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Plastic brain structure changes associated with the division of labor and aging in termites

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Abstract

Division of labor is a prominent feature of social insect societies, where different castes engage in different specialized tasks. As brain differences are associated with behavioral differences, brain anatomy may be linked to caste polymorphism. Here, we show that termite brain morphology changes markedly with caste differentiation and age in the termite, *Reticulitermes speratus*. Brain morphology was shown to be associated with reproductive division of labor, with reproductive individuals (alates and neotenic reproductives) having larger brains than nonreproductives (workers and soldiers). Micro-computed tomography (CT) imaging and dissection observations showed that the king's brain morphology changed markedly with shrinkage of the optic lobes during their long life in the dark. Behavioral experiments showed that mature primary kings lose visual function as a result of optic lobe shrinkage. These results suggested that termites restructure their nervous systems to perform necessary tasks as they undergo caste differentiation, and that they also show flexible changes in brain morphology even after the final molt. This study showed that brain morphology in social insects is linked to caste and aging, and that the evolution of the division of labor is underpinned by the development of diverse neural systems for specialized tasks.

KEYWORDS
aging, brain, division of labor, phenotypic plasticity, social insects, termites

1 | INTRODUCTION

As brain size and structure covary with behavioral differences, brain morphology may be a good predictor of behavioral repertoire and cognitive capacity, and may reflect species-specific cognitive challenges (Barton & Harvey, 2000; Gronenberg, 2001). However,

identifying correlations between overall brain sizes and cognitive ability is difficult because differences in ecological and evolutionary backgrounds among species complicate interpretations (Chittka & Niven, 2009; Logan et al., 2018). Intraspecific comparative studies on the diversity of brain morphology and behavioral repertoire represent a powerful approach to understand the association between brain and behavior (Reyes et al., 2022). Phenotypic plasticity within the same species is an ideal system for comparative brain–behavior research. In

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particular, caste polymorphism and polyethism in eusocial insects enable us to study the diversity of brain morphology and behavioral repertoire among individuals within the same colony with the same genetic background. Division of labor implies that individuals within a colony specialize in particular tasks, such as brood care, foraging, nest building, or defense. Division of labor is often considered the reason for the ecological success of social insects, which account for ~75% of the world's insect biomass (Hölldobler & Wilson, 1990).

Termites clearly show division of labor, with persistent individual specialization occurring, in which caste-specific morphology is coupled with caste-specific behaviors. Sexual division of labor is also common in termites, where soldiers are all female in some species, all male in some others, and both in the remaining species (Matsuura, 2002, 2006; Roisin, 2001). Division of labor between major and minor workers in termitids is also associated with sex (Noirot, 1985). It has also been shown that the behavior of colony members can change over a lifetime. Age-related behavioral change, called age polyethism, is also common in termites (Crosland et al., 1997; Šobotník et al., 2012; Yanagihara et al., 2018). In particular, alates (winged imagoes) show marked changes in behavior over their lifespan, flying from their natal nest, searching for mates by walking on the ground, founding new colonies to become primary kings and queens, producing and raising a first brood, and then exclusively engaging in reproduction (Figure 2a). The external and internal morphologies of the alates change during this process as the required tasks change (Chouvenc, 2019; Inagaki et al., 2020; Nutting, 1969), which can be linked to varying cognitive demands and brain morphological changes.

Richard (1969) summarized research on the nervous system and sense organs of termites and reported that brain size increased with growth, and mature soldiers had larger brains than individuals of the same age in the reproductive line, based on the data from *Zootermopsis nevadensis* and *Kalotermes flavicollis* (Richard, 1969). In *Zootermopsis* termites, it has been observed that nymphs, which will develop into winged imagoes and disperse from the nest, exhibit larger optic lobes than pseudergates (Sean O'Donnell et al., 2021), while pseudergates show the highest relative investment in antennal lobes and mushroom bodies compared with immature instars and later castes such as soldiers and reproductives (S. O'Donnell et al., 2022). Studies conducted on these primitive termite species indicate that the allocation of resources in different brain regions is associated with the perceptual and cognitive demands specific to each caste. Nevertheless, the precise correlation between the size of a specific brain region and an individual's sensory or cognitive abilities remains unestablished. Moreover, there is limited knowledge regarding the changes in brain size and function, particularly when the required abilities shift with age following the final molt. It has been observed that the optic lobe of old imagoes diminishes in *K. flavicollis* (Richard, 1969). However, the precise process of the age-related brain degradation and whether individuals with diminished optic lobes experience a loss of vision remain unknown.

In this study, we first investigated whether brain morphology differs among castes in the subterranean termite *Reticulitermes speratus*. Unlike *Zootermopsis* and *Kalotermes*, which exhibit a linear caste differentiation pathway, *R. speratus* possesses bifurcated pathways and a unique reproductive system known as asexual queen succession (AQS) (Matsuura et al., 2009). We compared the brain morphology among castes (primary reproductives, nymphoid reproductives, ergatoid reproductives, nymphs, workers, and soldiers) and also examined sexual dimorphism within each caste. Primary reproductives, as alates, leave the parental nest to establish new colonies, while neotenic reproductives inherit reproduction within the nest and are not exposed to the external environment, resulting in significant differences in required sensory and cognitive abilities. Furthermore, in this species, both morphological and behavioral sexual dimorphism is known not only in reproductive pathways but also in sterile castes, suggesting the possibility of sexual dimorphism in brain morphology. Then, we investigated morphological changes in the brains of primary kings with aging, coupled with a behavioral assay to determine whether their vision is functional after years spent in the dark. In AQS species, queens are inherited through parthenogenesis, while kings have remarkably long lifespans. Within this extended lifespan, how does the nervous system of the king change, and does their sensory ability, particularly vision, alter with these changes? This study focuses on the variations in the king's nervous system and the potential alterations in their sensory ability, specifically visual capabilities, throughout their long lifespan.

2 | MATERIALS AND METHODS

2.1 | Termites

The breeding system of the subterranean termite *R. speratus* is characterized by asexual queen succession (AQS) (Figure S1) (Matsuura, 2017; Matsuura et al., 2009; Yashiro & Matsuura, 2014). Termite colonies are typically founded by a pair of primary reproductives (adult winged forms), that is, a king and a queen. In AQS species, queens produce their neotenic replacement queens asexually but use normal sexual reproduction to produce other colony members. This system enables primary queens to increase their reproductive output while avoiding inbreeding as long as primary kings survive. Therefore, the primary queen can be considered genetically immortal until the colony dies, because neotenic queens are also replaced by subsequent cohorts of parthenogenetically produced neotenic queens. When the primary king dies, the neotenic king differentiates and takes over reproduction, but such inbreeding colonies do not last long and eventually come to an end. Thus, the AQS system exerts strong selective pressure for the longer lifespan of the primary kings (Matsuura, 2011). This system provides an ideal opportunity to examine changes in brain morphology and function during a very long adult lifespan.

To investigate changes in brain morphology associated with caste differentiation, we compared the brain sizes of nonreproductive castes (workers and soldiers) and reproductive castes (ergatoids, nymphoids and alates) of *R. speratus*. We prepared worker, soldier, and alate samples by collecting individuals from 11 colonies from pine forests in Kyoto, Shiga, Osaka and Fukui, Japan (see Datasets). Ergatoids and nymphoids were induced in the laboratory by isolating workers and nymphs from the colony and maintained in 90 mm Petri dishes

filled with a diet consisting of brown rotted pinewood and cellulose (Matsuura & Nishida, 2001) at 25°C under conditions of constant darkness. We observed 7–21 individuals for each sex of each caste in at least three colonies (see Datasets).

To examine age-related morphological changes in the brain, we compared the brain morphologies of primary kings at different ages: 0, 1, 2, and 3 years, and mature (>5 years). We prepared 0-year-old primary kings by collecting male alates from field colonies and allowing them to fly and shed their wings in the laboratory. To obtain 1–3-year-old primary kings, we prepared incipient colonies by pairing a male and female dealate in the laboratory and maintaining them at 25°C under constant darkness until the experiments (Matsuura & Nishida, 2001). We collected mature primary kings by sampling the royal chambers of large field colonies with a number of secondary queens. These primary kings were at least 5 years old because a newly founded colony cannot grow into a large mature colony with a large number of secondary queens within 5 years (Matsuura et al., 2018). We examined 10–16 primary kings for each age class (see Datasets).

2.2 | Brain size measurement

The brain was dissected from the head capsule using phosphate-buffered saline (PBS) containing 0.01% (w/v) Triton X-100. Immediately after dissection, the brain was positioned ventral side down at the center of the dissection dish, and dorsal-view images of the brain were captured using a digital camera (FX380; Olympus, Tokyo, Japan). The obtained brain images were processed using ImageJ Fiji v2.0.0 for manual outlining of the brain. The brain size, including width, height, and area, was measured using ImageJ Fiji v2.0.0 (Schindelin et al., 2012). To enhance the contrast of the brain image, a 2.5% saturation adjustment followed by gamma correction ($\gamma = 1.5$) was applied. The cross-sectional area of the brain, as viewed from the dorsal side, was calculated based on the outlined shape. Specifically, two points representing the greatest concave curvature around the optic lobe were manually selected, and the area enclosed by a line connecting these two points was measured as the optic lobe area. Following the image acquisition, the dissected brains were fixed with 4% paraformaldehyde, transferred to methanol, and used for subsequent immunostaining procedures.

2.3 | Micro-CT imaging of the brains of kings

We performed micro-computed tomography (CT) imaging of male alates and mature primary kings collected in the field (see Datasets). Termites were fixed with Bouin's solution and dehydrated by passage through a graded ethanol series. The specimens were then stained with 2.5% (w/v) sodium iodine. Micro-CT was performed at MST (Foundation for Promotion of Material Science and Technology of Japan, Tokyo, Japan) using an X-ray microscope (ZEISS Xradia 520 Versa X-ray Microscope; Zeiss, Oberkochen, Germany) with the following scanning parameters: X-ray tube voltage = 50 kV for male alates and 70 kV for mature kings; and voxel size = 5 μm for male alates and 6 μm for mature kings. Volumetric

data were processed and analyzed using VGSTUDIO MAX (Volume Graphics, Heidelberg, Germany), SYNAPSE VINCENT (Version 5.3.0001; FUJIFILM Corporation, Osaka, Japan) and ImageJ (version 1.53r) software [25].

2.4 | Immunostaining of the brain

Male alates and mature primary kings were collected in the field (see Datasets). To stain neuronal fibers in the brains, mouse anti-Fas2 antibody (1/1,000 dilution) and anti-mouse IgG-Cy3 antibody (1/1,000 dilution) (Jackson ImmunoResearch, West Grove, PA, USA) were used as the primary and secondary antibodies, respectively. Neuronal nuclei were detected by staining with 4',6-diamidino-2-phenylindole (DAPI). The brains were treated with a tissue-clearing reagent (methyl salicylate) and images were obtained by confocal microscopy (SP8; Leica Microsystems, Wetzlar, Germany). The images were processed using ImageJ Fiji v2.0.0 (Schindelin et al., 2012).

2.5 | Behavioral assay

To elucidate the effects of optic lobe shrinkage on behavior, we used male alates and mature primary kings obtained from two different colonies (see Datasets). First, a king was aspirated and transferred to the center of a piece of white paper measuring 210 × 297 mm. A transparent glass wall (H52 × W75 mm) or black wall (glass covered with black paper on its reverse side) was presented in front of the king (Figure 4a, Videos S1 and S2). Vision was scored as follows: 0, the king did not stop and hit the wall; 0.5, the king stopped at the wall but kept walking and hit it; 1, the king stopped and avoided hitting the wall (Figure 4b). After 10 repetitions, the type of wall was changed and the assay was repeated a further 10 times for each king and wall type. The white paper was replaced after every assay.

2.6 | Statistical analysis

To examine the differences in brain size according to reproductive status, caste, or age, generalized linear mixed models (GLMMs) were used. Brain size was treated as a response variable and reproductive status (0 or 1), caste and age were treated as explanatory variables. Colony and caste were treated as random effects in analyses of differences in brain size according to reproductive status. Colony was treated as a random effect in analyses of differences in brain size according to caste or age. We performed multiple pairwise comparisons for each caste or age, and p-values were adjusted by Bonferroni correction.

Ordinal logistic models were used to examine the differences in visual scores of the male alates and mature primary kings. The visual score was treated as a response variable, and age and colony were treated as explanatory variables. To evaluate the colony effect, a likelihood ratio test comparing models with and without

the effects of colony was performed. All statistical analyses were performed using R v.3.6.3 (R Core Team, 2020). Graphs were prepared using Matplotlib v.3.0.3 in Python v.3.6.4 (Hunter, 2007; Rossum & Drake, 1995).

3 | RESULTS

3.1 | Differences in brain size among castes

Brain size was significantly larger in the reproductive castes (alates, nymphoids, ergatoids) than nonreproductive castes (workers and soldiers) (Figure 1b,c; Table S1). Alates had the largest brain size among all castes, and alate brains were significantly larger than those of workers (GLMM, likelihood ratio test followed by multiple pairwise comparisons of castes with Bonferroni correction, $\chi_1^2 = 42.97$,

$p < 0.001$ in males and $\chi_1^2 = 10.74$, $p < 0.05$ in females; Figure 1b,c) and soldiers ($\chi_1^2 = 14.99$, $p < 0.001$ in males and $\chi_1^2 = 14.26$, $p < 0.001$ in females; Figure 1b,c). The nymphoid brains were also significantly larger than those of workers ($\chi_1^2 = 16.26$, $p < 0.001$ in males and $\chi_1^2 = 16.04$, $p < 0.001$ in females; Figure 1b,c) and soldiers ($\chi_1^2 = 8.360$, $p < 0.05$ in males and $\chi_1^2 = 11.70$, $p < 0.001$ in females; Figure 1b,c). Similarly, ergatoids also had larger brains than workers ($\chi_1^2 = 18.10$, $p < 0.001$ in males and $\chi_1^2 = 11.50$, $p < 0.01$ in females; Figure 1b,c). Therefore, reproductive individuals had larger brains than nonreproductive individuals regardless of the differentiation pathway (worker or nymph pathway) (Figure 1a). Among reproductive castes, the size of the optic lobe of alates leaving the nest was significantly larger than that of ergatoid or nymphoids taking over reproduction in the nest ($p < 0.001$ in males and $p < 0.001$ in females, Figure S2).

Although soldiers differentiate from workers, there was no significant difference in brain size between these two castes ($\chi_1^2 = 1.311$,
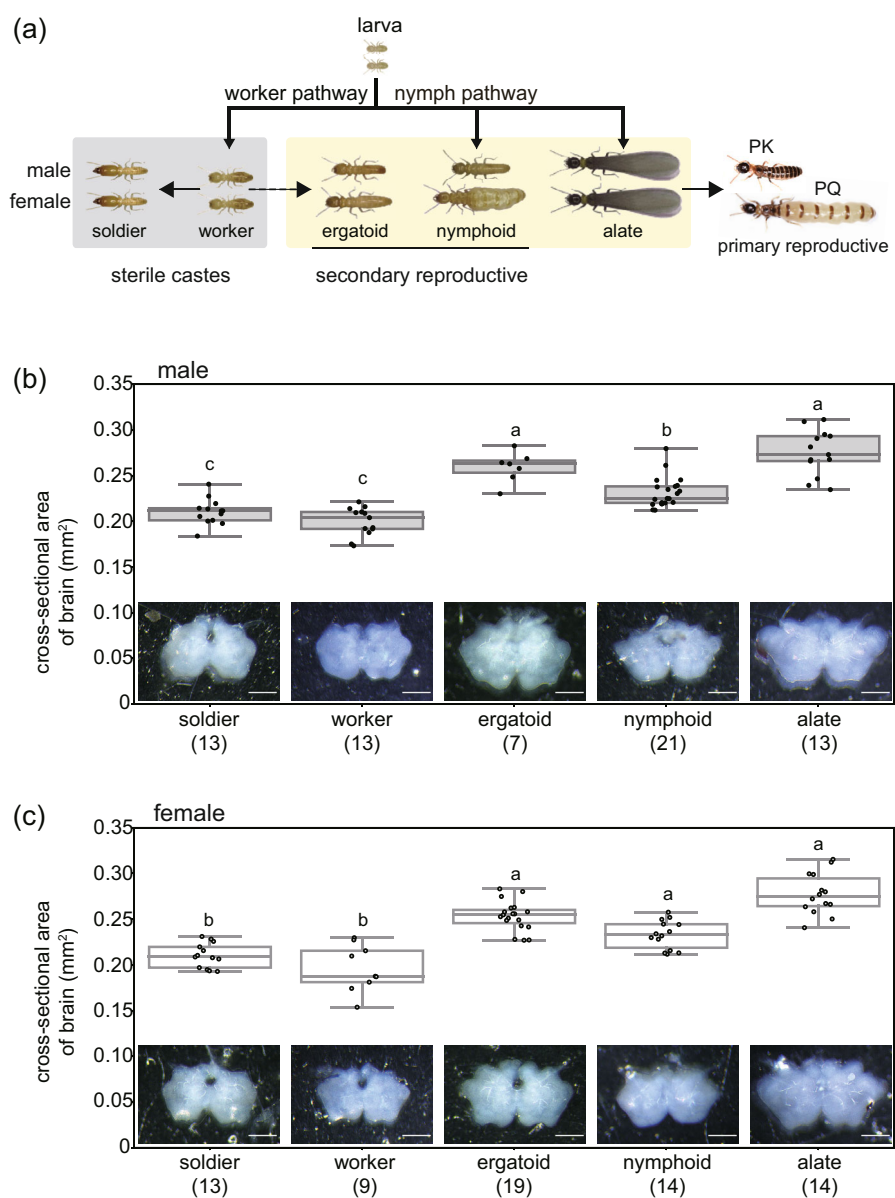

FIGURE 1   Comparison of brain morphology between castes. (a) Caste differentiation pathways of the termite, *Reticulitermes speratus*. Larvae differentiate along either the worker or nymph pathway. (b, c) Comparison of brain size between castes in males (b) and females (c). Dorsal images of dissected brains are shown below each graph. Sample size is shown in parentheses. Different letters indicate significant differences between castes within each sex ($p < 0.05$). Scale bars: 0.2 mm.

$p > 0.05$ in males and $\chi_1^2 = 2.952$, $p > 0.05$ in females; Figure 1b,c), indicating that molting alone cannot account for the differences in brain size. There were no significant differences in brain size between sexes in all castes (workers: $\chi_1^2 = 0.3639$, $p > 0.05$; soldiers: $\chi_1^2 = 0.0019$, $p > 0.05$; ergatoids: $\chi_1^2 = 0.5287$, $p > 0.05$; nymphoids: $\chi_1^2 = 0.3556$, $p > 0.05$; alates: $\chi_1^2 = 0.9344$, $p > 0.05$).

3.2 | Morphological changes in the brains of kings with aging

Whole-body micro-CT imaging demonstrated morphological differences in the nervous system between male alates and mature primary kings (Figure 2b–e); the brain and thoracic ganglion size decreased
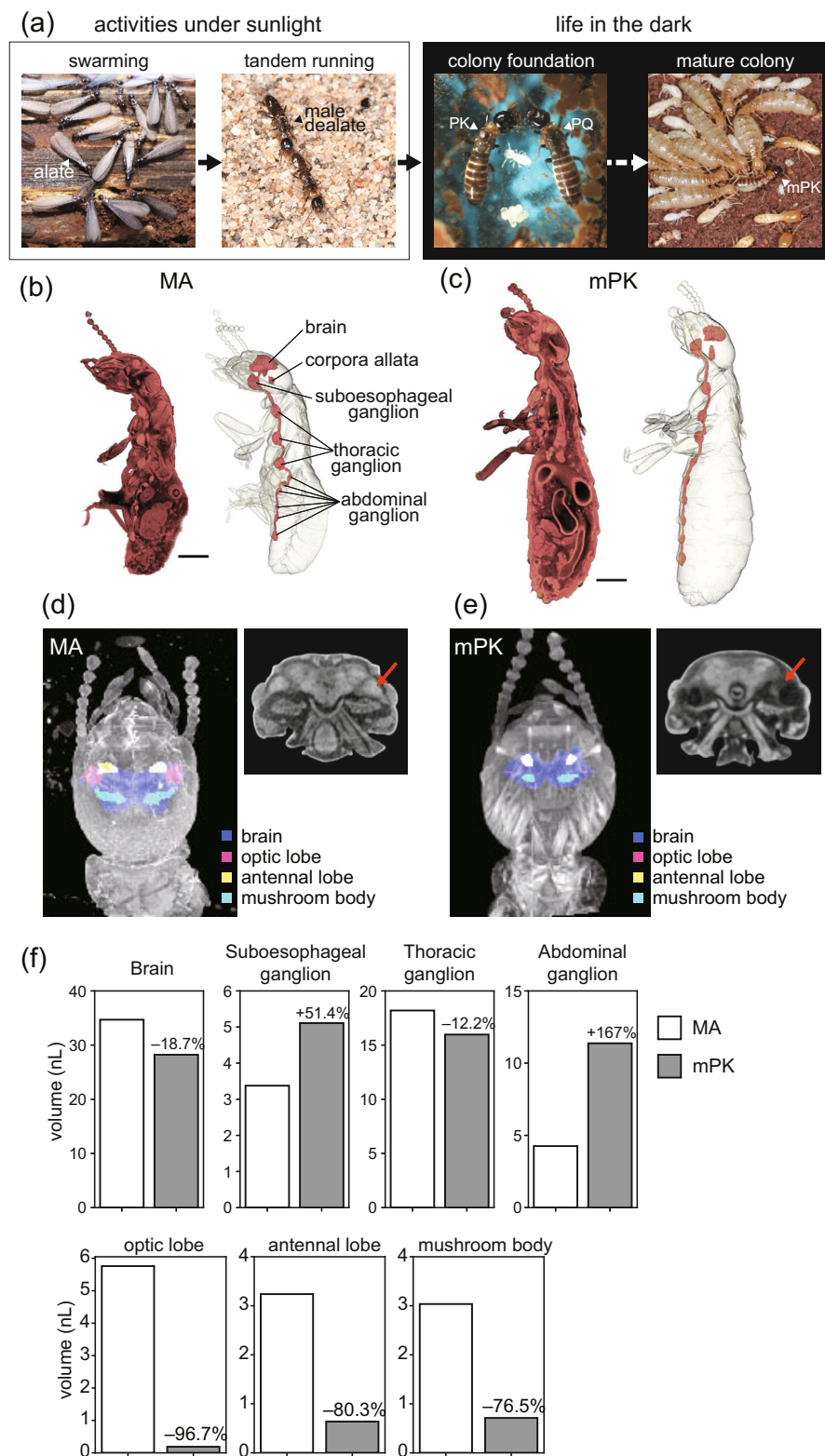

FIGURE 2 Visualization of changes in brain morphology with aging of primary kings using micro-CT. (a) Life of a primary king from swarming under sunlight to reproduction in the dark. (b, c) Whole-body micro-CT slice (left) and anatomical position of the nervous system (right) in MA (b) and mPK (c). Scale bars: 0.5 mm. (d, e) Three-dimensional dorsal views (left) and coronal slices (right) of MA (d) and mPK (e). Brain regions are color coded. The position of the optic lobe is indicated by red arrows. (f) Comparison of brain region volumes between MA and mPK. Size of mPK relative to MA is shown on each bar for mPK. PK, primary king; PQ, primary queen; mPK, mature primary king; MA, male alate.

FIGURE 3 Changes in brain size with aging of primary kings. (a) Comparison of whole brain size among different age classes from male alate (MA) to mature primary king (mPK). (b, c) Confocal microscopy images showing the nuclei of neurons (DAPI; cyan) and neuronal fibers (anti-Fas2; magenta) of the MA brain (b) and mPK brain (c). The upper and lower figures show ventral side views of whole brains and magnified views of optic lobes, respectively (scale bars: 50 μm). (d) Comparison of optic lobe size. (e) Comparison of brain sizes excluding optic lobe. Error bars indicate standard error of the mean. Sample size is shown at the top of each graph. Different letters indicate significant differences among age classes ($p < 0.05$).
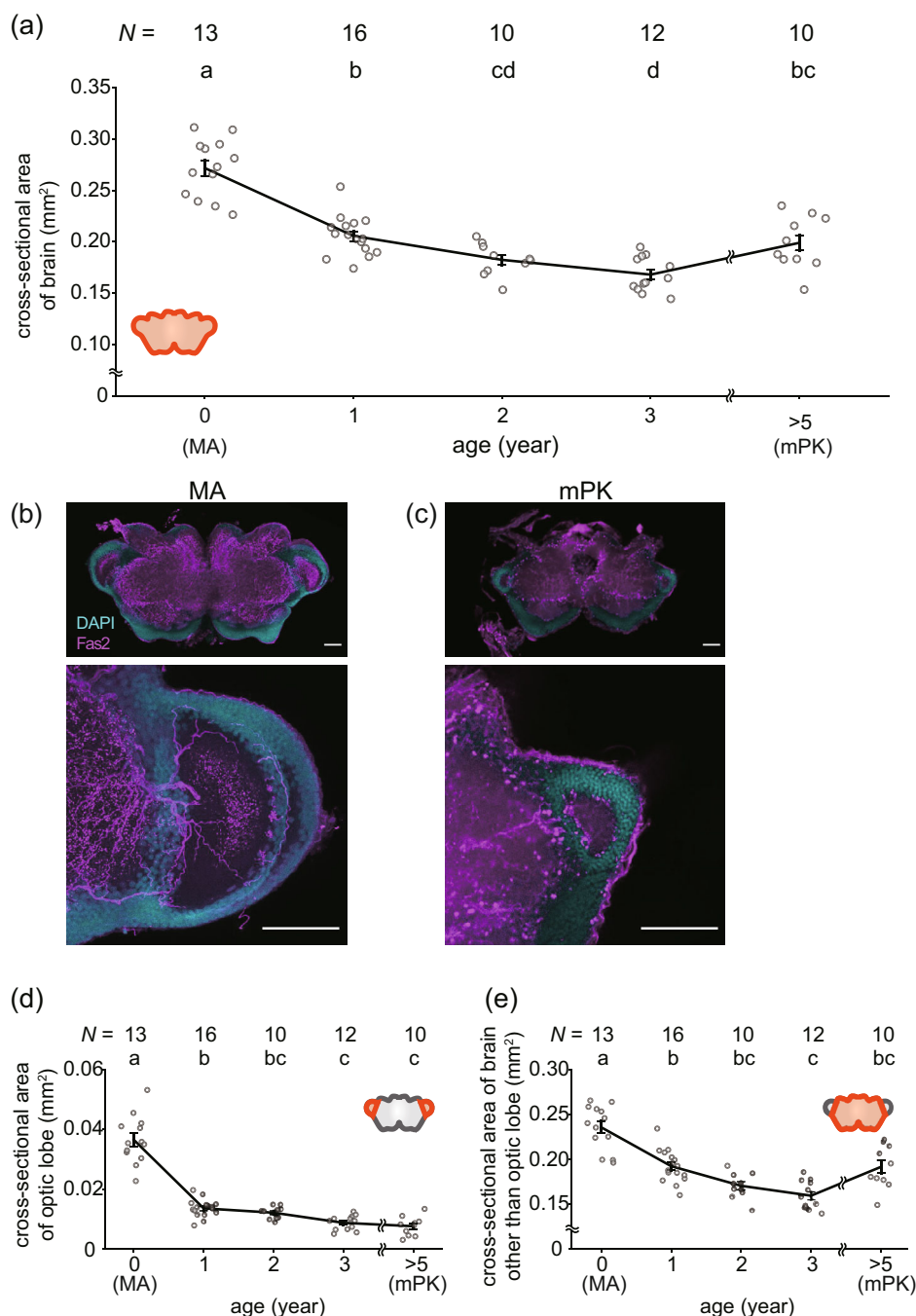
with age in mature primary kings, while suboesophageal ganglion and abdominal ganglion size increased (Figure 2f). Brain size was reduced overall, with a particularly marked reduction seen in the size of the optic lobe (Figure 2f). Next, we compared the dissected brains of kings aged 0–3 years with those of mature kings (aged ≥5 years) under a stereomicroscope. The brain size of older kings was significantly smaller compared with male alates (immediately after swarming) (GLMM, likelihood ratio test followed by multiple pairwise comparisons of age with Bonferroni correction, 1-year-old kings: $\chi_1^2 = 12.99$, $p < 0.01$; 2-year-old kings: $\chi_1^2 = 19.23$, $p < 0.001$; 3-year-old kings: $\chi_1^2 = 23.73$, $p < 0.001$; mature kings: $\chi_1^2 = 18.68$, $p < 0.001$, Figure 3a; Table S2).

Notably, the optic lobes were smaller in the mature kings compared with male alates (Figure 3b,c). Male alates had significantly larger optic lobes than older kings (1-year-old kings: $\chi_1^2 = 21.40$, $p < 0.001$; 2-year-old kings: $\chi_1^2 = 20.11$, $p < 0.001$; 3-year-old kings: $\chi_1^2 = 25.60$, $p < 0.001$; mature kings; $\chi_1^2 = 29.94$, $p < 0.001$, Figure 3d; Table S2). The cross-sectional area of the brain, except for the optic lobes, also shrank after colony foundation. Compared with male alates, the brain size without optic lobes was significantly smaller in the older kings (1-year-old kings: $\chi_1^2 = 9.83$, $p < 0.05$; 2-year-old kings: $\chi_1^2 = 17.32$, $p < 0.001$; 3-year-old kings: $\chi_1^2 = 21.24$, $p < 0.001$; mature kings: $\chi_1^2 = 12.26$, $p < 0.01$, Figure 3e).

(a)

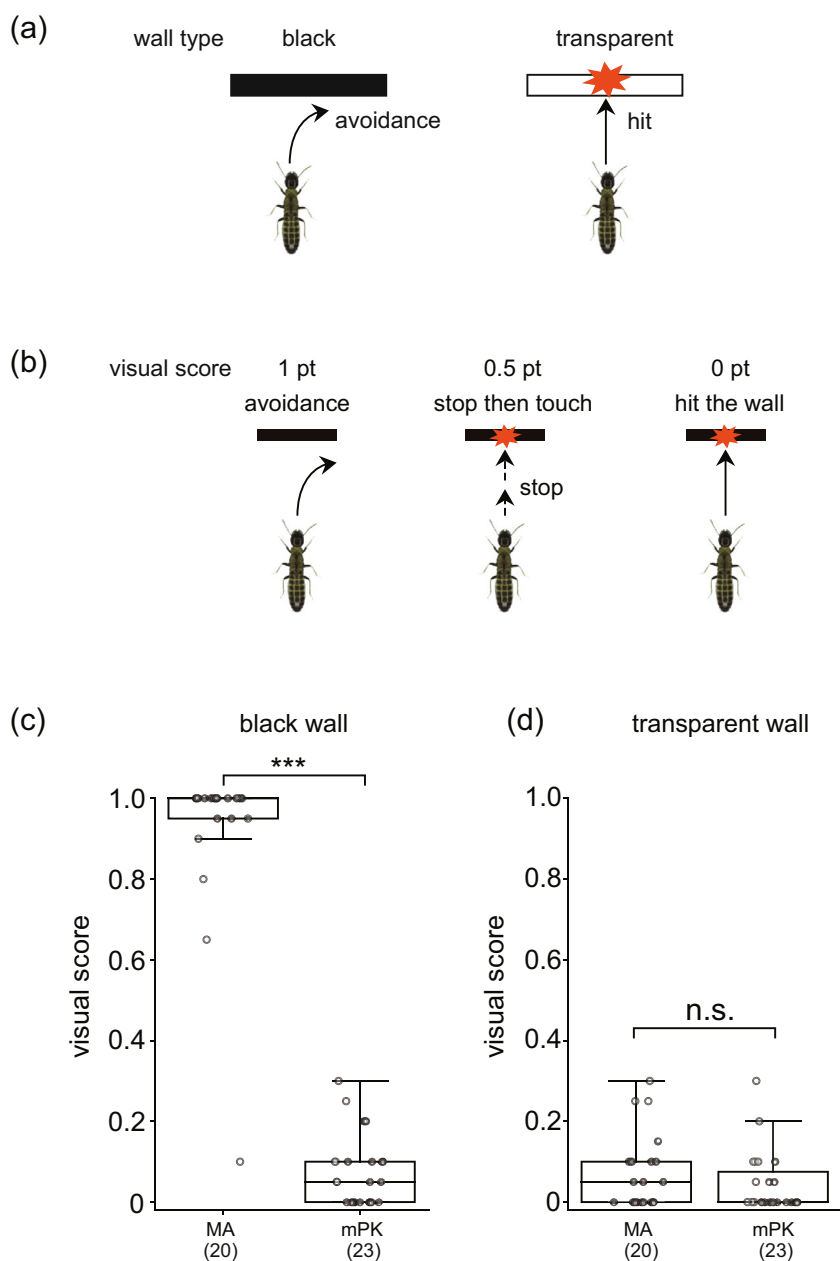(b)

(c) black wall

(d) transparent wall

FIGURE 4 Visual function test of kings of different age classes. (a) Schematic illustration of the visual function test. (b) Criteria for visual score. (c, d) Comparison of visual scores between MA and mPK against a black wall (c) and transparent wall (d). Sample size is shown in parentheses. ***, $p < 0.001$; n.s., not significant. MA, male alate; mPK, mature primary king.

3.3 | Visual function according to age

Male alates recognized and avoided hitting the black wall (Video S1), while mature primary kings hit the black wall. The visual score of mature kings was significantly lower than that of male alates when the black wall was presented (ordinal logistic model, likelihood ratio test, $\chi_1^2 = 14.76$, $p < 0.001$; Figure 4c). On the other hand, both male alates and mature kings failed to avoid the transparent wall ($\chi_1^2 = 0$, $p = 1$, Figure 4d; Video S2).

4 | DISCUSSION

Caste polymorphism and caste polyethism are prominent features of social insects. If brain differences underlie caste-specific behavior, the morphology of the brain could influence caste polymorphism. In non-reproductive castes, there was no significant difference in brain size (cerebral ganglion) between workers and soldiers (Figure 1b,c), consistent with earlier comparative studies of brain morphology in *Hodotermopsis sjostedti* (Ishikawa et al., 2008). In the present study, we demonstrated that brain morphology was associated with reproductive division of labor, where individuals of reproductive castes (primary and secondary reproductives) showed larger brain sizes than nonreproductive castes (workers and soldiers). Although both ergatoids (worker-derived neotenic reproductives) and soldiers develop from workers, ergatoids had 1.3-fold larger brains than soldiers. In most *Reticulitermes* termites, workers are ontogenetically totipotent immatures with the potential to become neotenic reproductives, even though they deviated from the reproductive pathway (nymph pathway) (Figure 1a). We found that ergatoids have brains as large as

those of nymphoids (nymph-derived neotenic reproductives), indicating that reproductive totipotency of workers must involve brain plasticity as well as the developmental plasticity of reproductive organs. Among the three types of reproductives (ergatoids, nymphoids, and alates), the optic lobe size of alates was significantly larger than the other two. This result, in line with previous findings in *Zootermopsis* (Sean O'Donnell et al., 2021), can be attributed to the necessity for alates to possess vision for activities outside the nest, while ergatoids and nymphoids do not rely on vision to inherit reproduction within the nest.

Founding reproductives (primary kings and queens) show marked changes in behavior after colony foundation (Figure 2a). We found that the brain of the primary king changed in shape and shrunk by up to 39% with aging (Figure 3a), with the optic lobe shrinking by 76% (Figure 3d). In addition, brain shrinkage with aging was also found in primary queens (Figure S3). Kings with reduced optic lobes showed decreased visual responses (Figure 4c). These results indicated that both caste-specific differences and age-related plastic changes in brain morphology were tightly coupled with differences in behavior among termite colony members, which may underlie the division of labor. Adult-stage atrophy of an organ that is no longer used occurs in a wide variety of insects. Flight muscle degeneration after dispersal is well known in insects, including aphids (Hemiptera) (Johnson, 1953), crickets (Orthoptera) (Tanaka, 1991), and mosquitoes (Diptera) (Hocking, 1952), which allows re-allocation of energy and protein for ovarian development and oocyte provision. Flight muscle histolysis is observed in founding pairs of termites after colony foundation, and muscle protein is recycled for reproduction and brood provisioning (Inagaki et al., 2020; Myles, 1988). Atrophy of the optic lobes and consequent loss of visual function in termite primary kings are likely to be functional adaptive changes, as they no longer use vision after colony foundation. It is known that maintaining neural tissue and information processing required for maintaining visual function incurs a significant energy cost (Niven & Laughlin, 2008). For instance, photoreceptors in adult blowflies consume approximately 10% of the total energy (Laughlin et al., 1998). Degeneration of the optic lobes allows kings to save maintenance costs and re-allocate energy and protein to other necessary functions.

Interestingly, the brain size of the field-collected mature kings was larger than that of the younger kings, except for the optic lobe (Figure 3e). In *R. speratus*, the asexual queen succession (AQS) system leads to an increasing number of neotenic queens through parthenogenesis as the colony grows, resulting in mature colonies that frequently possess a significant population of physogastric queens, sometimes exceeding 200 in number. In this context, the primary king assumes a crucial role in identifying queens in need of copulation and engaging in mating with multiple queens. Such a task requires specific sensory and cognitive abilities associated with mating behavior. Moreover, due to the multiple-site nesting behavior of *Reticulitermes* termites, the location of the royal chamber moves from one piece of wood to another in large mature colonies. Consequently, the capacity to navigate and relocate to the next royal chamber location is essential. In future studies, understanding the precise location and

functional implications of the enlarged region can provide deeper insights into the adaptive significance of the observed brain size differences.

Termites (Isoptera) and social Hymenoptera (ants and some bees and wasps) show striking convergence in terms of social organization, characterized by reproductive division of labor where few individuals within a colony reproduce; the majority of colony members forgo their own reproduction. Despite their apparent similarities in terms of social organization, the social lives of both groups evolved independently and differ in fundamental ways, including in terms of developmental processes (hemimataboly in Isoptera and holometaboly in Hymenoptera). In termites, workers and larvae still show great flexibility in their caste options, whereas in social Hymenoptera, reproductive division of labor occurs among adults. Nevertheless, these phylogenetically divergent insects share the common feature of caste-related differences in brain morphology. In social Hymenoptera, the brain structure of adults (queens and workers) can change after cuticular sclerotization in association with behavioral changes (Fahrbach et al., 1998; Gronenberg et al., 1996; Kühn-Bühlmann & Wehner, 2006; Sean O'Donnell et al., 2004). This convergence provides significant sociobiological insight showing that variation of brain morphology among colony members is important for establishing division of labor.

In conclusion, our comparative study of brain morphology showed that brain size is associated with the reproductive division of labor and age-related behavioral changes in termites. Here, we focused on the total brain size, but as the brain is composed of functionally independent units, it is also necessary to examine the relationships between the sizes of individual compartments and their functional demands. Indeed, we showed that the size of the optic lobe, which is one of the brain compartments, is correlated with the requirement for visual function in primary kings. In addition, previous work demonstrated that the mandibular motor neurons that regulate mandibular movements are significantly larger in soldiers than workers in other termite species, including *H. sjostedti*, *Mastotermes darwiniensis*, *Neotermes koshunensis*, *Coptotermes formosanus*, and *Nasutitermes takasagoensis* (Ishikawa et al., 2008). Furthermore, in social hymenopterans, the calyces, which are involved in higher order information processing, such as sensory integration, learning, and memory, are smaller in younger nurse bees than older and more experienced foragers among honey bee workers (Durst et al., 1994; Withers et al., 1993). These observations suggest that brain compartment size reflects the functional requirements for individual castes in social insects. Therefore, further studies are required to investigate the detailed relations between individual brain compartment sizes and task performance, which will provide novel insight into how the morphological specialization of the brain underlies the behavioral differentiation responsible for the development of social behavior in social insects.

AUTHOR CONTRIBUTIONS

Tomoki Ishibashi: conceptualization, data curation, formal analysis, investigation, visualization, writing—original draft and writing—review and editing; A.S.M. Waliullah: data curation, formal analysis,

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.