1.12, P=0.293). No significant changes in pupil size were observed in the right eye of guinea pigs across different age groups (P>0.05, repeated measures ANOVA). Regarding eccentricity, the left pupil (Figure 8C) showed a decrease (P<0.001) in 12-week-old guinea pigs after drug administration.

Pilocarpine group After 2% pilocarpine eye drops were applied to the left eye of two-week-old guinea pigs, the pupil size (Figure 7G) decreased from a baseline value of 3.71±0.12 mm, reaching a minimum of 2.64±0.13 mm at 30min. After 2h, the pupil gradually began to enlarge and returned to baseline values at 6h (t=1.83, P=0.101). For twelve-week-old guinea pigs, after the application of pilocarpine eye drops, the pupil size (Figure 7H) decreased from a baseline value of 5.48±0.12 mm to a minimum of 4.27±0.13 mm at 18min. The pupil began to constrict further at 4h and returned to baseline values at 6h (t=-0.48, P=0.644). No significant differences in the right

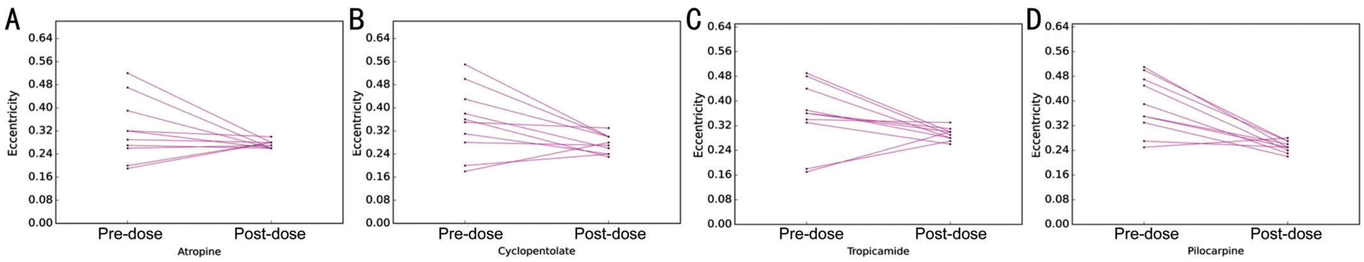

Figure 8 Changes in pupillary eccentricity in guinea pigs before and after drug administration A: 1% atropine group (t=-2.51, P=0.012); B: 1% cyclopentolate group (t=-2.75, P=0.034); C: 1% tropicamide group (t=-2.12, P=0.021); D: 2% pilocarpine group (t=-2.19, P=0.041).

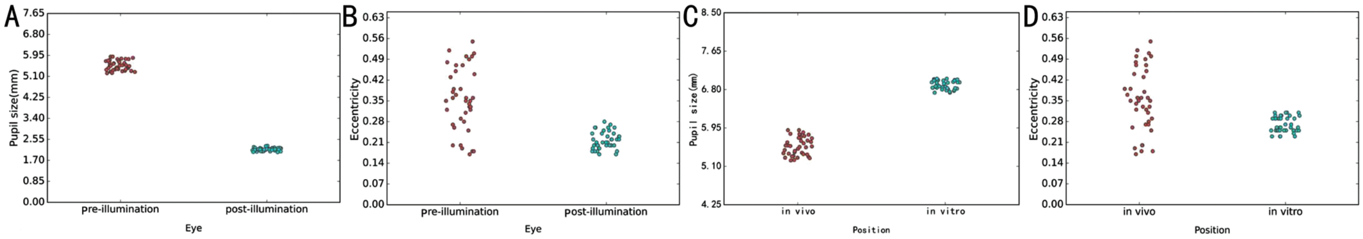

Figure 9 Parameters of the pupil of the eye in 12-week-old guinea-pigs A: 500 lx light causes guinea pig pupils to shrink (P<0.001); B: Guinea pig pupil eccentricity became smaller after light exposure (P<0.001); C: Pupil diameter of the 12-week-old guinea pig eye in the live and isolated state; D: Pupil eccentricity in the eye of the 12-week-old guinea-pig *in vivo* and in the isolated state.

pupil size of either two-week-old or twelve-week-old guinea pigs were observed before and after drug application (P>0.05, repeated measures ANOVA). After drug application in twelve-week-old guinea pigs, the left pupil eccentricity (Figure 8D) decreased from 0.39±0.09 to 0.25±0.02.

Changes in Pupillary Parameters of Guinea Pig Eyes *in vivo* and *in vitro* In the 12-week-old guinea pig eye *in vitro*, the pupil diameter (Figure 9C) enlarged from 5.54±0.22 to 6.90±0.10 mm, and the pupil eccentricity (Figure 9D) decreased from 0.35±0.11 to 0.27±0.03.

DISCUSSION

Our study systematically investigated pharmacological and light-driven pupillary accommodation in guinea pigs across developmental stages, revealing several key findings with implications for myopia research and ocular pharmacology. Below, we contextualize these results within the broader literature, address their clinical relevance, and discuss limitations and future directions.

Comparative Analysis with Other Animal Models Guinea pigs exhibit a robust direct PLR but lack a detectable indirect PLR, contrasting with humans and some other mammals. This difference may stem from fundamental disparities in binocular vision and cortical processing. Unlike humans, guinea pigs have laterally positioned eyes with limited binocular overlap, which likely reduces the neural integration required for consensual light reflexes[7,16]. Similar observations have been reported in mice, which also show minimal indirect PLR[17], suggesting that this may be a common feature in rodents. However, chimpanzees and rhesus monkeys show a more human-like PLR effect, which may be related to the fact that they have a more complex nervous system that relies on the coordination of the midbrain anterior parietal region of the parietal with the parasympathetic pathway of the motoneurons[18-19].

The pharmacological findings of the present study are consistent with those in chickens[20] and monkeys[21], in which muscarinic modulators similarly affected pupil size and regulation. However, guinea pigs showed age-dependent sensitivity to atropine, with older animals showing faster and more pronounced dilation.

Clinical Relevance for Myopia Research The age-related differences in drug sensitivity underscore the potential of guinea pigs as a model for studying pediatric myopia interventions. Atropine's prolonged effect in older guinea pigs mirrors its use in children for myopia control[22], while the rapid action of tropicamide aligns with its clinical utility for short-term cycloplegia[23]. Notably, the absence of indirect PLR in guinea pigs implies that their use in studies of binocular vision or cortical light processing may be limited, but their strong direct PLR and pharmacological responses make them ideal for investigating peripheral mechanisms of myopia progression.

Our system of non-contact measurement methods has an accuracy (0.01 mm) comparable to traditional methods such as pupillometry[24], providing a less invasive alternative to longitudinal studies. However, the applicability of the system to other species remains to be further validated.

Methodological Considerations and Limitations Although our non-contact method minimized handling stress, it was unable to assess intraocular pressure or axial length, parameters that are critical for myopia development. In future studies, we intend to supplement guinea pig studies on these parameters in conjunction with other devices in ophthalmology to provide a more comprehensive understanding of ocular changes. In addition, the lack of indirect PLR in guinea pigs may reflect methodological limitations, although our awake, nonanesthetized protocol reduced this concern.

Another limitation is that the study focused on short-term drug effects and did not explore the effects of long-term medication. In the future, we will further explore the effects of long-term drug administration on the biological parameters of guinea pig eyes. However, the guinea pig provides a valuable model for studying local mechanisms of eye regulation, especially drug- and light-induced pupillary responses. Although limitations remain, these findings provide some assistance for targeted studies of cholinergic signaling and the pathogenesis of myopia.

In conclusion, this study systematically investigated the pharmacological and light-driven pupillary accommodation in guinea pigs across developmental stages, revealing key insights into their ocular physiology. The findings demonstrate that guinea pigs exhibit a direct PLR with light intensity-dependent responses, while indirect PLR remains undetectable. The differential effects of muscarinic modulators on pupillary responses underscore the critical role of cholinergic signaling in ocular accommodation, with age-related variations in sensitivity. A novel non-contact measurement methodology achieved a precision of 0.01 mm for pupillary quantification, enhancing accuracy in ocular studies.

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Conflicts of Interest: Wu H, None; **Lu N,** None; **Qiao ZB,** None; **Cheng C,** None; **Luo WT,** None; **Wan T,** None; **Qiao T,** None; **Di Y,** None.

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