Original Research

Growth and Behavior of Congenitally Anophthalmic Lee–Sung Pigs

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Circadian rhythm is usually regulated by the environmental light–dark cycle. Congenitally anophthalmic miniature pigs provide a valuable model for the study of factors affecting circadian rhythms in the absence of visual exposure to the light–dark cycle. This study investigated the growth and daily behavior patterns of Lee–Sung pigs with congenital anophthalmia. Growth in 5 Lee–Sung pigs (LSP) with congenital anophthalmia (LSP-A) and 10 normally developed pigs (LSP-N) was assessed when they were 1 through 6 mo old. Behavioral studies using digital video recording were completed in 6 sexually mature LSP (3 LSP-A and 3 LSP-N). MRI showed that LSP-A lose their vision because of a lack of retinal input and optic chiasm development. LSP-N and LSP-A did not differ in body weight or size at 2, 4, and 6 mo of age. Behavior and activity pattern studies showed that both LSP-A and LSP-N were active mainly during daylight, but LSP-A spent significantly more time exploring their environment during the day (28%) and night (10%) than did LSP-N. This study revealed that growth performance was similar between LSP-A and normal pigs, but their behavior and activity patterns differed. LSP-A showed circadian rhythm abnormalities similar to those in blind humans. This study provides basic data on LSP-A as a model for studying compensatory cross-modal brain plasticity and hormone regulation in the absence of retinal input is deficient and for understanding the role of circadian rhythm regulation.

Abbreviations: LSP-A, Lee–Sung pig with congenital anophthalmia; LSP-N, normal LSP; MAC, microphthalmia, anophthalmia, and coloboma

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In 2017, 253 million people were visually impaired worldwide; 217 million of them had amblyopia, whereas 36 million of them were blind.^{8,47} The prevalence of anophthalmia, a birth defect whereby a baby is born absent one or both its eyes, ranges from 0.6 to 4.2 per 100,000 births.^{33,41} Absence of neural activity in the visual system of humans and mice with anophthalmia leads to a reductions in optic radiation, the lateral geniculate nucleus, and primary visual cortex due to cross-modal plasticity.²⁸ Because circadian rhythmicity is synchronized by the light–dark cycle, loss of sensory input from the retina due to anophthalmia leads to abnormal circadian rhythmicity in the absence of retinohypothalamic projection and a neural pathway to the pineal gland.³ Studying the relationship between cross-modal rewiring and circadian rhythmicity in anophthalmic animal models is an important way for us to understand the degree of neural plasticity that animals experience during profound blindness and changes to behavior which inevitably occur.

More than 80 genes are associated with microphthalmia, anophthalmia, and coloboma (MAC). These genes are categorized into 4 groups, depending on the effects expressed. The largest of these groups comprises genes related to transcription; TGFβ–BMP signal molecules and retinoic acid pathway members are assigned to another 2 groups. The remaining genes are assigned

to the last group.³⁷ Clinical findings indicate that mutations of these MAC-related genes are associated with syndromic microphthalmia, anophthalmia, and coloboma, including abnormalities in ocular development, in sclerocornea and corneal opacity, as well as endocrine regulation.^{31,42,43} For example, mutation of SOX2, OTX2, STRA6, BMP4, HCCS, or PORCN causes abnormal regulation of the endocrine system, leading to growth retardation.⁴² To increase the value of animal modeling, it is essential to understand how genetic mutations are being expressed in the species used.

The simulation of human disease in animal models involves adopting animals with congenital or induced pathologies that are similar to those in humans for biomedical and behavioral testing and observation. The results of such testing and observation help inform the development of human treatments.^{24,39,45} Developing suitable animal models is key to successful basic medical research. This process includes the selection of an appropriate animal species for modeling human afflictions. For example, although mice and rats are often used in animal models, they are considerably different from humans in terms of physiology, anatomy, and behavior patterns during the light–dark cycle.¹⁴ Because most mice and rats are nocturnal animals, their use in simulating abnormal circadian rhythms in humans and diurnal animals is not optimal.¹⁴ In contrast, pigs (*Sus scrofa*) are highly suited to simulating human circadian rhythmicity and for ocular developmental research.^{17,20,22,39,40}

Many animal models have been developed for eye disease studies. Zebrafish with congenital genetic defects and mice and rats with gene knockouts can all present symptoms of

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anophthalmia,¹¹ and they have been widely studied. However, their genetic and anatomic structures are vastly different from those in humans. For example, physiologically speaking, rodents lack a macula in the center of the retina, so they cannot be used for simulating Stargardt-like macular dystrophy.³⁰ However, ocular development, physiology, and anatomy are similar between pigs and humans, so adopting pigs in such animal models has structural advantages.^{7,18,38} In addition, ocular diseases induced in pigs closely mirror those in humans, such abnormalities in retinal structure.¹⁹ Moreover, pigs are well suited for use in studies of eye injury, tissue transplantation, and retinal pigment epithelial cell development (such as stem cell development).³² In regard to animal models that simulate human disease, the size and ratio of light-receiving rods and cones in pigs' eyes are close to those of humans; through transgenic techniques, pig models of retinitis pigmentosa and cone-rod retinal dystrophy have been developed.²⁴ A study evaluating intraocular pressure and the growth and death of retinal ganglion cells revealed that pigs are suitable for studying glaucoma.³⁹ Moreover, pigs have been evaluated as a relatively feasible species for corneal transplantation research.²¹

Black-coated Lanyu pigs are miniature pigs native to Taiwan. Under the "Developing Pigs for Medical Research" policy, the Department of Animal Science and Technology, National Taiwan University, and the Livestock Research Institute started conservation programs in 1975 and 1980, respectively, to establish inbred laboratory Lanyu pig herds. In 1983, National Taiwan University mated Lanyu sows with Landrace boars to selectively breed white-coated miniature Lee-Sung pigs (LSP, 75% Lanyu and 25% Landrace). Subsequently, full-sib and half-sib breeding programs were created for selective breeding of the inbred Lee-Sung pig strain.²⁷ In 2008, 3 LSP with phenotypic defects (anophthalmia, aniridia) appeared in the selectively bred population. Test mating experiments confirmed that anophthalmia and aniridia are inherited defects in LSP.

Recently, gene editing combined with induced pluripotent stem cell techniques and blastocyst complementation phenomenon showed that producing human tissues in pigs is feasible.⁴⁸ If anophthalmic LSP can be applied in blastocyst complementation studies, producing customized eye materials or tissues and organs for patients in need of transplantation should be feasible. Furthermore, mutation of MAC-related genes in the TGF β -BMP signal pathway causes not only ocular defects but also skeletal disorders;^{4,49} therefore, investigating whether anophthalmic LSP have growth abnormalities is important to these types of study. Given these points, understanding how the absence of ocular tissue in LSP affects cross-modal plasticity, growth performance, daily behavior, and activity patterns is crucial for any future developments in this area. This study aimed to investigate images of the anatomy of the optic chiasm of LSP, assess the growth performance of anophthalmic LSP, and establish baseline information regarding the effect of anophthalmia on circadian rhythmicity by observing normal and anophthalmic LSP.

Materials and Methods

All research protocols including anesthesia and analgesia, animal care procedures, and technical and scientific methods were approved by the IACUC of the National Taiwan University (IACUC no. NTU106-00039). All animals were raised in a pig house (no. 10) that has been ISO9001-2015-certified (certification no. FS686535; BSI, Taipei, Taiwan) and conforms to IACUC requirements regarding animal housing.

MRI analysis. Both eyes of 3 sexually mature normal (LSP-N) and 3 congenitally anophthalmic (LSP-A) pigs underwent

MRI analysis. Animals were obtained from the Department of Animal Science and Technology, National Taiwan University. Anesthesia was induced by using Zoletil (3 mg/kg IM; Virbac Animal Health, Fort Worth, TX) and xylazine (1 mg/kg IM) and maintained with isoflurane. The head of each pig was positioned within 480 mm $\times$ 550 mm radiofrequency coils and placed in the magnet bore. MR images were acquired on a 3.0-T MRI system (Achieva, Philips, Amsterdam, Netherlands) equipped with a 75-mT/m shielded gradient insert. The parameters used to acquire MRI data were as follows: 3D gradient-echo sequences; acquisition bandwidth, 25 kHz; repetition time/echo time, 32/1.9 ms; flip angle, 30°; 8 averages; field of view, 200 mm $\times$ 200 mm $\times$ 90 mm; acquisition matrix, 200 $\times$ 200 $\times$ 90; voxel resolution, 1 mm $\times$ 1 mm $\times$ 1 mm. Neither LSP-A nor LSP-N candidates underwent contrast MRI. MR images were analyzed by using Horos medical imaging software (version 1.1.7, Horos Project.org).

Assessment of growth performance. A total of 15 piglets from 5 litters in 2017 (5 with congenital bilateral anophthalmia [LSP-A] and 10 with normal eye development [LSP-N]) were tested for growth performance after birth. LSP are an inbred population, with LSP-A being derived from LSP-N through spontaneous mutation. As noted previously, the derivation of LSP-A may involve mutation of MAC-related genes associated with transcription, TGF β -BMP signaling molecules, and the retinoic acid pathway.³⁷ Piglets were selected from 5 litters of variable sizes (that is, 8, 7, 5, 4, and 4 piglets). One LSP-A piglet each was obtained from the litters of 8, 5, and 4 piglets, whereas 2 piglets were obtained from the remaining litter of 4. Piglets were weaned at 56 d. They were provided 50 g of creep feed during lactation. After 10 wk, pigs were transferred to a growth-finishing unit. Each postweaning pen had floor space of 4 m² dedicated to a single pig. Room temperature was recorded and kept at 26 to 29 °C through ventilation. Environmental enrichment was provided by adding balls and steel link chains, as suggested by the IACUC. All pigs were fed a commercial chow (Fopco, Taipei, Taiwan) containing corn cereal, wheat bran, soybean, sunflower meal, and field pea protein. This diet contained 3.05 Mcal metabolic energy, 14% crude protein, 2.5% crude fat, a minimum of 0.75% of digestible lysine, and 0.48% methionine. Pigs were fed at 0830 and 1630 daily and allowed unrestricted access to water throughout the experiment. Growth performance tests were conducted monthly from 1 to 6 mo of age. The piglets were fed an average of 100 g of feed daily for the first 3 mo. After 3 mo, feed was increased to an average of 150 g daily.

Items assessed for growth performance included weight, body length, withers height, and chest girth. Body length was measured from the base of the ear to the base of the tail. Withers height was the vertical distance from the shoulder blade to the ground when a pig stood normally on all 4 feet. Chest girth was the chest circumference at the pig's armpits.

Behavior and activity patterns. The circadian rhythmicity of locomotor activity in mammals is synchronized to light.^{9,12,34} To understand whether a lack of light stimulus affects circadian rhythmicity in pigs with anophthalmia, we used digital video recording to assess the amount of time pigs spent either resting or active during a 24-h cycle. Video recordings were manually analyzed offline by a trained observer using Excel software (Microsoft, Redmond, WA). Pigs were considered resting when they were sitting or lying down for more than 15 to 20 min, yet still exhibited occasional tail wagging and had quick stimuli response times. They were considered asleep when lying down with eyes closed for more than 1 h.²³ Activity included time spent drinking water, eating, excreting, and moving in the pen.

Figure 1. (A) Axial T1-weighted MR image shows normal eyes (arrows) and optic chiasm (asterisk) in a normal Lee-Sung pig. Bilateral optic nerves connect to the optic chiasm from both eyes. (B) At the same anatomic position in a congenitally anophthalmic Lee-Sung pig, the equivalent axial T1-weighted MR image shows residual disorganized fibrous tissue (arrow heads) and an absence of the optic chiasm.

Figure 2. (A) Body weight, (B) body length, (C) withers height, and (D) chest girth in LSP-N and LSP-A from 1 through 6 mo of age. Triangles, LSP-N; squares, LSP-A

These experiments involved 6 sexually mature LSP, 3 LSP-A and 3 LSP-N. Each pig was placed in a pen that measured 120 cm × 180 cm. Video tracking recording was conducted for 24 h daily for 3 d, and the results were processed with Smart 3.0

software (Panlab, Barcelona, Spain) to obtain each pig's hourly activity levels.

Statistical analysis. Growth performance variables were compared between LSP-A and LSP-N by using *t* tests to learn

Table 1. Body weight, body length, withers height, and chest girth of LSP-N and LSP-A at 2, 4, and 6 mo of age

		2 mo			4 mo			6 mo		
		Mean	1 SD	<i>P</i>	Mean	1 SD	<i>P</i>	Mean	1 SD	<i>P</i>
Weight (kg)	LSP-A	8.7	1.1	—	14.7	2.8	—	23.1	4.3	—
	LSP-N	8.8	1.2	0.902	14.0	1.8	0.547	20.6	2.9	0.210
Body length (cm)	LSP-A	64.6	1.4	—	74.4	8.8	—	91.8	8.1	—
	LSP-N	62.5	2.7	0.121	73.0	5.1	0.688	86.3	5.8	0.152
Withers height (cm)	LSP-A	29.0	2.0	—	35.6	2.2	—	40.3	5.7	—
	LSP-N	29.1	2.0	0.930	36.2	2.0	0.616	38.5	2.5	0.402
Chest girth (cm)	LSP-A	46.1	2.3	—	55.7	5.1	—	64.7	7.6	—
	LSP-N	46.1	2.6	1.000	54.4	3.7	0.576	64.3	5.3	0.906

Data for LSP-A were compared with those for LSP-N by using *t* tests.

Figure 3. Research on the behavior pattern of LSP-N and LSP-A during day and night. Recordings with rests longer than one minute are recorded as resting behavior (gray vertical lines). A, B, and C represented LSP-A, whereas D, E, and F stood for LSP-N. The 24 h are divided into 4 time periods: 00:00 to 05:59, 06:00 to 11:59, 12:00 to 17:59, and 18:00 to 23:59. The gray bars in each time period represent the time pigs conducted resting behavior.

Table 2. Total time that LSP-A and LSP-N spent resting during 4 time periods

Time period		Total resting time (min)		
		Mean	1 SD	P
0000-0559	LSP-A	323	19	0.0037
	LSP-N	351	7	—
0600-1159	LSP-A	195	36	0.044
	LSP-N	241	53	—
1200-1759	LSP-A	195	30	0.0059
	LSP-N	249	42	—
1800-2359	LSP-A	281	25	0.055
	LSP-N	310	36	—

whether, under the same feeding conditions, growth performance differed between the 2 types of pigs. Similarly, behavior pattern observations adopted *t* testing to analyze whether daytime and nighttime activity levels in pigs with congenital anophthalmia differed from those in pigs with normal eyes. Differences between LSP-A and LSP-N were acknowledged as statistically significant at *P* values less than 0.05.

Results

Brain MRI. On axial T1-weighted MR images of LSP-N, the eye globes and optic chiasm were present, and the bilateral optic nerves were connected with the optic chiasm (Figure 1 A). Bilaterally in LSP-A, the globes and optic chiasm were absent, with spaces filled with residual intraorbital tissue (Figure 1 B). The bilateral optic nerves were difficult to identify and, due to the lack of the optic chiasm, they were not connected to the brain.

Growth performance. Many genes are associated with syndromic anophthalmia and microphthalmia, and the mutation of genes associated with endocrine regulation can cause growth retardation. To understand whether LSP-A demonstrate growth retardation and to predict genes involved in inducing anophthalmia, we assessed growth performance (that is, body length, withers height, chest girth, and body weight) in LSP that were 1 to 6 mo old (Figure 2). None of these parameters differed significantly at any time point between LSP-N and LSP-A ($P \geq 0.05$; Table 1).

Rest patterns. The LSP tested in this study were fed at 0830 and 1630 daily. To understand the effect of the lack of visual stimulus and thus circadian rhythmicity in LSP-A, we used digital cameras to record the 24-h behavior patterns of 3 LSP-N and 3 LSP-A. The 24-h periods were divided into 4 recording periods, and results from each recording period were summed. Results revealed that from 0000 to 0559, LSP-A spent 89.7% of the time (total, 323 ± 19 min) resting, whereas LSP-N rested for 97.5% of the available time (351 ± 7 min; $P = 0.0037$). From 0600 to 1159, LSP-A spent 54.8% of the time resting (195 ± 37 min), whereas LSP-N rested for 68.6% (241 ± 53 min; $P = 0.0440$). From 1200 to 1759, LSP-A spent 54.8% of the time resting (195 ± 30 min) compared with 70.1% (249 ± 43 min) for LSP-N ($P = 0.0059$). From 1800 to 2359, LSP-A spent 77.8% of the time resting (281 ± 25 min), whereas LSP-N rested for 86.9% (311 ± 36 min; $P = 0.0548$; Figure 3). The total amounts of time that LSP-N and LSP-A spent on various activities during the day and night are shown in Table 2. The accumulated resting time of LSP-A was significantly less ($P < 0.05$) than that of LSP-N, suggesting that due to the lack of light stimulus to the eyes, bilaterally anophthalmic LSP had altered circadian rhythmicity.

Activity tracing. Digital video recording over 24-h periods revealed that for a total of 3 h before, during, and after feeding at 0830 and at 1630, the trace density for LSP-N and LSP-A around the feeding trough was higher than at other times (Figures 4 and 5). During nonfeeding hours (from 0900 to 1500 and from 1800 to 2400), the activity traces of LSP-A were more complex than those of LSP-N. Although LSP-A were unable to receive any light stimuli (to regulate their circadian rhythmicity), activity traces for LSP-A from 0100 to 0400 showed these pigs at the same location; that is, in a resting state. Like LSP-A, LSP-N often rested at the same location during nonfeeding hours (Figure 5), particularly from 2300 to 0600 (that is, the next day), when their traces typically showed them to be sleeping at the same location. However compared with traces from LSP-N, LSP-A movement patterns were more complex (Figure 4).

Discussion

Anatomically speaking, a third of the cortical surface of the human brain is involved in processing visual functions. When vision is lost at birth, a massive reorganization of visual projections takes place after an absence of visual exposure.¹³ Studies involving visual deprivation in cats and rodents demonstrate compensatory colonization of the visual cortex by other senses through plastic rearrangement of thalamocortical projections and corticocortical connection.¹³ In addition, neonatal loss of vision drives not only structural but also functional changes in the remaining sensory systems of the brain.³⁶ Many animal studies indicate that the timing of developmental changes at which visual loss occurs is of prime importance to the strength of cross-modal plasticity. In addition, the visual experience of the individual in early life is required for the brain to develop normal functional architecture.²⁵

In the present study, LSP-A miniature pigs were confirmed through MRI to have congenital absence of visual input and loss of the optic chiasm. LSP-A possessing a congenitally blind brain offer a unique model for gaining insight into the influence that visual experience has over brain development and its functional architecture. In addition, LSP-A provide a unique model for understanding compensatory neural plasticity and cross-modal reorganization among auditory, tactile, and olfactory sensation processing after early visual deprivation.

Developmental defects of the eye include microphthalmia, anophthalmia, and coloboma. To date, genetic studies have revealed at least 82 genes related to the occurrence of these conditions. However, many genes that result in ocular developmental defects have yet to be identified, and more than these 82 genes likely are involved.³⁷ Genes related to MAC, when classified by attribute, include transcription factors and members of the TGF β -BMP signaling and retinoic acid pathways. Transcription factors include the products of HMG-box domain-containing SOX2 gene; homeodomain-containing factors such as OTX1, PAX6, VSX2, PITX3, RAX, MITF, and SIX6; the products of paired-domain genes such as PAX6, forkhead family member FOXE3, zinc-finger gene SALL2, and basic helix-loop-helix gene ATHO7; TGF β -BMP signaling proteins (BMP4, BMP7, GDF6, and GDF3); retinoic acid pathway components including ALDH1A3, STRA6, RARB; and the products of other genes such as SHH, ABCB6 (retinoic acid pathway), ADAMTSL4, C12orf57, TENM3 (ODZ3), PXDN, YAP1, HMGB3, and CRIM1.³⁷ Mutation of the TGF β -BMP family members GDF3 and GDF6 causes ocular and skeletal disorders, such as MAC and defective tail formation in zebrafish models.^{4,49} Deletion of BMP4 in humans causes partial agenesis of the corpus callosum, cerebellar hypoplasia, and developmental delays.⁵ The BMP7 deficient/mutant mice model exhibits renal dysplasia,

Figure 4. A 24-h trace recording of LSP-A; the green square is the trough, the red square is the area of the pen (120 cm by 180 cm), and the blue area is the water-drinking device.

developmental defects in craniofacial structures, and anophthalmia at birth.^{16,50} Mice with a targeted, preconditional mutation in *Smoc1* express small body size, cleft palate and high perinatal mortality that accurately recapitulate the human phenotype.³⁵ Of all patients with MAC, 34% have other systemic comorbidities. For example, patients with anophthalmia or microphthalmia in both eyes have approximate possibilities of 56% and 54% for neurologic defects, respectively, and 11% and 32% chance of musculoskeletal or facial defects, respectively.^{41,46} Other defects include abnormalities in the cardiovascular system, urinary system, reproductive system, and integumentary system.⁶ However, heterogenous mutations in *GDF6* have also been reported in the nonMAC skeletal disorder Klippel-Feil syndrome, which is characterized by congenital fusion of vertebrae in a fetus.⁴⁴ In addition to *Smoc1*, mutation of *SOX2*, *OTX2*, *STRA6*, *BMP4*, *HCCS*, or *PORCN* causes abnormal regulation of the endocrine system, leading to growth retardation and small body size.^{42,43} The evidence suggests, however, that in most cases of MAC-associated symptoms, growth abnormalities have not been present. The degree of variable expression and incomplete penetrance of mutations in patients and animal models illustrate how complex the genetic inheritance of these MAC-associated phenotypes is. In the present study, LSP-A and LSP-N showed no significant differences in growth performance and body type, consistent with findings from human patients with bilateral anophthalmia. In addition, our current data shows that LSP-A achieve a normal

growth rate and body size, indicating that the anophthalmia of LSP-A may not reflect mutation of *Smoc1*, *SOX2*, *OTX2*, *STRA6*, *BMP4*, *HCCS*, or *PORCN*.

The circadian rhythmicity of people with congenital anophthalmia is affected. Most children with bilateral anophthalmia have sleep disorders because they lack the normal control provided through retinal input over circadian rhythm.¹² In addition, the salivary melatonin rhythm that regulates circadian rhythmicity disappears or perhaps does not manifest.³ Past studies in which heart and respiratory rates have been monitored indicate that other stimuli, such as sound, temperature, and stress, can also regulate circadian rhythmicity.^{2,29} In addition, the integrative effects of light and food can affect the circadian rhythm of locomotor activity in mice.³⁴

The present study shows that the amount of time during which they were active was greater for LSP-A than LSP-N at night (1800 to 0600 the next day), especially from 1800 to 2400, and during the daytime. These results reflect that the circadian rhythmicity of LSP-A is not as distinct as that of LSP-N. A possible inference is that the lack of light input to the eyes results in a decline in the distinctness of circadian rhythmicity in LSP-A. Moreover, given that LSP-A were almost always resting from 0000 to 0600 compared with 1800 to 2400 indicates that other environmental stimuli, such as sound and temperature, must be playing a role in governing behavior (Figure 3). These data suggest that vision deprivation at birth caused LSP-A to express

Figure 5. A 24-h trace recording of LSP-N; the green square is the trough, and the red square is the area of the pen (120 cm × 180 cm), and the blue area is the water dispenser.

abnormal circadian rhythms. In the future, LSP-A could be applied to study hormone regulation, stress responses, and genes involved in circadian rhythms when visual input is absent.

Many studies have shown that human eye developmental defects were comorbid with neurodevelopmental disorders in the brain.^{41,46} One group¹ found 3 patients with bilateral anophthalmia that had not only an absence of the optic chiasm but also hypoplasia of the posterior optic pathway. Studies adopting MRI to obtain human brain images and the metabolic status of patients with eye developmental defects revealed that the brain metabolism of these patients was significantly different from that of normal brains. Brain function also differed, including differences in the levels of choline, glutamate, myo-inositol, and total creatine in cerebral gray matter and in subcortical auditory responses in the hypodermis. Other studies in animals and humans have shown that the absence of vision causes extensive structural changes in the visually deprived cortex and other regions of the brain. These studies have further demonstrated that the visually deprived cortex becomes responsive to a wide variety of nonvisual sensory inputs.²⁵ In addition, ectopic cross-modal subcortical connections have been revealed in anophthalmic animals, but similar connections do not occur in sighted or sight-deprived animals.^{10,15,26}

Through our MR images of LSP-A, we verified an absence of the optic chiasm and optic nerve, meaning that no light stimulus can enter the brain. These characteristics could be beneficial to

future neuroplasticity studies. However, to study compensatory cross-modal plasticity in the brain, further MRI analyses of LSP-A brain images are needed.

In conclusion, whether changes in circadian rhythmicity among LSP-A are similar to those in anophthalmic humans requires further investigation. Our study has shown that the growth performance, abnormal circadian rhythmicity, and the lack of the optic chiasm and nerve in LSP-A are all similar to the consequences anophthalmic humans experience, thus indicating that LSP-A are suitable for modeling circadian rhythm regulation and compensatory cross-modal plasticity studies of the brain.

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