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The performance of cleaner wrasse, *Labroides dimidiatus*, in a reversal learning task varies across experimental paradigms

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Testing cognitive performance in controlled laboratory experiments is a powerful tool for understanding the extent and evolution of cognitive abilities in non-human animals. However, cognitive testing is prone to a number of potential biases, which, if unnoticed or unaccounted for, may affect the conclusions drawn. We examined whether slight modifications to the experimental procedure and apparatus used in a spatial discrimination and reversal learning task affected performance outcomes in the bluestreak cleaner wrasse, *Labroides dimidiatus* (hereafter 'cleaners'). Using two-alternative forced choice tests, fish had to learn to associate a food reward with a side (left or right) in their holding aquarium. Individuals were tested in one of four experimental treatments that differed slightly in procedure and/or physical set-up. Cleaners from all four treatment groups were equally able to solve the initial spatial discrimination test. However, groups differed in their ability to solve the reversal learning task: no individuals solved the reversal task when tested in small tanks with a transparent partition separating the two options, whereas over 50% of individuals solved the task when performed in a larger tank, or with an opaque partition. These results clearly show that seemingly insignificant details to the experimental set-up matter when testing cognitive performance, and might significantly influence the outcome of experiments. When designing the methodology for comparative cognitive tests, care should be taken to ensure that all groups understand and can respond to the relevant cue to avoid misinterpretations.

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Key Words: Cognitive performance, coral reef fish, *Labroides dimidiatus*, methodology, two-alternative forced choice test, spatial learning

Abstract

Testing cognitive performance in controlled laboratory experiments is a powerful tool for understanding the extent and evolution of cognitive abilities in non-human animals. However, cognitive testing is prone to a number of potential biases, which, if unnoticed or unaccounted for, may affect the conclusions drawn. We examined whether slight modifications to the experimental procedure and apparatus used in a spatial discrimination and reversal learning task affected performance outcomes in the bluestreak cleaner wrasse, *Labroides dimidiatus* (hereafter ‘cleaners’). Using two-alternative forced choice tests, fish had to learn to associate a food reward with a side (left or right) in their holding aquarium. Individuals were tested in one of four experimental treatments that differed slightly in procedure and/or physical set-up. Cleaners from all four treatment groups were equally able to solve the initial spatial discrimination test. However, groups differed in their ability to solve the reversal learning task: no individuals solved the reversal task when tested in small tanks with a transparent partition separating the two options, whereas over 50% of individuals solved the task when performed in a larger tank, or with an opaque partition. These results clearly show that seemingly insignificant details to the experimental set-up matter when testing cognitive performance, and might significantly influence the outcome of experiments. When designing the methodology for comparative cognitive tests, care should be taken to ensure that all groups understand and can respond to the relevant cue to avoid misinterpretations.

Introduction

Cognition is broadly defined as the way organisms acquire, store, process and act upon information obtained from their environment (Shettleworth 2010). Determining the extent to which species or individuals integrate and respond to this information has long been a topic of scientific interest, with one main goal of understanding the origins of human cognitive capacities (see Penn, Holyoak & Povinelli, 2008; Shettleworth, 2012; Burkart et al., 2014; Bolhuis, 2015). To this end, many researchers have adopted a comparative approach when studying the evolution of cognition: by testing a variety of species in the same cognitive tasks, researchers aim to understand how cognitive skills are distributed across taxa, and why (Emery & Clayton, 2004; MacLean et al., 2012; Salwiczek et al., 2012; Burkart et al., 2014; Gingins & Bshary, 2016). Similarly, testing cognitive performance across different sexes, ages and/or populations, allows researchers to explore the physiological, ontogenetic, and environmental mechanisms underlying within-species cognitive differences (Brown & Braithwaite, 2005; White & Brown, 2015; Carazo et al., 2014; Noble, Byrne & Whiting, 2014; Wismer et al., 2014).

Cognitive abilities are typically assessed in terms of presence/absence of a certain cognitive process like transitive inference or imitation learning, or through the speed at which individuals learn the correct solution in a specific context. While these approaches are very useful for documenting differences and similarities across groups, studying cognition is prone to a number of potential biases. First, inherent differences in motivation, perception or past experiences can affect the performance of different species and/or individuals in the same task (Rowe and Healy 2014). Second, extrinsic factors including differences in housing or rearing conditions and experimental manipulation are known to affect the behavioural responses of laboratory subjects in performance tests (Sorge et al., 2014; e.g. Gaffney, 2014). Third, the ecological relevance of the experimental paradigm used may also facilitate or impede performance, which can greatly impact heterospecific comparisons. Indeed, Lotem & Halpern (2012) suggested that the mechanisms involved in data-acquisition (e.g. attention, motivation) and in learning are tuned jointly. As a result, some species might be prompted to make stronger associations with certain types of cues and as a consequence learn faster in certain contexts. Whether in a comparative context or not, failing to notice or account for these biases may lead to inappropriate conclusions with regards to the behavioural or cognitive abilities of a group.

The degree to which slight modifications to the experimental paradigm (i.e. how the experiment is set up and conducted) influences the cognitive performance of test subjects has rarely been explored. Yet, the few existing examples suggest this question warrants further investigation. For instance, assessing the presence or absence of a specific cognitive trait can be strongly influenced by the experimental paradigm used. Indeed, four separate laboratory studies found no evidence for prosocial behaviour in chimpanzees (*Pan troglodytes*) (Silk et al., 2005; Jensen et al., 2006; Yamamoto & Tanaka, 2010; Vonk & Povinelli, 2011), prompting these authors to conclude that our most closely related primates are indifferent to the welfare of others. However, these studies involved complicated apparatuses that required pushing levers or pulling strings in order to receive or distribute a reward. When Horner et al. (2011) subsequently tested chimps in a token exchange task (and facilitated communication between test subjects), they found supporting evidence for prosocial behaviour, suggesting that the experimental paradigm used to detect this specific cognitive ability is of importance. Similarly, the presence of inequity aversion in primates is an ongoing debate, with separate studies find contrasting results. Using a token-exchange task and creating inequities in terms of the quality of the reward that each subject would receive, capuchin monkeys (*Cebus paella*) (Brosnan & de Waal, 2003) and chimpanzees (Brosnan, Schiff & Waal, 2005) have been argued to display inequity aversion. When the rewards were unequal, subjects were more likely to reject the lower reward and less likely to participate. However, several criticisms arose, arguing that the mere presence of a greater reward (Wynne, 2004), or the frustration caused by having previously received a greater reward (Roma et al., 2006; Dubreuil, Gentile & Visalberghi, 2006), might explain why primates rejected the lower reward in these experiments. Further experiments revealed that several factors can indeed affect inequity aversion in primates (Wolkenten, Brosnan & Waal, 2007; Brosnan et al., 2010). For instance, it appears that a task is necessary to emulate inequity aversion (Wolkenten, Brosnan & Waal, 2007), which was not performed in several studies that found contrasting results (Roma et al., 2006; e.g. Bräuer, Call & Tomasello, 2006). Inequity aversion abilities in primates are still debated today (Engelmann et al., 2017), clearly exemplifying the impact that the experimental paradigm used can have in assessing cognition.

Ecological relevance of the task may also affect the outcome and interpretation of cognitive tests. For instance, Salwiczek et al. (Salwiczek et al., 2012) compared the ability of capuchin monkeys (*Cebus paella*), chimpanzees (*Pan troglodytes*), orangutans (*Pongo spp.*) and cleaner wrasse (*L. dimidiatus*) to associate a rewarding versus ephemeral food source with the colour, shape and pattern of the plate on which the food was placed, a foraging task designed to mimic the ecological context experienced by cleaner wrasse on coral reefs. They found that cleaner wrasse outperformed all three primate species in this task (Salwiczek et al., 2012). However, after adjusting the task to be more relevant to the foraging context experienced by primates (i.e. rewarding ephemeral food associated with food colour rather than plate characteristics), the capuchins performed as well as the wrasse (Prétôt, Bshary & Brosnan, 2016). Ecological relevance in the experimental paradigm used was thus crucial for species to perform at their best in this task. Although the salient cue may be obvious to the experimenter, it is important to consider how and why it may be (mis)interpreted by the test subject when designing the task. Drawing robust conclusions from cognitive tests thus relies on the experimenter's ability to account for the many factors that might influence a species' performance in a given task.

Here, we asked whether variations in the experimental paradigm used to test spatial discrimination and reversal learning affected the speed at which bluestreak cleaner wrasse *Labroides dimidiatus* (hereafter 'cleaners') learned to solve these cognitive tasks. Cleaners are small coral reef fish whose ecological function is to remove ectoparasites off the surfaces of so-called "client" heterospecific fishes. The complexities of this cleaning mutualism are such that cleaners have emerged as a model system for testing strategic sophistication in vertebrates with primitive brains (Bshary & Würth, 2001; Bshary & Grutter, 2006; Bshary, 2011; Pinto et al., 2011; Gingins et al., 2013; Soares et al., 2014). Numerous studies have investigated decision-making in cleaners using flat Plexiglas feeding plates attached to levers which are lowered into the experimental tanks. Laboratory experiments mimicking ecologically relevant scenarios suggest that cleaners are able to solve foraging tasks using Plexiglas plates in the laboratory similarly to how they would in nature with client fishes (e.g. Bshary & Grutter, 2002a, 2006; Pinto et al., 2011; Gingins et al., 2013; Wismer et al., 2014). Cognitive tasks that are less ecologically oriented, such as spatial discrimination, have also used these feeding plates (Gingins & Bshary, 2016). In these spatial discrimination tests, the fish must learn to associate one side of the tank (i.e. left or right) with a food reward. When a cleaner chooses the correct side there are, in principle, two options regarding the unrewarding plate: stay in the tank, or be removed. The latter option might interfere with learning if cleaners perceive the removal of the unchosen plate as the loss of a client foraging opportunity. If so, cleaners might choose the side of the tank where they observed a plate leaving in the previous trial, rather than the side which offered a reward. Other extrinsic factors in the design of these tests may also influence the outcome including partition colour and tank length (i.e. distance swam before a decision must be made). We compared cleaner performance in four variations of a standard spatial discrimination and reversal learning test to evaluate the extent to which slight modifications to experimental procedure and apparatus affected cleaners' performance in solving these tasks. Two treatments involved a modification of an ecologically-relevant procedure (plate behaviour: "stay" or "lift'), which might affect the relevant cue learned by the cleaner. The remaining two treatments involved modifications to the experimental apparatus, namely the colour of the partition separating the two plates (transparent or opaque) and tank size (large versus small). Changes in partition colour and tank size may affect cleaner performance by accentuating the separation between the two discreet choices and/or giving the fish a longer time (i.e. distance) to swim before a choice was made.

Methods

Experiments were conducted at the Lizard Island Research Station (14°40'S 145°28'E), Australia, in August 2014 and September 2015. A total of 32 adult cleaner wrasse *L. dimidiatus,* were caught with monofilament barrier nets (10 mm stretch) and hand nets on the reefs surrounding Lizard Island, brought back to the research station within 1 hour of capture and housed in individual aquaria with a constant flow of seawater directly from the reef. Fish were each provided with a PVC tube for refuge (2 cm diameter; 10-15 cm length), and fed daily with mashed prawn smeared over the surface of Plexiglas plates (approx. 8 x 8 cm). Some individuals were used in experiments testing other cognitive abilities (biological market, feeding against preference; (see Wismer et al., 2014; Gingins & Bshary, 2016; Wismer, Grutter & Bshary, 2016) prior to their use in our experiments. However, none of the fish were tested for spatial discrimination or in tasks where they were likely to develop a side bias. Therefore, we assume that participation in previous experiments did not influence their performance in our experiments. Fish were habituated to our experimental set-up over three consecutive days before trials commenced. All experiments were carried out in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and under the approval of the Queensland Government (Australia) Department of Agriculture and Fisheries Animal Ethics Committee (AEC Proposal Reference Number: CA 2012/05/611). All field activities were covered by a general Queensland Fisheries Permit (2014: # 82440; 2015: #149800) and GBRMPA (2014: # G11/33857.1; 2015: #G14/36625.1) permit granted to the Lizard Island Research Station.

Cognition tests

The experimental paradigm used in our experiments was spatial discrimination, whereby fish had to learn to find a food reward based on its location (left or right) in their home tank. The methods were modeled after a previous study designed to compare the performance of cleaners with closely related species (Gingins & Bshary, 2016). The basic experimental protocol was as follows (Fig. 1): Subjects were simultaneously presented with two identical Plexiglas plates, placed next to each other approximately 10 cm apart. Between the two plates, a vertical Plexiglas partition was inserted to ensure fish could access only a single plate and allow the experimenter to determine when a definitive choice had been made. One of the two plates had an accessible food reward (mashed prawn) smeared on the back, whereas the second plate offered no food reward. At the beginning of each day of experiments, the tank was divided into a holding (approx. 1/3 of tank length) and an experimental (approx. 2/3 of tank length) compartment using an opaque barrier (Fig. 1), and the fish was given 30 minutes to acclimate. For each trial, the barrier was lifted to allow the subject full access to the tank and the plates. A choice (left or right) was noted when the tip of the fish's snout first passed the threshold of the Plexiglas partition. The fish was given approximately 5 seconds to eat the food item and/or explore the experimental compartment before the barrier was placed back in the tank. The fish was kept in this holding compartment until the next trial. The trial was considered null (not taken into account) if the fish did not make a choice within 5 minutes. Fish were tested 20 times per day (2 sessions of 10 trials) for a maximum of 100 trials (10 sessions). The task was considered solved when an individual chose the rewarding plate at least 9/10 times within a single session, 8/10 times in two consecutive sessions, or 7/10 times in 3 consecutive sessions. The location of the rewarding plate was randomly assigned to the right-hand side of the tank for half of the fish (n = 16), and to the left-hand side for the other half (n = 16). The location of the rewarding plate was constant throughout all the trials. All individuals that solved the initial spatial discrimination task within 10 sessions were further tested in a reversal task. Here, food plates were changed, the location of the available food plate was reversed, and trials proceeded as above. The procedures for the reversal experiment were the same as described above for the initial spatial discrimination task.

Experimental treatments

Thirty-two cleaner wrasse were assigned to one of four variations of the cognition test protocol described above (8 individuals per treatment). In the first treatment ("clear/lift"), the vertical partition placed between the two plates was transparent ("clear"). Thus, fish could see both plates at all times, and did not necessarily perceive the partition. As soon as the fish chose a plate, the second one was removed from the tank ("lift"), which prevented the fish from accessing both plates during the trial (see Fig. 1). The fish were able to see the unselected plate being removed. Treatment two ("clear/stay") used the same clear partition as in the first treatment. However, here, the experimenter only removed the second plate when the incorrect choice (i.e. the unrewarding detractor plate) was chosen. When an individual chose the correct rewarding plate, the detractor plate remained in the tank ("stay"), and could be inspected by the cleaner. In treatment three ("opaque"), the partition separating the two food plates was made of opaque Plexiglas, which prevented the fish from seeing the second plate once a choice had been made. Here, the experimenter also removed the unchosen plate as in the "clear/lift" treatment, but the fish could not see this removal happening. These three treatments were all conducted in white plastic aquaria (L = 37 cm; W = 29 cm; H = 30 cm). Treatment four ("large tank") was carried out as described in the "clear/lift" treatment, but in a longer (L = 62 cm; W = 26 cm; H = 37 cm), glass aquarium. This setup allowed for a larger distance between the holding compartment and the plates, and thus the fish had to swim further before making a choice. Water height was maintained at approximately half of the tank height (~15cm) in all treatments.

Statistical analysis

The number of trials to complete the task is a right-censored (i.e. maximum 100 trials), ordinal variable. Therefore, we used survival analyses to compare the number of trials needed to solve the spatial discrimination and reversal task among the four treatments (see Gingins &

Bshary, 2016). Our data did not meet the assumption of proportional hazards (assessed with cox.zph() from the "survival" R package), and thus non parametric log-rank tests were performed. Post-hoc planned pairwise comparisons were made for the reversal experiments, and p-values were adjusted using Holm's sequentially rejective multiple test procedure (Holm, 1979). All statistics were performed in R 3.3.0 (R Core Team, 2013), and the package "survival" was used for the log-rank tests (Therneau, 2014). All data and code for the analyses are deposited in the figshare data repository (DOI: 10.6084/m9.figshare.5032334).

Results

All cleaners, with the exception of three individuals (two from "clear/stay", one from "large tank"), solved the spatial discrimination task within 100 trials (range : 10-90; Clear/Lift, mean = 37.5; Clear/Stay, mean = 40; Opaque, mean = 45; Large Tank, mean = 41.43). The performance of *L. dimidiatus* in this task did not differ across treatments (log-rank test: $\chi^2_3 = 1.9$, $P = 0.595$; Fig. 2a).

In contrast, fewer fish solved the reversal test within the allocated 100 trials: 0/8 fish from the "clear/lift" and 0/6 fish from the "clear/stay" treatments solved the task, whereas 5/8 fish from the "opaque" and 4/7 fish from the "large tank treatment" solved it (range : 20-90; Opaque, mean = 74; Large Tank, mean = 40). The difference in the performance of fish in this task was significantly different among treatments (log-rank test: $\chi^2_3 = 12$, $P = 0.007$; Fig. 2b). Post-hoc pairwise comparisons revealed that cleaners performed significantly worse in the clear/lift treatment than in the opaque ($P = 0.028$) or the large tank treatment ($P = 0.030$). We found no significant difference between the opaque and the large tank treatments ($P = 0.706$). We did not run pairwise comparisons involving the clear/stay treatment because the outcome was exactly the same as in the clear/lift treatment (Fig. 2).

Discussion

We asked whether modifications to the experimental paradigm of typical two-alternative forced choice tasks (spatial discrimination and reversal learning) affects the speed of learning in the cleaner wrasse *L. dimidiatus*. We found that cognitive performance can indeed, be impaired or enhanced depending on how the experiment is implemented. Although differences in the experimental procedure and apparatus did not affect performance in the initial spatial discrimination task (Fig. 2a), the performance in reversal learning depended on the specific paradigm used (Fig. 2b). This suggests that the ability to form an initial association between food and tank location did not depend on the subtleties of the experimental design. However, the ability to form new associations in the reversal task were impeded or facilitated by specific aspects of the experimental paradigm employed. Below, we discuss potential factors which may explain our results.

Increased distance to choice

Increasing the distance swam by the cleaners before a choice was made (i.e. large tank treatment) improved performance in the reversal learning task relative to the clear/lift and clear/stay treatments (Fig. 2b). Cleaners were conditioned to associate the removal of the barrier with the presence of a food reward. Consequently, cleaners typically darted out of the holding compartment immediately after the removal of the barrier. A longer travel distance between the holding compartment and the food plate translates into a longer computation time between the visual input of the task (i.e. plates) and the decision made. Trade-offs between speed and accuracy in both individual and collective decision making have been documented across a range of taxa (see Franks et al., 2003; Chittka, Skorupski & Raine, 2009; Latty & Beekman, 2011). Although performance was similar across all treatments in the initial spatial learning task, it is possible that this longer computation time provided to fish in the large tank treatment facilitated the break-down of a previously learned association, which is a prerequisite for success in the reversal task.

Visibility of the separating barrier

We found that the use of an opaque partition to separate the left from the right plate significantly improved the performance of cleaners in the reversal task (Fig. 2b). Interestingly, one could argue that having visual access to the unchosen plate via a transparent partition would facilitate learning, since the fish directly observes the removal of the rewarding plate following an incorrect choice, which should reinforce the negative association. However, transparent materials, such as the partition we used, are virtually non-existent in the natural world, and animals might have difficulties perceiving such a solid, yet transparent, object. In our experiments, we attributed a choice to the moment the fish's head passed on one side of the partition. If the individual was unable to perceive the transparent material, this criterion may not have been appropriate for determining a true choice by the fish: an individual may have attempted to approach the rewarding plate from the opposite side (i.e. from the side of the non-rewarding plate) and consequently run into this invisible partition. This scenario is likely given that fish generally prefer to swim close to structures such as tank walls, especially in situations which may induce anxiety (Maximino et al., 2010). Thus, an individual might have "known" which plate offered the food reward, but effectively "chose" the detractor plate because it approached the plates from the wrong side of the aquarium, not realizing the transparent partition would prevent it from accessing its preferred choice. This issue is intuitively more likely to occur in the reversal experiment. During the spatial discrimination experiment, individuals would have become conditioned to approaching the plates from the side initially offering the reward. Although the rewarding side was switched in the reversal experiment, the side preference for the approach would likely carry over from the first experiment even if fish did not receive a reward. This likely explains the improved performance of cleaners in the opaque partition treatment, where the fish were able to clearly perceive the separation between the options. In other words, our criteria for what constituted a correct choice may have favored opaque plates. If the choice criteria had been for the fish to touch the plate, it is possible that learning speed would have been similar for both opaque and transparent barriers, since many subjects did not actually touch the non-rewarding plate but went straight against the partition in the transparent treatments.

The role of ecology

In nature, client fishes that have access to several cleaning stations are less likely to return to the same cleaner if they had been ignored during their previous visit (Bshary & Schäffer, 2002). Ignoring a client can thus have negative consequences for cleaners, and we had hypothesized that cleaners might also associate the removal of the unchosen plate as the loss of a foraging opportunity (i.e. a potential client leaving the cleaning station to seek service elsewhere). Removing the unchosen plate in view of the cleaner might have thus impaired their ability to associate the positive feedback of the reward with their decision. As a result, we expected that removing the unchosen plate only when individuals made the wrong decision (i.e. the clear/stay treatment) would facilitate learning for cleaners in this task. We did not find evidence supporting this prediction, suggesting that the nature of cleaner-client interactions had little influence on performance in the spatial discrimination task. Spatial discrimination is not of ecological relevance to cleaners, and they do not excel in this ability compared to other wrasse species (Gingins & Bshary, 2016). It would therefore be interesting to repeat our manipulation in an ecologically relevant task: Cleaners appear to form negative associations between their decisions and the departure of a client fish in nature (Bshary & Grutter, 2002b), or feeding plates in the lab (Bshary & Grutter, 2005). One could thus repeat the study with different colours or patterns to identify the rewarding plate, rather than by its location.

Conclusion

Our study provides clear evidence that modifications in the way a cognitive test is designed or executed can have a significant impact on the subject's test scores. If different species or individuals respond differently to the experimental paradigm, this could affect interpretations derived from comparisons among groups (Rowe & Healy, 2014; Morand-Ferron, Cole & Quinn, 2016). The importance of good experimental design in the field of comparative cognition cannot be overstated. In our experiments, even seemingly small details such as the colour of certain material and arena size had a significant impact on the outcome of one of our cognitive tests. Given this finding, it is perhaps unsurprising that independent studies aimed at testing the same cognitive abilities using different methodologies find different results (see introduction). In comparative cognition, it may be virtually impossible to design experiments in which variation in performance exactly reflects variation in cognition, particularly across species. A good understanding of the ecology of each species, and hence the cognitive challenges they naturally face, may help researchers avoid many sources of bias in individual performance. Nevertheless, biases may still go unperceived by researchers. Whether researchers are interested in comparing cognitive performance across species, populations, or individuals, it remains important to increase the number and the diversity of groups tested, to test subjects in a variety of tasks, and to reflect upon the potential influence of each species' ecology before drawing general conclusions about the cognitive abilities of a given group.

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Figure 1(on next page)

The four different experimental paradigms (i.e. treatments) used in the experiments

Diagram of the four different experimental treatments used in our two alternative forced choice tasks. At the beginning of each trial, an opaque barrier (dotted line) separating the holding and the experimental compartments was lifted. In all treatments, the fish was presented with two identical plates separated by a small partition. One of the plates consistently had a food reward located at the back of the plate (i.e. invisible from the front). In this illustration, the correct choice (i.e. the rewarding plate) is always located on the left-hand side of the tank. The solid arrows (green = correct; red = incorrect) illustrate the fish's decisions, and the dashed arrows show which plate is removed following the initial choice. In all treatments except for Clear/Stay, the unchosen plate was removed immediately after a choice was made. In the Clear/Stay treatment, the unchosen plate was only removed when the fish made an incorrect choice. The partition placed between the two plates was always transparent (grey line), except in the Opaque treatment (black line). In the Large Tank treatment, the experiments were performed exactly as in the Clear/Lift treatment, but in a longer tank. Tank sizes are drawn to scale.

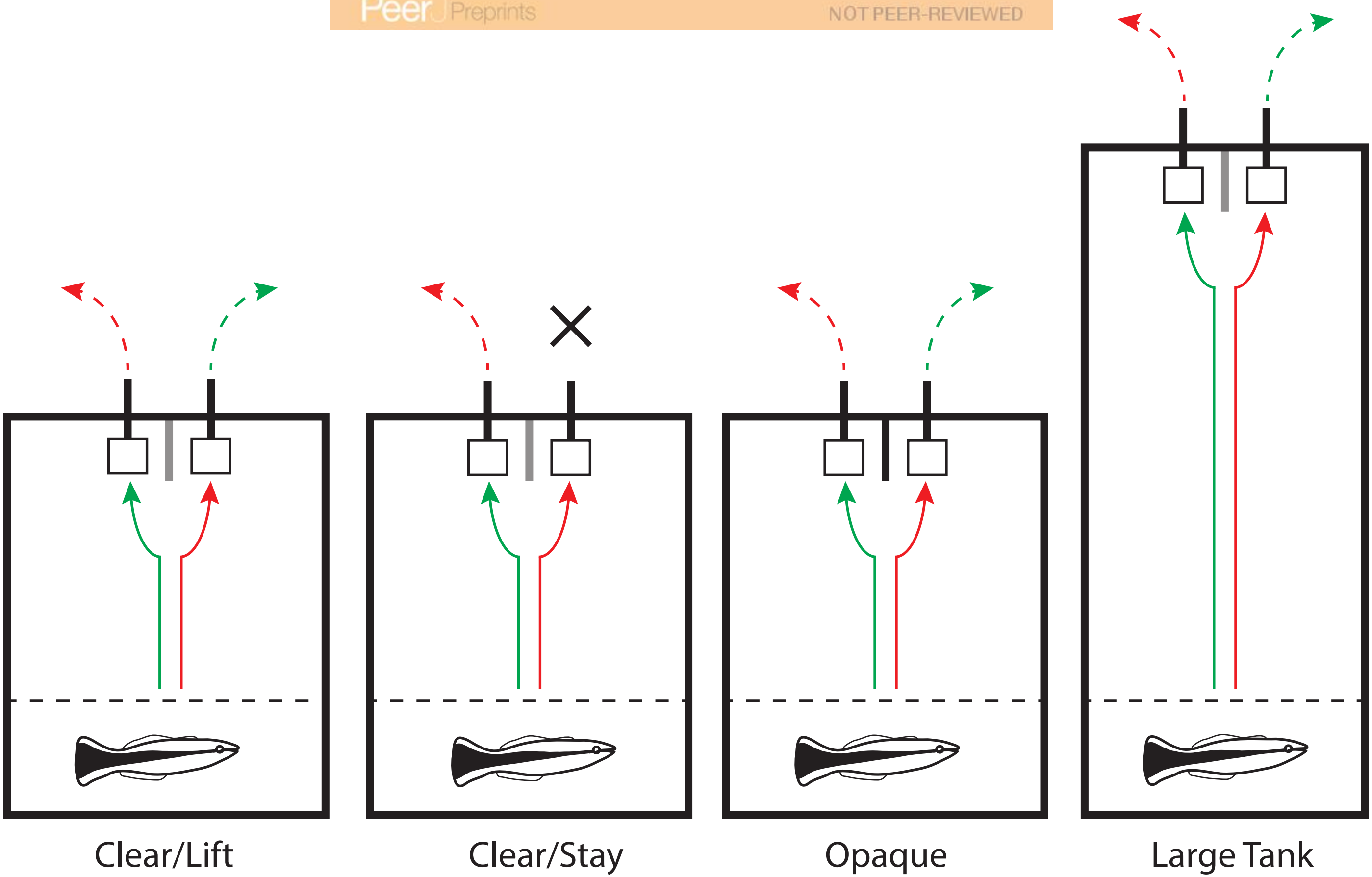

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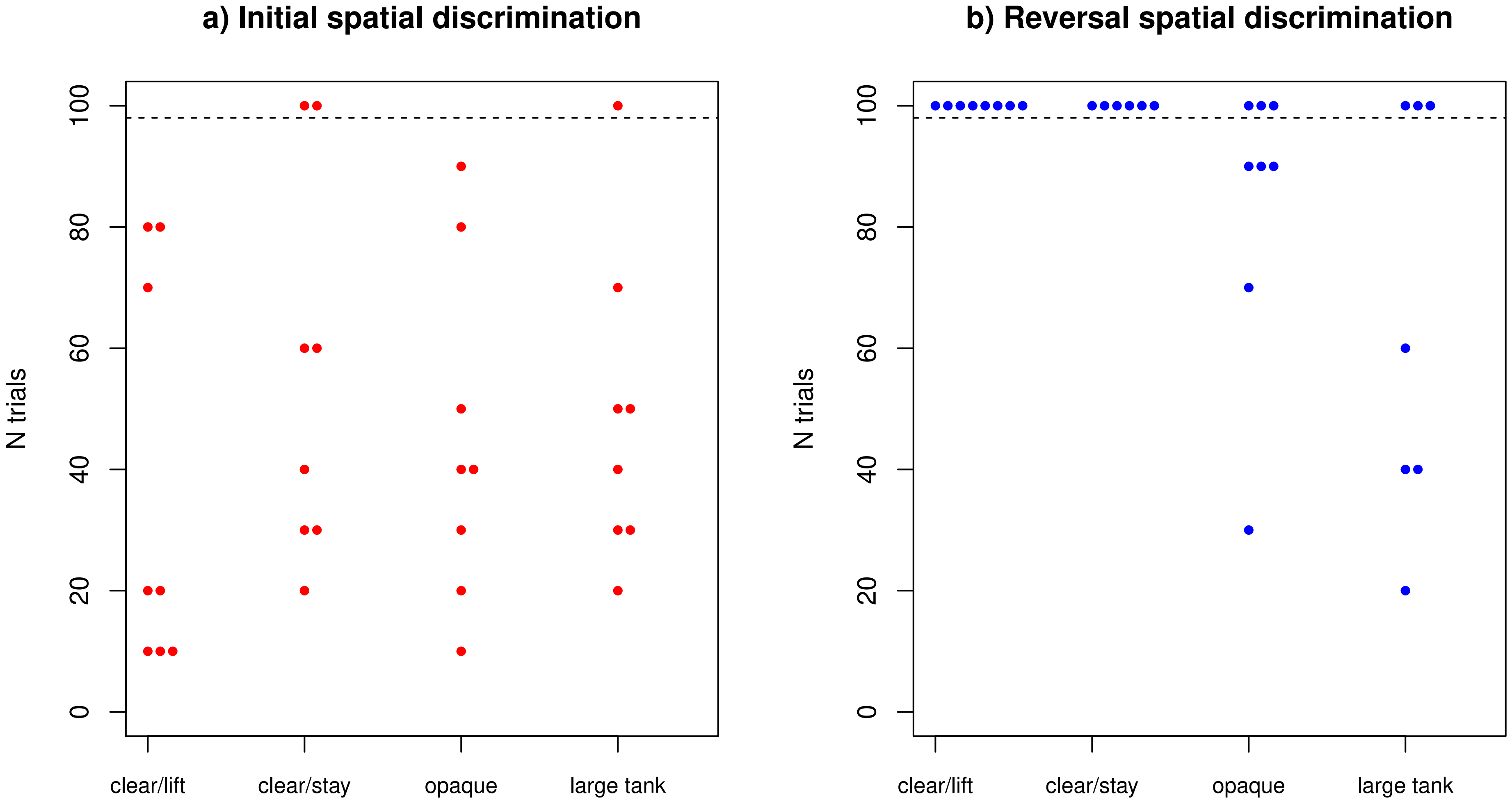
Figure 2(on next page)

Learning speed

Number of trials required to solve the task in a) the initial spatial discrimination task and b) the reversal spatial discrimination task. Each dot represents one individuals tested. All individuals depicted above the dotted line failed to solve the task within the 100 allocated trials. The three individuals that did not solve the initial task were not tested in the reversal learning task.


a) Initial spatial discrimination
b) Reversal spatial discrimination
N trials
0
20
40
60
80
100
clear/lift
clear/stay
opaque
large tank