Research

Spatial relational memory in 9-month-old macaque monkeys

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This experiment assesses spatial and nonspatial relational memory in freely moving 9-mo-old and adult (11–13-yr-old) macaque monkeys (*Macaca mulatta*). We tested the use of proximal landmarks, two different objects placed at the center of an open-field arena, as conditional cues allowing monkeys to predict the location of food rewards hidden in one of two sets of three distinct locations. Monkeys were tested in two different conditions: (1) when local visual cues marked the two sets of potentially baited locations, so that monkeys could use both local and spatial information to discriminate these locations from never-baited locations; and (2) when no local visual cues marked the two sets of potentially baited locations, so that monkeys had to rely on a spatial relational representation of the environment to discriminate these locations. No 9-mo-old or adult monkey associated the presence of the proximal landmarks, at the center of the arena, with the presence of food in one set of three distinct locations. All monkeys, however, discriminated the potentially baited locations in the presence of local visual cues, thus providing evidence of visual discrimination learning. More importantly, all 9-mo-old monkeys tested discriminated the potentially baited locations in absence of the local visual cues, thus exhibiting evidence of spatial relational learning. These findings indicate that spatial memory processes characterized by a relational representation of the environment are present as early as 9 mo of age in macaque monkeys.

The existence of multiple memory systems subserved by different neural substrates is a widely accepted view of memory organization in the mammalian brain (Cohen and Eichenbaum 1993; Milner et al. 1998; Eichenbaum 2000). Declarative (relational) memory was originally defined as the type of memory sensitive to lesion of the medial temporal lobe (the hippocampus in particular), whereas nondeclarative memory encompasses a set of disparate memory processes that are not sensitive to medial temporal lobe damage (Cohen and Squire 1980; Squire 1992). In rodents, declarative memory has been intensively investigated with tasks that assess spatial relational abilities in freely moving individuals (Olton and Samuelson 1976; O'Keefe and Nadel 1978; Morris 1981; Barnes 1988; Lavenex and Schenk 1995, 1996, 1997, 1998; Schenk et al. 1995; Lavenex et al. 1998). These tasks are readily learned and can easily be adapted to the ecological and ethological characteristics of different species (Lavenex et al. 1998) including humans (Overman et al. 1996), thus enabling the comparative evaluation of learning and memory processes (Banta Lavenex et al. 2001). Spatial learning tasks in which subjects can move about freely in a controlled environment, in contrast, have only rarely been used to study memory processes in monkeys (but see Rapp et al. 1997; Rehbein and Moss 2002; Ludvig et al. 2003; Ma et al. 2003; Hampton and Murray 2004).

We have adapted an experimental design originally developed to assess spatial cognition in rodents (Lavenex and Schenk 1995, 1997; Lavenex et al. 1998) in order to study the emergence of memory processes in macaque monkeys. We sought to determine whether 9-mo-old monkeys are capable of relying on relational representations of the environment in order to successfully forage for food in an open-field arena. The goal of these experiments was not to compare the overall performance of juvenile monkeys with that of adults, because relative performance is not germane to determining whether or not an individual possesses defined cognitive processes. Indeed, if an animal is capable of discriminating one or several goal locations in the absence of local cues (i.e., in relation to distant environmental cues) (Morris 1981), the animal, by definition, must be using a relational memory representation (i.e., an allocentric representation of space) (O'Keefe and Nadel 1978), regardless of its performance relative to other individuals. We therefore tested 9-mo-old and adult (11–13-yr-old) monkeys in order to compare the behavioral strategies used by monkeys of different ages to forage for food in our experimental conditions.

Our task allows freely moving monkeys to forage for preferred food located in two arrays of three distinct locations among 18 potential locations distributed in an open-field arena (Figs. 1, 2) (i.e., on any given trial, food was located in only three of 18 locations). Nine-month-old ($n = 5$) and adult ($n = 3$) monkeys were given three trials per day, 5–7 d per wk. The location of the food remained the same between trials (1-min interval) within a daily session, but changed pseudo-randomly between the two arrays between sessions (24-h interval). For half of the sessions, one specific object placed at the center of the arena (the central object) was associated with the presence of food in one array of three distinct locations (Fig. 2A). On alternate days, a different object placed at the center of the arena was associated with the presence of food in the other array of three distinct locations (Fig. 2A). Thus, the central object could be used as a conditional cue to predict which array of three distinct locations contained food on any particular day (i.e., nonspatial relational learning).

The experiment was conducted in two successive phases. During the first phase, the local cue condition, potentially baited locations were marked by local cues; the two sets of potentially baited locations were marked by different colored cups (which were different in color from the central objects) (Fig. 2A). In this

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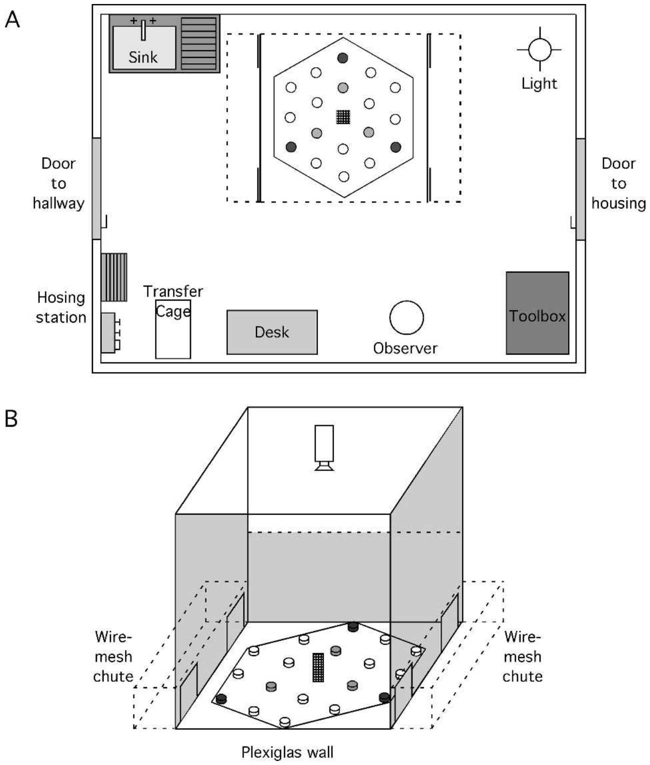

Figure 1. Schematic representation of the testing environment for the nine-month-old monkeys. (*A*) Aerial view of the experimental room. (*B*) Three-dimensional view of the testing arena. Eighteen plastic cups were regularly distributed on a hexagonal board (1.2 m in diameter for 9-mo-olds and 2.1 m in diameter for the adults) placed in a square testing arena (1.3 m W × 1.3 m D × 1.3 m H for 9-mo-olds and 2.2 m W × 2.2 m D × 2.2 m H for the adults). Remotely operated sliding doors at each corner of the arena allowed the animals to go in and out of the arena from wire-mesh chutes located along both sides. The front panel, the roof, and the top half of the back panel (dashed lines in *A*; clear in *B*) were made of Plexiglas, allowing a clear view of distant environmental cues; two opaque side panels (solid lines in *A*; gray shading in *B*) provided visual barriers between the open-field arena and the wire-mesh holding chutes. Two different objects could be placed alternately at the center of the arena and used to predict which array of three potentially baited locations was baited. We precluded the reliance on an egocentric representation of space by alternating pseudo-randomly between four different entrances into the testing arena. See main text for detailed description of the experimental room and procedure. Adult monkeys were tested in a similar, albeit larger experimental room.

condition, monkeys could rely on a visual guidance strategy to locate the potentially baited locations and therefore exhibit evidence of visual discrimination. During this phase, the monkeys were also free to associate the presence of the central object with the presence of food in one array of three distinct locations. In the middle of this local cue phase, monkeys were subject to a reversal of the relation between the central objects and the baited arrays in order to assess their reliance on the central object to predict which array was baited. Monkeys were also subject to a single dissociation probe trial during which the position of the colored cups was shifted 60° from their usual spatial locations and none of the cups was baited (Fig. 2B). The aim of this probe trial was to assess the monkeys' reliance on local cues (i.e., visual guidance/discrimination) versus spatial information (i.e., a spatial relational representation) to discriminate potentially baited locations from never-baited locations. During the second phase, the spatial relational condition, no local cues marked the potentially baited locations; all cups were the same neutral color (Fig. 2C). In this case, monkeys had to rely on a spatial relational representation of the environment to discriminate these locations. The central objects could still be used to predict which array of three distinct locations was baited. During both phases, we precluded the reliance on an egocentric representation of space by alternating pseudo-randomly between four different entrances into the testing arena.

In sum, this task was designed to evaluate several types of cognitive and memory processes in freely moving 9-mo-old and adult monkeys: (1) visual discrimination learning in the local cue condition; (2) spatial relational learning in the spatial relational condition; and (3) nonspatial relational learning in both the color and spatial conditions, as evaluated by the monkeys' use of the central object to predict which array was baited. Furthermore, because the baited locations changed pseudo-randomly between the two arrays between sessions, the monkeys' choices during the first trial of the session could be analyzed to evaluate their long-term (24 h) memory for color or spatial information. Similarly, because monkeys were given three trials per daily session, their choices could also be analyzed to evaluate their short-term (1-min) memory for color or spatial information.

Results

Relational memory in the local cue condition

We first focused on establishing whether 9-mo-old and adult monkeys used the central objects to predict which array of three distinct locations was baited in the local cue condition (i.e., nonspatial relational learning). If monkeys inferred the predictive value of the central objects, they should organize their foraging to search the array of baited locations associated with a particular object. We classified the monkeys' choices as follows: "Correct", the opening of a colored cup at a baited location; "Incorrect", the opening of a colored cup at a potentially baited location that was not baited that particular day; and "Other", the opening of a neutral cup at a never-baited location.

Evaluation of the 9-mo-old monkeys' choices for the first daily trial of seven daily sessions preceding the reversal procedure (only the first trial of each session was included to exclude any possible effects of learning within a daily session) did not reveal any evidence that the 9-mo-olds were using the central objects to predict which array of three distinct, marked locations was baited (Fig. 3A). For the first choice, 9-mo-old monkeys discriminated potentially baited locations from never-baited locations, but chose potentially baited locations on the nonbaited array as frequently as baited locations ($F_{(2,57)} = 6.064$, $P = 0.0041$; Correct = Incorrect > Other, $P < 0.01$). For the first four choices, 9-mo-old monkeys continued to choose potentially baited locations on the nonbaited array as frequently as baited locations, and significantly fewer never-baited locations ($F_{(2,57)} = 52.7$, $P < 0.0001$; Correct = Incorrect > Other, $P < 0.0001$).

Similarly, adult monkeys did not rely on the central objects to predict which array of three distinct marked locations was baited (Fig. 3B). For both the first choice and the first four choices, adult monkeys discriminated potentially baited from never-baited locations, but chose potentially baited locations on the nonbaited array as frequently as baited locations (First choice: $F_{(2,33)} = 5.617$, $P = 0.008$; Correct = Incorrect > Other, all $P < 0.01$; First four choices: $F_{(2,33)} = 219.943$, $P < 0.0001$; Correct = Incorrect > Other, all $P < 0.0001$).

We further tested whether 9-mo-old and adult monkeys inferred the predictive value of the central objects by reversing the relation between the objects and the baited arrays, and assessing whether the monkeys' behavior was perturbed. No 9-mo-old or

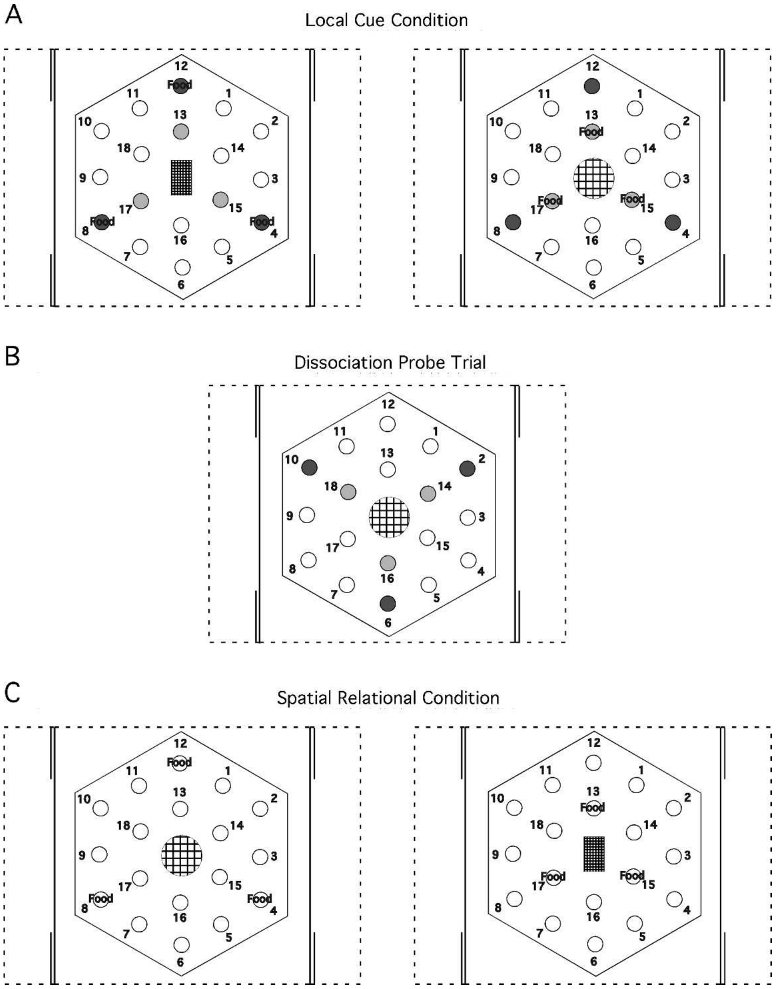

Figure 2. Schematic representation of the arena in the different conditions. (*A*) Local cue condition, green cups at locations 4, 8, and 12 on the outer array were baited when a yellow bottle was present at the center of the arena, whereas red cups at locations 13, 15, and 17 on the inner array were baited when a blue ball was present at the center of the arena. (*B*) Dissociation probe trial, colored cups were shifted 60° from the correct spatial locations. Green cups were at locations 2, 6, and 10, and red cups were at locations 14, 16, and 18. Neutral cups were at locations 4, 8, 12, 13, 15, and 17. No food was present. (*C*) Spatial relational condition, neutral cups at locations 4, 8, and 12 were baited when a blue ball was present at the center of the arena, whereas neutral cups at locations 13, 15, and 17 were baited when a yellow bottle was present at the center of the arena.

adult monkey tested was affected by the reversal of the relation between objects and arrays (data not shown). Altogether, these data indicate that monkeys, irrespective of their age, did not use the central objects to predict which array of locations was baited.

Long-term (24 h) and short-term (1 min) memory of the baited locations

We next evaluated whether monkeys' choices were influenced by their memory of the locations, in both the local cue and spatial relational conditions, which were baited during the previous session (24 h earlier). If monkeys remembered which array of three distinct locations was baited the previous day and used this knowledge to guide their choices (i.e., to choose the same locations as had been baited on the previous day), we would expect the number of correct choices to be higher when the baited array was the same as during the previous session, as compared with when the alternate array had been baited during the previous session (Lavenex et al. 1998). Because the monkeys' choices could obviously be influenced by learning within a session (i.e., from trial 1, to trial 2, to trial 3), we considered only the first trial of each session for this analysis. Group and individual analyses did not reveal evidence that any 9-mo-old or adult monkey relied on its long-term (24 h) memory of the baited locations to guide its choices in either the local cue or spatial relational condition (data not shown).

We also evaluated whether monkeys' choices were influenced by their memory of the locations that were baited during the previous trial(s) within a daily session (1-min intertrial interval). Monkeys performed three trials per session, during which the baited locations remained the same. If monkeys remembered which array of three distinct locations was baited during the previous trial and used this knowledge to guide their choices, we would expect the number of correct choices to increase within a daily session (Lavenex et al. 1998). Our analyses did not reveal evidence that any 9-mo-old or adult monkey relied on its short-term (1 min) memory of the baited locations to guide its choices in either the local cue or spatial relational condition (data not shown).

Foraging strategies

After determining that neither the monkeys' short-term (1 min) nor long-term (24 h) memory of the baited locations influenced their choices among potentially baited locations, we analyzed the strategies they used to discriminate potentially baited locations from never-baited locations. For these analyses, each location was classified into one of five categories with respect to whether it was potentially baited and its position in the open-field arena: "Pot IN", the three potentially baited locations at the corners of the inner hexagon (Fig. 2A,C; locations 13, 15, 17); "Pot OUT", the three potentially baited locations at the corners of the outer hexagon (locations 4, 8, 12); "Equ IN", the three never-baited locations at the corners of the inner hexagon (locations 14, 16, 18; denoted "equivalent" because of their position at one of the three corners of the hexagon topologically equivalent to the position of the potentially baited locations); "Equ OUT", the three never-baited locations at the corners of the outer hexagon (locations 2, 6, 10); and "Other", the never-baited locations on the sides of the outer hexagon (locations 1, 3, 5, 7, 9, 11). Analyzing the monkeys' choices with respect to these five categories allows us to characterize the strategies that 9-mo-old and adult monkeys relied on to discriminate the baited locations. In the spatial relational condition, monkeys must remember these locations in relation to distant environmental cues (i.e., form a spatial relational representation of the environment) in order to discriminate Pot IN from Equ IN, or Pot OUT from Equ OUT, which are located at topologically equivalent locations at the corners of the inner and outer hexagon, respectively.

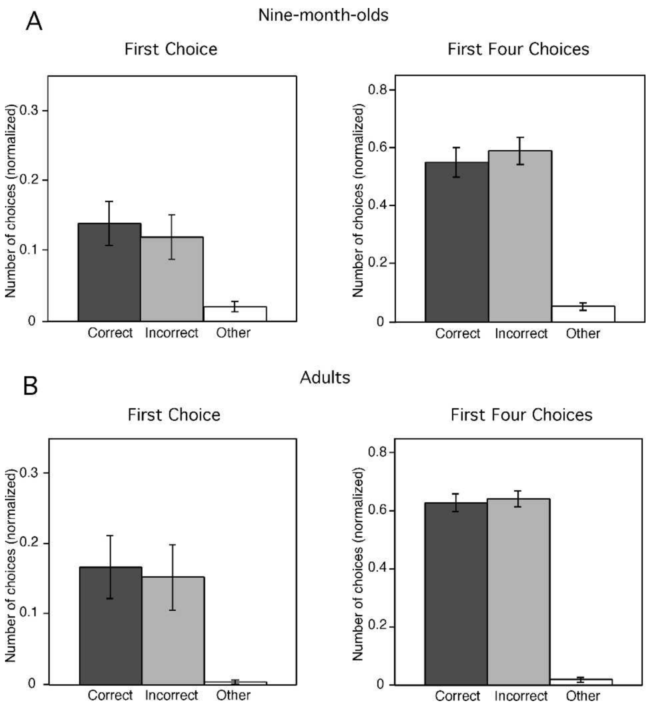

Figure 3. Nine-month-old (*A*) and adult (*B*) monkeys' choices in the local cue condition. Average number of Correct (baited locations), Incorrect (potentially baited locations not baited), and Other (never-baited) locations for the first and first four choices in the first trial of the day for the sessions preceding the reversal of the relation between the central objects and baited arrays. The number of choices in each category (n) is normalized according to the probability of making that choice (n/3 for Correct and Incorrect choices and n/12 for Other choices).

Local cue condition

We first evaluated the monkeys' discrimination of the potentially baited cups in the presence of local cues marking these locations. For the first choice (i.e., the first cup opened during an individual trial) in the local cue condition, 9-mo-old monkeys chose potentially baited locations more often than never-baited locations (Fig. 4A; $F_{(4,16)} = 19.986$, $P < 0.0001$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.005$; Pot IN > Equ IN = Equ OUT = Other, all $P < 0.005$). In addition, they exhibited a preference for the potentially baited locations on the outer array as compared with those on the inner array (Pot OUT > Pot IN, $P = 0.0249$). For the first four choices (i.e., the first four cups opened during an individual trial), 9-mo-olds continued to choose potentially baited locations more often than never-baited locations (Fig. 4A; $F_{(4,16)} = 254.467$, $P < 0.0001$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.0001$; Pot IN > Equ IN = Equ OUT = Other, all $P < 0.0001$), and exhibited a preference for the potentially-baited locations on the outer array as compared with those on the inner array (Pot OUT > Pot IN, $P = 0.0423$). Thus, 9-mo-old monkeys discriminated potentially baited locations from never-baited locations, and demonstrated a preference for locations on the outer array.

For the first choice in the local cue condition, adult monkeys chose potentially baited locations more often than never-baited locations (Fig. 4B; $F_{(4,8)} = 5.656$, $P < 0.0001$; Pot IN > Equ IN = Equ OUT = Other, all $P < 0.0001$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.05$). In addition, they chose potentially baited locations on the inner array more often than potentially baited locations on the outer array (Pot IN > Pot OUT, $P = 0.0002$). For the first four choices, adult monkeys again chose potentially baited locations more often than never-baited locations (Fig. 4B; $F_{(4,8)} = 342.582$, $P < 0.0001$; Pot IN > Equ IN > Equ OUT > Other, all $P < 0.0001$; Pot OUT > Equ IN > Equ OUT > Other, all $P < 0.0001$), and potentially baited locations on the inner array more than potentially baited locations on the outer array (Pot IN > Pot OUT, $P = 0.0305$). Thus, adult monkeys discriminated potentially baited locations from never-baited locations, and demonstrated a preference for locations on the inner array.

Dissociation probe trial

During the local cue phase, monkeys were subject to a probe trial during which the colored cups were shifted 60° from their standard spatial locations (Fig. 2B) and no food was present. This trial was the first of the day (24 h after the last trial of the previous session) and was followed by three regular trials. This trial was designed to evaluate the monkeys' reliance on local and/or spatial information to discriminate the baited locations. We analyzed the first choice (i.e., the first cup opened), the first four choices (i.e., the first four cups opened), and all the choices (i.e., all of the cups opened) made by each monkey during the probe trial. We classified the monkeys' choices with respect to whether they made a "Color" choice (i.e., following local information, a red [Color In] or green [Color Out] cup placed at a never-baited "equivalent" location at the corners of the inner or outer hexagon, 2, 6, 10, 14, 16, 18), a "Spatial" choice (i.e., following spatial relational information, a neutral cup placed at a potentially baited "correct" spatial location on the inner [Space In] or outer [Space Out] array, 4, 8, 12, 13, 15, 17), or "Other" choices (the never-baited locations on the sides of the outer hexagon, 1, 3, 5, 7, 9, 11).

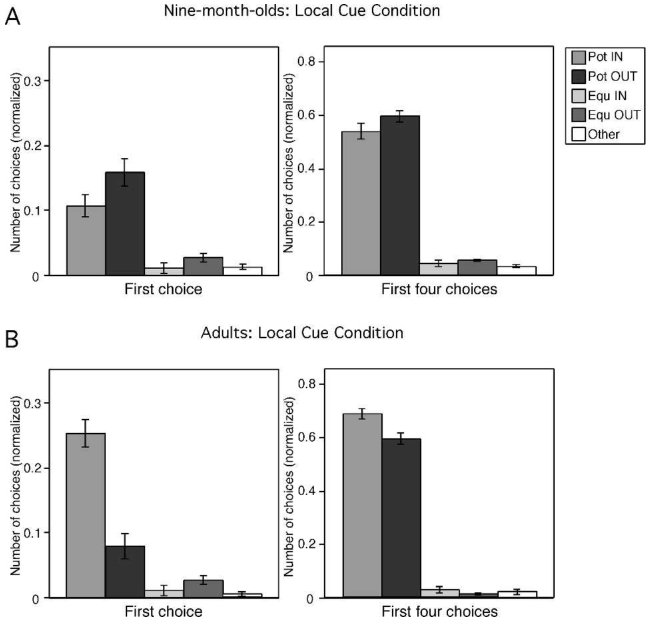

Figure 4. Nine-month-old (*A*) and adult (*B*) monkeys' strategy in the local cue condition. (Pot IN) Potentially baited locations at the corners of the inner hexagon (locations 13, 15, 17 see Figure 2); (Pot OUT) potentially baited locations at the corners of the outer hexagon (locations 4, 8, 12); (Equ IN) never-baited locations at the corners of the inner hexagon (locations 14, 16, 18); (Equ OUT) never-baited locations at the corners of the outer hexagon (locations 2, 6, 10); (Other) never-baited locations on the sides of the outer hexagon (locations 1, 3, 5, 7, 9, 11). The number of choices in each category (n) is normalized according to the probability of making that choice (n/3 for Pot IN, Pot OUT, Equ IN, Equ OUT, and n/6 for Other).

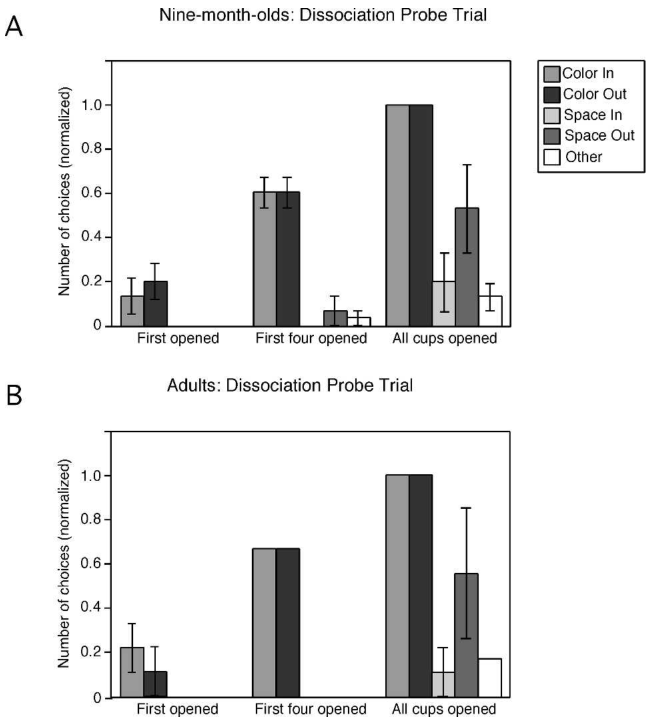

Figure 5. Nine-month-old (*A*) and adult (*B*) monkeys' choices in the dissociation probe trial (no food present). (Color In) Red cups at never-baited locations at the corners of the inner hexagon (locations 14, 16, 18, see Figure 2); (Color Out) green cups at never-baited locations at the corners of the outer hexagon (locations 2, 6, 10); (Space In) neutral cups at correct spatial locations at the corners of the inner hexagon (locations 13, 15, 17); (Space Out) neutral cups at correct spatial locations at the corners of the outer hexagon (locations 4, 8, 10); (Other) neutral cups at never-baited locations on the sides of the outer hexagon (locations 1, 3, 5, 7, 9, 11). The number of choices in each category (n) is normalized according to the probability of making that choice (n/3 for Color In, Color Out, Space In, Space Out, and n/6 for Other).

For their first choice, all 9-mo-old monkeys opened colored cups (Fig. 5A). No 9-mo-old opened neutral cups at the correct spatial locations ([Space In] or [Space Out]). For their first four choices, 9-mo-old monkeys opened more colored cups than any other cups ($F_{(4,16)} = 26.848$, $P < 0.0001$; Color In = Color Out > Space In = Space Out = Other, all $P < 0.0001$). When considering all of the cups opened, 9-mo-old monkeys chose locations marked by a local cue significantly more than any other location, but they also discriminated the correct spatial locations on the outer array from the never-baited locations ($F_{(4,16)} = 17.479$, $P < 0.0001$; Color IN = Color OUT > Space OUT > Space IN = Other, all $P < 0.05$). Thus, although 9-mo-old monkeys exhibited a strong preference for local information (i.e., colored cups), they exhibited clear evidence of relying on spatial relational information to discriminate potentially baited locations and guide their choices in the open-field arena.

For the first choice and the first four choices, adult monkeys opened exclusively colored cups (Fig. 5B); no monkeys chose neutral cups at correct spatial locations. For all choices, adult monkeys discriminated different locations ($F_{(4,8)} = 9.703$, $P = 0.0037$). They chose colored cups more than neutral cups located at the correct spatial locations on the inner array and other cups at never-baited locations (Color In = Color Out > Space In = Other, all $P < 0.05$), but did not choose colored cups significantly more than neutral cups located at the correct spatial locations on the outer array (Color In = Color Out = Space Out, all $P > 0.05$). Adult monkeys, however, did not choose cups at the correct spatial locations on the outer or inner array more often than other cups at never-baited locations (Space Out = Space In = Other, all $P > 0.05$). In contrast to the 9-mo-olds, there is no unequivocal evidence to suggest that adult monkeys relied on spatial relational information to discriminate the potentially baited locations in the local cue condition.

Spatial relational condition

We further evaluated the monkeys' discrimination of the potentially baited cups in absence of local cues marking these locations. For the first choice, 9-mo-old monkeys chose potentially baited locations on the outer array more often than never-baited locations (Fig. 6A; $F_{(4,16)} = 4.819$, $P = 0.0096$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.01$) or potentially baited locations on the inner array (Pot OUT > Pot IN, $P = 0.0231$). As a group, they did not choose potentially baited locations on the inner array more often than never-baited locations (but see the analysis of individual behavior, below). Similarly, for the first four choices, 9-mo-old monkeys chose potentially baited locations on the outer array more often than never-baited locations (Fig. 6A; $F_{(4,16)} = 8.007$, $P = 0.001$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.0005$) or potentially baited locations on the inner array (Pot OUT > Pot IN, $P = 0.0035$).

For the adults, in contrast, group analyses did not reveal any evidence that they discriminated the potentially baited locations from the never-baited locations in absence of local cues (Fig. 6B; First choice: $F_{(4,8)} = 1.608$, $P = 0.2628$; First four choices: $F_{(4,8)} = 3.201$, $P = 0.07570$); but see the analysis of individual behavior, below.

In sum, 9-mo-old monkeys discriminated potentially baited locations on the outer array (Pot OUT) from never-baited locations on the outer array (Equ OUT and Other) in the spatial relational condition. These results indicate that 9-mo-olds were capable of using spatial relational information to discriminate potentially baited locations. In contrast, adult monkeys, as a group, did not exhibit evidence of the use of spatial relational information to discriminate the potentially baited locations in

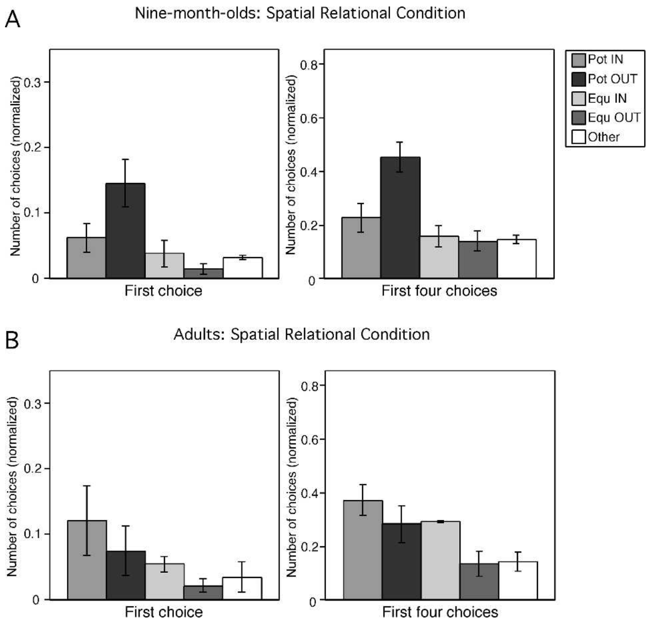

Figure 6. Nine-month-old (*A*) and adult (*B*) monkeys' strategy in the spatial relational condition. Abbreviations as in Figure 4.

the spatial relational condition. This experiment, however, comprised only three adult individuals and the lack of a group effect may simply be due to differences in individual strategies. We therefore also analyzed the behavior of each individual separately and present data for representative 9-mo-old and adult monkeys in the spatial relational condition.

Individual behaviors

We first present the sequence of cups opened (raw data) for each monkey in one typical session per testing condition (Table 1) in order to help the reader get a better sense of the animals' actual behavior during testing. We then present analyzed data from two adult monkeys (Freebird, 13-yr-old and Bouillon, 11-yr-old) in

Table 1. **Sequence of cups opened for each monkey in one typical session per testing condition**

	Local cue	Spatial relational
Freebird	Inner	Inner
(13-years-old)	T1: 17, 8, 12, 13, 15, 4, 14	T1: 17, 8, 9, 16, 15, 4, 3, 14, 2, 1, 13, 12, 18
	T2: 13, 12, 15, 4, 17, 8	T2: 13, 12, 18, 11, 10, 17, 8, 16, 15, 4, 14, 1
	T3:13, 12, 15, 4, 17, 8	T3: 13, 12, 1, 1, 2, 3, 15, 4, 16, 7, 17, 8, 9, 18
	Outer	Outer
	T1: 15, 16, 4, 17, 8, 18, 12, 13, 11	T1: 16, 15, 4, 3, 14, 2, 1, 12, 13, 18, 9, 17, 8, 7
	T2: 13, 12, 17, 8, 15, 4, 14	T2: 17, 8, 16, 15, 5, 4, 14, 13, 12, 18, 10, 9, 7
	T3: 17, 8, 15, 4, 13, 12	T3: 13, 12, 18, 9, 17, 8, 16, 15, 4, 14
Bouillon	Inner	Inner
(11-years-old)	T1: 13, 12, 17, 8, 15, 4	T1: 8, 17, 10, 11, 12, 1, 2, 4, 3, 15, 13, 18, 9, 16, 7, 6, 14
	T2: 13, 12, 15, 4, 17, 8	T2: 14, 13, 12, 18, 17, 16, 15, 4
	T3: 17, 8, 13, 12, 15, 4, 11	T3: 15, 4, 14, 13, 12, 18, 17, 16, 3, 9
	Outer	Outer
	T1: 4, 13, 12, 17, 8, 15, 3, 2	T1: 13, 12, 18, 10, 17, 8, 16, 15, 4, 3, 14, 9
	T2: 13, 12, 17, 8, 4, 15	T2: 12, 14, 2, 3, 4, 15, 16, 17, 8
	T3: 4, 15, 13, 12, 17, 8	T3: 12, 13, 18, 17, 8, 15, 4, 14
Tambourine	Inner	Inner
(13-years-old)	T1: 17, 8, 15, 4, 13, 12	T1: 17, 7, 16, 6, 15, 4, 3, 14, 13, 12, 11, 10, 18, 9, 8, 1
	T2: 12, 13, 15, 4, 17, 8	T2: 7, 16, 17, 18, 11, 12, 13, 1, 14, 2, 3, 15, 4, 5, 6, 10
	T3: 13, 17, 8, 12, 15, 4	T3: 2, 14, 15, 5, 16, 6, 4, 3, 1, 13, 12, 11, 10, 18, 9, 17, 8, 7
	Outer	Outer
	T1: 15, 4, 17, 8, 13, 12	T1: 2, 13, 8, 7, 17, 16, 15, 4, 9, 10, 11, 12, 1, 3, 5, 18, 14
	T2: 12, 13, 17, 8, 15, 4	T2: 14, 15, 4, 3, 11, 18, 10, 9, 17, 8, 7, 16, 6, 5
	T3: 15, 17, 8, 13, 12, 4	T3: 18, 17, 7, 16, 15, 5, 4, 3, 14, 2, 1, 12, 13, 11, 9
Tyson	Inner	Inner
(9-months-old)	T1: 8, 4, 15, 12, 13, 17, 5, 9	T1: 3, 4, 15, 6, 7, 8, 9, 18, 11, 12, 13, 16, 17, 9, 5, 1, 14
	T2: 13, 12, 17, 8, 15, 4	T2: 17, 16, 18, 10, 11, 13, 15, 8, 9, 7, 4, 15
	T3: 8, 4, 15, 12, 13, 17	T3: 17, 4, 15, 14, 1, 1
	Outer	Outer
	T1: 8, 17, 15, 4, 13, 12	T1: 8, 9, 17, 7, 16, 5, 4, 3, 15, 14, 2, 13, 12, 11, 10
	T2: 12, 13, 15, 4, 17, 8	T2: 8, 17, 9, 18, 10, 16, 5, 15, 4, 3, 14, 2, 1, 13, 12
	T3: 13, 12, 17, 8	T3: 17, 8, 13, 11, 12, 16, 5, 15, 4
Qutie	Inner	Inner
(9-months-old)	T1: 8, 6, 5, 4, 15, 14, 13	T1: 10, 9, 8, 6, 4, 2, 12, 11, 1, 5
	T2: 15, 8, 4	T2: 8, 6, 4, 2, 17, 11, 12, 7, 5, 14, 1, 16
	T3: 18, 17, 8, 13, 16, 4, 12	T3: 4, 12, 8, 3, 14
	Outer	Outer
	T1: 8, 4, 13, 12	T1: 18, 10, 8, 6, 4, 2, 12
	T2: 5, 17, 8, 4, 13, 18, 15	T2: 9, 8, 5, 4, 3, 2, 1, 12, 13, 17
	T3: 5, 4, 12	T3: 9, 8, 5, 4, 2, 6, 12
Rascal	Inner	Inner
(9-months-old)	T1: 8, 17, 4, 15, 12, 13	T1: 16, 18, 12, 11, 10, 8, 6, 4, 15, 3, 2, 1, 13, 14, 9, 7, 17
	T2: 2, 12, 13, 17, 18, 6, 4, 15	T2: 8, 7, 5, 15, 16, 12, 10, 9, 6, 4, 3, 2, 1, 14, 13, 11, 18, 17
	T3: 8, 17, 12, 13, 6, 15, 4	T3: 15, 17, 6, 4, 18, 12, 2, 3, 16, 9, 10, 7, 5, 1, 14, 13
	Outer	Outer
	T1: 2, 12, 13, 4, 15, 8	T1: 14, 12, 5, 4, 16, 2, 11, 18, 8
	T2: 8, 17, 16, 15, 4, 12, 13	T2: 14, 16, 7, 8, 6, 4, 17, 18, 12
	T3: 15, 14, 8, 17, 4, 12, 13	T3: 12, 13, 9, 8, 15, 1, 18, 6, 14, 3, 5, 7, 4
Papillon	Inner	Inner
(9-months-old)	T1: 8, 7, 4, 15, 13, 12, 17	T1: 15, 1, 12, 13, 11, 10, 8, 6, 4, 2, 7, 1, 11, 9, 14, 5, 17
	T2: 15, 13, 12, 8, 17	T2: 3, 2, 12, 11, 10, 8, 16, 15, 4, 14, 13, 5, 9, 17
	T3: 12, 13, 8, 7, 6, 17, 15, 4	T3: 10, 11, 12, 2, 3, 5, 16, 7, 8, 9, 13, 15, 14, 1, 6, 4, 18, 17
	Outer	Outer
	T1: 17, 8, 12, 13, 15, 4	T1: 14, 1, 12, 4, 8
	T2: 13, 8, 12, 4, 15	T2: 4, 2, 12, 14, 1, 11, 10, 8
	T3: 4, 13, 12, 15, 8	T3: 15, 3, 2, 12, 13, 9, 8, 17, 6, 5, 4
Sugar	Inner	Inner
(9-months-old)	T1: 1, 12, 10, 8, 13, 9, 7, 15, 16, 15, 4, 14	T1: 12, 18, 13, 8, 17, 4, 15
	T2: 12, 13, 14, 4, 15, 9, 8, 17	T2: 12, 13, 8, 17, 4, 5, 16, 18, 2, 15
	T3: 12, 13, 8, 17, 15	T3: 12, 13, 17, 4, 15
	Outer	Outer
	T1: 12, 13, 15, 8, 17, 2, 4	T1: 12, 9, 8, 4, 15, 1
	T2: 12, 13, 8, 17, 15, 4	T2: 12, 4, 8
	T3: 12, 4, 13, 15, 8	T3: 12, 8, 4

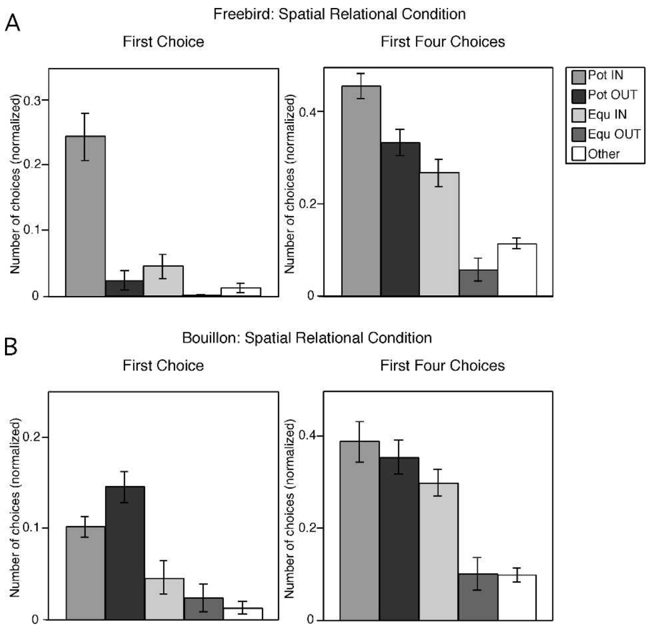

Figure 7. Individual adult monkeys' strategy in the spatial relational condition. (*A*) Freebird, 13-yr-old; (*B*) Bouillon, 11-yr-old. Abbreviations as in Figure 4.

the spatial relational condition and briefly discuss the results of the third adult (Tambourine, 13-yr-old). For the 9-mo-olds, we present data from Tyson, whose behavior is representative of three other 9-mo-olds in the study, Qutie, Rascal, and Papillon. The fifth 9-mo-old, Sugar, exhibited notable differences in strategy that we will discuss in more detail below.

Freebird (13-yr-old)

Freebird exhibited clear evidence of spatial relational learning in absence of local cues marking the potentially baited locations (Fig. 7A). For the first choice, he chose potentially baited locations on the inner array more than any other location ($F_{(4,36)} = 21.776$, $P < 0.0001$; Pot IN > Pot OUT = Equ IN = Equ OUT = Other, all $P < 0.0001$). For the first four choices, he again chose potentially baited locations on the inner array over all other locations, but also chose potentially baited locations on the outer array more than never-baited locations on the outer array ($F_{(4,36)} = 33.834$, $P < 0.0001$; Pot IN > Pot OUT = Equ IN > Equ OUT = Other, all $P < 0.01$). Of particular importance regarding evidence of spatial relational learning is Freebird's preference for potentially baited locations on the inner array (Pot IN) as compared with equivalent locations on the inner array (Equ IN), and his preference for potentially baited locations on the outer array (Pot OUT) as compared with equivalent and other locations on the outer array (Equ OUT, Other), thus demonstrating his use of spatial relational information to discriminate potentially baited locations.

Bouillon (11-yr-old)

Similarly, Bouillon exhibited clear evidence of spatial relational learning in absence of local cues marking the potentially baited locations (Fig. 7B). For the first choice, he chose potentially baited locations on the inner and outer array more than never-baited locations ($F_{(4,36)} = 12.639$, $P < 0.0001$; Pot IN = Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.019$), thus demonstrating his use of spatial relational information to discriminate potentially baited locations on both the inner and outer array. For the first four choices, he chose potentially baited locations on the inner array, potentially baited locations on the outer array, and equivalent locations on the inner array with equal frequency, and more often than never-baited locations on the outer array ($F_{(4,36)} = 14.001$, $P < 0.0001$; Pot IN = Pot OUT = Equ IN > Equ OUT = Other, $P < 0.0001$). Again, of specific interest is Bouillon's ability to discriminate potentially baited locations on the outer array from equivalent and other locations on the outer array, signifying his use of spatial relational information to discriminate potentially baited locations.

Tambourine (13-yr-old)

Although Tambourine discriminated potentially baited locations from never-baited locations in the local cue condition (data not shown), he did not discriminate different types of locations in absence of local cues marking the potentially baited locations (data not shown) (First choice: $F_{(4,36)} = 0.834$, $P = 0.5123$; First four choices: $F_{(4,40)} = 1.830$, $P = 0.1420$). Tambourine's contrasting results likely skewed the adult group data and highlight the importance of performing separate analyses for each individual, in particular when using few subjects, in order to provide experimental evidence for the existence, or absence, of particular cognitive processes.

Tyson (9-mo-old)

For the first choice in the spatial relational condition (Fig. 8A), Tyson chose potentially baited locations on the outer array more often than never-baited locations ($F_{(4,36)} = 11.438$, $P < 0.0001$; Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.0001$) or potentially baited locations on the inner array (Pot OUT > Pot IN, $P = 0.008$). Tyson also chose potentially baited locations on the inner array more often than never-baited locations at equivalent corners of the inner and outer arrays (Pot IN > Equ IN = Equ OUT, all $P < 0.01$). For the first four choices, Tyson chose potentially baited locations on the inner and outer arrays more than never-baited locations on the outer array ($F_{(4,36)} = 6.072$, $P = 0.0008$; Pot IN = Pot OUT > Equ OUT = Other, all $P < 0.05$),

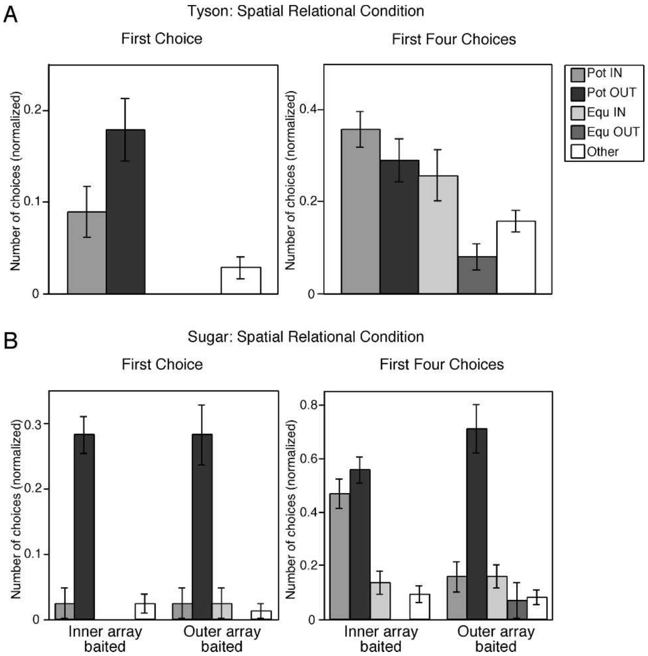

Figure 8. Individual 9-mo-old monkeys' strategy in the spatial relational condition. (*A*) Tyson; (*B*) Sugar. Note the difference in choices made by Sugar when the inner and outer arrays were baited. Abbreviations as in Figure 4.

and equivalent locations on the inner array more than equivalent locations on the outer array (Equ IN > Equ OUT, $P = 0.0079$). Tyson chose potentially baited locations on the inner and outer array, and equivalent locations on the inner array with approximately the same frequency (Pot IN = Pot OUT = Equ IN). Of particular importance regarding evidence of spatial relational learning is Tyson's preference for potentially baited locations on the inner array (Pot IN) as compared with equivalent locations on the inner array (Equ IN) for the first choice, and his preference for potentially baited locations on the outer array (Pot OUT) as compared with equivalent and other locations on the outer array (Equ OUT, Other) for both the first choice and first four choices. Analyses for three other 9-mo-olds (Qutie, Rascal, Papillon) yielded similar results. In sum, Tyson, as well as three other 9-mo-old monkeys, exhibited clear evidence of spatial relational learning.

Sugar (9-mo-old)

Sugar exhibited a more sophisticated behavior in the spatial relational condition. For the first choice, she chose potentially baited locations on the outer array more often than any other location ($F_{(4,36)} = 51.738$, $P < 0.0001$; Pot OUT > Pot IN = Equ IN = Equ OUT = Other, all $P < 0.0001$). For the first four choices, Sugar chose more potentially baited locations on the outer array than potentially baited locations on the inner array, and discriminated potentially baited locations on both the inner and outer arrays from never-baited locations ($F_{(4,36)} = 25.421$, $P < 0.0001$; Pot OUT > Pot IN > Equ IN = Equ OUT = Other, all $P < 0.02$). Analyzing her behavior with respect to which array was baited (Inner vs. Outer) did not reveal any effect for the first choice (Fig. 8B; $F_{(4,32)} = 0.133$, $P = 0.9690$), but her first four choices revealed an interaction between choice and array ($F_{(4,32)} = 4.765$, $P = 0.0039$). When the outer array was baited, Sugar chose mainly potentially baited locations on the outer array ($F_{(4,16)} = 16.109$, $P < 0.0001$; Pot OUT > Pot IN = Equ IN = Equ OUT = Other, all $P < 0.0001$), whereas when the inner array was baited, Sugar chose potentially baited locations on the outer and inner arrays with equal frequency ($F_{(4,16)} = 30.110$, $P < 0.0001$; Pot IN = Pot OUT > Equ IN = Equ OUT = Other, all $P < 0.0001$).

In sum, Sugar exhibited a tendency to open potentially baited cups located on the outer array for her first choice. However, Sugar's second, third, and fourth choices were influenced by which array was baited. For example, Sugar typically sampled a potentially baited location on the outer array first (most often location 12, but occasionally location 4 or 8). If that location was baited, she then restricted her search primarily to potentially baited locations on the outer array. In contrast, if that location was not baited, Sugar proceeded by searching potentially baited locations on both the inner and outer arrays. These results provide evidence of flexible use of relational information outside of the spatial domain in a 9-mo-old monkey.

Summary of the results

All adult and 9-mo-old monkeys tested exhibited visual discrimination learning. Two of three adults and all 9-mo-old monkeys tested exhibited spatial relational learning. These findings indicate that spatial relational memory is present as early as 9 mo of age in rhesus monkeys, and is largely similar in 9-mo-old and adult animals. In contrast, no adult or 9-mo-old monkeys exhibited evidence of relational learning based on the objects placed at the center of the arena.

Discussion

The aim of this study was to assess spatial and nonspatial relational learning in 9-mo-old and adult monkeys in order to obtain evidence of the maturity of relational memory processes in 9-mo-old monkeys. We found that (1) all 9-mo-old monkeys tested exhibited spatial relational learning, and (2) one 9-mo-old exhibited nonspatial relational learning. These findings demonstrate that spatial memory processes characterized by a relational representation of the environment are present as early as 9 mo of age in rhesus monkeys.

Spatial relational learning

In the local cue condition, 9-mo-old monkeys readily discriminated the two sets of potentially baited locations marked by different local cues (green and red cups) from the never-baited locations (neutral cups). These findings were expected, as visual discrimination abilities have been well documented in neonate monkeys (Harlow 1959; Harlow et al. 1960; Bachevalier et al. 1993). The animals' choices revealed that they were highly selective and opened almost exclusively potentially baited cups at fixed locations marked by local cues. The dissociation probe trial demonstrated that 9-mo-old monkeys relied primarily on the local cues (i.e., the colored cups) to guide their choices. They did, however, discriminate between neutral cups at correct spatial locations and neutral cups at never-baited locations on the outer array. These results indicate that 9-mo-old monkeys had learned the location of potentially baited cups in relation to distant environmental cues, even in the presence of local cues (Lavenex and Schenk 1995, 1997).

The monkeys' selectivity for potentially baited cups decreased in the absence of local cues marking these locations (compare the number of potentially baited cups opened in the first and first four choices in Fig. 4, local cue condition, and Fig. 6, spatial relational condition; see also Table 1). This suggests that the colored cups provided strong cues facilitating the discrimination of the potentially baited locations (Lavenex and Schenk 1995) and may have helped the monkeys to learn the potentially baited locations in relation to distant environmental cues (Lavenex and Schenk 1997). What is important, however, is not the monkeys' overall selectivity in the local cue or spatial relational conditions, nor what cues they used to establish a spatial relational representation, but rather their use of a behavioral strategy dependent on a spatial relational representation of the testing environment (O'Keefe and Nadel 1978; Morris 1981; Lavenex and Schenk 1995, 1996, 1997, 1998; Schenk et al. 1995; Lavenex et al. 1998). Testing in the spatial relational condition demonstrated unequivocally that 9-mo-old monkeys could discriminate the potentially baited locations in absence of local cues. We did not attempt to determine what distant environmental cues monkeys used to establish a spatial relational representation of the environment (O'Keefe and Nadel 1978; Schenk et al. 1995). We did, however, preclude the reliance on an egocentric representation of space by alternating pseudo-randomly between four different entrances into the testing arena (O'Keefe and Nadel 1978; Morris 1981; Schenk et al. 1995; Lavenex and Schenk 1998; Eichenbaum 2000), and eliminated the reliance on uncontrolled local cues by rotating the board on which the cups were located between trials (Lavenex and Schenk 1995, 1996, 1997, 1998; Lavenex et al. 1998). The primary and critical finding of this study, therefore, is that all 9-mo-old monkeys tested exhibited a behavioral strategy dependent on a spatial relational representation of the environment.

Finally, we observed a difference in foraging strategy between 9-mo-old and adult monkeys. In both the local cue and spatial relational conditions, 9-mo-olds exhibited a preference for cups located on the outer array, whereas adults exhibited a preference for cups located on the inner array. Based on our observations in these experiments, we believe that the 9-mo-olds' foraging strategy was influenced by their natural reluctance to venture in the middle of an open environment (Bauman et al. 2004). They organized their foraging by staying closer to the walls and approached the cups located on the inner array from the outside of the board. This resulted in the opening of a proportionally higher number of cups located on the outer array (whether or not they were baited), which were easily reached from the periphery. In contrast, the adults were not fearful of the open space and organized their foraging to minimize travel distance. They organized their search from the middle of the board and approached the cups located on the outer array from the center. This resulted in the opening of a proportionally higher number of cups located on the inner array (whether or not they were baited), which were more easily reached from the center. Consequently, we do not believe that such a difference in foraging strategy reflects a fundamental difference is the spatial relational representation of the environment by monkeys of different ages.

Relational representation of information

Although spatial relational learning has been the model of choice to study the neurobiological basis of declarative memory processes in rodents (O'Keefe and Nadel 1978; Nadel and Eichenbaum 1999), it is well established that relational memory processes extend beyond the spatial domain in both human and nonhuman species (Squire 1992; Eichenbaum 1999). These experiments were therefore also designed to include two possible ways in which to evaluate nonspatial relational learning in freely moving 9-mo-old and adult (11–13-yr-old) monkeys.

Central objects

First, the objects placed at the center of the arena could be used as conditional cues to predict which array of three distinct locations was baited on a particular day. No 9-mo-old or adult monkey tested exhibited any evidence of relational learning based on the central objects. This finding is unexpected, especially because the central objects provided the only information enabling maximal foraging efficiency. The salience of the central objects is unlikely to explain why monkeys failed to use them to predict food locations, as monkeys were often observed exploring the objects manually or orally. One possible explanation, however, is that these objects were changing pseudo-randomly between sessions, so monkeys relied instead on stable features of the environment to guide their behavior, as is observed in rodents (Lavenex et al. 1998). Indeed, we did not use any shaping procedures to force the monkeys to focus their attention on these cues and the number of choices monkeys were allowed to make was not limited. In terms of reward contingency, the monkeys were correct half of the time if they focused their search on potentially baited cups, but more importantly, they found all of the food rewards during every single trial whether or not they were selective. The cost associated with opening nonbaited cups was quite low and might not have been sufficient to push the animals to be more selective.

Cup sampling

Second, because three locations were always baited simultaneously, monkeys could also predict which array was baited after sampling one of the six potentially baited locations. One 9-mo-old monkey, Sugar, exhibited evidence of nonspatial relational learning in the spatial relational condition. Sugar typically sampled a potentially baited location on the outer array for her first choice. If that outer location was baited, she then restricted her search to potentially baited locations on the outer array. In contrast, if that location was not baited, Sugar proceeded by searching potentially baited locations on both the inner and outer arrays.

These findings may be explained by two different behavioral strategies. First, Sugar might have learned that locations 4, 8, and 12 on the outer array were always baited simultaneously, and when the outer array was baited, the inner array (locations 13, 15, and 17) was not. Inversely, when locations 13, 15, and 17 were baited, locations 4, 8, and 12 were not. Again, besides the obvious requirement of relying on a spatial relational representation of the environment to discriminate the potentially baited locations (from never-baited locations), this behavior would require relying on a nonspatial relational representation to code the reinforcement contingencies: If X+, then Y+, Z+, and M−, N−, O−; whereas, if X−, then Y−, Z− and M+, N+, O+. Alternatively, Sugar did not necessarily need to associate X with Y and Z, and M with N and O. Another strategy was to systematically sample a potentially baited cup located on the outer array and if that location was baited, refrain herself from sampling the adjacent cup on the inner array and proceed to the next baited cup on the outer array. In this case, the representational demands would have been the following: If X+, then M−; if Y+, then N−, if Z+, then O−; as well as, if X−, then M+, if Y−, then N+, if Z−, then O+.

A trial-by-trial analysis of Sugar's behavior revealed that she often searched a cup located on the outer array first, and then searched the adjacent cup located on the inner array, suggesting the second strategy (if X−, then M+). It is important to note, however, that Sugar tended to go around the board rather than across it to move from one location to the next. It is thus possible that after finding the first potentially baited cup on the outer array not baited, Sugar simply did not refrain herself from opening the other potentially baited cups on the outer array as she approached the baited cups on the inner array from the outside of the board, a behavior consistent with the first strategy (if X−, then M+, N+, O+). Although the two strategies are difficult to distinguish based on the available data, it is clear that this 9-mo-old monkey modified her behavior and acted differentially based on conditional information acquired within a trial. These results are consistent with the flexible expression of behavior dependent on a relational representation of information, outside of the spatial domain.

Spatial relational system

Some have argued that the spatial relational system is separate from and more fundamental than other relational systems (O'Keefe and Nadel 1978; Nadel 1991; Nadel and Hardt 2004). This experiment showed that 9-mo-old and adult monkeys can rely on a spatial representation encoding stable features of the environment to predict food locations. In this task, monkeys were not taught which cues were relevant, as is generally the case in laboratory experiments. Naturalistic tasks, which do not require long pretraining procedures, are more likely to tell us about the fundamental cognitive processes that are sensitive to natural selective pressures and that may be responsible for the evolution of a particular cognitive trait (Lavenex et al. 1998) and its underlying brain structures (Banta Lavenex et al. 2001). Our results support the view that spatial relational representations might be more fundamental, as all 9-mo-olds tested exhibited spatial relational learning and only one exhibited nonspatial relational learning. However, because adult monkeys did not exhibit evidence of nonspatial relational learning in our experimental conditions, we are unable to evaluate whether spatial and nonspatial relational memory processes are indeed separate and/or exhibit different developmental timelines.

Short-term and long-term memory for color or spatial information

A detailed analysis of choices did not reveal any evidence that the monkeys' behavior was influenced by their short-term (1 min) or long-term (24 h) memory of the baited locations in either the local cue or spatial relational condition (data not shown). These findings contrast with previous results in free-ranging squirrels tested in the spatial relational condition, which showed that squirrels remembered the locations of the food rewards in the previous session (>24 h) and searched for food preferentially where they had found it previously (Lavenex et al. 1998). Similarly, squirrels' selectivity (i.e., the number of correct choices) increased between daily trials, demonstrating that they also relied on their short-term (1-min intertrial interval) memory to improve their foraging efficiency. The present results should not be interpreted as evidence that monkeys do not have short-term or long-term memory of the baited locations. Obviously, both 9-mo-old and adult monkeys discriminated potentially baited cups that contained food half of the time from never-baited cups, thus demonstrating evidence of long-term memory for color and spatial information. What is more surprising is that the monkeys' selectivity did not increase between trials within a daily session, suggesting that monkeys approached each trial as a new trial independent from the previous one. However, the monkeys were behaving freely without any restriction as to the number of choices they could make, and without any correction procedure to stress the solution to the task or require them to perform maximally (Eichenbaum et al. 1994; Rapp et al. 1996). Based on the results observed in squirrels, one would likely infer that the reward contingencies of the task were simply not adequate to elucidate similar cognitive processes in monkeys. Although this task was designed to evaluate whether these memory processes exhibit different developmental timelines, we are unable to address this issue.

Emergence of memory functions in monkeys

Numerous tasks have been used to study the emergence of declarative memory processes in nonhuman primates (Alvarado and Bachevalier 2000). The choice of specific paradigms has been dictated by findings that overall performance on the task is sensitive to medial temporal lobe damage in adult individuals. There are, however, some inadequacies that confound the interpretation of these studies.

First, findings that performance in tasks known to be hippocampus dependent in adults improves gradually throughout postnatal development (Harlow 1959; Rudy et al. 1993; Overman et al. 1996; Hayne et al. 2000; Malkova et al. 2000; Overman and Bachevalier 2001) have been viewed as evidence that declarative memory exhibits a protracted development that parallels the postnatal maturation of brain structures subserving this type of memory in adult individuals (Nadel and Zola-Morgan 1984; Alvarado and Bachevalier 2000). This interpretation neglects the fact that differences exist in motivation and/or physical abilities at various ages, as well as the fact that other brain areas, such as the frontal cortex, exhibit significant postnatal maturation (Rosenberg and Lewis 1995; Luciana and Nelson 1998; Lambe et al. 2000). These factors likely affect performance levels (Alvarado and Bachevalier 2000; Corkin 2001), and thus make the simple comparison of overall performance levels an invalid measure of memory processes in developing individuals (Aadland et al. 1985; Overman et al. 1996).

Second, and most importantly, those studies failed to specify the fundamental types of information representations that must be used to perform their particular tasks, an aspect critical to implicating specific cognitive and memory processes (Cohen and Eichenbaum 1993). This has led to inconsistent findings regarding the age at which specific memory processes are believed to emerge (Alvarado and Bachevalier 2000). One specific example is visual-recognition memory as assessed by the visual paired-comparison and delayed-nonmatching-to-sample tasks. Although performance in both tasks is affected by hippocampal lesions in adult monkeys (Zola et al. 2000), neonatal lesions have produced apparently discrepant results (Pascalis and Bachevalier 1999; Resende et al. 2002). Neonatal aspiration lesion of the hippocampal formation impairs performance in the visual paired-comparison task, but not in the delayed-nonmatching-to-sample task (Pascalis and Bachevalier 1999). Interestingly, neonatal neurotoxic lesion of the hippocampus does not impair visual paired-comparison performance (Resende et al. 2002). Although both tasks were designed to evaluate visual recognition memory, the behavioral strategies used to perform these tasks must be different (Pascalis and Bachevalier 1999). These specific behavioral strategies must therefore be elucidated in order to infer the fundamental cognitive processes that are actually being evaluated.

Evaluation of other tasks used to assess the emergence of memory functions in monkeys (spatial delayed-nonmatching-to-sample, biconditional discrimination, transverse patterning, oddity task) suggests that relational memory processes are present by 1 yr of age, although juvenile monkeys do not exhibit adult-like performance until they reach 2–3 yr of age (Alvarado and Bachevalier 2000). It is clear, however, that the comparison of overall performance between juvenile and adult monkeys is not appropriate, by itself, to draw inferences about the emergence of specific memory functions. The fact that juvenile monkeys can perform these tasks is sufficient to demonstrate the existence of particular cognitive processes, as long as these processes can be defined operationally in terms of the type of representation of information necessary to solve these particular problems. Similarly, if overall performance in a particular behavioral task is also dependent on the function of other brain structures, such as the frontal cortex, the fact that performance is not adult-like until a certain age is not sufficient evidence to infer the functional immaturity of the medial temporal lobe structures. In sum, what is needed to study the emergence of memory processes is a clear definition of the behavioral strategies that must be used to solve a particular task, as well as of the type of information representation underlying these behavioral strategies.

Conclusions

Our findings demonstrate that spatial memory processes characterized by a relational representation of information are present as early as 9 mo of age in macaque monkeys. This study emphasizes the need to evaluate the emergence of memory processes in relation to the representational demands of the task, rather than simply comparing the overall levels of performance between developing and adult individuals.

Materials and Methods

All protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Davis, and were in accordance with the NIH guidelines for the use of animals in research.

Experimental subjects

Subjects were eight macaque monkeys (*Macaca mulatta*): five 9-mo-olds (two females, Sugar and Qutie, and three males, Tyson, Rascal, and Papillon), and three adult males (Tambourine, 13-yr-old; Freebird, 13-yr-old; and Bouillon, 11-yr-old). Nine-month-old monkeys were born at the California National Primate Research Center (CNPRC) and housed with their mothers in standard home cages (61 cm W × 66 cm D × 81 cm H) from birth until they were 6 mo old. Mothers were then removed, and subjects were housed in pairs. Subjects were separated from their cage mate prior to testing, but remained in auditory contact even during testing. Monkeys were tested on the foraging task described below beginning at 7.5–9.5 mo-of-age: Sugar, 225-d-old; Tyson, 274-d-old; Qutie, 244-d-old; Rascal, 293-d-old; Papillon, 250-d-old.

Adult monkeys were born and raised at the CNPRC. They were maternally reared in 2000 m^2 outdoor enclosures and lived in large social groups until ~1 yr prior to testing. At that time, each adult male was moved indoors and maintained in a large chain-link enclosure (2.13 m W × 3.35 m D × 2.44 H), which housed a group of animals including the adult male and six mother–infant pairs.

Monkeys were not subject to any food or water restriction, except that they did not receive their regular morning rations until after testing was completed. Monkeys were tested at the same time each day (5–7 d a week), between 7:30 and 10:30 a.m.

Apparatus

The apparatus consisted of a hexagonal board (1.2 m in diameter for 9-mo-olds, 2.1 m in diameter for adults) made of white acrylic plastic, on which 18 plastic cups (7.5 cm in diameter) were arranged in a regular pattern (Figs. 1, 2). The hexagonal-shaped board, on which the cups were distributed, was mounted on wheels, which allowed it to be rotated about its central axis (see below). The plastic cups were inverted so that the monkeys had to lift them or turn them over to obtain the food reward beneath. Two different conspicuous objects could be placed at the center of the board (central objects); the presence of each object was predictive of the presence of food in one of two sets of three distinct locations (Figs. 1, 2; see below). The arena was surrounded by three clear Plexiglas panels (front panel, roof, and top half of the back panel; dashed lines in Figs. 1, 2) allowing clear view of distant environmental cues distributed in the experimental room (Fig. 1). Objects permanently located in the experimental room for the duration of testing included two doors located on each side of the room; one door connecting the experimental room to the monkeys' housing room, and another door leading to a hallway; a flood lamp (turned off) attached to a 2-m high metal stand; a red Craftsman tool cabinet (64 cm W × 48 cm D × 92 cm H); a metal stool on which the experimenter sat during testing; a metal/wood desk (69 cm W × 32 cm D × 60 cm H); a gray metallic transfer cage (30 cm W × 50 cm D × 42 cm H) used to transport the monkeys between their home cages and the holding chutes at the beginning and the end of each session (always placed at the same location while the animal was in the testing arena); a hosing station including two faucets and a green rubber hose on a metal reel; a stainless-steel sink (85 cm W × 45 cm D × 90 cm H). Vertically sliding doors at each corner of the arena could be remotely operated (from the front of the arena) allowing the animals to go in and out of the arena from wire-mesh chutes located along both sides of the arena. Opaque side panels (solid lines in Figs. 1A, 2; gray shading in Fig. 1B) provided visual barriers between the open-field arena and the wire-mesh holding chutes. Monkeys had full access to distant environmental cues from within the arena and from the holding chutes (Fig. 1). Two rows of fluorescent lights attached to the ceiling provided even illumination of the entire experimental room. All testing was videotaped with a video camera located above the testing arena, which could be operated by remote control from the observer's location.

Procedure

Pretraining and acclimation

Monkeys were trained to open the cups during a pretraining phase that took place in their home cages for the 9-mo-olds and temporary holding cages (61 cm W × 66 cm D × 81 cm H) for the adults. Monkeys were gradually trained to displace a plastic cup to retrieve a grape hidden underneath. They received 20 trials per day until they successfully retrieved the fully covered grape in less than 30 sec in all trials; this phase lasted up to 5 d. Following pretraining, monkeys received one 5-min acclimation session per day for 5 d, during which they were free to explore the open-field arena. During this phase, there was no food or cups present in the arena. The acclimation phase was aimed at habituating the monkeys to the testing environment prior to the beginning of the actual experiments.

Testing

The task required monkeys to find a preferred food (grapes) located in one of two different arrays of three distinct locations (Fig. 2: either the inner array, locations 13, 15, 17, or the outer array, locations 4, 8, 12). Monkeys were given three trials per day (with a 1-min intertrial interval), 5–7 d per week. Testing took place over a 27–33-d period for all 9-mo-old monkeys. Monkeys behaved freely, without any negative reinforcement to shape their behavior; they were allowed up to 5 min to complete a trial. They were coaxed in and out of the open-field arena from the holding chutes located on each side (Fig. 1). From the holding chutes, the monkeys were unable to see the arena while the experimenter replenished the food under the cups. Monkeys entered the arena from one of the four entrances located at the corners of the arena, which was chosen pseudo-randomly for every trial by the experimenter. The orientation of the entire apparatus remained fixed in relation to distant environmental cues within the experimental room (Fig. 1). The board on which the cups were distributed was rotated 60° before each trial in order to make irrelevant any noncontrolled local cues, such as olfactory traces (Lavenex and Schenk 1998). The rewards (and local cues if present) were always placed in the exact same locations in relation to distant environmental cues (Figs. 1, 2).

The location of the food (baited array) remained the same between trials (1-min interval) within a daily session, but changed pseudo-randomly between the two arrays between sessions (24-h interval). One particular central object was associated with the presence of food in one particular array of three locations, and could thus be used to predict which array of three distinct locations contained food (see below). The first central object was a yellow plastic bottle (25 cm high, 10 cm in diameter) and was associated with the presence of food under the three cups at locations 4, 8, and 12 on the outer array (Fig. 2A). The second central object was a blue ball (25 cm in diameter) and was associated with the presence of food under the three cups at location 13, 15, and 17 on the inner array. The experiment was conducted in two successive phases, and included one additional probe trial.

Local cue condition

During the first phase, monkeys' choices were assessed to determine their ability to find food at fixed locations marked by local cues, i.e., colored cups. During this phase, the monkeys were free to associate the presence of the central object with the presence of food in one array of three distinct locations. When the yellow bottle was present, the three green cups located at positions 4, 8, and 12 on the outer array were baited. When the blue ball was present, the three red cups located at positions 13, 15, and 17 on the inner array were baited. The particular array that was baited changed pseudo-randomly between days, but remained the same within a daily session.

Nine-month-old monkeys were tested for 18–20 d in the local cue condition (Qutie, Rascal, Papillon = 18 d or 54 trials, Sugar and Tyson = 20 d or 60 trials). We included results from the last 10 d in the analysis of short-term and long-term memory, as well as for the analysis of foraging strategies. Adult monkeys had a total of 20 d (i.e., 60 trials) of experience in the local cue condition, and we included the last 10 d in our analyses.

Dissociation probe trial

Monkeys were tested on a single probe trial, during which the locations of the colored cups were shifted 60° from their usual spatial locations and none of the cups were baited (Fig. 2B). This probe trial took place as the first trial of the 12th or 13th day of testing in the color condition and was followed by three standard local cue trials. The aim of this probe trial was to assess the monkeys' reliance on local versus spatial information to discriminate potentially baited locations from never-baited locations. During local cue trials, potentially rewarded locations were covered by a colored cup, so that both local and spatial information were coherent (Fig. 2A). During the dissociation probe trial (Fig. 2B), the colored cups covered the never-baited locations 2, 6, 10, 14, 16, and 18 and neutral cups covered the normally potentially baited locations 4, 8, 12, 13, 15, and 17. As monkeys entered the arena, they encountered the same pattern of cups distributed throughout the arena, but the absolute locations of the colored cups within the arena were changed, thus rendering the local and spatial relational information incoherent. The monkeys were thus faced with two competing strategies to determine where the food rewards might be hidden (although no food reward was actually present for this trial), either (1) under the colored cups at spatially "incorrect" locations, or (2) under neutral cups at "correct" spatial locations.

Spatial relational condition

No local cues (i.e., no colored cups) marked the potentially baited locations (Fig. 2C). In this case, the animals could not discriminate between potentially baited and never-baited locations based on local features. Instead, monkeys had to rely on a spatial relational representation of the environment to discriminate these locations (i.e., coding the goal locations in relation to distant environmental cues). The objects, placed at the center of the arena, could still be used to predict which of the two arrays of three distinct locations was baited.

Nine-month-old monkeys were tested for either 6 or 12 d in the spatial relational condition (Qutie, Rascal, Papillon = 6 d or 18 trials; Sugar and Tyson = 12 d or 36 trials). We included Qutie, Rascal, and Papillon's last five sessions, and Sugar and Tyson's last 10 sessions in the analysis of the data in the spatial relational condition. Juvenile monkeys were all between 8.5–10.5 mo-of-age at the end of testing: Sugar, 258-d-old; Tyson, 307-d-old; Qutie, 271-d-old; Rascal, 320-d-old; Papillon, 277-d-old. Adult monkeys had a total of 11 d (i.e., 33 trials) of experience in the spatial relational condition, and we included the last 10 d in the analysis.

Data analysis

For all local cue and spatial relational trials, we reported the first and the first four choices made (i.e., cups opened) by each monkey. For the dissociation probe trial, we reported the first, first four, and all choices made (i.e., cups opened) by each monkey. Because animals developed unique strategies to find the food locations, we also analyzed individual behaviors to detect evidence of learning. We present individual data from several subjects. In this case, statistical analyses were performed within subject across daily sessions.

For each analysis, we normalized the number of choices of a particular type based on the probability to make that choice. In the analysis of pre-reversal performance in the local cue condition, the number of Correct choices was divided by three (as there were three baited locations, out of 18 potential locations), the number of Incorrect choices by three (three unbaited locations) and the number of Other choices by 12 (12 never-baited locations). For the analyses of the standard trials based on five categories of choice, the number of choices of Pot IN (locations 13, 15, 17), Pot OUT (locations 4, 8, 12), Equ IN (locations 14, 16, 18), Equ OUT (locations 2, 6, 10) was divided by three and the number of Other choices (locations 1, 3, 5, 7, 9, 11) was divided by six. For the analysis of the probe trial based on five categories of choice, the number of choices of Color In (locations 14, 16, 18), Color Out (locations 2, 6, 10), Space In (locations 13, 15, 17), and Space Out (locations 4, 8, 12) was divided by three and the number of Other choices (locations 1, 3, 5, 7, 9, 11) was divided by six. Statview 5.0.1 statistical software was used to perform all statistical analyses.

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