Spontaneous alternation behavior in *Paramecium*

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Spontaneous alternating behavior (SAB), the tendency of an organism to successively turn left and right in a maze, is a standard metric of short-term memory in developmental and psychopharmacological studies. Although SAB is perhaps the most phylogenetically widespread behavior in motile lifeforms, conflicting data on SAB exist for several sets of closely related taxa. For example, previous studies provide contradictory evidence concerning the existence of spontaneous alternation behavior in the protozoan *Paramecium*. However, these studies tested different species, using very different experimental designs. Using a single, factorial design for both previously tested species, *P. multimicronucleatum* and *P. caudatum*, we found that, in fact, the two species do not differ in their alternation behavior; specifically, they alternate in mazes with short tracks, but not in mazes with long tracks. These results are in accord with alternation studies on other taxa, and they fully resolve the apparent contradictions in the earlier studies on *Paramecium*. They also indicate that caution should be used in interpreting variable results across species tested with different experimental designs and suggest that, in at least some taxa, SAB may not be strictly dependent on the use of short-term memory.

Spontaneous alternation behavior (SAB) is the nonrandom tendency of an organism to choose to turn in the direction opposite to that of its previous turn in the absence of reward or reinforcement (Dember & Richman, 1989). Although the underlying mechanism of SAB has been controversial over the years, most researchers agree that the ability to alternate choices requires that the organism remember its previous choice (Hughes, 2004). As a result, SAB is a standard metric of short-term memory that has been used in hundreds of psychopharmacologic and developmental studies in mice and rats (Lalonde, 2002). Recently, however, some authors have begun to question the utility of SAB and other maze-based measures of memory (Wolfer, Staglar-Bozicevic, Errington, & Lipp, 1998), noting the potential for noncognitive factors such as thigmotaxis and residual odor cues to influence subject behavior in mazes.

Perhaps the most phylogenetically general behavior of motile life forms, SAB has been reported in animals ranging from primitive flatworms (Aderman & Dawson, 1970; Shinkman & Hertzler, 1964) to mammals (Dashiell & Bayroff, 1931), in protozoans (Lepley & Rice, 1952), and even in sperm cells (Brugger, Macas, & Ihlemann, 2002). It is unusual for closely related species to differ in such an apparently conservative (and fundamental) trait, and yet there are several instances in which SAB has been reported in one species but not observed in a close

relative. Some examples include paramecia (Lachman & Havlena, 1962; Lepley & Rice, 1952), planarians (Aderman & Dawson, 1970; Rice & Lawless, 1957; Shinkman & Hertzler, 1964), and chickens (Cogan, Jones, & Irons, 1979; Hayes & Warren, 1963; Hughes, 1989a). To date, little attention has been paid to this curious situation, whose interpretation is further complicated by the extreme diversity of experimental designs used to study alternation behaviors.

For example, Lepley and Rice (1952) and Lachman and Havlena (1962) used paramecia to test Hull's (1943) model of reactive inhibition. Although neither study found support for reactive inhibition, Lepley and Rice did find unambiguous evidence for SAB in *P. multimicronucleatum* (84.7% alternation, $N = 1,115$, $\chi^2 = 535.9$, $p < .001$), whereas Lachman and Havlena reported equally compelling evidence that SAB does not occur in *P. caudatum* (47% alternation in single-forced-turn trials, $N = 100$, $\chi^2 = 0.36$, $p = .55$; 48.5% alternation in multiple-forced-turn trials, $N = 800$, $\chi^2 = 0.72$, $p = .40$). Lester (1968) and Hughes (1989b) noted the conflicting evidence provided by these studies but, like Lachman and Havlena, did not comment on the basis of this discrepancy. Shinkman and Hertzler (1964) speculated that the differences might have been due to differences in the experimental design of the two studies.

It is thus unclear whether or not these species truly differ in SAB. Not only did Lepley and Rice (1952) and Lachman and Havlena (1962) use different species of *Paramecium*, but, as Shinkman and Hertzler (1964) noted, their experimental designs differed in numerous other ways as well (Table 1). Of these many parameters, only track length and, to a limited extent, runway length have been systematically tested with respect to SAB (see Figure 1 for the maze terminology used in this article). SAB

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Table 1
Summary of Differences in Experimental Design in Lepley and Rice (1952) and Lachman and Havlena (1962)

Parameter	Lepley and Rice	Lachman and Havlena
<i>Paramecium</i> species	<i>P. multimicronucleatum</i>	<i>P. caudatum</i>
Maze construction	Etched in glass with hydrofluoric acid	Carved in paraffin layer on glass
Choice arm	Straight (T-maze)	Bent medially (Y-maze)
Track length	1 mm	6 mm (estimated)
Runway length	3 mm	6 mm (estimated)
Segment width	0.08 mm	1.6 mm
<i>n</i> per trial	43 (mean; some trials with >100 individuals)	1

Note—Restricted to differences in the basic spontaneous alternation behavior experiment and control—that is, mazes with either one or no forced turn. See Figure 1 for a description and illustration of maze terminology. Track and runway lengths were not explicitly provided by Lachman and Havlena and were approximated from their Figure 1.

consistently decreases as track length increases, as would be expected if SAB requires organisms to “remember” the direction of their previous turn. Runway length does not normally influence SAB, except in several species of insects (Hughes, 1989b). Of the remaining differences between Lepley and Rice’s study and that of Lachman and Havlena listed in Table 1, maze segment width seems the most likely to affect SAB expression, although in a perhaps artifactual fashion. Specifically, a maze segment may be so wide that it is effectively perceived by the organism not as a *path* but, rather, as an *area*, in which directional choices are unlimited.

In this study, we used a single experimental setup to determine whether or not SAB is expressed in *P. caudatum* and *P. multimicronucleatum* and how track length and segment width influence this behavior in these species. Our results clearly show that the interspecific differences reported by Lepley and Rice (1952) and Lachman and Hav-

lena (1962) reflect differences in their experimental design, rather than in the behavior of the species themselves.

METHOD

Subjects

P. caudatum and *P. multimicronucleatum* were obtained from Berkshire Biological Laboratories (Westhampton, MA). Each paramecium was used in only one trial. Fifty paramecia were used in each control group and each treatment group.

Apparatus

Mazes were constructed in a fashion similar to that in Lachman and Havlena (1962). A microscope slide was dipped into a container of melted paraffin wax, allowed to cool and dry, and then dipped again. Paraffin on one side of the slide was then scraped off, leaving a level layer of paraffin (0.5 mm deep) on the other side into which the maze was etched. Mazes varied in three parameters (refer to Figure 1 for maze terminology): segment width (narrow, 0.5 mm; wide, 1.5 mm), track length (short, 3 mm from choice point; long, 6 mm from choice point), and presence of forced turn (forced turn, the maze included an extra 3-mm-long runway connected at right angles to the start of the track; control, a standard T-maze, with no runway). Thus, four experimental mazes and four control mazes were used in this study. Unfortunately, we were not able to duplicate the maze dimensions of either earlier study; Lepley and Rice (1952) used a nitric acid etching technique that apparently allowed them to produce narrower and shorter maze elements than is possible with the paraffin method, and the only dimension supplied by Lachman and Havlena was segment width.

All forced turns were to the right, as in Lepley and Rice (1952) and Lachman and Havlena (1962).

Procedure

Prior to each trial, a maze was rinsed and then carefully flooded with filtered, aged culture media. One paramecium was transferred to the reservoir at the entrance to the maze with a 1.5-ml pipette and observed until it entered either arm of the choice turn or until 15 min had elapsed, in which case the trial was terminated and excluded from analyses. Trials were observed under a dissecting microscope at 10X magnification with bottom lighting. Mazes were placed atop a room temperature water bath to minimize heating during the trial.

Statistical Analysis

A chi-square test was used to compare the proportion of paramecia turning left versus right in each species with the a priori expected 50:50 ratio for SAB. Neither species of *Paramecium* showed any

Figure 1. General maze terminology used in this study. **Runway:** straight section of maze leading from starting reservoir to **forced turn** (90° turn to the right). **Track:** straight section of maze leading from the forced turn to a **choice turn** (90° turn either to the left or the right). **Choice arm:** straight section of maze centered at and perpendicular to the far end of the track. Runways, when present, were 3 mm long; track lengths were either 3 or 6 mm. All the segments (i.e., runway, track, and choice arms) were either 0.5 or 1.5 mm wide.

directional bias in the control T-mazes (*P. caudatum*, $\chi^2 < 0.01$, $p = .94$, $N = 157$; *P. multimicronucleatum*, $\chi^2 = 0.09$, $p = .77$, $N = 188$; see Figure 2). Therefore, each species was similarly evaluated for SAB against an a priori 50:50 null expectation. We used three-way contingency analyses to determine whether maze arm choice was affected by maze length or maze width for each species.

RESULTS

Virtually all the paramecia completed the trials in the wide mazes, but the narrow mazes seemed to be somewhat more difficult for the paramecia, especially *P. caudatum*, to complete during the 15-min trial (Figures 2 and 3). Overall, both species of *Paramecium* showed significant SAB (*P. caudatum*, $\chi^2 = 6.68$, $p < .01$, $N = 163$; *P. multimicronucleatum*, $\chi^2 = 8.00$, $p < .005$, $N = 200$; Figure 3). However, SAB in all the short-track maze treatments represented the only deviation from random choice (Figure 3). Track length significantly affected SAB in both *P. caudatum* ($G = 8.81$,

$p = .01$, $df = 2$) and *P. multimicronucleatum* ($G = 19.82$, $p < .01$, $df = 2$; see Figure 3): Paramecia exhibited SAB only in the short tracks. In contrast, maze width did not influence the occurrence of SAB in either species (*P. caudatum*, $G = 0.25$, $p = .88$, $df = 2$; *P. multimicronucleatum*, $G = 0.20$, $p = .91$, $df = 2$; see Figure 3).

DISCUSSION

The present study clearly demonstrates that the expression of SAB does not differ between *P. caudatum* and *P. multimicronucleatum* and provides an unambiguous explanation for the diametrically opposite results in Lepley and Rice (1952) and Lachman and Havlena (1962) with respect to basic SAB in these species. Lepley and Rice observed SAB in *P. multimicronucleatum* because they used mazes with a very short track between the forced and the free turns. Lachman and Havlena did not observe SAB in *P. caudatum* because they used mazes with a much longer track between the forced and the free turns.

At 3 mm, our short track was three times longer than that in Lepley and Rice (1952), and correspondingly, we observed considerably lower rates of SAB than did Lepley and Rice for *P. multimicronucleatum* (72% vs. 84.7%). Lachman and Havlena (1962) did not give explicit segment lengths (although the fact that the *width* of their maze segments was 60% greater than the *length* of Lepley & Rice's track requires that their track was at least several times longer than that in Lepley & Rice), but approximations from their Figure 1 suggest that their track was about 6 mm long. This is the same as our long track, and in both cases, SAB was completely absent.

Maze width differences, in contrast, did not affect SAB in either species and are, thus, unlikely to have played a role in the earlier results. Although the methods of Lepley and Rice (1952) and Lachman and Havlena (1962) differed in several other respects (Table 1), we did not test the effects of these other parameters, and in any case, the closely equivalent performance of the two species across the three studies, once track length is accounted for, suggests that these other differences were relatively unimportant.

Although distance commonly is not evaluated as an experimental variable in SAB studies, those studies that do so nearly always report that SAB decreases as track length increases (Hughes, 1989b). The standard explanation for this inverse relationship follows from a memory-based interpretation of SAB: When given a choice, an organism will turn in the direction opposite of its previous turn *if it can remember the direction of that turn* (Kupfermann, 1966). If it does not remember what it did last time, its next choice will be random. The longer the distance between successive turns, the greater the probability that the organism will "forget" the direction of its previous turn (Hughes, 1989b).

A couple of points deserve mention here. First, in the argument above, *distance* logically would seem to be a proxy for *time*. That is, presumably, the main reason an organism is more likely to forget something over longer distances is because of the increasing time it takes to travel

Figure 2. Percentage of *Paramecium* turning left at the choice point in control mazes. (A) Trials with *P. caudatum*. (B) Trials with *P. multimicronucleatum*. Short, track length 3 mm; long, track length 6 mm; narrow, segments 0.5 mm wide; wide, segments 1.5 mm wide. Each bar refers to a sample size of 50 completed trials, unless otherwise indicated by a number at the base of the bar.

Figure 3. Percentage of *Paramecium* turning left at the choice point in experimental mazes. All forced turns were to the right; therefore, left turns represent alternation. (A) Trials with *P. caudatum*. (B) Trials with *P. multimicronucleatum*. Short, track length 3 mm; long, track length 6 mm; narrow, Segments 0.5 mm wide; wide, Segments 1.5 mm wide. Each bar refers to a sample size of 50 completed trials, unless otherwise indicated by a number at the base of the bar.

longer distances, not because of the greater distance itself. Second, no particular mechanism of “memory” is implied in this context. That is, *any* mechanism (e.g., central vs. peripheral neural control or even explicit neural control vs. mechanical influences, such as differential muscle fatigue) that causes current actions to be influenced by previous actions could potentially lead to SAB in general and to the distance effect in particular.

Note further that it is the track length *relative to the size of the organism*, not the absolute track length, that matters here. Thus, one might expect that larger individuals would show relatively more SAB than would smaller individuals running mazes of the same size. For example, Taylor (1995) reported SAB in four species of jumping spiders, with no significant differences among the species. However, two of the species Taylor used, *Portia fimbriata* and *Trite planiceps*, are considerably larger (up to 10 mm

long) than the other two, *T. auricoma* and *Helpis minitabunda* (less than 8 mm long) (Zabka, 1988). An analysis of Taylor’s data shows that, as was predicted, large spiders were more likely to exhibit SAB than were smaller spiders (one-tailed $p = .052$, Fisher’s exact test).

Similarly, one might predict that within a species, younger individuals would be less likely to express SAB than would adults to the degree that juveniles are smaller (and perhaps slower) than adults. We know of only two species in which adults and demonstrably smaller juveniles were tested under the same maze conditions: boxelder bugs *Boisea trivittatus*, (Dingle, 1961) and big-headed bugs *Alydus eurinus* (Dingle, 1962). In both cases, adults, but not juveniles, showed SAB.

Although we did not specifically investigate the proximate mechanisms of SAB in *Paramecium*, we feel it is worth reiterating Jensen’s (1959) observations on swimming behavior in *Paramecium*. Jensen (1959) noted that a free-swimming *Paramecium* moves forward in a constant spiral, which would make it difficult, if not impossible, for the organism to maintain a consistent left–right frame of reference as it travels through the maze. Thus, although the specific patterns shown by *Paramecium* are similar to those found in other animals (i.e., SAB that decreases as track length increases), the underlying mechanism, at least for *Paramecium*, is not likely to involve short-term memory, no matter how broadly interpreted.

In conclusion, the differences reported by Lepley and Rice (1952) and Lachman and Havlena (1962) do not reflect species differences in the expression of SAB. Rather, they reflect an important difference in the experimental design of the two studies—specifically, the distance between the forced and the free turns in the maze. The great range of motile life forms in which SAB has been observed indicates that studies reporting the absence of SAB in a particular species should be considered carefully. Because SAB is a context-dependent behavior, negative results may be identifying a set of conditions inappropriate for eliciting SAB in that species, rather than identifying a species that does not exhibit SAB.

Unfortunately, despite the apparent simplicity of the SAB concept, virtually every study investigating this behavior invokes a unique experimental design that makes strong cross-study comparisons difficult to impossible. Thus, more studies such as the present one, in which multiple species are tested using a single, factorial experimental design, will be needed to properly understand the true phylogenetic generality of, and the underlying mechanisms responsible for, spontaneous alternation behavior.

Furthermore, the fact that *Paramecium* exhibit standard distance-dependent SAB, despite the improbability that paramecia can store and use information concerning their previous choices, suggests that noncognitive cues may play a stronger role in SAB than is currently realized. This, of course, does not imply that short-term memory plays no role in maze navigation, but it is consistent with Hughes’s (2004) concern that SAB may not be the most reliable metric of short-term memory.

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