

Research article

Open Access

Way-marking behaviour: an aid to spatial navigation in the wood mouse (*Apodemus sylvaticus*)

Pavel Stopka^{1,2} and David W Macdonald*²

Address: ¹Biodiversity Research Group, Department of Zoology, Charles University, Vinicna 7, Prague 2, Czech Republic and ²Wildlife Conservation Research Unit, Department of Zoology, University of Oxford, South Parks Road, Oxford, UK

Email: Pavel Stopka - pstopka@natur.cuni.cz; David W Macdonald* - david.macdonald@zoology.oxford.ac.uk

* Corresponding author

Published: 2 April 2003

Received: 13 June 2002

BMC Ecology 2003, 3:3

Accepted: 2 April 2003

This article is available from: <http://www.biomedcentral.com/1472-6785/3/3>

© 2003 Stopka and Macdonald; licensee BioMed Central Ltd. This is an Open Access article: verbatim copying and redistribution of this article are permitted in all media for any purpose, provided this notice is preserved along with the article's original URL.

Abstract

Background: During their movements in the wild, wood mice (*Apodemus sylvaticus*) distribute small objects, such as leaves or twigs, which are often visually conspicuous. Our experiments demonstrate that these marks serve as points of reference during exploration. Way-marking, as we call it, may diminish the likelihood of losing an "interesting" location, perhaps following disturbance by, for example, a predator or conspecific. Way-marks, being readily portable, may be a less confusing method of marking ephemeral sites than scent marks. They may also be a safer option for local navigation insofar as scent marks can easily be detected by a predator.

Results: In an experiment, conspicuous natural candidate way-marks were removed from a simple arena and wood mice were given white plastic discs instead. The wood mice picked up these discs and re-distributed them about their arena; as the mice moved, they repeatedly re-positioned the discs and usually spent a considerable time near recently repositioned discs. Analysis revealed a statistically significant association between the location of places in which the mice had positioned way-marks and the subsequent pattern of their movements. In a separate analysis, based on the context in which each behaviour occurred, we used the components and sequences of wood mouse behaviour to deduce the motivation behind each activity. One set of behaviour patterns, the elements of which were closely linked by the high transition probabilities amongst them, were interpreted as linked elements of exploration; whenever the mice transported a disc it was in association with these exploratory behaviours. This evidence that transporting discs is set in the motivational context of exploratory behaviour supports the conclusion that way-marking is part of the wood mouse's system of spatial orientation.

Conclusion: We conclude that way-marking – a behaviour not previously described in mammals other than humans – serves solely as an aid to spatial navigation during exploration.

Background

All animals face at least occasional problems of orientation. Especially for vertebrates, this can involve periods of almost continuous decisions. Much remains unknown about the processes involved in spatial orientation, and the behaviour patterns through which navigation is

achieved. As a general model, mammals are thought to use fixed external cues as points of reference during their movements [1]. This model holds that an individual's spatial references are updated, with respect to the array of fixed land-marks, so that it is able to return to its refuge, feeding places, or other places of interest. This system

(termed 'path integration') is generally thought to be especially important to central-place foragers, which sometimes have to return home even in the absence of external cues [1]. Return by path integration is, however, never precise and, depending on the species, systematic errors can occur in navigation. Indeed, it remains possible that path integration and landmark guidance are independent processes.

The problem of finding one's way around, especially while exploring, is a pressing one for all mammals. It is certainly so for the subject of this paper, the wood mouse (*Apodemus sylvaticus*), because this small, nocturnal rodent often occupies home ranges that are vast in comparison to its body size [2]. Wood mice are rodents and amongst this Order several studies have already revealed differences between species in their orientation behaviour, although these do not necessarily indicate that different orientation systems are involved. In captivity hamsters (*Mesocricetus auratus*) use landmarks and dead reckoning (i.e. path integration) to find their way back to their den from a food source [1]. Amongst meadow voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus*) the sexes differ in their performances of tasks involving orientation in water mazes and circular arenas, and these differences are associated with hormonal changes and especially with levels of oestradiol [3]. Laboratory rats (*Rattus norvegicus*) use the geometry of surfaces for navigation and can remember a number of locations in space by their relations to surrounding landmarks; while navigating, rats attach greater priority to landmarks than, for example, the smell of food pellets, or the scent left on the arms of the maze [4]. Mice (*Mus musculus*) and hamsters give priority to stable visual references and path integration over scent-marks when these cues are contradictory [1].

In the case of the wood mice, the problem of navigation would seem, to a human observer, to be particularly challenging because their relatively huge home ranges encompass environments – for example, expanses of ploughed fields or uniform cereal crops – that seem particularly homogeneous. The need for a reliable mechanism of orientation in this species is particularly obvious when one observes both the fine-grained spatial complexity of, and temporal shifts in, the distribution of their food plants [5,6]. The reality of temporal shifts in the ecology of wood mice is illustrated dramatically by the impact of harvest on the dispersions of both their food and of predatory risk [7]. While spatio-temporal instability of the habitat in the agricultural mosaic is particularly evident on farmland [8] it seems likely that good navigational skills were similarly at a premium throughout the evolution of this species. Indeed, there is evidence that wood mice use magnetic orientation [9,10]

Radio-tracking studies in cereal fields makes it clear that wood mice do not always confine their movements to a restricted network of paths [5,11], although during exploratory searches in the vicinity of a nest, wood mice have been observed to travel repeatedly on trails [12]. Jamon proposed that these trails develop from following scent left during earlier visits; he dusted wood mice with fluorescent pigments, which revealed trail-following to be non-random. However, Benhamou [13], watching tagged wood mice from an observation tower, found that their movements fitted a first-order correlated random walk model. That is, any future turn depended on the current direction with some certain probability p . Although different, these results are not necessarily contradictory; indeed, it seems likely that, while in the vicinity of a fixed resource such as a burrow, the mice might use regular trials and perhaps scent, whereas, when travelling widely in search of ephemeral supplies of food, they might use a different pattern of movement and a different means of navigation. Indeed, Blackwell [14] used a random diffusion model that provided support for the hypothesis that an individual switches, in a random way, between different diffusion processes on the basis of different motivations.

Earlier work, therefore, has raised the question of how wood mice navigate and also introduced the idea of relating movement patterns to the animal's motivation. However, so far as we are aware, there has been no demonstration of either how wood mice navigate while using ephemeral resources or, for any mammal, any attempt to combine an analysis of motivation and corresponding spatial variables to reveal the properties of an animal's movements.

In this paper, we firstly describe a new behaviour, during which wood mice distribute what appear to be visual markers while they forage. We were alerted to this phenomenon because we noticed accumulation of small objects where wood mice had been active in the field. Secondly, we use video-surveillance in experimental enclosures to record simultaneously both behaviour and movements, and thereby to demonstrate that this behaviour functions as an aid to navigation. Similar behaviour has not previously been observed except amongst humans. Lastly, using the same video records and, having defined behaviour patterns from an ethogram, we used Markov chain analysis [15] to reveal that this new behaviour, which we call way-marking, does indeed fit contextually within the motivational category of exploratory behaviour.

Results

Testing the directionality of way-marking

The wood mice moved about the arena, sometimes making short, convoluted movements as they investigated an

area in detail, and sometimes making longer (>10 cm), more directed movements (see below). When a mouse had begun to investigate a small area of apparent interest, it would scan the arena, seemingly looking for a disc, to which it would then move, and then carry that disc directly back to the place of current interest and position it there. Thereafter, while the mouse continued to be active in that place, and having drifted away from the disc, it would intermittently make a directed movement straight back to the disc. These movements back to the disc were associated with scanning, sometimes while the mouse reared on its hind legs, apparently checking that it was en route to the disc. When the mouse finally left that place of temporary interest the disc would be left behind, but once the mouse started to investigate a new place it might return to the original disc, or to any other, and transfer it to the new place of temporary interest. In every case that a disc was moved, it was picked up after the mouse had scanned, apparently looking for a disc, and in every case, having picked up the disc, it was carried to the new place of interest in a directed movement of more than >10 cm (which we define as an exploratory movement, see Methods below). Thus, at the start of an experiment, when all the discs were in the centre of the arena, the mice scanning to find a disc invariably had to make their way back to the centre to find one; thereafter, progressively, all the discs became scattered around the arena, in places that had previously been the foci of temporary investigation, and so when a mouse needed a disc it would generally pick up the nearest one it could find. The wood mice were also seen to move directly towards a disc when, following a 'false alarm', they had dashed for cover – in this case, a combination of directed movements and scanning gave the clear impression that they used the discs to find rapidly the place from which they had been disturbed.

Overall, the mice did not carry the discs in any particular direction; specifically, they did not carry them predominantly towards the nest box, as might have been predicted if the behaviour was motivated to collect them as potential bedding material. Indeed, the prevailing angles of transferral were random with respect to the likely candidate visual reference point, the nest-box (Rayleigh test $p > 0.2$; $n = 14$). Rather, the locations to which the wood mice carried the discs, and the direction they took while moving them, depended solely on the current focus of their activity. In short, the mice transferred the discs to the sites at which they were temporarily active.

Testing the directionality of exploratory movements

During exploratory (> 10 cm) movements, both the angle between the mouse's trail and the disc, and the angle between the trail and the nest-box, were significantly different from random. However, the results of the Rayleigh test were markedly more significant for disc-egocentric-angle

($p < 0.0001$) than for the angle with the nest-box position ($p < 0.05$; see Tab. 1). Therefore, of the disc and the nest box, we deduce that, during exploration, the disc was the more important landmark. From Fig. 1 it is striking that movement with reference to the disc was non-random and directional, whilst the distribution of nest-box angles, although non-random, was non-directional. In short, while the mice operated in an area of temporary interest, they occasionally made exploratory movements and, when they did so, these were highly directional with respect to the discs – essentially leading the mouse back to the disc which served as a spatial focus of activity. The fact that the trajectories of exploratory movements were also related to the location of the nest box, although much less strongly than they were to the disc, may have arisen because of the general vector of the mouse's movements, which invariably involved forays starting from, and ending at, the nest box.

Analysis of behavioural sequences and the motivational affiliations of way-marking

From the videotapes, and using the behaviour patterns listed in Figure 2, we sampled the distributions of frequencies of transition between different behaviours during movements, and found no significant variations between male and female individuals (MANOVA, $p = 0.773$, $n = 20$; 18 behavioural elements). This lack of significant difference between the sexes applied when contact ($p = 0.322$) and non-contact ($p = 0.237$) behaviours were treated separately. However, during inter-sexual interactions, the male and female interacting subjects did differ significantly in the rates at which they groomed ($p = 0.016$) and sniffed ($p = 0.036$) each other (this asymmetry is in accord with our earlier work [16]). Frequencies of agonistic behaviour patterns were too low (for both sexes) for analysis in our model. The probabilities with which individuals of both sexes performed different behaviours are given in Fig. 2. Both sexes frequently moved discs, but there was no significant difference between the sexes in the frequency of this behaviour.

Analysis of stationarity using cumulative bout-length plots according to the procedures of ref. [15] revealed that while the mice were active only the following behaviour patterns were stationary (in the statistical sense of ref.[15]), and sufficiently abundant for further testing: self-grooming, immobile, sniff-ground, scanning (= scanning-while-walking), walking, scanning-while-immobile, rear-up. Analysis of the distributions of bout-lengths for each of these behaviours revealed that those that were largely performed when the wood mouse was immobile (i.e. self-grooming, immobile, sniff-ground) were exponential, in the terminology of Haccou & Meelis [15]. That is, these three elements were performed randomly in terms of their bout length distribution (Fisher's omnibus

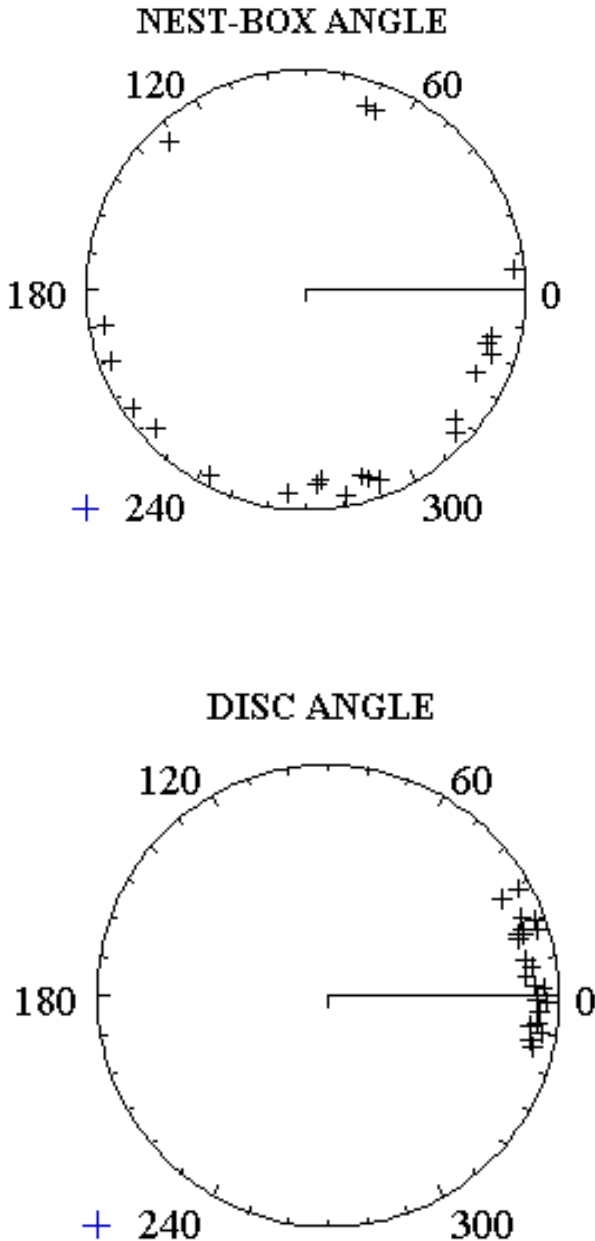


Figure 1
Graphical representation of directionality based on the 22 mean angle values of the walks across the arena with reference to either disk position (above) or the nest-box position (below). Above: 0 indicates position of the discs and data points represent the angle between actual movement of individual wood mice and the position of the disc. Below: 0 represents the position of the nest-box and data points represent the averages of movement in relation to the nest-box position.

for Kolmogorov-Smirnov tests: $p_{(\text{SELF-GROOMING})} = 0.75$; $p_{(\text{IMMOBILE})} = 0.5$; $p_{(\text{SNIFF-GROUND})} = 0.2$.

In contrast, the distributions of behaviour patterns that occurred during actual movements did not meet the foregoing [15] definition of exponentiality (and Shapiro-Wilk's tests confirmed that the deviations from exponentiality were not caused simply by each behaviour having a fixed minimum duration). The p-values indicating the deviations from exponentiality for behavioural elements scanning, walking, scanning-while-immobile, rear-up combined from 20 individual focal samples (10 males + 10 females) using Fisher's omnibus procedure are $p = 0.01$, $p = 0.0001$, $p = 0.001$, $p = 0.001$ respectively. Since the Chi-squared test for sequential dependency revealed significant p-values it can be concluded that there is evidence for the first-order sequential dependencies ($p < 0.01$). Furthermore, the individual Chi-square values for different behaviours revealed that these sequential dependencies were between triplets of behaviours associated with long distance movements (i.e. scanning, walking, scanning-while-immobile, rear-up). In contrast, the Chi-square values for triplets of behaviours including combinations of behaviours self-grooming, immobile, sniff-ground, did not reach the critical value of $p = 0.05$ and, therefore, could not be considered as sequentially linked with other activities. To illustrate the interpretation of these analyses, based on the observed probabilities of transitions of one behaviour to another, one example would be that, given that behaviour immobile has already started, the probability that behaviour self-grooming will be the next behaviour is approximately the same as the probability that self-grooming will not be the next behaviour. Since the independence of other processes was formally proved using tests for deviations from exponentiality and tests for sequentiality, this probability is not multinomial (i.e. it does not depend on the proportions of other states). Exponentiality here means that the bout length (e.g. of self-grooming) does not depend on the duration or presence of the previous act(s) and, therefore, relations between probabilities of this processes can be treated independently from other behaviours.

Furthermore, the analysis of bout-length distribution using Darling's test revealed that behaviours walking ($p > 0.01$) and scanning-while-walking ($p > 0.001$) deviated from exponentiality due to the presence of mixtures of exponentials. An analysis using log-survivorship plots revealed that the distributions of both these behavioural elements were nearly identical for c. 95 % of their distributions. The simplest model that encompasses these various probabilistic relationships based on the average values of transition rates between the elements of the wood mouse ethogram is that the mice switch intermittently between two states (behaviour patterns): walking

Table 1: Output of the Rayleigh analysis (i.e. Z and P values) of angles of trails with either the disc or the nest-box.

	N (ind.)	Mean - β	Z - values	P - values
disk	22	3.1	21.581	< 0.0001
nest-box	22	293.5	4.3166	< 0.05

Legend: N – number of individuals, mean - β – angle between the trail (i.e. where mouse moves) and disc position or nest-box position.

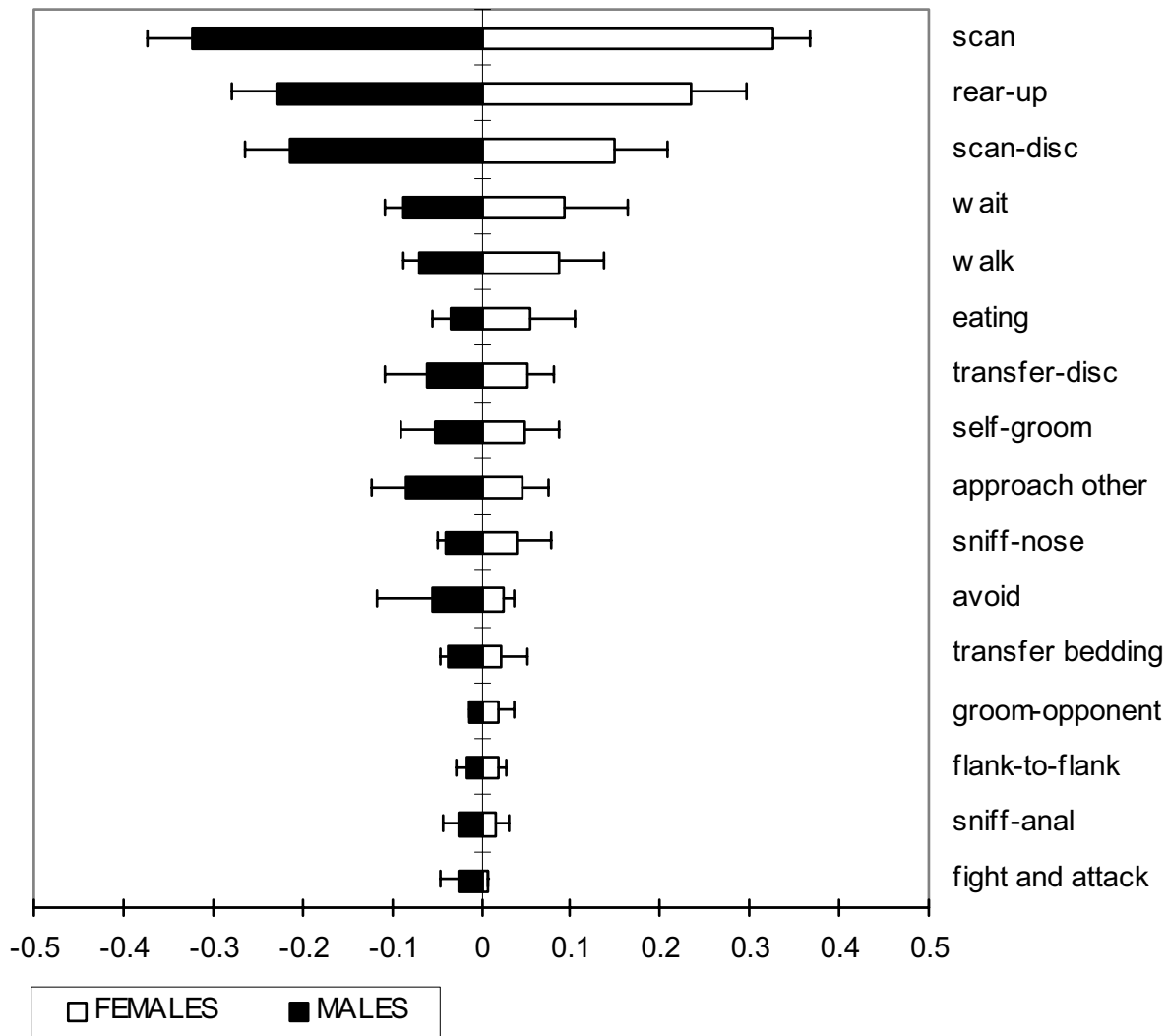


Figure 2
Average weighted probabilities of occurrence of different behaviour patterns

and scanning-while-walking, and that they also switch less frequently to a third state, the behaviour rear-up. Exploration by wood mice – considered here as the combination of behaviours associated with movements around the experimental arena of more than 10 cm – is therefore not comprised of an entirely random sequence of behaviour patterns. Although it may involve some elements that do occur at random, for the most part exploration by wood mice is, statistically, described by a two-state model where one mixed state 'scanning while immobile' frequently switches to another mixed state, 'scanning while walking'. The fact that these behaviour patterns are linked together statistically suggests that they are linked motivationally; since wood mice only moved discs during these exploratory movements, and since exploratory movements were oriented with respect to the current position of a disc, the function of the discs would strongly appear to be itself part of the same motivational constellation of behaviours that comprises exploration.

Discussion

In the field, our curiosity was aroused when we noticed accumulations of small objects – shells, leaves etc in the vicinity of wood mouse activity, and pilot studies in captivity revealed that these mice seemed eager to transfer plastic discs around their enclosures as they explored. Several hypotheses might explain this behaviour, but simply watching the mice caused us to suspect that they were using these objects as visual reference points to help them orient. This hypothesis was plausible insofar as, although they are macrosmatic [1] and nocturnal, wood mice have good vision [17]. Furthermore, considering the relatively large areas encompassed by their home ranges, the apparent recurring similarities in the landscape they inhabit, it seemed likely that wood mice would benefit if they could use some sort of 'book-mark' to help them quickly 'find their place'. In particular, these animals are often startled, briefly dash for cover, and then need quickly to get back to what they were doing before the 'false alarm'. To test quantitatively what seemed likely from watching the mice, we measured first the directions in which they carried experimental plastic discs, and then the directions and angles at which the mice themselves moved with respect to these discs. The results demonstrate that the wood mice transport the discs to locations in which they are currently interested and then orient their own exploratory movements to and from these discs. The behaviour of the wood mice involved short movements, meandering, sniffing, digging and probing, interspersed with occasional longer (>10 cm) more directed movements – which we defined as exploratory. It was these exploratory movements that were so clearly oriented with respect to the discs, and indeed when a disc was carried by a mouse this was invariably done during these exploratory movements. On this basis we deduced that the discs serve as way-

marks, providing a portable signpost that enables the mouse to orient its movements around places of ephemeral interest. A different, but loosely parallel function, may be served by the particular pattern of urine marking sometimes used by red foxes (*Vulpes vulpes*) and termed 'book-keeping' by Henry [18]. In this case the foxes are excavating small caches of food in very uniform leaf litter, and at risk of wasting time searching fruitlessly at the odour of a cache they have already emptied – in these circumstances they mark with urine on each cache site as they empty it, a behaviour thought to provide them with a signal, or *aide memoire*, that the site has been used and does not merit further exploration. The situation for the wood mice described here is different: they use a visual mark as a portable and ready-reference to focus their meanderings in an area of current interest – by analogy, this is precisely how a human might tackle the problem of searching efficiently in a homogeneous environment – for example by placing a cane (or series of canes) in the ground as a reference point from which to search for a set of keys dropped on a lawn. Interestingly, the wood mice used a visual way-mark and, so far as we know, not a specific scent mark [see, for example, [19]]. We suggest that the durable and diffusing nature of scent, added to the fact that it is detectable by predators and incurs production costs, may make it less suitable for this function. Indeed, the same way-marking function (but in that case helping them to avoid wasting time on duplicated searches of an area) might also benefit the aforementioned foxes without necessarily contradicting the book-keeping interpretation of their behaviour.

Having garnered experimental evidence that way-marking is a plausible functional explanation of the wood mouse's habit of transporting discs, we then sought further support for this interpretation by seeking to elucidate the motivational context in which this behaviour occurred. The study of mammalian movement using Random walk modelling [20] and random searching theories [21] has enjoyed a recent revival, with ingenious applications of theory to real situations [14]. Furthermore, mathematical techniques for elucidating motivational links through the probabilities of transition from one behaviour to another are now available [15]. The statistical intricacy of these techniques (described under Methods below) may seem impenetrable (although they are clearly explained by Haccou & Meelis [15], with a simple primer in [22]) but the principle is simple: the pattern of sequences and durations in which behaviour patterns occur can reveal linkages between them. In this case, we identified a particular family of linkages associated with directed movements of greater than 10 cm that we judged to be exploratory. This analysis revealed that when exploring the mice switched between spurts of movement, including scanning, and short pauses when they stood, still and erect, and scanned their surroundings. The facts that discs were transferred

only during movements of this sort, and that these movements were, at least sometimes, oriented very precisely around a disc, is further evidence that the discs function as part of the wood mouse's system of spatial orientation.

Conclusions

We conclude that the most likely explanation of our evidence is that wood mice use visually conspicuous objects (in this case, plastic discs) as way-marks that function in their spatial orientation. These way-marks serve as portable signposts. While investigating a place of current interest, the meanderings of the mice were intermittently interrupted by highly directed movements back towards the way-mark, as if along the spokes of a wheel with the way-mark at its hub. The directionality of the egocentric angles of the mouse's movement to the disc was evidence of navigation using visual objects to highlight areas of activity. In addition, if the mouse was disturbed, perhaps by a predator alarm, the way-mark enabled it to orient quickly back to the place where it had previously had a temporary focus of interest (for example for digging or collecting bedding). Furthermore, the invariable association of disc transferrals, and of movements towards a disc, with exploratory (>10 cm) movements confirmed the motivational context of way-marking behaviour within a linked set of exploratory behaviours. As far as we are aware, this is the first description of the use of portable way-marks by a non-human mammal.

Methods

Subjects

All experiments strictly followed the requirements for the use of animals in research and followed ethical guidelines [23]. We drew subjects from a total of 50 adult wood mice caught in Longworth live-traps in several rural localities near Oxford, UK. Males and females of similar weight (20 – 26 g) were selected for transport to the laboratory; the remaining animals were immediately released at their place of capture. Selected animals were released following each experiment (checks of body condition, injury and weight revealed that none was disadvantaged by captivity). All males were scrotal and all females were reproductive (according to vaginal smears with abundant cornified epithelia); no female was pregnant at the outset (as judged by the absence of vaginal plugs).

Each subject was individually marked with a black Nyanzol-D stain on its back to render it identifiable under video surveillance. Before the experiment, animals were individually caged with ad libitum food (grain and pellets) and water. The next day, four males and four females – each coming from different trapping sites – were placed in a wooden enclosure (2.2 × 2 × 1.8 m) in a separate room, illuminated by natural light from one window (the walls of the arena were sufficient tall that the window was

out of view and hence only incident light without a directional source illuminated the arena). The enclosure was a topographically simple environment, and contained one black plastic nest-box with two entrances in the shape of an equilateral triangle (base = 20 cm), which was partly hidden under the substrate (natural ground cover collected from sites in the vicinity of the trapping grids). Bedding material (hay) was scattered every day before 11:00 am (when no animal was active).

A total of three colonies, each of four male and four female adults, was observed for 15 days each. Ten plastic discs, each 5 cm in diameter and weighing 1.5 g, were placed randomly in the centre of the arena. Set of discs were replaced every fifth day (i.e. three times in each 15 days trial). At the time of disc-exchange, the substrate was raked and scattered to disperse or eradicate the signs of previous digging. Preliminary trials with two nest-boxes had revealed that, invariably, wood mice shared one nest-box, so just one nest-box was provided; this was the only conspicuous landmark within the topography of the arena. Special consideration was given to the presentation of food to minimise aggression and stress, and to minimise cues for spatial reference. Therefore, we sank a large open food-box (10 × 10 × 2.5 cm) below the level of substrate in the enclosure, so it was visible only at close quarters.

All observations were continuously recorded using a CCD video-camera (Sanyo, VCB 3312P) attached to a time-lapse video-recorder (Sanyo, TLS-924P) – a technique already proven valuable in the study of this species [24]. The enclosure was illuminated with a plain infra-red bulb producing light of wavelengths believed to be outside normal mammalian vision [25] so that the video could record throughout the hours of darkness (the light levels in the room, via moonlight from the un-curtained window approximated that outside). Observations were recorded at 12.5 frames per second and analysed at 6.25 frames per second. A single microphone (20–60 kHz) was attached next to the camera to monitor any noise that might influence the behaviour of the wood mice. Observers did not enter the room, other than to feed the animals or replace the discs as described.

Data analysis and experimental design

All individual behaviours were analysed from videotapes using Video Pro – analysis system v. 4.0 [26]. Elements of the wood mouse ethogram were available from our previous work [27] and other studies [28,29]

(i) Testing the directionality of disc transferral

Our approach was greatly influenced by the naturalistic observations made during pilot studies. These had suggested to us that wood mice were positioning visual markers so that they could function as way-marks. Our

hypothesis, therefore, was that the mice were carrying visual markers to a location at which they were currently exploring, and then using these marks as portable, temporary reference points. An alternative hypothesis was that the mice were moving the discs in directions determined by another factor – perhaps perceiving them as super-normal bedding stimuli and carrying them back to the nest-box, or perhaps using them as spatial reference points with respect to the location of a visual reference point.

Each of these possibilities leads to different predictions regarding the direction in, and angles at, which the mice carry the discs. If the mice were treating the discs as bedding, then the prevailing direction in which they were carried should have been towards the nest box. We designed the arena to have only one likely candidate reference point (the nest box), and measured the angle between the prevailing direction along which the mouse transferred the disc and the nest box. A systematic relationship between these angles and the position of the nest box would be predicted if the mice were positioning the discs with respect to this static visual reference point. On the contrary, if the mice moved the experimental discs to places where they had a current interest, and without reference to the nest box or any other fixed point, then the distribution of these angles would be random.

We measured these angles for all individuals that transferred a particular disc more than three times; this filter reduced our sample size to 14 individuals, but was necessary to obtain chains that were sufficiently long for Markov analysis and to increase statistical power while reducing the chance that the observed movements were random. Since we were interested in biologically relevant deviations from the random pattern with respect to the position of the nest-box, the only way to test this hypothesis was to analyse a series of angles of consecutive transferrals for each individual. For each of the 14 qualifying individuals we measured from 4 to 9 angles, and an average value per individual was used for calculating Rayleigh statistics [30].

(ii) Testing spatial referents of wood mouse movements

Preliminary analysis revealed that movements were bimodal, either short (less than 10 cm) or long (more than 10 cm) distance. Short distance movements were usually erratic and associated with local activity. Long-distance movements (classified in our ethogram as walk) were generally interspersed between two clearly identifiable behaviour patterns characteristic of the wood mouse's ethogram: scan and rear-up. Both these behaviour patterns appear likely candidates to function in spatial navigation. To discover how they were navigating, we tested two hypotheses. First, that the mice were positioning the

discs with respect to a landmark (the nest box), in which case we predicted that the mouse movements would be non-random and that there would be a systematic and directional relationship between the mouse's movements, the placement of the discs and the nest box. Second, that the mice were using the discs as local reference points, in which case we again predicted that the mouse movements would be non-random, but in this case systematically related to the position of the nearest disc. To distinguish these predictions, for each individual wood mouse we selected from the video record the first five long-distance (>10 cm) movements in the arena after emergence from the nest-box. The angles between the position of an individual before movement ($I_{X,Y}$) the position of the nearest disc ($D_{X,Y}$) and the position of next stop after walk ($S_{X,Y}$) were used in trigonometric calculation of egocentric-angles. The Rayleigh test [30] was employed to determine whether the sample of 22 mean directions (5 angles per individual; $N = 22$) differed significantly from random with respect to the mouse's trail (that is, the line between $I_{x,y}$ and $S_{x,y}$). Similarly the egocentric angle between the trail and nest-box was calculated for the same individuals and situations, to determine whether the position of the nest-box served as a point of reference.

Testing the motivational basis of way-marking

To discover the motivational affiliation of way-marking we used the methods described by Haccou & Meelis [15]. These involved analysis of the sequence of elements of the wood mouse's ethogram (e.g. walk, scan etc – as listed in Figure 2) and, deducing from the pattern of transitions from one behaviour pattern to another, the motivational links between them (an introduction to this complicated methodology is given in Macdonald et al. [22]). Analyses of these sequences of behaviour patterns, transcribed from the video tapes of mouse behaviour, enabled us to calculate the transition matrices, matrices of transition probabilities and transition rates described in [15]. Confirmation that the properties of the data were appropriate for this analysis necessitated, as a pre-requisite, several tests as outlined by Haccou & Meelis [15]. These involved (i) the inspection of each record for stationarity using cumulative bout-length plots to ensure that deviations from random alternatives to our hypotheses were not caused by lack of stationarity, (ii) use of the Kolmogorov-Smirnov test to test bout lengths of all behavioural elements for deviations from exponentiality, (iii) the simultaneous employing of Darling's test and Shapiro-Wilk's test to find out whether deviations from exponentiality were caused by convolutions of exponentials or by the presence of lags (caused, for example, by a minimum duration) [15].

On the basis of our preliminary results, we expected that some behavioural elements would deviate from exponentiality. Therefore, we followed the guidance of Haccou &

Meelis [15] and treated the sequence of behaviour patterns as a semi-Markov chain. The methodology then requires testing each record for sequential dependencies (using a Chi-squared test). This means that for each act A, we tested whether the preceding act X influenced the chance of transitions to the following act Y [15]. The result is a complete matrix of the probabilities per unit of time of switching from one behaviour (or 'state') to another behaviour (a different 'state'). These results are known as state-space models and can most easily be imagined as flow diagrams where the flux is defined on the basis of transition tendencies. Several behaviour patterns may be linked by a high likelihood of transitions between them and, intuitively, these linked constellations may have shared motivational bases. Statistically, and following [15], the overall results from testing deviations from a random pattern of transitions are presented using p-values obtained from combination procedures: when the values of test statistics are not distributed normally Fisher's omnibus procedure was used to combine the results from numerous observations, whilst the Sum test was used to combine values of test statistics with normal or asymptotically normal distribution (as, for example, those obtained using Darling's test).

Authors' contributions

This project grew, from a continuing collaboration with respect to the behaviour of wood mice, and its motivational bases. PS conducted the experiments and statistical analyses, and both authors participated in design, interpretation and writing.

Acknowledgements

We warmly acknowledge The Royal Society / NATO for awarding a fellowship to P. Stopka. We are also grateful to Paul Johnson for his statistical help and Ruth Feber, Tim Guilford and John Flowerdew for their helpful discussions. Additional support came from a grant from the Ministry of Education Youth and Sport of the Czech Republic (VZ 1131004) to PS and the People's Trust for Endangered Species to DWM.

References

- Etienne AS, Maurer R and Séguinot V **Path integration in mammals and its interaction with visual landmarks** *J Exp Biol* 1996, **199**:201-209
- Tew TE and Macdonald DW **Dynamics of space use and male vigour amongst wood mice, *Apodemus sylvaticus*, in the cereal ecosystem** *Behav Ecol Sociobiol* 1994, **34**:337-345
- Galea LAM, Kavaliere M and Ossenkopp KP **Sexually dimorphic spatial learning in meadow voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus*)** *J Exp Biol* 1996, **199**:195-200
- Cheng K **Rats use the geometry of surfaces for navigation** *In: Cognitive processes and spatial orientation in animal and man* (Edited by: Ellen P, Thinus-Blanc C) Dordrecht: Martinus Nijhoff Publishers 1987,
- Tew TE, Todd AI and Macdonald DW **Arable habitat use by wood mice (*Apodemus sylvaticus*)**. *Microhabitat J Zool Lond* 2000, **250**:305-311
- Tattersall FH, Macdonald DW, Hart BJ, Johnson P, Manley W and Feber R **Is habitat linearity important for small mammal communities on farmland?** *J Appl Ecol* 2002, **39**:643-652
- Tew TE and Macdonald DW **The effects of harvest on arable wood mice, *Apodemus sylvaticus*** *Biological Conservation* 1993, **65**:279-283
- Macdonald DW, Feber RE, Tattersall FH and Johnson PJ **Ecological experiments in farmland conservation** *In: The Ecological Consequences of Environmental Heterogeneity* (Edited by: Hutchings MJ, John EA, Stewart, AJA) *British Ecological Society Symposium*. Blackwell Scientific Publications 2000, 357-378
- Mather JG and Baker RR **A demonstration of navigation by small rodents using an orientation cage** *Nature* 1980, **284**:259-262
- JG Mather and Baker RR **Magnetic sense of direction in wood-mice for route-based navigation** *Nature* 1981, **291**:152-155
- Todd IA, Tew TE and Macdonald DW **Arable habitat use by wood mice (*Apodemus sylvaticus*)**. *Microhabitat J Zool Lond* 2000, **250**:299-303
- Jamon M **An analysis of trail following behaviour in the wood mouse, *Apodemus sylvaticus*** *Anim Behav* 1994, **47**:1127-1134
- Benhamou S **An analysis of movements of the Wood mouse *Apodemus sylvaticus* in its home range** *Behav Processes* 1990, **22**:235-250
- Blackwell PG **Random diffusion models for animal movement** *Ecol Model* 1997, **100**:87-102
- Haccou P and Meelis E **Analysis based on a (semi-)Markov description** *In: Statistical analysis of behavioural data (An approach based on time-structured models)* Oxford: Oxford University Press 1996, 256-304
- Stopka P and Macdonald DW **The market effect in the wood mouse *Apodemus sylvaticus*: selling information on reproductive status** *Ethology* 1999, **105**:969-982
- Szél A, Csorba G, Caffè AR, Szél G, Rohlich P and van Veen T **Different patterns of retinal cone topography in two genera of rodents, *Mus* and *Apodemus*** *Cell Tissue Research* 1994, **276**:143-150
- Henry JD **The use of urine marking in the scavenging behaviour of the red fox (*Vulpes vulpes*)** *Behaviour* 1977, **62**:82-105
- Brown RE and Macdonald DW **Social odours in mammals** Clarendon Press, Oxford, UK 1985,
- Bovet P and Benhamou S **Spatial analysis of movements using a correlated random walk model** *J Theor Biol* 1988, **131**:419-433
- Dusenbery DB **Ranging strategies** *J theor Biol* 1989, **136**:309-316
- Macdonald DW, Stewart PD, Stopka P and Yamaguchi N **Measuring the dynamics of mammalian societies: an ecologist's guide to ethological methods** *In Research techniques in Animal Ecology: controversies and consequences* (Edited by: Boitani L & Fuller TK) Columbia Press, New York 2000, 332-388
- Dawkins MS and Gosling M **Ethics in research on animal behaviour** *Animal Behaviour Monograph* 1992,
- Lambin X **Social relations in *Apodemus sylvaticus* as revealed by video observation in the wild** *J Zool Lond* 1988, **216**:587-593
- Lythgoe JN **The ecology of vision** Oxford: Clarendon Press 1979,
- Noldus Information Technology **The Observer, Support Package for Video Analysis. Reference Manual, Version 4.0 for Windows Edition** Noldus, Wageningen, The Netherlands 1997,
- Stopka P and Macdonald DW **Signal interchange during mating in the wood mouse (*Apodemus sylvaticus*): the concept of active and passive signalling** *Behaviour* 1998, **135**:231-249
- Flowerdew JR **Field and laboratory experiments on the social behaviour and population dynamics of the wood mouse (*Apodemus sylvaticus*)** *J Anim Ecol* 1974, **43**:499-511
- Montgomery WI **Intra- and interspecific interactions of *Apodemus sylvaticus* (L.) and *A. flavicollis* (Melchior) under laboratory conditions** *Anim Behav* 1978, **26**:1247-1254
- Lehner PN **Handbook of ethological methods** Cambridge: Cambridge University Press 1996,