Title

Group-level matching behavior in phototaxis of acoel flatworm, *Praesagittifera naikaiensis*.

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Abstract

The matching law, which posits that animals allocate their responses in proportion to the rate of reinforcement, has been supported across diverse animal taxa. Although originally formulated in the context of operant choice, matching also applies to time allocation in foraging and to Pavlovian responses, indicating its generality across behavioral domains. However, empirical evidence has thus far been largely limited to vertebrates and arthropods. Addressing the broader applicability of this principle requires extending investigations beyond these taxonomic groups, across a wider phylogenetic spectrum. Here, we examined phototactic behavior in the acoel flatworm *Praesagittifera naikaiensis*, a species that acquires nutrients through photosynthesis by symbiotic algae and exhibits positive phototaxis. Using a custom-designed T-maze in which the number of illuminated LEDs varied across arms, we found that the animals distributed themselves in proportion to relative brightness, consistent with matching behavior. Moreover, prior exposure to light for twenty-four hours attenuated this pattern. This manipulation was intended to induce a state of nutritional sufficiency, and the resulting decline in phototactic responses suggests that internal physiological states can modulate even seemingly reflexive locomotor behaviors.

Introduction

All behavior takes time. Thus, animals must constantly allocate their finite time across possible actions (Baum, 2013). How do animals determine the distribution of their own behavior by allocating their investment over time?

In addressing this question, the matching law has proven empirically reliable across a wide range of circumstances and species. Put simply, it states that the rate of responding is proportional to the rate of reinforcement for each option. Among vertebrates, matching behavior has been observed across numerous taxa, including pigeons (Herrnstein, 1961), rats (Belke & Belliveau, 2001), humans (Kollins et al., 1997), zebrafish (Kuroda et al., 2021), horses (Dougherty & Lewis, 1992), coyotes (Gilbert-Norton et al., 2009), and possums (Bron, 2003) and so on. Although most studies have focused on vertebrates, matching behavior has also been reported in invertebrate, for example, in place learning of fruit flies (Zars & Zars, 2009). While matching behavior has been primarily examined in operant choice settings, it also holds under non-operant conditions, such as concurrent variable-time (VT) schedules (DeCarlo, 1985). Similarly, matching under VT schedules has been observed in carpenter ants (DeCarlo & Abramson, 1989). Moreover, in Pavlovian conditioning that do not requires responses contingent with reinforcement, strength of CR has been shown to match the relative amounts of US presentations between two CSs (Harris & Carpenter, 2011).

The observation that matching occurs across a wide range of behavioral domains suggests that it may represent a ‘default pattern’ of action selection in animals. Indeed, Gallistel et al. (2007) demonstrated that even naïve mice exhibit matching in foraging behavior, independent of prior training. Among invertebrates, the natural foraging behavior of bees has been theoretically shown to conform to the matching law, and to constitute an optimal strategy under certain ecological conditions (Thuijsman et al., 1995). This raises a

fundamental question: through which evolutionary changes in nervous systems, body plans, or behavioral repertoires did this principle emerge? Matching behavior has been reported across both operant and non-operant, as well as innate and learned behaviors.

However, empirical evidence to date has been limited to vertebrates and arthropods. Answering this question requires extending investigations beyond these taxonomic groups, across a broader phylogenetic spectrum. Thus, to evaluate the generality of matching as a behavioral principle, it is critical to examine whether similar patterns emerge in non-model organisms with diverse body plans and nervous systems.

Acoelomorphs provide a unique opportunity to test the generality of laws derived from behavioral psychology, particularly in the context of early bilaterian evolution. These animals possess a bilaterally symmetrical, worm-like body plan, yet lack a through-gut or anus, reflecting a structurally simple organization. Notably, the degree of neural centralization varies across species, ranging from forms with little centralization to those possessing a 'commissural brain' structure at the anterior pole (Gavilán et al., 2016; Perea-Atienza et al., 2015). Despite their anatomical simplicity, genomic analyses have revealed the near-complete presence of major bilaterian signaling pathways, including Wnt, TGF-β, Notch, Hedgehog, and FGF. This suggests that simplicity in morphology does not imply genetic primitiveness (Schiffer et al., 2024). Taken together, these features make acoelomorphs a promising model for investigating what kinds of behavioral capacities emerged alongside the evolution of brains and gene regulatory networks.

The growing interest in acoelomorphs was initially driven by debates surrounding their phylogenetic position. While they were once thought to be closely related to flatworms, molecular phylogenetic analyses have revealed that they are more distantly related, leading to the establishment of a new phylum (Baguñà & Riutort, 2004). The phylogenetic placement of acoelomorphs remains contentious. Some studies suggest that they represent a basal lineage of all bilaterians (Cannon et al., 2016; Hejnol et al., 2009; Ryan, 2013), whereas others argue that they are a derived clade within Deuterostomia, forming a sister group to Xenoturbellida (Mertes et al., 2019; Philippe et al., 2011, 2019). In either case, given their unique nervous system organization and body plan, examining behavioral complexity in acoelomorphs may offer unparalleled insights into the generality of behavioral principles across evolutionary lineages.

Praesagittifera naikaiensis, a species of acoel flatworm, is endemic to the Seto Inland Sea of Japan (Yamasu, 1982). It inhabits shallow marine environments, ranging from intertidal zones to depths of approximately one meter, and can be readily collected in the field (Hikosaka-Katayama et al., 2020). Owing to these advantages, *P. naikaiensis*, together with other species in *Convoluta* family, has attracted attention as a suitable model organism in studies of acoela. Indeed, various research has been conducted on this species, including its neural architecture (Sakagami et al., 2021), sensory receptor (Sakagami et al., 2024), and genome sequence (Arimoto et al., 2019). *P. naikaiensis* exhibits positive phototaxis, which is mediated by light detection through its ocelli. Because it derives nutrients from photosynthetic symbiotic algae, approaching a light source is likely to facilitate nutrient acquisition through enhanced photosynthesis. However, aspects such as the quantitative adjustment of behavioral choices and modifications in response to internal states shaped by prior experience remain largely unexplored.

In this study, thus, we investigated whether the phototactic behavior of *P. naikaiensis* follows the matching law with respect to light intensity. Using a custom-built T-maze, we varied the number of lit LEDs (0 to 3) on each of the two choice arms and examined whether individuals preferentially moved toward the brighter side. In Experiment 1, the proportion of choices closely tracked the proportion of lit LEDs, consistent with matching behavior. In Experiment 2, we tested whether this matching was modulated by prior exposure to light. Individuals were continuously exposed to LED illumination for 24 hours prior to the experiment, a treatment intended to induce a 'satiated 'state, given that *P.*

naikaiensis obtains nutrients through the photosynthesis of its symbiotic algae. By examining whether such exposure altered choice behavior, we aimed to assess whether internal states—such as nutritional status or algal activity—influence phototactic decision-making. These two experiments allowed us to test whether phototactic responses in *P. naikaiensis* follow the quantitative pattern predicted by the matching law, and whether this behavior is modulated by prior exposure to light.

Materials and Method

Subject and housing: Acoel flatworms, *P. naikaiensis*, were used (Figure 1a) were collected from public beaches in Hiroshima and Okayama prefectures in Japan, according to the collecting spots described in Hikosaka-Katayama (2020). The rearing system was based on the method described by Hikosaka (2015). The animals were group-housed in an acrylic aquarium (25 cm W × 20 cm D × 20 cm H), initially filled with seawater from the collection sites and gradually replaced with artificial seawater (Delphis, Japan). The salinity was maintained at 30–35‰, based on that of the natural habitat. The algal culture medium KW21 (ISC Inc., Japan) was diluted at a ratio of 1:4000 and added. The bottom was covered with coral sand. The water temperature was kept at 20–24°C using a air conditioner in the room. The light cycle was controlled at 12D:12L. No additional food was provided, and the animals were acclimated for at least 7 days prior to experimentation.

Apparatus: a custom-made T-maze was built using a 3D printer (Figure 1b). The maze was printed with translucent PETG filament to facilitate phototaxis behavior, and was filled with artificial seawater during experiments. The initial arm was 10 cm long and 3 cm wide, leading to two branching choice arms, each 5 cm in length. The maze was 3 cm deep and was filled with artificial seawater to a depth of approximately 2.5 cm. At the end of each choice arm, three white bullet-shaped LEDs (6500-7000K) were mounted using utility wax, and were arranged adjacently with a 2 mm spacing between each unit. The LEDs were controlled via an Arduino microcontroller (Arduino LLC, USA). The apparatus is freely available and may be modified or reused without restriction. The 3D design file is shared on GitHub (https://github.com/HeathRossie/stlfiles).

General *procedure and analysis*: two experiments were conducted in a dark room during the light phase of the light–dark cycle. Each session began by gently placing *P. naikaiensis* individuals into the initial arm of a T-maze using a 1 mL dropper to avoid injury. The number of individuals per session ranged from 41 to 137, depending on how many could be collected with the dropper (Supplementary Table). Several LEDs were illuminated on each side, and *P. naikaiensis* were expected to move within the maze according to the relative light intensity on the left and right arms. Each session was terminated 1 hour after the last individual had been placed in the maze. At the end of the session, the final positions of all individuals were recorded using a smartphone camera.

The x–y coordinates of *P. naikaiensis* were manually digitized using ImageJ (NIH, USA). Coordinates were obtained based on the final positions of individuals. Each individual was categorized into one of three groups: left choice, right choice, or no-choice. A left or right choice was defined as movement beyond the width of the initial arm (3 cm), measured from the central axis, into either side of the choice zone. Individuals that did not cross this threshold were classified as no-choice. Choice rates were calculated excluding no-choice individuals. If matching behavior is present, the proportion of left and right choices should correspond to the relative number of lit LEDs on each arm. All statistical analyses and data visualizations were conducted using R v.4.1.0.

Experiment 1: *P. naikaiensis* individuals that had gathered at the bottom of the aquarium were gently collected using a 1 mL dropper and immediately transferred to the experimental room for testing.

LED lighting conditions included all 16 possible combinations of illumination on the left and right choice arms, ranging from (0,0) to (3,3), representing the number of LEDs on the left and right arms, respectively. All sessions were conducted on separate days.

Experiment 2: prior to the experiment, *P. naikaiensis* were continuously exposed to white LED light (300 lumens; LTC-LC08U-KN 06-0910, OHM, Japan) for 24 hours. This treatment was intended to induce a satiated state, as the animals primarily obtain nutrients through photosynthesis by their symbiotic algae. The satiation procedure was conducted in a 200 mL glass cup covered with plastic wrap to prevent salinity increase due to evaporation.

A subset of the LED lighting conditions used in Experiment 1 was employed in this experiment. Specifically, eight combinations were selected to include all possible ratios of lit LEDs between the left and right arms: (2,3), (3,2), (3,1), (1,3), (1,2), (2,1), (0,3), and (3,0).

Result

Experiment 1: Group-level matching in phototactic locomotion

The final choice proportions closely matched the proportion of lit LEDs, indicating matching behavior (Figure 2a). The Pearson correlation was significant($t(14) = 9.74$, $p < .0001$), with an explained variance of $R^2 = .871$, indicating the strong linear relation between choice distribution and light intensity.

To further examine the determinants of choice behavior, we conducted a multiple linear regression with the proportion of right-arm choices as the dependent variable. The independent variables included the proportion of lit LEDs in the right arm, the total number of lit LEDs, and their interaction term. The model revealed a significant interaction ($F(1,12) = 39.48$, $p < .0001$), with a positive regression coefficient for the interaction term ($\beta = 0.22$). This indicates that the degree of matching increased as the total number of lit LEDs increased. In other words, matching behavior approached a more complete form under high-luminance conditions, whereas slightly under-matching was observed when fewer LEDs were lit (Figure 2a).

To evaluate whether stimulus intensity affected no-choice behavior, we performed a regression analysis examining whether the total number of lit LEDs predicted the proportion of no-choice responses (Figure 2b).The rationale for this analysis was that a lower number of lit LEDs might have failed to provide sufficient control to elicit phototaxis. However, the analysis revealed no significant relationship between the total number of lit LEDs and the proportion of no-choice responses ($F(1,14) = 3.72$, $p = 0.074$), suggesting that no-choice behavior was not simply due to insufficient light intensity to elicit phototaxis.

Experiment 2: Prior light exposure reduces matching behavior

The matching behavior observed in *P. naikaiensis* arises from phototactic movement. But does this behavior reflect the behavioral history of being exposed to light? To address this question, we tested whether pre-exposure to light for 24 hours would attenuate the matching response.

As predicted, matching behavior was attenuated by light pre-exposure, resulting in an under-matching pattern (Figure 3a). While the choice rate remained positively correlated with the proportion of lit LEDs, the strength of this relationship was weaker than in Experiment 1. Indeed, the linear regression revealed that the correlation coefficient did not

reach significant ($t(6) = 2.150$, $p = .075$). The explained variance was $R^2 = .435$ indicating a reduced correlation compared to the Experiment 1($R^2 = .871$).

As in Experiment 1, the no-choice rate was not related to the total number of lit LEDs (Figure 3b). This suggests again that a lower number of LEDs did not fail to elicit phototaxis. However, the overall no-choice rate was higher than in Experiment 1 ($t(11.04) = 5.22$, $p = 0.0003$). This indicates that light pre-exposure reduced the animals ʼsensitivity to the stimulus, leading to a general decline in phototactic behavior.

Discussion

Taxis has traditionally been regarded as a reflexive and mechanistic form of behavior, typically characterized by rigid control with little capacity for adjustment. In this study, we aimed to test whether phototactic locomotion in *P. naikaiensis* results in matching behavior, in which the distribution of choices corresponds proportionally to stimulus intensity. Furthermore, we examined whether this behavioral pattern could be modulated by the animalʼs prior exposure to light.

The distribution of choices was clearly proportional to the relative number of lit LEDs, indicating the presence of matching behavior. The degree of matching became more complete as the total number of lit LEDs increased, whereas a slight under-matching pattern was observed under conditions with fewer lit LEDs. This trend may reflect increased discriminability under high-luminance conditions, in contrast to what would be predicted by the Weber–Fechner law, which claims the discriminability is proportional to the ratio of stimulus strengths. Importantly, this pattern was not attributable to a failure in light detection under low-luminance conditions, as the total number of lit LEDs did not affect the rate of no-choice responses. In sum, *P. naikaiensis* exhibited matching behavior in its phototactic choices under the T-maze condition. However, this pattern was modulated by prior light exposure, which resulted in under-matching and increased no-choice behavior. These findings suggest that phototaxis in P. naikaiensis is not a rigid reflex, but rather a flexible behavior influenced by internal physiological states.

The ecological significance of matching behavior is typically discussed in relation to optimality in behavioral strategies. In operant choice, formal models have derived the matching law from the optimization of feedback functions linking reinforcement rates to response rates, and from its connection to optimal foraging theory (Baum, 1981; Staddon, 2016). Empirically, matching has been observed as a near-optimal behavioral pattern in pigeons (Hinson & Staddon), monkeys (Iigaya et al., 2019), and human visual foraging (Kobayashi et al., 2024a, 2024b). But what about non-operant behavior? In C. elegans, a member of the phylum Nematoda, solitary strains are known to distribute themselves across food patches in proportion to bacterial density, effectively maintaining a ratio-based foraging pattern (Gloria-Soria & Azevedo, 2008). Haley and Chalasani (2024) have argued that this distribution is consistent with the matching law. Notably, the behavior in *C. elegans* appears to be elicited through chemosensory processing and driven by the salience of environmental stimuli, rather than being the result of operant reinforcement. This suggests that matching can emerge even in stimulus-driven behaviors. In our study, *P. naikaiensis* exhibited choice patterns that were consistent with the matching law. Despite their phylogenetic uniqueness, their behavioral allocation followed a lawful distribution similar to those observed in more extensively studied taxa.

Matching behavior can deviate depending on environmental conditions or internal states. For example, uncertainty regarding food availability can lead to over-matching in pigeons (Anselme et al., 2022). In both humans and mice, under-matching has been interpreted as a consequence of "compressed" decision-making policies, which serve to reduce cognitive costs (Bari & Gershman, 2023). Theoretically, deviations from perfect matching have been explained in terms of over- or underestimation of reinforcer utility (Baum, 1974; Kubanek,

2024). In this context, the under-matching observed in *P. naikaiensis* may also reflect a state-dependent deviation, modulated by pre-exposure to light and the resulting changes in internal physiological conditions.

Given the less centralized nervous system of Xenacoelomorpha, deviations from matching behavior are unlikely to be achieved through ‘complex’ computational processes. Instead, reductions in matching behavior may be mediated by bodily mechanisms. In *Symsagittifera roscoffensis*, a species in the same family as *P. naikaiensis*, symbiotic algae are not located within host cells but are embedded in the intercellular spaces of subepidermal tissues (Arboleda et al., 2018; Bailly et al., 2014). Although the precise routes of nutrient transfer remain unknown, it is presumed that the algae release photosynthetically derived amino acids and products into these intercellular spaces. Phototaxis in *P. naikaiensis* is likely driven by a pair of rhabdomeric eyes, where photoreceptor cells are concentrated and presumably connected to motor circuits that produce directed movement toward light. The phototactic behavior shown in our study suggests an interaction between the metabolic system supporting nutrient uptake via algal photosynthesis and the sensory-motor system. Understanding how such integration is achieved in the nervous system of acoelomorphs is a key issue for elucidating the evolution of behavior in early bilaterians.

Flexible adjustment in phototactic behavior has also been reported in *S. roscoffensis*. In intertidal environments, individuals face the risk of excessive sunlight exposure when lying on surface substrates, which can cause damage to their symbiotic algae (Straka and Rittmann, 2018). However, it remained unclear whether *S. roscoffensis* actively avoids harmful light by inhibiting phototaxis (Serôdio et al., 2011; Nissen et al., 2015). Recently, Thomas et al. (2024) conducted behavioral experiments in which *S. roscoffensis* were exposed to excessive light levels. The animals were able to escape by burrowing into the sand, and indeed, they did so. Our findings are consistent with their observation, in that pre-exposure to light attenuated phototactic movement toward the light source. These results collectively suggest that although taxis has traditionally been regarded as a reflexive movement, its flexible modulation in response to environmental context deserves renewed attention.

Placing our findings in a broader context, our results resonate with early behavioral studies by Charles Darwin on earthworms (Darwin, 1892) and Herbert Spencer Jennings on protists (Jennings, 1906). Darwin observed that earthworms pull leaves and small stones into their burrows. Interestingly, he noted that under warmer conditions, this behavior became sloppier, whereas under colder conditions, the burrow entrances were covered more carefully, presumably to block cold air. He interpreted this variation not as a failure of reflex but as evidence that the worms adjusted their actions according to necessity. Similarly, Jennings described how even unicellular organisms such as Paramecium and Amoeba exhibited state-dependent modulation of taxis behavior. In line with these observations, *P. naikaiensis* also exhibited matching behavior that was attenuated following light pre-exposure, suggesting that internal states modulate even seemingly reflexive phototactic behavior. Reed (1982) later highlighted the significance of Darwin’s work, arguing that identifying the external and internal conditions under which behavior is adaptively modulated is a central agenda for evolutionary psychology.

Lastly, it is important to acknowledge a limitation of the present study. Standard formulations of the matching law are typically applied at the level of individual behavior, where an organism's choice distribution is shown to vary proportionally with reinforcement ratios across conditions (e.g., Herrnstein, 1961). In contrast, our experiments with P. naikaiensis revealed matching behavior at the group level. Extending this line of research to examine the individual level decision making within this taxon would be a valuable direction for future studies.

Funding
No funding support was used in this study.

Declaration of generative AI
The authors used ChatGPT 4.0 for language-proofing to refine the manuscript written by non-native English speakers. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Figure 1: (a) Photographs of acoel flatworms, *Praesagittifera naikaiensis*. Top: a single individual (×240). Bottom: several individuals attached to the inner wall of the aquarium. (b) Photograph of the custom-built T-maze apparatus used in the experiments. Individual P. naikaiensis were introduced into the initial arm at the start of each session.

Figure 2: (a) Choice behavior of *P. naikaiensis* showed a proportional relationship with the relative number of lit LEDs on each arm, indicating matching behavior. (b) The total number of lit LEDs had no significant effect on the rate of no-choice responses

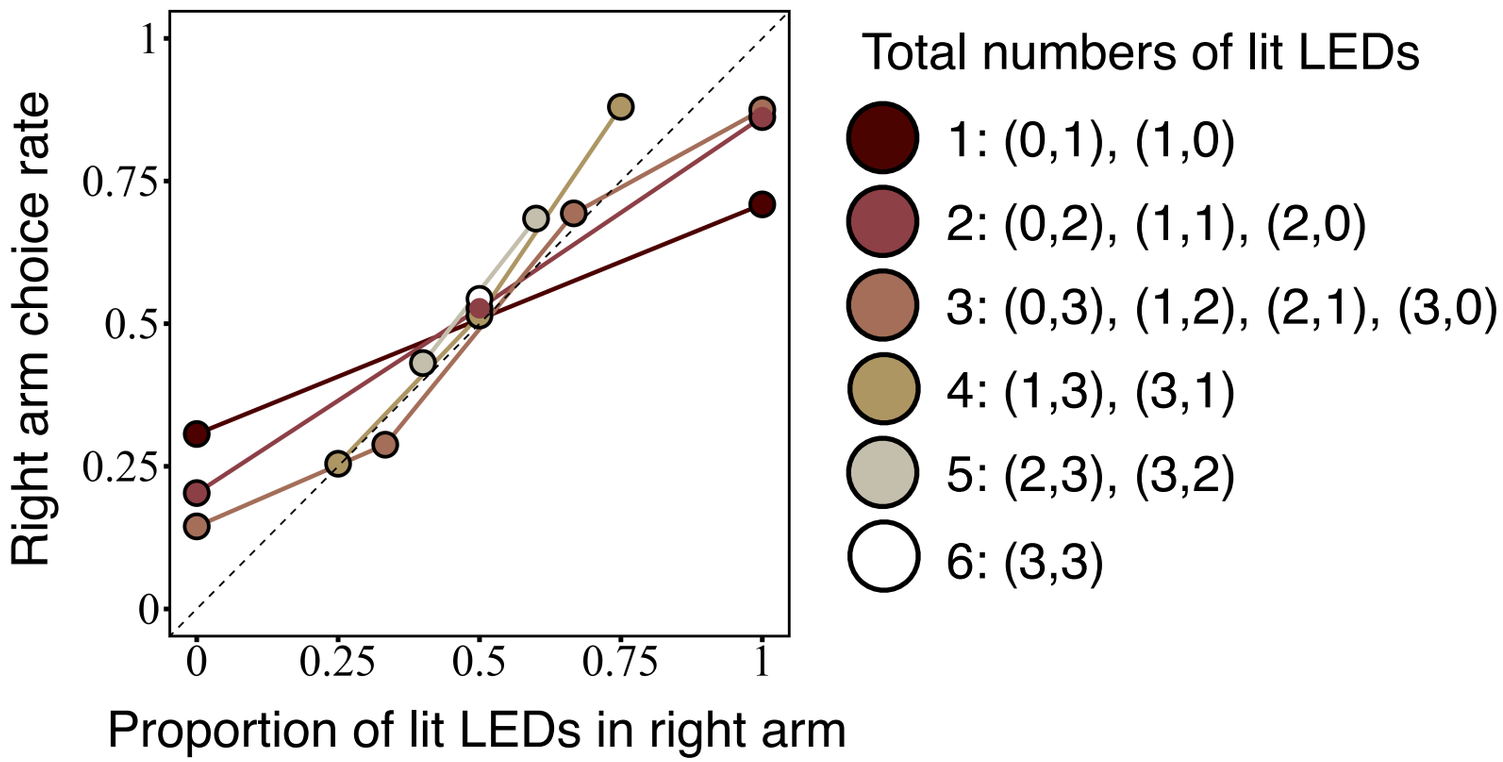
Figure 3: (a) Choice behavior deviated from strict matching and exhibited under-matching following 24-hour light pre-exposure, suggesting a modulatory effect of internal state. (b) The rate of no-choice responses increased after light pre-exposure.

Supplementary Table: summary of the number of *P. naikaiensis* individuals used under each LED illumination condition across both experiments.

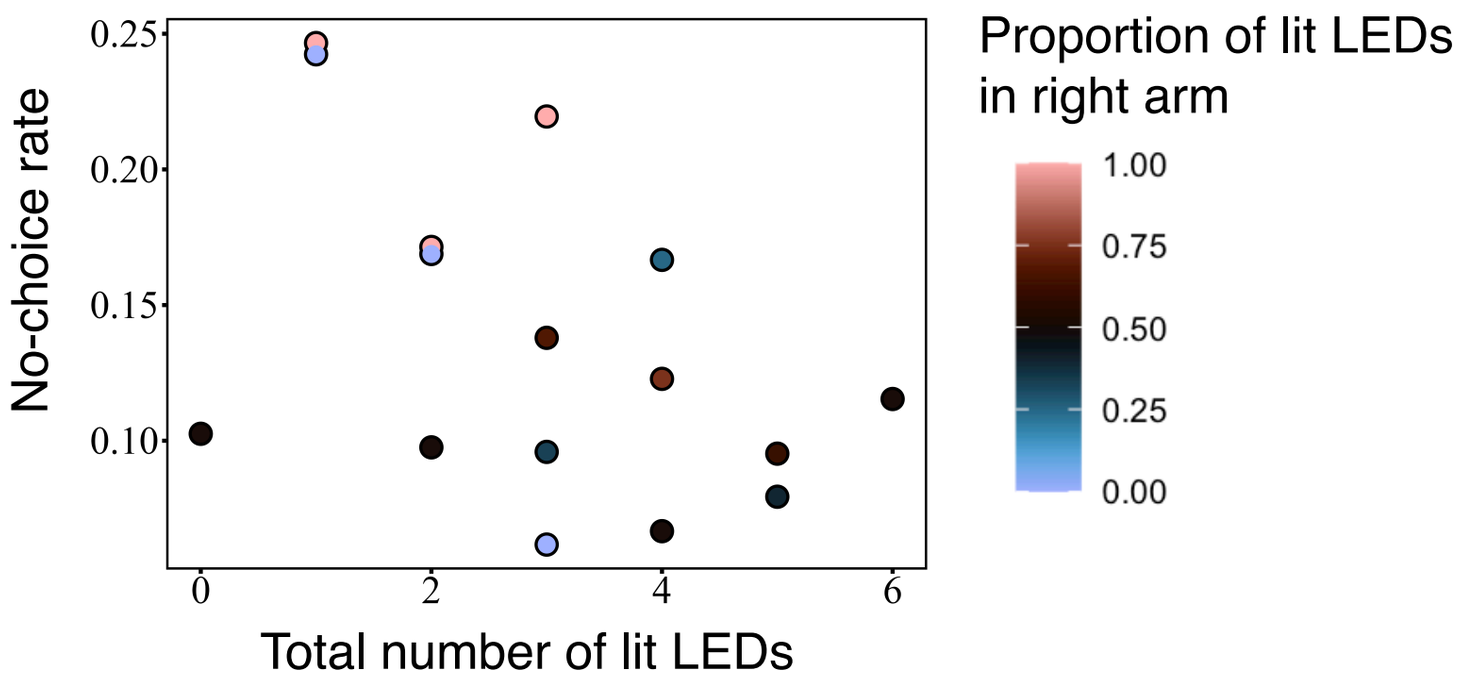
(a)

(b)

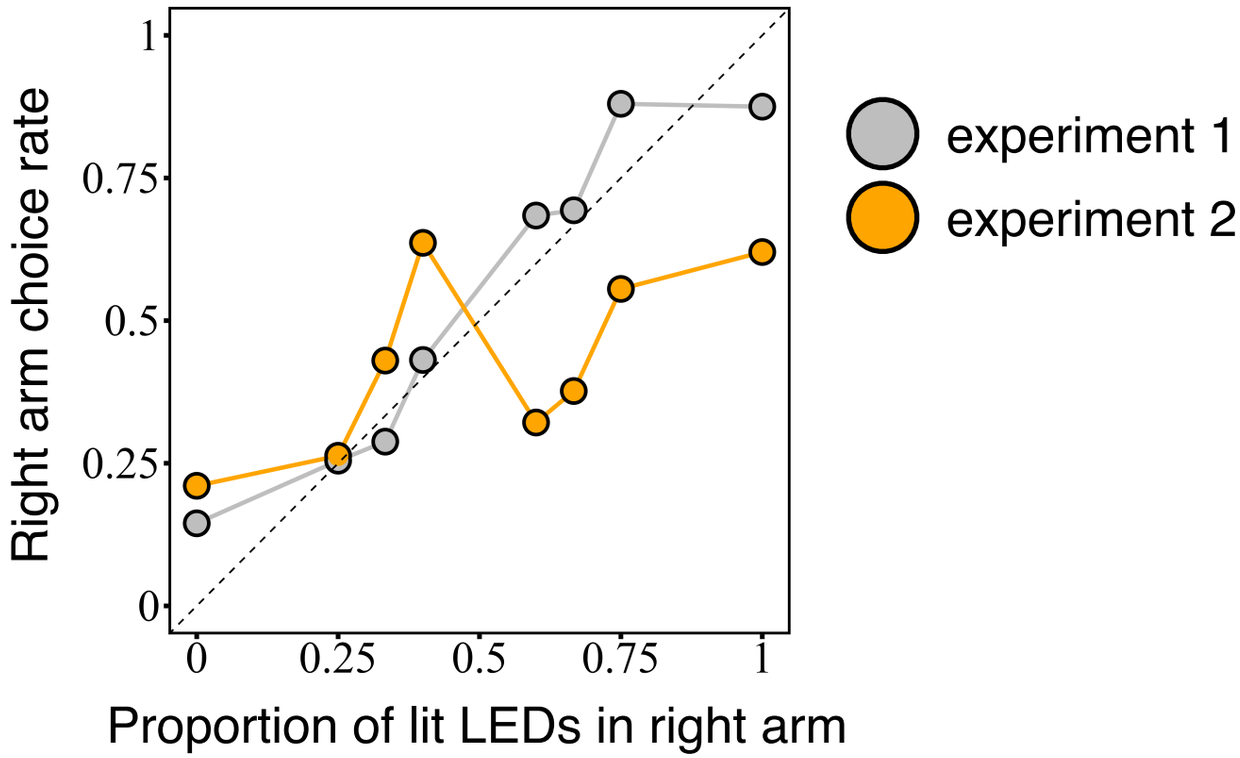
(a)


Right arm choice rate
Proportion of lit LEDs in right arm
0
0.25
0.5
0.75
1
Total numbers of lit LEDs
1: (0,1), (1,0)
2: (0,2), (1,1), (2,0)
3: (0,3), (1,2), (2,1), (3,0)
4: (1,3), (3,1)
5: (2,3), (3,2)
6: (3,3)

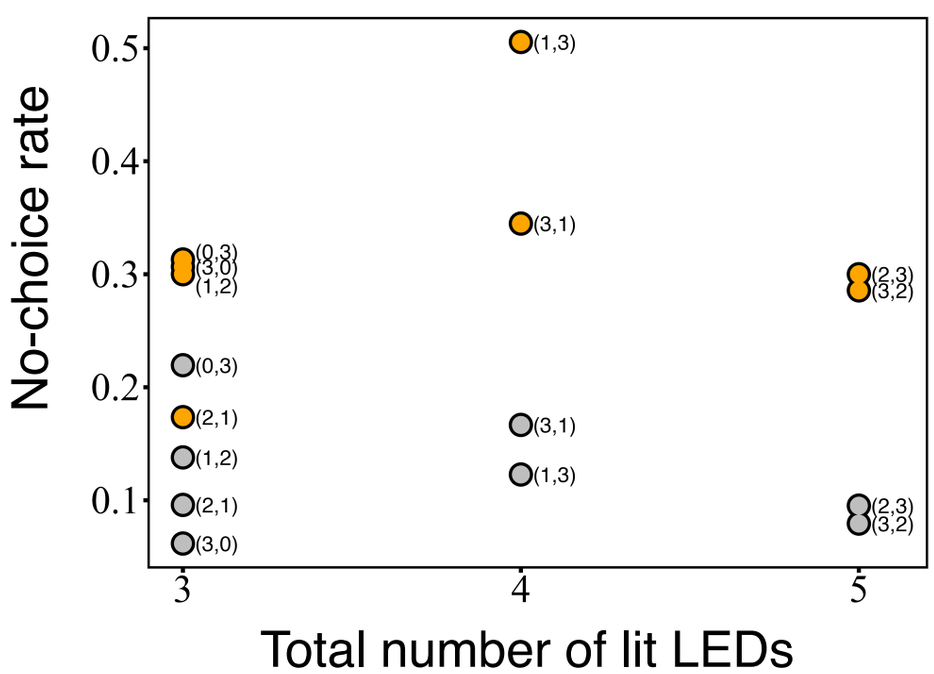
(b)


No-choice rate
Total number of lit LEDs
0.10
0.15
0.20
0.25
0
2
4
6
Proportion of lit LEDs in right arm
1.00
0.75
0.50
0.25
0.00

(a)


Right arm choice rate
Proportion of lit LEDs in right arm
0
0.25
0.5
0.75
1
experiment 1
experiment 2

(b)


No-choice rate
Total number of lit LEDs
0.1
0.2
0.3
0.4
0.5
3
4
5
(1,3)
(3,1)
(0,3)
(3,0)
(1,2)
(2,3)
(3,2)
(0,3)
(2,1)
(1,2)
(2,1)
(3,0)
(3,1)
(1,3)
(2,3)
(3,2)