Coordination between binocular field and spontaneous self-motion specifies the efficiency of planarians’ photo-response orientation behavior

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Eyes show remarkable diversity in morphology among creatures. However, little is known about how morphological traits of eyes affect behaviors. Here, we investigate the mechanisms responsible for the establishment of efficient photo-response orientation behavior using the planarian *Dugesia japonica* as a model. Our behavioral assays reveal the functional angle of the visual field and show that the binocular field formed by paired eyes in *D. japonica* has an impact on the accurate recognition of the direction of a light source. Furthermore, we find that the binocular field in coordination with spontaneous wigwag self-motion of the head specifies the efficiency of photo-responsive evasive behavior in planarians. Our findings suggest that the linkage between the architecture of the sensory organs and spontaneous self-motion is a platform that serves for efficient and adaptive outcomes of planarian and potentially other animal behaviors.

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Behavior based on visual cues is common in the animal kingdom and is achieved by a process that comprises receiving optical signals through visual neurons, integrating them in the brain, and making a decision about the appropriate response. The morphology of eyes is diverse among creatures in different ecological niches and during evolution, suggesting that the morphological traits of eyes adapt to match animals' visual behaviors. In particular, the photo-response orientation behavior of simple animals is one of the strongest ecological factors directly and indirectly contributing to biomass migration and reproduction. However, the mechanisms responsible for the accuracy of photo-perception, those underlying the architectural features of the eyes, and the internal processing mechanisms contributing to efficient directional movements remain largely unknown although they are highly relevant to many questions regarding the physiology and evolution of visual properties.

The planarian *Dugesia japonica* (phylum Platyhelminthes) belongs to an evolutionarily basal group of animals possessing a pair of simple eyes and a brain, and it exhibits robust evasive behavior in response to light (known as negative phototaxis). Planarian eyes, which share genetic similarities with vertebrate eyes, are composed of two cell types: pigment cells that are arranged into a semilunar eyecup, and visual bipolar neurons that consist of cell bodies, rhabdomeres, and axons. These axons form a hemidecussation and project directly to the brain. It was suggested that although the planarian eye is nondioptric and cannot recognize images, the visual neurons respond to light from only one side due to shading by a pigment cup.

The planarian brain consists of several structural domains that are defined by a complex set of genes and neural networks. The combination of behavioral assays quantifying many parameters and RNAi to knockdown neuron-specific genes has demonstrated that neural networks in planarians strictly regulate distinct behaviors via the corresponding sensory organs and brain neurons in response to specific environmental stimuli. For example, using region-specific RNAi (Readyknock) of a neuron-specific gene, 

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(synaptosome-associated protein of 25 kDa), it was shown that planarians receive light signals from visual neurons, and that these signals may be processed in the brain. It was also reported that GABAergic neurons in the brain might be involved in the information-processing of the light signals transmitted from visual neurons, as revealed by RNAi experiments to knockdown the 

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gene, which encodes a rate-limiting enzyme for the synthesis of GABA. In addition to behaviors performed in response to a single environmental stimulus, planarians exhibit decision-making behaviors in response to simultaneously provided environmental stimuli, indicating that planarians decide upon behavioral strategies by integrating multiple external signals in their brain. However, the mechanisms underlying robust photo-response orientation behavior have not yet been elucidated.

More recent work showed that planarians sway their heads horizontally, and this swaying motion (called the wigwag self-motion) is sustained even after removal of the head from the rest of the body, in the absence of any environmental cues or spatial information, indicating that this motion occurs spontaneously independent of brain activity. Interestingly, although planarians have a behavioral trait of proceeding along a wall, the angle and the frequency of the wigwag self-motion determine the probability of staying near the wall or leaving it even in the absence of any environmental cues and are optimized for sustaining the proper distance from the wall, suggesting that the brain-independent spontaneous self-motion in planarians plays crucial roles in some adaptive behaviors, such as hiding in a concave space. However, the relationship between spontaneous self-motion and environmental stimulus-associated directional movement behavior in planarians has not yet been investigated.

Therefore, planarians, with their simple eyes, brain, robust behavioral properties, and evolutionary position, provide unique models for investigating the pivotal functions of the perception of the light direction in bilaterians. Here, using rigorous photo-response orientation behavior assays with the planarian *D. japonica* we obtained insight into how the architecture of the simple eyes enables precise photodetection and demonstrated that *D. japonica* possesses an anterior binocular field, which in association with spontaneous self-motion contributes to establishing efficient and adaptive behavioral outcomes.

**Results**

**D. japonica has an anterior binocular field.** When we stained the cell bodies of visual neurons, the dendrites and axons of visual neurons, and pigment cells of planarian eyes (Fig. 1a) using specific markers, the structure of the planarian visual system was visualized (Fig. 1b, c). The monocular visual field (\(\alpha\)) of a planarian eye was greater than 170° (172.6 ± 2.1°), and was slightly tilted anteriorly relative to the body’s lengthwise axis in the horizontal plane, as revealed by three-dimensional reconstructions of the pigment cup and rhabdomere outline (Fig. 1d). The eyes are oriented obliquely relative to the anterior–posterior axis at an angle (\(\beta\)), which provides a binocular field of approximately 40° (37.8 ± 4.1°, mean ± standard deviation (SD), \(n = 28\)) on the anterior side (Fig. 1e).

In order to investigate the accuracy of the recognition of the light direction by *D. japonica*, we developed a photo-response orientation assay specializing for analyzing the orientation of movement relative to the incident light, named the orientation assay with one light source (OA1L), and examined the trajectories of the movement of illuminated animals. Animals strongly avoided light (Fig. 1f). However, OA1L revealed that animals did not move straight away from the light source; trajectories fell into two clusters that were twin-tailed. The difference between the angles of the escape trajectories toward the left versus toward the right of *D. japonica* was 50.6 ± 3.7° (Fig. 1g, Supplementary Fig. 1), which was consistent with the geometric arrangement of the blind field (51.5 ± 13.7° = 2(180° – \(\alpha + \beta\))) on the posterior side (Fig. 1h), indicating that the direction of movement away from the light source strongly correlated with the eye architecture in planarians. These data indicate that this novel assay is sufficiently sensitive to enable measurements of the angle of the visual field, and also that the morphological features of the eyes affect photo-response orientation behavior in planarians.

**Planarians evaluate the difference of the eyes’ input.** To investigate the interaction of optical signals received by the left and right eyes, we inhibited the activity of the visual neurons using an inhibitor of ionic influx (lidocaine) to alter the perception of light intensity between the two eyes. Control planarians administered lidocaine at a position adjacent to the visual neurons and showed spontaneous self-motion relative to the incident light, named the orientation assay with one light source (OA1L), and examined the trajectories of the movement of illuminated animals. Animals strongly avoided light (Fig. 1f). However, OA1L revealed that animals did not move straight away from the light source; trajectories fell into two clusters that were twin-tailed. The difference between the angles of the escape trajectories toward the left versus toward the right of *D. japonica* was 50.6 ± 3.7° (Fig. 1g, Supplementary Fig. 1), which was consistent with the geometric arrangement of the blind field (51.5 ± 13.7° = 2(180° – \(\alpha + \beta\))) on the posterior side (Fig. 1h), indicating that the direction of movement away from the light source strongly correlated with the eye architecture in planarians. These data indicate that this novel assay is sufficiently sensitive to enable measurements of the angle of the visual field, and also that the morphological features of the eyes affect photo-response orientation behavior in planarians.
planarians with removal of both eyecups was dramatically perturbed: they showed random directional trajectories, although their visual neurons and locomotor activity were intact (Fig. 2b, Supplementary Fig. 2a, b). The trajectory of left-eyecup-removed planarians, in which the input signal intensity of the left eye would be strengthened, was significantly biased in the right direction, which was consistent with the behavior of planarians with lidocaine anesthesia of the right eye. Conversely, the trajectory of right-eyecup-removed planarians was significantly biased toward the left. When we treated the eyecup-less right eye with lidocaine to decrease the signal input, the orientation of movement reverted toward normal, with less-biased orientation toward the left.

Furthermore, menashi mutant planarians, which naturally lack eyecups (although their visual neurons are intact), did not show normal photo-response orientation behavior (Supplementary Fig. 2c, d). This result is consistent with the behavior of tryptophan hydroxylase (tph)(RNAi) planarians, which lack melanin pigmentation in the eyecup. The above data indicated that planarians recognized the light direction by assessing the laterality of the signal inputs received by the two eyes, and the input value of the light in each eye is approximated by the eye's...
were perturbed (Both), indicating that the lidocaine treatment efficiently inhibits the activity of visual neurons. Trajectories of the group administered lidocaine to both eyes were perturbed (Both), indicating that the lidocaine treatment efficiently inhibits the activity of visual neurons. Trajectories of the group administered lidocaine to the left eye were biased toward the left (Left), whereas a greater light angle caused a weaker correlation (blue line). Since the input signal value of planarian eyes is approximated by the surface area of the eye receiving light, as represented by a sine function\(^{10,19}\), the light inputs of the left and right eyes are obtained as \(L(\theta t)\) and \(R(\theta t)\), respectively (Supplementary Fig. 3a, b). The minimum difference in the signal input value (response threshold) corresponding to the light angle of 130°, which is the borderline light angle for inducing a body movement reaction, was calculated by the subtractive formula \([L(\theta t) - R(\theta t)]\) (Fig. 3b). The result obtained indicated that planarians are induced to turn their bodies away from the light source at a difference of more than 0.5 (maximum input assumed to be 1.0) between the light inputs received by the two eyes. Our results indicate that the variation of trajectories caused by the arrangement of the pigment eyecup shield inevitably creates a blind area and the response threshold (Figs. 1 and 3a, b). Therefore, to further evaluate the parameters of the response threshold, we performed another photo-response orientation behavior assay, named the “orientation assay with two light sources (OA2L)” to exclude a blind area in planarians. Trajectories showed bow-tie shaped composite plots (Supplementary Fig. 3c), which indicated that the animals escaped in paths perpendicular to both of the two light sources without a blind field on the posterior end, and supported the notion that planarian recognize the light direction by determining the input difference between the two eyes. Simulation analysis with different values of the response threshold showed that the response threshold of 0.5 resulted in trajectories that accorded with the actual trajectories, in contrast to those at a response threshold of 0.6 (Supplementary Fig. 3d, e). This result indicated that the response threshold affects the photo-response orientation behavior in planarians and the value estimated by performing OA2L is consistent with the results of the regression analysis (Fig. 3a, b).

When we tested OA1L with \(D.\ japonica\) possessing a super-numerary eye\(^{16}\), the planarians showed normal evasive behavior (Supplementary Fig. 4a, b), suggesting that the brain to which visual neurons project, not the eyes, may evaluate the disparity between the signal input and the response threshold. To identify the brain neural networks that define the response threshold for achieving light-avoidance behavior, we performed OA1L using planarians treated with RNAi of neurotransmission-related genes (Fig. 3c). Although both the synaptotagmin (syt) and \(\text{snap}25\) genes are widely expressed in the brain (Fig. 3d), syt(RNAi) planarians and \(\text{snap}25\)(RNAi) planarians showed slightly different photo-response orientation behavior (Fig. 3c). The syt(RNAi) planarians could not recognize the direction of the light and showed random movements, whereas \(\text{snap}25\)(RNAi) planarians did not move toward the light source; instead, \(\text{snap}25\)(RNAi) planarians functioned properly. Rose plots in the lower panels show the histogram of the orientation (angle) distribution of movement of eyecup-removed planarians, determined as the average for individuals used for this assay.

Values were less than 0.005 in the lidocaine-treated and eyecup-removed planarians, but not in the right eyecup-removed and lidocaine-treated group, relative to the control. Arrows indicate rays of light. \(n = 22\text{–}32\)

Binarized response via the GABAergic neural network. To examine the coefficient of determination for the induction of body turning based on the value of the difference between left and right inputs, we measured the angle of a planarian’s turn away from the direction of the light (\(\theta t\)), and then performed regression analyses using data obtained at different angles of light. Plots of the turning angles of the body in response to light irradiated from various different angles indicated that planarian showed avoidance behavior to light received from an angle of less than 130° relative to the anterior–posterior body axis (red dots) (Fig. 3a). In contrast, when planarians received light from an angle of more than 130° (black dots), the turning angles were random. A regression analysis showed a correlation when using the turning angle with respect to a light angle of 130° or less (red line), whereas a greater light angle caused a weaker correlation (blue line). Since the input signal value of planarian eyes is approximated by the surface area of the eye receiving light, as represented by a sine function\(^{10,19}\), the light inputs of the left and right eyes are obtained as \(L(\theta t)\) and \(R(\theta t)\), respectively (Supplementary Fig. 3a, b). The minimum difference in the signal input value (response threshold) corresponding to the light angle of 130°, which is the borderline light angle for inducing a body movement reaction, was calculated by the subtractive formula \([L(\theta t) - R(\theta t)]\) (Fig. 3b). The result obtained indicated that planarians are induced to turn their bodies away from the light source at a difference of more than 0.5 (maximum input assumed to be 1.0) between the light inputs received by the two eyes. Our results indicate that the variation of trajectories caused by the arrangement of the pigment eyecup shield inevitably creates a blind area and the response threshold (Figs. 1 and 3a, b). Therefore, to further evaluate the parameters of the response threshold, we performed another photo-response orientation behavior assay, named the “orientation assay with two light sources (OA2L)” to exclude a blind area in planarians. Trajectories showed bow-tie shaped composite plots (Supplementary Fig. 3c), which indicated that the animals escaped in paths perpendicular to both of the two light sources without a blind field on the posterior end, and supported the notion that planarian recognize the light direction by determining the input difference between the two eyes. Simulation analysis with different values of the response threshold showed that the response threshold of 0.5 resulted in trajectories that accorded with the actual trajectories, in contrast to those at a response threshold of 0.6 (Supplementary Fig. 3d, e). This result indicated that the response threshold affects the photo-response orientation behavior in planarians and the value estimated by performing OA2L is consistent with the results of the regression analysis (Fig. 3a, b).

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caused broadened escape trajectories (Fig. 3c). To identify the neuronal subtype recognizing the laterality between the left and right inputs, we used RNAi planarians of genes encoding rate-limiting enzymes for neurotransmitter synthesis. RNAi planarians of choline acetyltransferase (chat) showed random trajectories, which were similar to the trajectories of syt(RNAi) planarians, whereas RNAi of the tyramine β-hydroxylase (tbh) and tyrosine hydroxylase (th) genes did not affect the photo-response orientation behavior. RNAi of glutamic acid decarboxylase (gad) caused broad trajectories, although gad(RNAi) planarians did not move toward the light source, a property that was the same as that of snap25(RNAi) planarians. When double staining analysis was performed, we found that GABAergic neurons co-express the syt and snap25 genes, but do not co-express other genes encoding...
indicated that a single 100-ms period for comparing the difference of left and right input, but is corresponded to a light angle of ±130° when the difference in the signal input value between the L and R eyes exceeded the response threshold to induce precise turning (vertical black solid line at 130°). b Input value to the left and right eyes (top) and subtractive difference in the left and right eyes (bottom) with a change in the light direction. The response threshold calculated using the subtractive formula \( |L(\theta) - R(\theta)| \) corresponding to the light angle of ±130° is 0.5 (red shaded) between the light input received by the two eyes. Maximum input is assumed to be 1.0. c The trajectories of control (GFP-dsRNA injected), syt(RNAi), gta(RNAi), tbt(RNAi), and th(RNAi) planarians and their histograms of the orientation (angle) distribution indicated by rose plots. Percentage of oriented movements in the range of four for every 90° interval on the rose plots. d Fluorescence immunohistochemistry of GAD proteins combined with fluorescence in situ hybridization of syt, snap25, chat, tbt, th genes. Percentages (mean ± SEM) of GAD-positive neurons co-expressing neurotransmitter-related genes are shown in the lower left corners. Scale bars, 50 µm. Inset scale bars, 5 µm. e Expression patterns of the planarian gad, GABAA-RBa, and GABAB-Ra genes in the eyes. Arrows indicate pigment eyecups. Scale bar, 20 µm. f The trajectories of control (GFP-dsRNA injected), GABAA-RBa(RNAi), and GABAB-Ra(RNAi) planarians and their histograms of the orientation (angle) distribution indicated by rose plots. Arrows indicate rays of light.
(Fig. 5g), and the difference in the angle between the left and right twin-tailed paths of *S. mediterranea* was markedly larger than that of *D. japonica* (Fig. 5h). The escape value of *S. mediterranea* was lower than that of *D. japonica* in OA1L (Fig. 5i). These results indicate that the angle of the binocular field in planarians is an important bio-architectural feature that enables precise recognition of the direction of incident light.

Spontaneous wigwag self-motion breaks illumination symmetry. Planarians recognize asymmetry in inputs to the two eyes when they are irradiated with light from an angle of approximately 15°–130° or an angle of approximately −15° to −130° relative to the anterior–posterior axis, corresponding to a difference in the signal input value of 0.5 or more (Fig. 3a, b). In addition, the value of the response threshold implied the existence of angles of illumination for which the light direction cannot be distinguished on the anterior side (front blind-like spot), since in the front blind-like spot the inputs to the two eyes are equal. In addition, the angle of the front blind-like spot differed depending on the angle of the binocular field (Fig. 5a). However, a regression analysis between the turn angle and the angle of incident light from −15° to 15° indicated that planarians clearly escaped from the light (Fig. 3a); therefore, we subsequently investigated the mechanisms by which planarians recognize the direction of light irradiated from the anterior end (from an angle of approximately −15° to 15°).

Although planarians only glide forward using cilia, in addition they constantly spontaneously sway (perform wigwag self-motion) of their head independent of brain function during movement (Fig. 6a). The distribution of the wigwag angle follows a normal distribution, and the SD of the change of head-angle during wigwag was ±18.7° (between inflection points indicated by SD) (Fig. 6b). The frequency of wigwag self-motions...
A binocular field of 40° is optimally tuned to achieve maximal efficiency of photo-response orientation behavior. a Subtractive difference of inputs from the two eyes with a change in the light direction and binocular field parameters (bf) of 0° (green), 40° (red), and 80° (blue) (upper panel). The value of the response threshold caused an angle that could not be distinguished from the light direction on the anterior side (front blind-like spot), and the angle of front blind-like spot differed depending on the angle of the binocular field. Filled colored areas of the graph indicate the angle greater than the response threshold. Lower panel shows higher magnification of the front blind-like spot. Double-headed arrows indicate the angle of the front blind-like spot for different angles of binocular field of 0°, 40°, and 80°. b Simulated trajectories of photo-response orientation behavior in planarians with bf of 0°, 40°, and 80°. n = 60. Arrows indicate rays of light. c The “escape value” was defined as the relative distance from the start line from which ideal planarians showed an escape value of 1, moving straight away from a light source. d The simulated escape value was plotted versus different angles of the binocular field. The actual angle of the binocular field of *D. japonica* is shown by a dashed line. n = 1000. (e) Head and eyes of *S. mediterranea* visualized by immunohistochemistry of TPH (magenta) and arrestin (green). Scale bars, 100 μm. f Violin plot showing the comparison of the angle of the binocular field between *D. japonica* and *S. mediterranea*. n = 21. g Actual trajectories of *S. mediterranea* in OAIL showing broader twin-tailed trajectories. Arrows indicate rays of light, n = 16. Scale bar: 1 cm. h Difference in the twin-tail angles of *D. japonica* and *S. mediterranea*. i Escape values of actual trajectories of *D. japonica* and *S. mediterranea*. Horizontal solid lines indicate median value, and horizontal dashed lines indicate average value in violin plots in f-h, and i, respectively. ***p < 0.005

follows a log-normal distribution, and the average frequency of wigwag self-motions was once per 0.77 s (Fig. 6c). In order to investigate the role of wigwag self-motion in the photo-response orientation behavior, we performed the simulation analysis without the wigwag parameter. The result showed that most animals succeeded in escaping from the light source with composite twin-tail-shaped trajectories, with an angle of 58.1° between the twin-tailed paths (Fig. 6d), which was consistent with the actual observed trajectories (Fig. 1e, f). However, the simulation showed that some individuals moved toward the light (Fig. 6d). Consistent with this simulation result that planarians cannot distinguish between light from the anterior and posterior sides, we found that planarians occasionally turned their bodies by approximately 180°, even when they were exposed to light from the posterior side (±150 to ±180 relative to the body’s anteroposterior axis), although in almost all cases planarians make direction corrections of only small angles (black dots) (Fig. 3a). These results indicated that the mechanism employed by planarians to avoid going toward light does not depend solely on the binocular system; in addition, wigwag self-motion is required. When the angle of the front blind-like spot is greater than that of the wigwag self-motion, planarians move toward the light source until they sense the laterality of the light input as a result of the wigwag self-motion. These differences between the angle of the front blind-like spot and the angle of the wigwag self-motion are consistent with the observation that some individuals moved toward the light source in the absence of a binocular field (bf = 0°) or in the presence of a wide-angle binocular field (bf = 80°) (Fig. 5b). The restriction of the wigwag self-motion by a lateral wall caused planarians to move toward the light source for
a longer time, whereas planarians in an open space promptly changed their orientation (Fig. 6e, f). This result indicates that this consistent escape behavior of planarians is due to the difference in the input signal between the two eyes becoming as small as the response threshold when light comes from an anterior direction (front blind-like spot: from an angle of approximately $-15^\circ$ to $15^\circ$) as when it comes from a posterior direction. In other words, planarians without wigwag self-motion cannot distinguish between light from the anterior and posterior sides. When the angle of the front blind-like spot is greater than that of wigwag self-motion, planarians lose the ability to detect the difference between the light inputs of the two eyes, and they may go toward the light source until they sense the laterality of the light input as a result of wigwag self-motion. The angle of the front blind-like spot ($\pm 15^\circ$, total $30^\circ$) of planarians possessing a binocular field of $40^\circ$ is less than the value of SD ($\pm 18.7^\circ$, total $37.4^\circ$) of the wigwag angle (Fig. 6g). In contrast, the angle of the front blind-like spot of planarians possessing either a $0^\circ$ or $80^\circ$ binocular field is greater than the value of SD of the wigwag angle. Thus, stochastically, 42% wigwag self-motion is larger than the front blind-like spot calculated using the standard normal distribution (see Methods). Since the frequency of wigwag motion is once every 0.7 s, planarians can turn away from the light within approximately 1.6 s even if planarians are facing the light source.
light source. In contrast, the probability that planarians possessing either a 0° or 80° binocular field would sway at larger angles than the front blind-like spot would be low, and consequently they would take more time to turn away from the light source.

Furthermore, we determined the optimal combination between the angles of the binocular field and wigwag angles for achieving efficient light-avoidive behavior, and the results obtained were consistent with the actual values found in D. japonica (Supplementary Fig. 5a). Moreover, S. mediterranea, which possesses a wider binocular field, showed wider wigwag angles, indicating that the binocular field is correlated with spontaneous wigwag self-motion (Supplementary Fig. 5b, c).

Discussion
Photo-response orientation behavior in planarians has been reported for more than a century, and the working principles have been explained, but with some missing details. Here, we developed novel planarian photo-response orientation behavior assays that enabled us to examine the functional angle of the visual field, revealing the angle of the binocular field on the anterior side and a blind field on the posterior side in planarians. These assays revealed the mechanisms underlying the recognition of light directions, providing criteria for evaluating the precision of the recognition of light directions in planarians. We revealed the significance of the binocular field using a simulation analysis and comparative behavioral analyses with different angles of the binocular field, and revealed that simulated planarians that lacked a binocular field (bf = 0°) or that had a broad binocular field (bf = 80°) were predicted to have reduced precision of recognition of the light orientation. Our results show that the slight angle between the two eyes of planarians represented a functional trait for producing a binocular field that enables more precise recognition of the direction of incident light. It was previously stated that the diagonal trajectory of escape-behavior relative to a ray of light might be due to the eyes’ obliqueness decreasing the fidelity of the recognition of light directions in planarians. In contrast, the results of this study indicate that the binocular field was acquired for establishing the robustness of the laterality of input signals between the eyes. Intriguingly, the eyes of multicellular planarian species, such as Polycelis sapporo, are also positioned at an angle relative to the anterior–posterior axis, and vertebrates show interspecific variations in the angles of their eyes, suggesting that the architectural basis for the recognition of light directions is conserved across diverse species of animals. A binocular field is involved in stereoscopic vision in vertebrates and in detecting moving targets in insects, but planarians possess a novel function of the binocular field, namely, forming a blind-like spot in which planarians cannot distinguish the difference between left and right light inputs.

Although the architecture of the eyes is diverse among animals, animals as evolutionarily early as multicellular organisms without any nervous systems, as well as unicellular organisms, had acquired screening pigments and a photosensory capacity in a single cell, indicating that the detection of the orientation of light direction by shielding against light coming from the opposite side represents an evolutionarily fundamental function of photosensing. Our results suggested that changing of planarian’s body orientation away from a light source that is induced when the difference in the ratio of the signal input value between the eyes is greater than the value of the response threshold might be determined by the inhibitory GABAergic neural pathway, as previously suggested. The integration of inputs from the two eyes in the brain may not only improve behavioral efficiency by allowing more modifications, such as decision-making, but may also bridge the gap between simple photo-response orientation behavior and more complex behavior, including animal navigation that requires cross interactions with other environmental cues, such as odor molecules and magnetic fields.

In the chemical source localization of cells and insects, random motility increases the precision of sensing a chemical source. Also, many planktonic organisms display spiral, conical, or horizontal motion during phototaxis, suggesting that intrinsic self-motions are a basis for the precise directional movement in the animals. The spontaneous self-motion of the planarian head, the wigwag self-motion, which was previously considered to disturb responsive behaviors, was shown here to be necessary for ensuring the binocular field-based accurate perception of the direction of light. These facts support the notion that intrinsic noise-driven excitability is generally employed for a number of sensory systems. Indeed, the suppression of the wigwag self-motion sufficiently explains some previous uninterpretable phenomena, i.e., that individuals occasionally did not respond to light or headed towards light. In S. mediterranea, the wigwag angle is greater than that in D. japonica, and our data suggested that simulated planarians without a binocular field (bf = 0°) could efficiently escape if they had a much wider angle of wigwag self-motions that was greater than the broad front blind-like spot. However, it was reported that planarians moving with wider wigwag angles may lose the ability to stay in concave spaces such as on a stone or fallen leaves in the natural environment, although this ability is critical for avoiding toxic sunlight and strong water flow, when planarians cannot sense the environmental cues during head regeneration. Thus, the wigwag self-motion and binocular field are strongly correlated and their angles presumably evolved in order to achieve multiple adaptive behaviors.

Planarians basically move straight ahead in the absence of any environmental stimulus, indicating that planarians keep moving straight when the difference of left and right light input is less than the response threshold. Therefore, accurate light direction-perception based on the linkage between spontaneous self-motion and the eye architecture is simply implemented by the two motional characteristics: turning movement at over the response threshold of left and right input difference and straight movement at below the response threshold of left and right input difference. Previously it was observed that planarians responded to a weak light, but not to narrow bands of the spectrum, indicating that photo-response orientation behavior in planarian may not employ mechanisms detecting the illuminance gradient based on the difference of photoreactive chemicals in the nervous system, although we have not ruled out the possibility that planarians detect the light intensity for other behaviors. Moreover, temporal comparison between the light intensities at a certain position at time t_n and a previous position at time t_{n-1} requires saving this information in a memory system for illuminance or signal intensities at each time point, but planarian does not need this processing. We propose that planarian’s spontaneous self-motion that generates behavioral noise and its sensory architecture are co-adapted to enable the efficient, robust, and adaptive outcomes of multiple behaviors with potential reduction of energy consumption in the nervous system.

Methods
Animals. Three species of planarians: a clonal strain of freshwater planarian (SS strain of D. japonica), Dugesia ryukyuensis (mushuni mutant strain) and S. mediterranea were used in the present study. They were cultured at 23°C in freshwater. Planarians that were 8 mm in length were used in all experiments. In
the lidocaine treatment, animals were placed on two pieces of filter paper on ice to paralyze them, and 5 mg ml⁻¹ lidocaine hydrochloride (Sigma) in 1.5% agarose gel (Takara) was then administered using a sharpened glass capillary under a microscope to block the activity of the visual neurons. The control group was treated with anesthesia just posterior to the eyes. Regarding eyepecu removal, after animals were paralyzed on ice, the pigmented eyecup was scraped out with a sharpened tungsten needle under a microscope. Behavior assays were performed 1 day after surgery, when visual neurons had healed and the pigmented cup had not yet regenerated (Supplementary Fig. 2a). Control animals were scraped at a position next to the right pigmented eyecup. All planarians were maintained and manipulated according to a protocol approved by the Animal Care and Use Committee of Kyoto University and Gakushuin University.

**Photo-response orientation behavioral assays.** All behavioral experiments were conducted in a dark room with only a red light, the wavelength of which was not sensed by planarians. The lid of a 9-cm plastic circular dish was placed on black paper to suppress reflection, and the central 8-cm portion of the dish (the arena) was used for the assay field. In OAIL, a light source with a condenser was set 30 cm away from the center of the assay field (yielding approximately 300 lux in the assay field). In OAA2, an additional light source of the same type was also set on the side of the assay field opposite the light source used for OAIL to remove the blind area (which inevitably affected variations in the orientation of movements due to the arrangement of the pigmented eyecup shield). In light-flash experiments, a light source was connected to an electric digital stimulator (Nihon Kohden SEN-8203). The light intensity in the assay field was measured using an illuminance meter (Topcon, IM-5). To confirm the uniform intensity of illumination of the assay field, pictures of the field were evaluated using ImageJ (National Institutes of Health). Planarians were kept in the dark for at least 60 min before the experiment, and then placed in random orientation in the center of the arena. Planarian behavior was recorded using a video camera (Sony HDR-CX700 or ILCE-7S) fixed above the assay field. Photo-response orientation behavior was recorded until animals crossed the edge of the arena (4 cm from the center), and tracking data were analyzed using a computer, SMART v2.0 behavior analysis software (Panlab), ImageJ, and R software.

**Statistical analysis.** Data plotting and statistical analyses were performed using R software. The precision index of orientation was expressed as the inverse of the statistical analysis were performed using a computer, SMART v2.0 behavior analysis software (Panlab), ImageJ, and R software.

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Statistical analysis. Data plotting and statistical analyses were performed using R software. The precision index of orientation was expressed as the inverse of the circular SD of average angles during movement using Eq. (1):

\[ \text{Precision index} = \frac{1}{\sqrt{-2 \log R}} \]  

where \( R \) is the sample mean resultant length. The probability of wigwag angle larger than the front blind-like spot was calculated using the standard normal distribution Eq. (2):

\[ F(x) = 2 \times \left( \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}} e^{-\frac{x^2}{2}} \, dx \right) \]  

where \( Z \) was the angle of front blind-like spot.

In order to analyze differences between the angles of the left and right tails, data on body angles were sorted into two groups: less than 180° as the left group, and greater than 180° as the right group, and the difference in the median value between the two groups was calculated. In order to measure body angle changes, we converted video files to image sequences. Images that showed the previous orientation (angle) relative to the orientation at the time point of a subsequent turning behavior were selected, head angles were measured using ImageJ, and body angle changes were calculated using these measurements. Data from at least three experiments were averaged to calculate the orientation (angle) of movement.

Photo-response orientation behavior was recorded until animals crossed the edge of the arena (4 cm from the center), and tracking data were analyzed using a computer, SMART v2.0 behavior analysis software (Panlab), ImageJ, and R software.

Computer simulation of planarian photo-response orientation behavior. All algorithms were implemented in Racket language. Output data were plotted using the Plot package. In order to generate a random number with a probability density that followed a normal distribution, Racket library math/distributions were used. The input signal was approximated with a sine function since the planarian pigmented eyecup is semicircular (Fig. 1b, c, Supplementary Fig. 3). The functions for input signal intensities to the two eyes were expressed using Eqs. (3) and (4), respectively, where \( \theta \) is the direction of light compared to the planarian orientation.

\[ L(\theta) = -\sin \left( \theta - \frac{b f}{2} \right) \]  

\[ R(\theta) = \sin \left( \theta + \frac{b f}{2} \right) \]  

In order to generate a random number with a probability density that followed a normal distribution, Racket library math/distributions were used. The input signal was approximated with a sine function since the planarian pigmented eyecup is semicircular (Fig. 1b, c, Supplementary Fig. 3). The functions for input signal intensities to the two eyes were expressed using Eqs. (3) and (4), respectively, where \( \theta \) is the direction of light compared to the planarian anterior–posterior body axis at time \( t \) and \( bf \) is a binocular field parameter. The range of \( \theta \) was limited to avoid the input signal intensity being a negative value. \( bf \) was assigned as 40 in the case of D. japonica or 60 in the case of D. mediterranea. If the difference in input signals was unacceptably large (over the response threshold \( \phi \)), planarians changed their body angles in proportion to the difference, formulated using Eqs. (5) and (6),

\[ \varphi_{t+1} = \varphi_t - 3 \left( R(\theta_t) - L(\theta_t) \right) - \tau \]  

\[ \left( |R(\theta_t) - L(\theta_t)| > \tau \land R(\theta_t) < L(\theta_t) \right) \]

\[ \varphi_{t+1} = \varphi_t + 3 \left( R(\theta_t) - L(\theta_t) \right) - \tau \]  

\[ \left( |R(\theta_t) - L(\theta_t)| > \tau \land R(\theta_t) > L(\theta_t) \right) \]

where \( \varphi \) was defined as the planarian direction of movement with \( \varphi = -\theta \). The wigwag frequency \( (k) \) of the simulation was generated by a random number generator with a probability density that followed a log-normal distribution. The log-mean \( (E(x)) \) and log-standard distribution \( (V(x)) \) of the actual wigwag frequency were calculated using Eqs. (7) and (8), respectively.

\[ E(x) = \exp \left( \mu + \frac{\sigma^2}{2} \right) \]  

\[ V(x) = \left( e^{\mu + \frac{\sigma^2}{2}} - 1 \right) e^{\mu + \frac{\sigma^2}{2}} \]  

where \( \mu \) is the mean, \( \sigma \) is the standard distribution.

An assigned value of 3 was used as a coefficient to approximate the angle of wigwag self-motions of the head. If the input difference was acceptable \( (|R(\theta_t) - L(\theta_t)| \leq \tau) \), the body angle did not change using Eq. (9):

\[ \varphi_{t+1} = \varphi_t \]  

Wigwag self-motion was considered to add a random parameter \( \xi \), which is a randomly generated parameter with a distribution that fits the normal distribution \( \xi \) (Fig. 6b, c) using Eqs. (10)–(12):

\[ \varphi_{t+1} = \varphi_t - 3 \left( R(\theta_t) - L(\theta_t) \right) - \tau + \xi \]  

\[ \left( |R(\theta_t) - L(\theta_t)| > \tau \land R(\theta_t) < L(\theta_t) \right) \]

\[ \varphi_{t+1} = \varphi_t + 3 \left( R(\theta_t) - L(\theta_t) \right) - \tau + \xi \]  

\[ \left( |R(\theta_t) - L(\theta_t)| > \tau \land R(\theta_t) > L(\theta_t) \right) \]

\[ \varphi_{t+1} = \varphi_t + 3 \left( R(\theta_t) - L(\theta_t) \right) - \tau \]  

\[ \left( |R(\theta_t) - L(\theta_t)| \leq \tau \right) \]

The coordinates of the predicted planarian position at time \( t \) were calculated using Eq. (13):

\[ \begin{bmatrix} x_{t+1} \ y_{t+1} \end{bmatrix} = \begin{bmatrix} x_t + \sin(\varphi_t) \ y_t + \cos(\varphi_t) \end{bmatrix} \]  

**Histology.** For whole-mount immunohistochemistry, planarians were stained using the following dilutions of antibodies: 1/2000 rabbit anti-planarian arrestin16, 1/2000 rabbit anti-planarian GAD18, 1/2000 mouse anti-planarian TPH119. The plasmids pBlueScript SK (--) containing planarian chat16, gad18, GABA−R-Ba, GABA−R-Ra, pCS2+ snap2521, ryb20, ribl20, and hCD6 DNAs were used as templates for synthesizing digoxigenin (DIG)-labeled antisense RNA probes (Roche Diagnostics). Planarians were treated with 1% HNO3, 50 mM MgCl2 solution for 5 min
at room temperature and fixed in 4% paraformaldehyde, 5% methanol, 50% PBS solution for 30 min at room temperature. Fixed animals were subjected to in situ hybridization with appropriate probes. For visualization of fluorescent color a TSA kit (Thermo Fisher Scientific) was used according to the manufacturer’s instructions. Cell nuclei were labeled with Hoechst 33342. Fluorescence was detected with a confocal laser scanning microscope (FV10i, Olympus).

RNA interference. Double-stranded RNAs (dsRNAs) were synthesized from appropriate cDNA clones. dsRNA was injected into the posterior intestinal duct of planarians. Total RNA was extracted from planarians using ISOGEN-LS (Nippon Gene), and first-strand cDNA was synthesized from 1 μg total RNA using a Quantitect Reverse Transcription Kit (Qiagen). Quantitative analysis of the amount of each gene product was performed using the real-time polymerase chain reaction (PCR) machine (7900HT, Thermo Fisher Scientific). Each reaction (10 μl) contained 1x Quantitect SYBR Green PCR Master Mix (Qiagen), gene-specific primer at 0.3 µM, and 1 µl diluted (1:20) cDNA solution as template. At least four expression experiments were performed for each RNAi experiment.

Reverse transcription and quantitative PCR analysis. Animals were divided into the head and body. The eye-rich fragments were collected surgically using a capillary glass pipette. Total RNA was extracted from planarians using ISOGEN-LS (Nippon Gene), and first-strand cDNA was synthesized from 1 μg total RNA using a Quantitect Reverse Transcription Kit (Qiagen). Quantitative analysis of the amount of each gene product was performed using the real-time polymerase chain reaction (PCR) machine (7900HT, Thermo Fisher Scientific). Each reaction (10 μl) contained 1x Quantitect SYBR Green PCR Master Mix (Qiagen), gene-specific primer at 0.3 µM, and 1 µl diluted (1:20) cDNA solution as template. At least four technical replicates and at least three biological replicates were done. The PCR primers used are listed in Table 1. Expression levels were normalized by GAPDH gene expression.

Data availability. The sequences of GABAA-Rβs and GABAB-Rα reported here have been deposited in the DNA Data Bank of Japan (DDBJ) and National Center for Biotechnology Information (NCBI) (Accession numbers LC035387 and LC035388). All materials except for some primary antibodies used in the present work described in the manuscript are available from standard commercial sources (Thermo Fisher Scientific, Sigma, Roche, Takara Bio) or from the corresponding author (T.I.). The anti-planarian arrestin and anti-planarian tryptophan hydroxylase used are an antisera and culture supernatant, respectively, and therefore the distribution of these antibodies might become restricted.

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Table 1 Oligonucleotide primer sequences used for qRT-PCR assays

| Names       | Sequences                                                                 |
|-------------|---------------------------------------------------------------------------|
| gad forward | 5′-AAAAAGTCATGGCTTTCTTATCTGAAATGGA-3′                                   |
| gad reverse | 5′-CACCACTGACACATCTTGTCT-3′                                              |
| GABAA-Rβ forward | 5′-TTTCCGTCTCAAACTTTATTTACCATC-3′                                      |
| GABAA-Rβ reverse | 5′-AGAACACAAATACAAACACACAGG-3′                                          |
| GABAB-Rα forward | 5′-TTATAATTGGTTGTGATGCT-3′                                               |
| GABAB-Rα reverse | 5′-GGAATTTCATCTTGTTCTCCCT-3′                                             |
| opsin forward | 5′-GGACAAAACCACAGAATAATTACCACAG-3′                                      |
| opsin reverse | 5′-ACATGGGAAAACATTACAGCCTCTT-3′                                         |
| snap25 forward | 5′-AGGCTGTATCTACATCTTGTGATC-3′                                         |
| snap25 reverse | 5′-ATATTTTGATCCATTTCATCTTCTTG-3′                                        |
| GAPDH forward | 5′-ACCAACACCTTGTGACCTCCCTTAG-3′                                        |
| GAPDH reverse | 5′-GATGGTTCCATCAACAGCTTTTC-3′                                           |

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