RESEARCH ARTICLE

Niche-specific cognitive strategies: object memory interferes with spatial memory in the predatory bat *Myotis nattereri*

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ABSTRACT

Related species with different diets are predicted to rely on different cognitive strategies: those best suited for locating available and appropriate foods. Here we tested two predictions of the niche-specific cognitive strategies hypothesis in bats, which suggests that predatory species should rely more on object memory than on spatial memory for finding food and that the opposite is true of frugivorous and nectivorous species. Specifically, we predicted that: (1) predatory bats would readily learn to associate shapes with palatable prey and (2) once bats had made such associations, these would interfere with their subsequent learning of a spatial memory task. We trained free-flying *Myotis nattereri* to approach palatable and unpalatable insect prey suspended below polystyrene objects. Experimentally naïve bats learned to associate different objects with palatable and unpalatable prey but performed no better than chance in a subsequent spatial memory experiment. Because experimental sequence was predicted to be of consequence, we introduced a second group of bats first to the spatial memory experiment. These bats learned to associate prey position with palatability. Control trials indicated that bats made their decisions based on information acquired through echolocation. Previous studies have shown that bat species that eat mainly nectar and fruit rely heavily on spatial memory, reflecting the relative consistency of distribution of fruit and nectar compared with insects. Our results support the niche-specific cognitive strategies hypothesis and suggest that for gleaning and clutter-resistant aerial hawking bats, learning to associate shape with food interferes with subsequent spatial memory learning.

KEY WORDS: Echolocation, Spatial memory, Object memory, Foraging behaviour, Predatory bats

INTRODUCTION

To better exploit nutritional resources, animals with different dietary requirements should make decisions about where to forage based on multiple sources of both real-time sensory information and stored information (i.e. memory). Across species, animals should do so using cognitive processes shaped through natural selection (Dukas and Ratcliffe, 2009; Shettleworth, 2010). For example, birds that store food rely more on spatial memory to relocate caches than closely related non-storing species that tend to rely more on food-associated cues such as colour and shape (Clayton and Krebs, 1994; McGregor and Healy, 1999). Although no species of bat is known to store food for future use, they depend upon spatial memory to relocate food and

feeding sites, mates and roosts. In some species, spatial memory is essential for migration (Griffin, 1970; Holland, 2007; Schnitzler et al., 2003; McGuire and Ratcliffe, 2011; Tsoar et al., 2011).

Stich and Winter (Stich and Winter, 2006) proposed the niche-specific cognitive strategies hypothesis for phyllostomid bats, predicting that nectar-feeding and fruit-eating species should rely more on spatial memory, while predatory species should rely more on object memory. Consistent with this hypothesis, in the nectivorous and frugivorous phyllostomid bats *Glossophaga soricina* and *Carollia perspicillata*, spatial memory during foraging takes precedence over other sources of sensory information (e.g. flower shape, olfaction, colour) once the bats have identified potential food resources (Carter et al., 2010; Thiele and Winter, 2005; Stich and Winter, 2006). For fruit- and nectar-feeding bats, relying on spatial memory to relocate stationary, nutritional resources such as ripe fruit and recently produced nectar should be advantageous. Similarly, remembering that a site has recently been depleted of food or associated with unripe or rotting fruit may lead to adaptive avoidance (Ratcliffe and ter Hofstede, 2005) [see Healy and Hurly (Healy and Hurly, 2004) for hummingbirds].

However, no experiments on the relative importance of spatial versus object memory have been reported from predatory bats. Predatory bats may benefit from prioritizing object memory over spatial memory, as moving prey are not expected to be as spatiotemporally predictable as fruit and flowers. While informative, the results of small-scale landmark-use experiments in predatory species do not provide clear-cut answers to this question (e.g. Jensen et al., 2005; Mueller and Mueller, 1979; Ratcliffe et al., 2005; Schnitzler et al., 2003; Surlykke et al., 2009). In the present study, we concur with Stich and Winter's (Stich and Winter, 2006) diet-specific predictions and extend this hypothesis to bats other than phyllostomids.

Natterer's bat, *Myotis nattereri* (Kuhl 1817), is a ~10 g vespertilionid bat that primarily hunts suspended arthropod prey (e.g. spiders and caterpillars), which vary in palatability, close to vegetation. *Myotis nattereri* is behaviourally flexible with respect to foraging strategy (Ratcliffe et al., 2006), aerially hawking prey close to vegetation and gleaning prey from substrate (Czech et al., 2008; Schnitzler and Kalko, 2001; Siemers and Schnitzler, 2000; Swift and Racey, 2002). It uses very short, broadband echolocation calls to resolve the echoes from suspended prey from those returning from clutter (Siemers and Schnitzler, 2000; Siemers and Schnitzler, 2004). It forages using a sequence of search flights and stationary hovering, continuously producing echolocation calls, including feeding buzzes (i.e. echolocation call rates $>100\,\mathrm{s}^{-1}$) at the end of an attack sequence (Melcón et al., 2007; Neuweiler, 2003; Siemers and Schnitzler, 2000; Swift and Racey, 2002).

Most species of gleaning bats rely on prey-generated sounds to detect and localize individual prey (reviewed in Ratcliffe, 2009; but see Geipel et al., 2013). Indeed, Siemers and Schnitzler (Siemers and Schnitzler, 2004) found that *M. nattereri* could not locate silent prey

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positioned directly on substrate using echolocation alone. These and similar results from other predatory bats raise the question as to whether predatory bats hunting in cluttered habitat use additional strategies for identifying potential profitable sites for foraging, such as spatial memory or through the association of nearby objects (e.g. specific plant species) with potential prey (i.e. object memory). Anecdotal observations of a single individual suggest that *M. nattereri* can associate nearby shapes with food (Siemers, 2001). In that case the bat associated a food dish of a particular shape with palatable mealworms. It has also been suggested that *M. nattereri*, in particular, and other bats, in general, may be able to discriminate between different plant species using spectral cues received through echolocation (Yovel et al., 2008; Yovel et al., 2011).

We tested the hypothesis that predatory bats rely more on object memory than on spatial memory for identifying potential prey patches than do frugivorous and nectivorous bats. We predicted, first, that unlike frugivorous and nectivorous bats, predatory *M. nattereri* would readily learn to associate different object shapes with the palatability of nearby prey under ecologically relevant conditions and that the learning of such associations would not be overshadowed by positional cues. And, second, that once having learned to associate nearby objects with palatable and unpalatable food, such learning would negatively impact (i.e. interfere with) subsequent spatial memory learning as compared with spatial memory learning in experimentally naïve *M. nattereri*. We monitored echolocation signals throughout our experiments (Fig. 1A) and recorded and analysed them for a subset of trials to describe the bats' echolocation behaviour and to determine whether there were any differences in echolocation behaviour between tasks. Our overall experimental procedure is described in Fig. 1B.

RESULTS
Object memory (Experiment 1)
Bats in Group 1 learned to associate shape with reward (Fig. 2A–C). These three bats readily transferred these associations across scenarios as the number of negatively reinforced shapes decreased from three to two and then to one (Fig. 2A–C).

In scenario 3, we found that on the majority of days (>6 days) and overall, each of the bats made more mistakes during the first approximately five trials of a day's session than the last approximately five trials (overall 72, 72 and 74% versus 88, 88 and 84%, respectively). We found no evidence that the bats' mistakes were related to the bats returning to the previously rewarded position. Specifically, over the 10 training days, each bat made <34% of it total errors at the position last rewarded, where 33.3% is the level predicted by chance given on our experimental design.

As described in the Materials and methods (see also Fig. 1B), following Experiment 2 (spatial memory), we reintroduced one of the bats from Group 1 to the third scenario of Experiment 1 (object memory). This bat recalled the positively reinforced shape despite an 11-day absence of object memory experiments and an intervening 10 days of spatial memory training (Fig. 2D).

Spatial memory (Experiment 2)
Bats in Group 1, having just completed the object memory experiment (three scenarios, 30 days total) and control trials (10 days), did not perform better than chance during the spatial memory experiment (Fig. 3A).

Please note the greater than chance performance in scenario 3 of Experiment 1 in the Group 1 bat tested after these spatial memory trials (Fig. 2D). This suggests that the lack of motivation observed during the last days of the spatial memory task for all three bats was
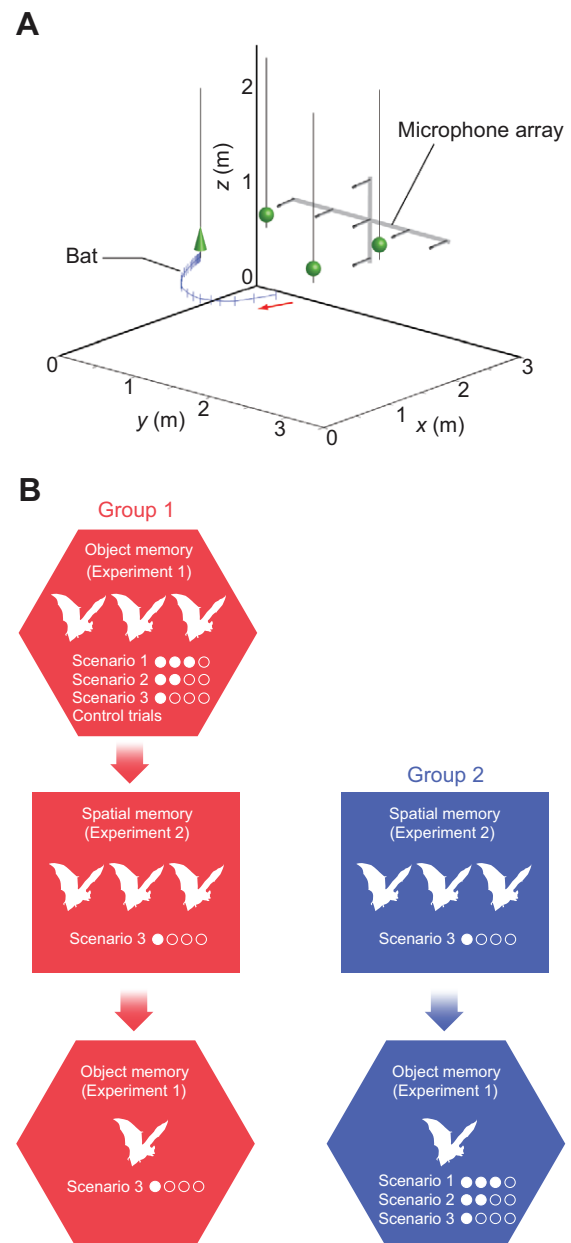

Fig. 1. Flight room, experimental setup and experiment flowchart.
(A) Flight room and experimental setup. Blue vertical and horizontal lines indicate echolocation calls and flight path of a single bat making a correct choice in Experiment 1 (red arrow indicates flight direction). (B) Experiment flowchart for Group 1 and Group 2 bats. Filled circles indicate the number of palatable prey available each trial; open circles indicate the number of unpalatable prey available each trial. Arrows indicate sequence order.

due to the difficulty the bats had in learning this task after experiencing the object memory task and not an overall loss of motivation to capture mealworms in the flight room or time in captivity.

Bats in Group 2 (previously experimentally naïve) learned where they could reliably find palatable prey (Fig. 3B).

Over the course of this experiment (10 days), each of the bats in Group 1 chose the correct position on <34% of all trials, while all bats in Group 2 chose the correct position >50% of all trials (Fig. 3A,B). Taking these as below and above threshold values, respectively, Group 2 bats performed significantly better than did
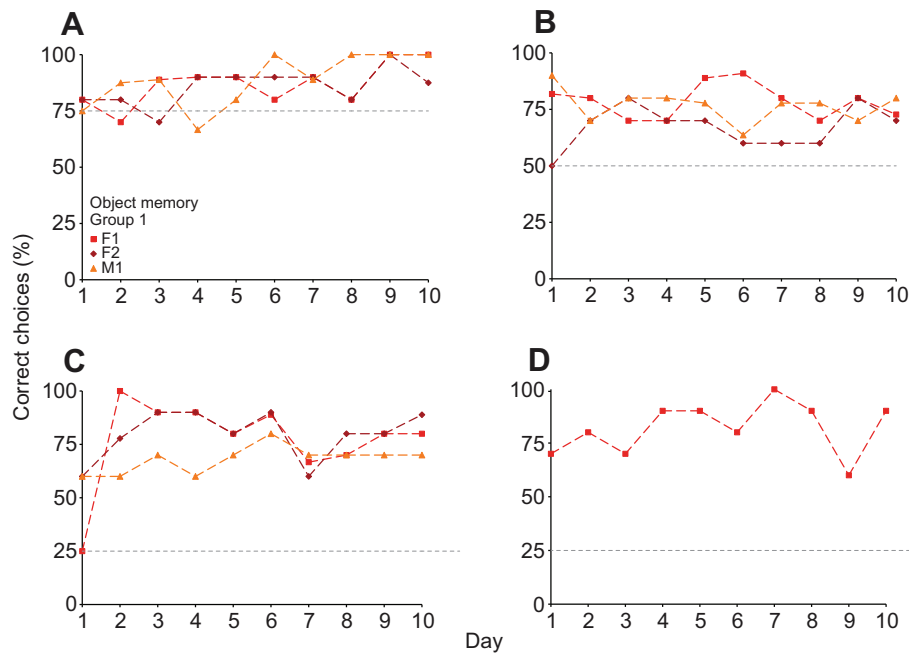
Fig. 2. Performance of Group 1 bats during object memory tasks (Experiment 1). Percentage of correct choices made over the course of ca. 10 trials each day for ca. 10 days for the three bats in Group 1. (A) Scenario 1: each bat was presented with three of the same, positively rewarded shape and a fourth different, negatively rewarded shape. Here the chance of taking a palatable mealworm without learning was 75% (dashed line). (B) Scenario 2: two positively and two negatively rewarded shapes. Chance was 50% (dashed line). (C) Scenario 3: three negatively rewarded shapes and one positively rewarded shape. Chance was 25% (dashed line). (D) After completing Experiments 1 and 2, one Group 1 bat was reintroduced to scenario 3 of Experiment 1.

Group 1 bats (Barnard's exact test, two-tailed, $P<0.04$) (Barnard, 1945). Similarly, the slope of the learning curve describing Group 2 was significantly steeper than that for bats in Group 1 (two-tailed test for difference between two population regression coefficients, $t=2.19$, $P<0.05$; Fig. 3C,D) (Zar, 1996).

Following Experiment 2, we introduced one Group 2 bat to scenarios 1–3 of Experiment 1 (Fig. 1B). This bat learned the object memory tasks (Fig. 4A–C) as quickly as did Group 1 bats (Fig. 2A–C).

Control trials (Group 1)

On day 2 of the control session, when all shapes had palatable mealworms suspended below them (that is, no negative reinforcement), two of the three Group 1 bats took mealworms from under the normally negative reinforced shape as their first choice

(Fig. 4D). These two bats then proceeded to take mealworms from each of the four positions, regardless of the shape above (Fig. 4D).

In darkness (day 6; Fig. 4D), and for those trials for which the monofilament lines between the palatable prey and shape had been dipped in quinine (day 9; Fig. 4D), bats performed as during experimental trials (Fig. 2A–C, Fig. 4D).

Echolocation

Bats produced echolocation calls in all trials, and always produced a buzz before taking a mealworm (Figs 5, 6, Table 1). We noted no difference in echolocation call design or emission behaviour between bats, experiments, or correct and incorrect choices (Table 1). In the approach phase (20–100 calls s^{-1}), bats produced echolocation call strobe groups (Table 1, Figs 5, 6).
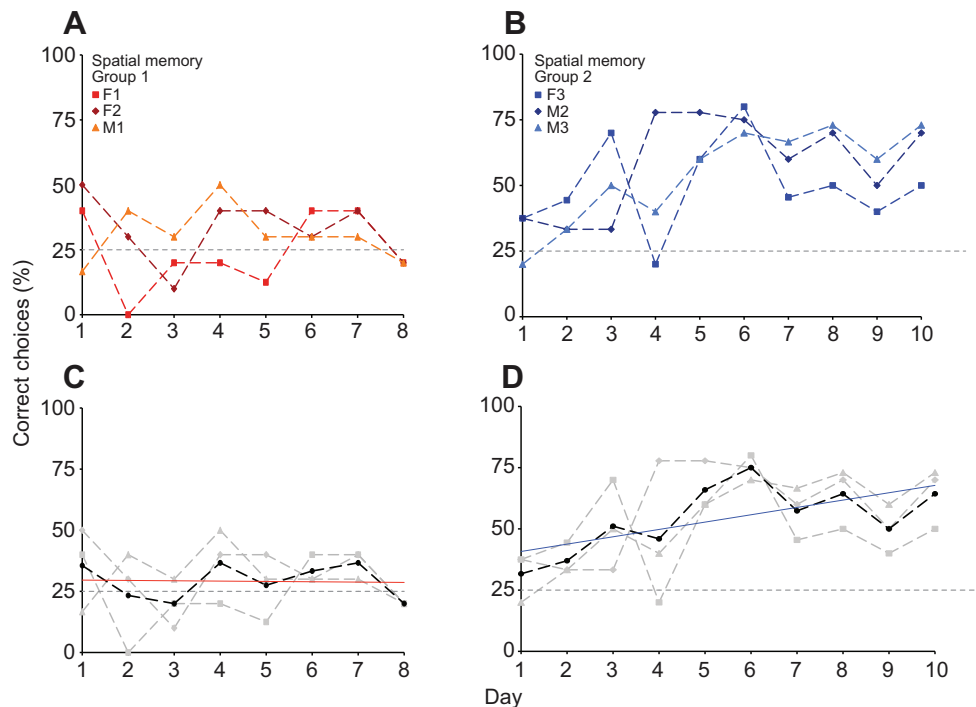

Fig. 3. Performance of Group 1 and Group 2 bats during spatial memory tasks (Experiment 2). (A) Group 1 performance. (B) Group 2 performance. (C) Group 1 overall performance (regression line). (D) Group 2 overall performance (regression line). Here, the chance of taking a palatable mealworm without learning was 25% (dashed line). Note that for two Group 1 bats (A), the experiment was stopped after 8 days, as these bats had lost their motivation to participate (took <5 mealworms on Day 8). We also stopped this experiment on Day 9 for the third bat (A), which had also started to make fewer capture attempts per unit time.
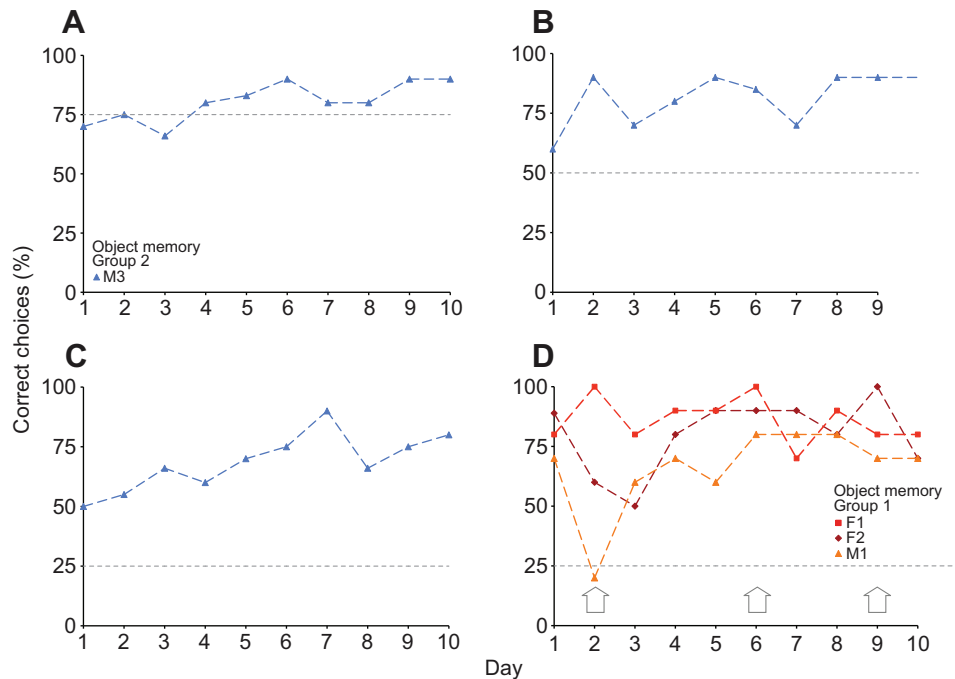
Fig. 4. Object memory performance after spatial memory task. (A–C) Performance of a single bat from Group 2 during scenarios 1–3 of Experiment 1, after the bat had completed the spatial memory task (Experiment 2). (D) After completion of Experiment 1, but before Experiment 2, Group 1 bats experienced a second round of scenario 3. We ran probe trials to ascertain to effects of negative reinforcement (quinine) and the possible use of chemical and/or visual cues for object discrimination. On day 2, all positions always had palatable mealworms hanging below them, that is, negative reinforcement was removed. On day 6, bats completed trials in darkness to exclude the use of visual cues. On day 9, the monofilament between the palatable mealworms and positively associated shapes were doused in quinine solution, twice over the course of each bat's trials that day (i.e. before trial 1 and before trial 5).

DISCUSSION

Myotis nattereri readily learned to associate 3D polystyrene shapes with the palatable and unpalatable mealworms suspended beneath them. After being introduced to the flight room, the three bats from Group 1 learned to take palatable prey suspended from objects of one shape and to avoid taking mealworms suspended from beneath a differently shaped object (see Results; Fig. 2A–C). These results

Fig. 5. Sound recording of echolocation call attack sequence from Fig. 1. (A) Spectrogram and (B) oscillogram as recorded on the centre microphone of the array. Buzz begins when call repetition rate exceeds 100 calls s^{-1}; during the buzz call peak frequency drops by an octave. p, pressure. (C) Plot of pulse interval (PI; time elapsed from start of one call to start of next) versus time to contact with target, illustrating the decrease in PI as the bat approaches the target. Red circles indicate two and three call strobe groups (Moss et al., 2006; Surlykke et al., 2009).

demonstrate that the bats, including the Group 2 bat that was successfully trained on shape after being trained on position (Fig. 4A–C), learned to associate object shape with the relative palatability prey suspended from it, supporting our first prediction.

Our second prediction was also supported: bats that had previously experienced the object memory experiment (Group 1) performed poorly in the spatial memory experiment relative to the experimentally naïve bats (Group 2). Indeed, bats in Group 1 did not perform any better than chance (Fig. 3A,C). Bats in Group 2, which experienced the spatial learning memory experiment first, however, readily learned the location of palatable prey (Fig. 3B,D). *Myotis nattereri* is thus able to form associations between location and prey quality, but formation of spatial associations is hindered by previously formed associations between object shape and prey quality.

While these experiments demonstrate only that object learning interferes with spatial memory in this predatory species, the results are roughly the opposite of what has been reported from nectivorous and frugivorous species. In *G. soricina* and *C. perspicillata*, spatial memory profoundly overshadows object memory learning (Carter et al., 2010; Stich and Winter, 2006; Thiele and Winter, 2005). Experiments with predatory bat species often randomize reward position to overcome the potential impact of spatial memory on cue–consequence associative learning (e.g. Page and Ryan, 2005). However, our results show that although object memory interferes with spatial memory, the reverse might not be the case. Following Experiment 2 (spatial memory), we reintroduced one of the bats from Group 1 to scenario 3 of Experiment 1 (object memory) and found that this bat recalled the shape–consequence associations (Fig. 2D). Similarly, one bat from Group 2 learned to associate object shape with reward (Experiment 1, all scenarios) after having learned the spatial memory task (Fig. 4A–C). We note that this last result does not mean that had this bat had 30–40 days of spatial memory training (rather than 10 days) it may not as readily have learned the object memory tasks.

Comparative evidence of how bats made successive choices within training days corroborates the hypothesis that object memory interferes with spatial learning in predatory bats. We found no tendency during the object memory experiment for predatory *M.*
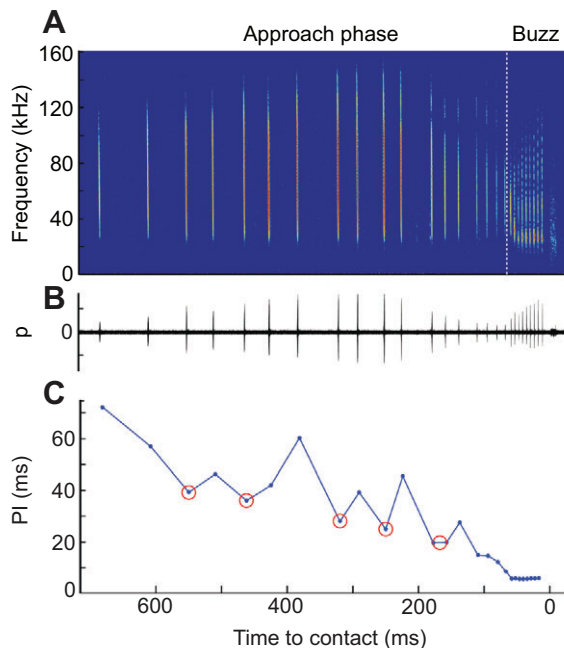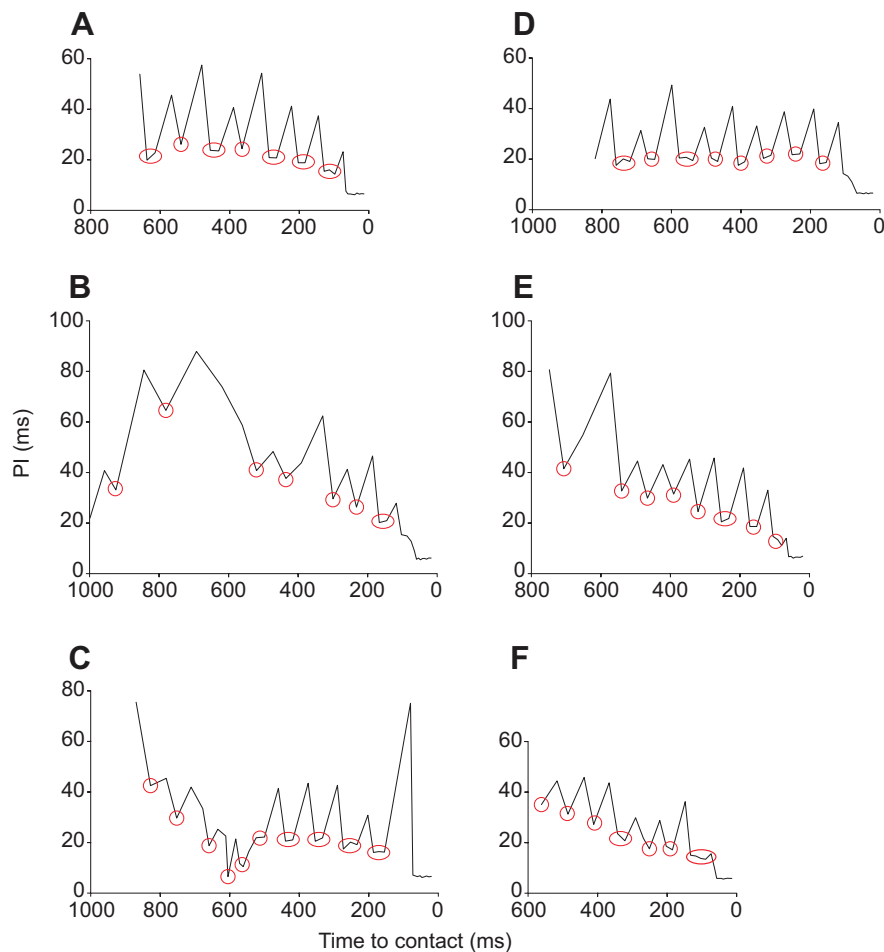
Fig. 6. Plots of pulse interval (PI) versus time to contact with mealworm. (A–C) Group 1 bats on day 7 of scenario 3, Experiment 1 (one for each bat, correct choice made in each instance). (D–F) Group 2 bats on day 7 of Experiment 2 (one for each bat, correct choice made in each instance). Note that PI always decreases as the bat approaches the target and in every instance there are multiple strobe groups. Strobe groups (circled in red) are defined as clusters of calls with a stable PI (up to 5% variation about the mean PI), separated by a PI of >1.2 times the mean PI of the strobe group (Moss et al., 2006; Surlykke et al., 2009).

nattereri to revisit the location of their previous correct choice more so than would be predicted by chance. This result also supports the idea that predatory bats will rely on object memory over spatial memory. Conversely, fruit- and nectar-eating bats often and consistently return to the site of previous reward, essentially ignoring shape and olfactory cues, despite reward position being consistently changed between trials (Carter et al., 2010).

One of our aims was to investigate *M. nattereri*'s ability to associate shape and position with positive and negative reinforcement under controlled but ecologically relevant conditions

(see Materials and methods). Specifically, we wanted to see whether we could train multiple bats to associate shape with reward, and exclude the possibility that Siemers' (Siemers, 2001) bat was an exceptional individual. In that study, one bat, among several, spontaneously learned to associate a particular food dish with food (Siemers, 2001). In our study, all bats tested (four of four) learned to associate a specific shape with palatable prey (Fig. 2A–D, Fig. 4A–C) under conditions specifically designed to mimic those found in nature and make learning easier. Our results allow us to dismiss the notion that the single bat observed by Siemers (Siemers,

Table 1. Echolocation behaviour of Group 1 bats in scenario 3 of the object memory experiment and Group 2 bats in the spatial memory experiment, recorded on day 7

	Object memory	Spatial memory
Call duration (ms)		
Approach	1.6 (1.6–2.5)	1.9 (1.8–1.9)
Buzz	0.8 (0.64–0.86)	0.8 (0.7–0.8)
Call peak frequency (kHz)		
Approach	61.6 (57–66.3)	57.9 (55.7–67.5)
Buzz	28.3 (21.8–33.8)	29 (20–35.4)
Call bandwidth (−10 dB from peak frequency, kHz)		
Approach	87.5 (82.3–89.6)	84.6 (80–87.8)
Call intensity (dB SPL r.m.s. @10 cm @57 kHz)		
Approach	90.3 (94.1)	92 (101)
Strobe groups		
Number of groups	8 (3–8)	7 (7–10)
Number of calls per group	3 (2–6)	3 (2–6)
Buzz phase		
Number of calls	9 (8–11)	9 (7–11)

Data are presented as medians (range), except for call intensity approach data, which are presented as medians (maximum).

2001) was itself exceptional. To our knowledge, ours is the first study to demonstrate that predatory bats can quickly associate novel shapes with food while in free flight.

We suggest that, given the choice, predatory bats are more likely to rely on object memory than spatial memory for finding profitable prey and avoiding unprofitable prey. Taken together with results from frugivorous and nectivorous species, our results support Stich and Winter's (Stich and Winter, 2006) niche-specific cognitive strategies hypothesis in bats. This hypothesis falls under the umbrella of the more general hypothesis of a cognitive continuum comprising closely related vertebrate species that differ in foraging behaviour and diet [e.g. non-storing birds and non-nectivorous birds versus food storing birds and hummingbirds, respectively (reviewed in Dukas and Ratcliffe, 2009; Shettleworth, 2010)]. For example, hummingbirds remember not only where flowers are in bloom but also which flowers have been depleted of nectar (Healy and Hurly, 2004). Because insects are not as spatiotemporally predictable as flowering plants, selective pressures for spatial memory in insectivorous species may not be as strong as for nectivorous and frugivorous species. Indeed, while the bats in Group 2 learned to associate position with food (Fig. 3B,D), learning was less profound than the immediate, almost inextinguishable, associations made in frugivorous and nectivorous phyllostomid bats (Carter et al., 2010; Stich and Winter, 2006; Thiele and Winter, 2005). For example, Thiele and Winter (Thiele and Winter, 2005) found that *G. soricina* developed a location preference after only eight consecutive rewards, while Stich and Winter (Stich and Winter, 2006) reported that *G. soricina* needed to have made several thousand choices before learning to ignore a spatial location.

Some bat-pollinated plants are acoustically conspicuous to facilitate their detection by nectar-feeding bats (von Helversen et al., 2003; von Helversen and von Helversen, 1999; von Helversen and von Helversen, 2003). Presumably, palatable insects have evolved no such cues, which for them would be distinctly maladaptive. Prey recognition for predatory bats relative to frugivorous and nectivorous species may thus be inherently more difficult, especially for those predatory bats hunting in clutter (Neuweiler, 1990; Schnitzler and Kalko, 2001; Schnitzler et al., 2003). For echolocating bats, differentiating between plant species may be more difficult than flower recognition, but not as difficult as detecting insects perched on vegetation (Geipel et al., 2013).

In an unpublished study, Denzinger and Schnitzler found that *M. nattereri* learned to discriminate between conifers and broad-leafed plants (described in Yovel et al., 2011). When some plant species are hosts for palatable insects, it may be advantageous for bats to remember and investigate these species more closely upon detection. Like most predatory bats, *M. nattereri* appears to be unable to resolve perched prey from the background using echolocation information alone, and must instead rely on another strategy, such as object memory, or prey-generated sounds to localize prey (Siemers et al., 2012). In our study, all bats always produced echolocation calls, including the terminal buzz, before taking a mealworm (Table 1, Figs 5, 6). That echolocation behaviour was consistent across bats, regardless of experiment or choice, suggests that *M. nattereri*'s echolocation behaviour while foraging is not object or spatial memory specific. During the approach phase of an attack, bats always produced echolocation calls in strobe groups (Table 1, Figs 5, 6). In the vespertilionid *Eptesicus fuscus*, strobe groups are produced before airborne targets are acquired in clutter, and are thought to sharpen the bat's auditory scene with respect to the shape and orientation of objects nearby (Moss et al., 2006; Surlykke et al., 2009).

Further investigation is required to determine whether the pattern we observed in *M. nattereri* (i.e. object memory learning interfering with spatial memory learning) is widespread across predatory bats. Further study is also needed to investigate whether predatory bats rely more on object memory than spatial memory in identifying prey patches, approaching those associated with palatable prey, while avoiding those associated with defended prey. However, in general it appears that attempts to control for the possible effects of spatial memory are more important in studies using frugivorous and nectivorous species (e.g. Carter et al., 2010; Stich and Winter, 2006; Winter and Stich, 2005; Winter and von Helversen, 2001) than for the design of similar studies using predatory species (Ratcliffe and Dawson, 2003; Ratcliffe and Fullard, 2005; Ratcliffe et al., 2005; ter Hofstede et al., 2008). In those studies using predatory bats, there was no evidence that bats relied on spatial memory over real-time sensory information (e.g. prey-generated sounds). Our results from Experiment 1 suggest something similar. As a related example, while the frog-eating phyllostomid *Trachops cirrhosis* has been observed to use spatial cues to find prey, its primary mode of prey detection is eavesdropping on prey-generated cues (R. A. Page, personal communication).

Our results suggest that *M. nattereri* and, by extension, predatory bats in general may rely on object memory more so than do frugivorous and nectivorous bats when searching for profitable prey patches. Like most other predatory bat species, *M. nattereri* hunts prey that are ephemeral in space and time. Predatory species would do well to recognize and associate plant types with prey availability and palatability, especially if hunting for still and silent prey. By using object memory before sweeping or landing on surfaces on which prey may be perched (Czech et al., 2008; Siemers and Schnitzler, 2000), many gleaning and behaviourally flexible bat species could limit what plant species they will investigate closely. Avoidance behaviour, however, apparently requires consistent reinforcement (Fig. 4D), and this may allow bats to reinvestigate plants once associated with defended prey that may now bear palatable arthropods (see Page and Ryan, 2006; Barber and Conner, 2007).

Recently it has been demonstrated that the small predatory phyllostomid bat *Micronycteris microtus* uses echolocation to detect still, silent dragonflies on background vegetation (Geipel et al., 2013). This feat had been thought to be impossible because of the masking effects of overlapping echoes from the would-be target and background clutter. This discovery also provides support for the niche-specific cognitive strategies hypothesis in bats. Geipel et al. (Geipel et al., 2013) showed that the bats they studied use a suite of traits (wings, body) to identify still and silent dragonflies perched on leaves. In this case, object learning is of the shape of the prey itself, through an extraordinary ability to resolve prey from background through echolocation. At a larger scale, object memory of specific plant species would allow bats to identify which species they should search closely for prey (using prey-generated sounds and/or echolocation) and which plant species to pass over. From a sensori-motor perspective, gleaning bats and those that take prey close to vegetation have much in common with nectar and fruit-eating bats. Most predatory phyllostomid bats are gleaners. In extending Stich and Winter's hypothesis from phyllostomid bats (Stich and Winter, 2006), the only family of laryngeal echolocating bats to include non-predatory species, to bats in general, we recognize that placing obligate open-air insectivores on a diet-based cognitive continuum would be neither straightforward nor, perhaps, meaningful. However, once an underestimated strategy, half or more of today's ca. 900 predatory bat species may glean some or all of their prey (Ratcliffe et al., 2006). For bats that glean from terrestrial

surfaces or hawk prey close to vegetation, recognizing plant species by shape, rather than position, may be vital.

MATERIALS AND METHODS

Study animals and shapes

We conducted free-flight experiments with wild-caught Natterer's bats, *M. nattereri*, to investigate the importance of associated shape and spatial position for food acquisition and avoidance in a predatory bat (Fig. 1). The subjects were six adults (three males, three non-lactating females) caught in Odense, Denmark (55°24′N, 10°23′E). Experiments were conducted at the University of Southern Denmark in Odense. Bats were released at capture site after experimentation.

Bats were housed individually or in same-sex pairs in 25 cm diameter, 35 cm high aluminium mesh cylinders with a soft cloth hung for each bat from the top for roosting. Bats were kept at a constant temperature of 20°C and relative humidity of ~50% on a reversed light schedule of 12 h low light, 12 h darkness. They acclimated to this light regime over the course of 7–10 days. Bats had continuous access to water. Throughout their time in captivity, each bat received 10–12 palatable mealworms per day. We flew bats individually in a screened, indoor flight room (3.5×3.0×2.5 m, length × width × height), illuminated by a red light bulb (25 W) suspended above the centre of the room. Prior to experiments, bats quickly accustomed themselves to taking mealworms suspended from cotton sewing threads distributed in a larger flight room. During experiments, mealworms hung ~1 m above the ground (~1.5 m below the ceiling) and ~10 cm below white polystyrene objects (Fig. 1A).

We used polystyrene objects of four different shapes: 8 cm diameter spheres, 12 cm diameter rings, cones (7.5×15 cm, base × height) and hearts (11×11 cm, height × width) (Fig. 1A). Monofilament (2 kg test fishing line, 0.2 mm diameter) was used to tether the objects to the ceiling and the mealworms to the objects. The position of each of the four ceiling-to-object tethers remained constant throughout the experiments (Fig. 1A). Each individual object, however, could be moved between tethers between trials. Because of differences between the four screened walls (e.g. size, a zipper, a post), bats could distinguish one wall from another, which would help them orient.

To mimic a naturally occurring insect chemical defence and thus render some mealworms unpalatable to the bats, 'unpalatable' mealworms were made so by dousing them with 10% w/v quinine sulphate aqueous solution. Quinine is a bitter-tasting, odourless alkaloid similar to those sequestered from host plants by some Lepidoptera as caterpillars (Nishida, 2002; Weller et al., 1999) and avoided by the vespertilionid bat *E. fuscus* (Ratcliffe et al., 2003). Trials commenced 1–2 h after lights off and a day's trials were completed when the bat had taken (i.e. eaten or dropped) 10 mealworms or rested for >20 min since its last take.

The six bats used in this study were divided into two groups, each comprising three individuals. These two groups had different experiences and their performance during the spatial memory task was compared to test for the predicted interference of object memory learning on the subsequent acquisition of the spatial memory task. Specifically, Group 1 bats first learned the object memory tasks and were then introduced to the spatial memory task. Group 2 bats were introduced to the spatial task while still experimentally naïve (Fig. 1B). Performance during the spatial memory task was compared to test for the predicted interference of object memory learning on the subsequent acquisition of the spatial memory task.

Object memory (Experiment 1)

In this experiment we used unadulterated and quinine-soaked mealworms suspended on fishing line to simulate, respectively, a foraging bat's encounters with hanging, palatable prey (e.g. spiders, caterpillars) and hanging, unpalatable prey (e.g. chemically defended caterpillars). These arthropods might be found suspended from different plant species in the wild. Our purpose was to determine whether bats would make associations between prey of low versus high palatability and the shape of objects from which these different prey groups were suspended. In this experiment the three shapes used were the sphere, the ring and the cone (described above). Each bat tested was assigned four objects of two different shapes, one shape with unpalatable prey underneath, and another shape with palatable prey suspended underneath (Fig. 1A). At least two of the objects, including the one the bat took a

mealworm from, were repositioned after every trial. This experiment lasted roughly 30 days and was divided sequentially into three 10-day, ca. 10 trials per day, scenarios, the first with three, the second with two, and the last with one positively reinforced shape. Therefore, the mere chance of the bat taking a positively reinforced mealworm (i.e. a palatable mealworm) declined from 75% in scenario 1 to 50% in scenario 2 and 25% in scenario 3.

In addition to assessing object learning in scenario 3, we also investigated whether bats were more prone to make mistakes in the first half of each daily session than the second half. Furthermore, to identify the potential use of spatial memory in subtle ways during the object memory scenarios, we explored whether bats were influenced by spatial position, specifically, whether bats made more mistakes at the previously rewarded position than at the other positions. We did this because such has been demonstrated to be the case in the frugivorous bat *C. perspicillata* and the nectivorous bat *G. soricina* (Carter et al., 2010; Thiele and Winter, 2005; Stich and Winter, 2006).

Spatial memory (Experiment 2)

The purpose of this experiment was to determine whether, and if so how quickly, each bat associated one of the four object positions with profitable prey and the other three positions with unprofitable prey. This experiment lasted ca. 10 days (ca. 100 trials per bat in total). We had intended to run the spatial memory task for an additional 20–30 days, but the poor performance of the Group 1 bats precluded this. For the sake of congruence, Group 2 bats experienced the spatial memory task for only 10 days as well.

For each bat, we chose a single position (a different one for each bat) at which profitable prey items were provided. During all trials, the remaining three positions always bore unprofitable prey. All objects had the same shape and while the rewarded position remained constant, at least two of the objects were moved between trials. Although to our eyes the objects were indistinguishable, we did this to ensure the bats were relying on spatial memory alone. We used four objects of the same shape for each bat, one on each of the four positions to simulate prey hanging from a single plant species. The objects used in this experiment were novel to the bats. Specifically, we used spheres for two of the bats in each group and polystyrene hearts for the bat in Group 1 that had already experienced spheres in Experiment 1 and also for one bat in Group 2. We suspended prey beneath shapes in both experiments so the bats would always have to take prey from beneath a shape, which demands different flight and, presumably, echolocation behaviour, than an attack on an unobstructed mealworm.

Control trials

Control trials (i.e. probe trials) were run over the course of 10 days using the bats from Group 1 to determine whether the bats made their shape–reward associations using information obtained through echolocation. These trials were run immediately following scenario 3 of Experiment 1. Control trials were conducted as for scenario 3 but with the following differences. On day 2, all shapes had palatable mealworms suspended beneath them (i.e. no negative reinforcement) to test for use of olfactory cues and the persistence of memory in the absence of negative reinforcement (Fig. 4D). On day 6, trials were run in complete darkness, to test for the use of vision (Fig. 4D). On day 9, all of the monofilament lines were dipped in quinine, to test for the use of olfactory cues (see Fig. 4D).

To determine whether object memory persisted after spatial memory training, one bat from Group 1 experienced scenario 3 of Experiment 1 after completing the spatial memory experiment (see Fig. 2D). Only one bat was so assigned because the other two bats from Group 1 had lost motivation during the spatial memory trials and so were hand-fed for 3 days and released.

Echolocation

We monitored echolocation throughout our experiments using an ultrasound detector (D240X, Pettersson Elektronik AB) to document echolocation call production. On day 7 of Experiment 1, scenario 3 for Group 1, and day 7 of experiment 2 for Group 2, we also recorded bats' echolocation calls (six bats, four sequences per bat; Fig. 1A, Figs 5, 6) using a cross-shaped array of seven quarter-inch G.R.A.S. microphones (see Fig. 1A) (for details, see Jakobsen and Surlykke, 2010; Elemans et al., 2011). We made and analysed these recordings to compare echolocation behaviour between tasks and successes versus failures.

To this end, we selected recordings from two trials for each bat (one palatable/correct choice, one unpalatable/incorrect choice). For each sequence, the bat's position was estimated at each call emission by triangulation of the differences in arrival times at the seven microphones (Fig. 1A). From these data, call intensity was estimated as described in Jakobsen and Surlykke (Jakobsen and Surlykke, 2010). Call intensity is flexible in vespertilionids and varies based on habitat and task (Jakobsen and Surlykke, 2010; Jakobsen et al., 2013). Pulse interval (time elapsed from onset of call to onset of next call) and call duration were measured from the oscillogram of the channel recording the highest intensity signals (Fig. 5B). We also measured each call's fundamental peak frequency and −10 dB bandwidth by measuring the frequency range 10 dB down from peak frequency on the power spectra (Table 1). Echolocation call strobe groups (Fig. 5C) were identified using criteria outlined in Moss et al. (Moss et al., 2006).

This research complied with the legal requirements of Denmark and all institutional guidelines. Animal capture and experimentation were approved by Skov-og Naturstyrelsen (Denmark) and carried out in accordance with the species-specific recommendations of the Canadian Council on Animal Care for bats (Canadian Council on Animal Care, 2003).

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