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Short-distance homing in a small mammal: the role of exteroceptive cues and path integration

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Summary. When hoarding food under IR light, the golden hamster returns to its nest by path integration after an active outward journey, and it is capable of compensating the angular component of a passive outward journey independently of auditory, olfactory, tactile and geomagnetic cues. If, however, peripheral visual cues are available, they predominate over information which is gained during the active or passive outward journey. Further experiments show the limitations of homing by path integration, which is open to cumulative errors and therefore needs to be complemented by other categories of information.
Key words. Hamster, golden; *Mesocricetus auratus*; homing, short distance; exteroceptive cues; path integration.

The question to what extent spatial orientation can occur without being controlled by exteroceptive information has preoccupied comparative psychologists since Watson's pioneering work[2] and has, more recently, been reformulated from an ethological and biocybernetic point of view[3-5]. Experimental evidence that this may be the case in higher vertebrates has to be gained either through the attempt to eliminate all possible directional cues from the environment, or through the more satisfactory demonstration that such cues can, on logical grounds, not explain the observed behavior.

Within the limited space of an experimental arena, golden hamsters (*Mesocricetus auratus* W.) hoard food by carrying it directly to their peripherally located nest site. As shown by previous experiments[6], some animals maintain significantly orientated homing itineraries in conditions which simulate long distance homing by a passive outward journey combined with the simultaneous elimination of visual, auditory, olfactory and tactile cues. This unexpected result opened up the question as to whether the animals orientate menotactically with respect to a still unknown external frame of reference, or whether they home by path integration. In the second case, their behavior would, most probably, be controlled by vestibular information[7,8] and could therefore be interpreted as inertial navigation[9,10].

The subjects, mostly adult females, remain in their own arena (∅ = 2.20 m), which is located in a cellar with heavy walls and no windows, throughout the experimental period. They are provided with a nest box which is fixed to the outside of the arena's peripheral wall. 12 doors are set in the base of this wall at an angular distance of 30° from each other; all, except one, which gives access to the nest box, are permanently closed. In each trial, a pile of hazelnuts is placed at the centre of the arena; the subject, having filled its cheek-pouches, returns to the periphery of the arena in search of the nest entrance. The animals's return path is coded in terms of its location at the moment it crosses each of six concentric and equidistant circles, within any of the 12 sectors of 30°, which subdivide the arena's floor. The most peripheral of these circles is located at a distance of 15 cm from the sidewall of the arena and delimits a 'peripheral zone'. In trials with an active outward journey, the animal walks from its nest exit (which leads into the arena) to the centre of the arena, following a bait which the experimenter moves in front of its nose. In trials with a passive outward journey, the animal goes directly from its nest exit into a transportation box (16 × 18 × 25 cm), which is then moved to the centre of the arena. The animal is released from the box when the latter has been rotated in such a way that the subject enters the central zone of the arena with its head pointing either towards the nest entrance or ±90° or 180° away from it.

The following procedures were used to eliminate or mask directional cues from the environment. Visual cues: Unless mentioned differently, all trials take place under IR light (peak emission at 940 nm, bandwidth = 45 nm[11]). Auditory cues: 'pink noise' (bandwidth = 45–20,000 Hz) is emitted from four (mobile) loudspeakers; at the same time, the sound-reflecting properties of the experimental room are altered at regular intervals by the displacement of panels covered with vetroflex and strongly-absorbing acoustical foam along the walls. Olfactory and tactile cues (on the floor of the arena): at regular intervals, a thick substrate of sawdust which covers the arena's floor is stirred thoroughly and then flattened out again. All kinds of cues within the arena: In most experiments with a passive outward journey, the technical procedures involved in 50% of the trials included the rotation of the arena by ±90° or 180° after the animal had left the nest; during the rotation of the arena, the subject was kept in the transportation box which was lifted above the arena's floor. If the animal continues to return towards the usual, i.e. 0°, location of the nestbox in absolute space, it can be assumed that (rotated) arena-based cues play no role.

It is important to keep in mind that, with the exception of experiment 1, current tests of circular statistics[12] were applied to the population of itineraries which were performed by one individual during a given experimental series. The Rayleigh test was used to examine whether a given subject was significantly orientated (with respect to the 12 sectors of 30°) when it crossed the six concentric circles on the arena's floor during its return itineraries in a given experimental situation (see the six vectors on fig. 2, 3 and 4). For each experimental series, the average orientation of one subject during the control and the experimental trials was compared by means of the Wheeler and Watson test. In the experiments 3a, b and 4b, this test was applied to the angular deviation of the animal's homing itinerary with respect to the actual (and not to the usual) position of the nestbox during the animals's return to the periphery of the arena. In all other experiments, the angular deviation of the subject's homing itineraries was calculated with respect to the usual or 0° position of the nestbox (i.e. its position when the arena had not been rotated). The results of this test are not mentioned unless significant.

Experiment 1 was originally designed to answer our basic question as to whether the hamster orientates in a given direction by means of allothetic[4] information or whether it homes by path integration. In the first part of the experiment, each subject performed four control trials in its own familiar arena A with passive outward journey and the elimination of visual, auditory, olfactory and tactile cues. During the 5th, experimental trial, the animal entered the transportation box as usual from its nest exit, but was then translocated to the centre of an adjacent, unfamiliar arena B, the floor of which had been covered with sawdust from arena A and therefore had a familiar odour. It was expected that, if the animals exhibited any systematic directional trend at all, they would either 1) behave according to the principle of inertial navigation and thus return to their nest box by inverting their outgoing trip, or 2) orientate with respect to a general external frame of reference and therefore go in a constant direction in absolute space[6]. As indicated by figure 1, 10 out of 11 subjects behaved according to the 2nd alternative: In arena B, these animals orientated in the same rough compass direction as in arena A and, consequently, moved away from the actual location of the nest box. This result led to experiment 2, where we examined the possibility that our subjects use a general, biologically relevant compass system. It is important to mention, however, that the hamster's tendency to choose a constant direction in absolute space is, in our experimental conditions, compatible with a 2nd interpretation, which brings us back to the hypothesis of path integration and will be discussed below.

Experiment 2 examined the hypothesis that the animals use the earth's magnetic field (EMF) as a compass system[13]. In addition to the usual elimination of various exteroceptive cues, the total intensity of the EMF was therefore reduced by a factor of ~ 10 at the level of the arena's floor. Figure 2 shows the orientation of one subject which was tested under these conditions. Clearly, the animal's capacity to return to the usual location of its nest remains unaffected in a very much weakened EMF. Similar data was obtained with 13 other subjects which hoarded food in different types of artificial EMF[14] and were at the same time deprived of visual, auditory, olfactory and tactile cues. These results suggested either that our attempts to mask all directional information from outside the arena had failed, or that the animal did not use this type of information at all.

The next step was therefore to test in a more exclusive manner the relative role of exteroceptive cues and of path integration. In *experiment 3*, the animals were tested under IR light in conditions which implied a general conflict between all possible (nonvisual) cues from the outside of the arena on the one hand, and information based on path integration on the other hand. Figure 3 illustrates the homing performance of one animal which a) was induced to walk actively to the centre of the arena, or b) translocated passively to it. On each experimental trial the arena was rotated before the animal had left its nest to enter either the peripheral zone of the arena (condition a), or the transportation box (condition b). In both conditions, the animal heads back to the new position of the nest box, i.e. to the place from where it has started its (active or passive) outward journey. By way of control, the same animal was also tested according to our usual technique (condition c), namely by entering the transportation box at the very beginning of the experimental trials, before the rotation of the arena. As in our

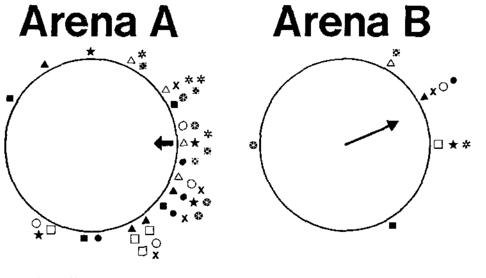

Figure 1. The directional choice within an unfamiliar arena. All trials involved the simultaneous elimination of visual, acoustical, olfactory and tactile cues. The circle on the left refers to four initial control trials, in which the animals were transferred from the nest exit (see arrow) to the center of their own arena A. The symbols indicate in which sector of 30° each subject entered the peripheral zone of arena A during its return to the nest in each control trial. The circle on the right refers to the experimental trial, in which the subjects were transferred from the nest exit leading into arena A to the center of an unfamiliar arena B. The symbols indicate in which sector of 30° each subject entered the peripheral zone of arena B. The vector represents the mean orientation of the 11 subjects upon their arrival at the peripheral zone ($p < 0.01$, Rayleigh test).

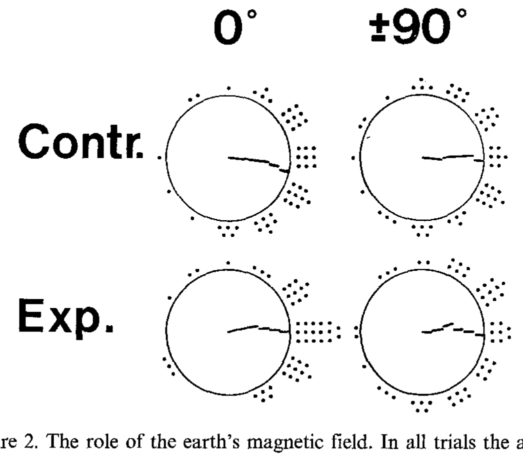

Figure 2. The role of the earth's magnetic field. In all trials the animal was transported to the center of the arena and tested under conditions of simultaneous elimination of visual, acoustical, olfactory and tactile cues. Upper circles: control trials in the natural EMF (total intensity: 0.45 G). Lower circles: experimental trials (total intensity at the center of the arena: 0.03 G). Circles on the left: the arena was not rotated (0°). Circles on the right: the arena was turned 90° in a clockwise or anticlockwise direction; the animal entered the transportation box before the arena was rotated. The six vectors ($p < 0.01$, Rayleigh test) within each circle represent the mean orientation of the subject when it crossed six concentric circles on the arena's floor. The dots around each circle show in which sector of 30° the subject entered, in each trial, the peripheral zone of the arena. The usual location of the nest box is always shown at the right.

previously mentioned results, the subject is not influenced in this case by the new position of the nest box, but returns to the usual location of its nest. The repetition of the experiments mentioned under a and b with seven subjects confirmed that in the above-mentioned test conditions, the animals return to the place in absolute space where they have started their outward journey. Thus, the hamster's return itineraries are controlled neither by cues from the more distant environment (see experiments 3 a and b), nor by arena-based information (see experiments 1, 2 and 3c), but by information which has been gained during the preceding outward journey. As expected on theoretical grounds (see below V) the homing performance is, in general, more accurate after an active outgoing trip than after a passive one.

Our experiments so far have all been carried out under IR light. A complementary line of research, in which we analyzed the specific role of external cues pertaining to particular sensory modalities, showed that visual cues from outside the arena play an important role and completely change the control of the animals' homing behavior: Whenever a subject gets lost and wanders around the arena under IR light, it immediately orientates in the direction of the usual position of the nest when the ordinary room lights are switched on. In *experiment 4* we assessed the relative role of visual cues and of path integration by repeating with six subjects experiment 3a under IR light (condition 1) and under ordinary room light (condition 2). Figure 4 illustrates through the behavior of one animal that the usual visual background strongly predominates over idiothetic[4] cues when both categories of information are presented in a conflicting manner consecutively to the rotation of the arena.

To summarize, our subjects return to the point of departure of their outward journey in the absence of visual cues, but orientate towards the usual position of their nestbox, i.e. in a specific direction in absolute space, if presented with visual cues from the more distant environment. Astonishingly enough, these results are valid irrespective of whether the outward journey is active or passive. However, a more detailed analysis of our results show that the relative importance and accuracy of path integration depends on the modality of the outgoing trip.

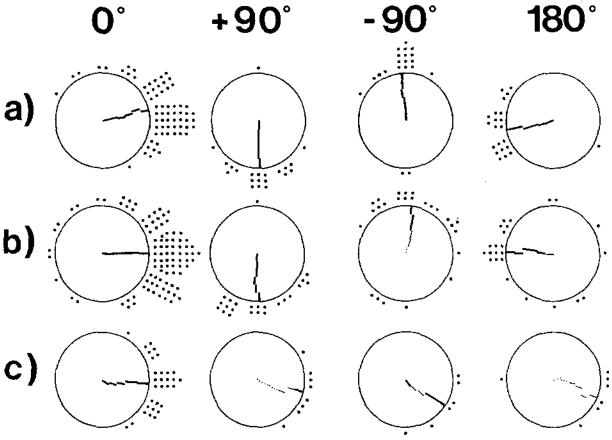

Figure 3. The influence of conflicting idiothetic and allothetic cues. All trials involved the simultaneous elimination of visual, olfactory and tactile cues. Circles a and b: during control trials, the arena was not rotated (0°), and the nestbox remained therefore in its usual position. During experimental trials, the arena was turned by ±90°, resp. 180°, before the animal had left the nest. a: active outward journey; b: passive outward journey. Circles c: same procedure as in b, except that the arena was turned after the animal had left the nest box. The six vectors within each circle are either nonsignificant (dotted lines), or significant at the level of 5% (solid lines) or 1% (heavy lines). For further explanations, see legend of figure 2.

Under *visible light*, idiothetic information is always overshadowed by the impact of visual cues; nevertheless, a secondary influence of path integration remains noticeable after an active outward journey, but not after a passive one (Teroni, unpubl. results). Under *IR light*, an active outward journey allows the animals to return to the point of departure of the complete hoarding trip from a feeding place located anywhere in the arena and therefore provides them with all the information necessary to home adequately (see below). After a passive outward journey, the hamsters' homing performance seemed to follow the same pattern in experiments 2 and 3, but not in experiment 1. It remains to be explained why, after their transfer to an unfamiliar arena, the majority of animals chose to go in the same direction in absolute space as they would have done in their own arena, instead of returning in the opposite direction by inverting their passive outward trip (see fig. 1).

Theoretically, homing by path integration involves double integrations, with respect to time, of the angular and the linear acceleration of the preceding outward journey; furthermore, this 2-fold computation must begin at the moment of departure, taking into account the initial location, orientation and velocity of the subject. The results of experiment 1 suggest that a passive outward journey leads to an incomplete path integration. During its transportation to the centre of the arena, the animal seems to integrate the angular, but not the linear component of the passive itinerary; the subsequent inversion of the angular component of the outgoing trip leads the animal in an apparently correct homing direction in its familiar arena, but also in the same direction in absolute space when placed in any other experimental arena, whatever the absolute point of departure of the attempted return path to the nest. This possibility of a partial path integration is in agreement with certain functional properties of the vestibular system. During its passive transportation to the food source the animal is informed, through the stimulation of the semicircular canals, of the angular component of the outward journey[10]. However, according to certain authors[15], the concomitant stimulation of the otoliths cannot be interpreted as translation in the horizontal dimension without additional, nonvestibular information. Recent experiments on the gerbil[16] as well as complementary controls on the golden hamster (unpubl. results) support this interpretation.

Further experiments 1) examined the limits of the hamster's capacity to assess the angular component of the outward journey and 2) dealt with the initialization of path integration at the beginning of a complete hoarding trip. 1a) Under IR light, our subjects maintain a correct homing direction after being

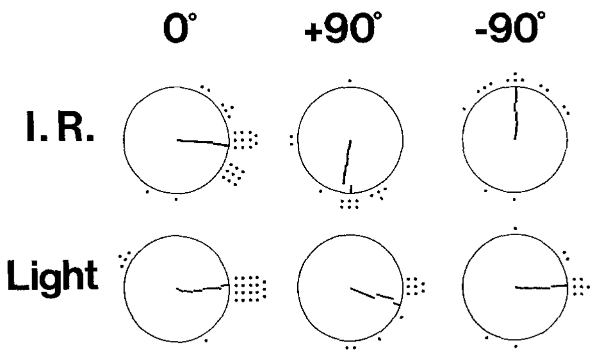

Figure 4. The role of path integration and of optical cues. All trials included an active outward journey and the elimination of olfactory and tactile cues. The animal was tested either under IR light (condition 1, upper circles), or under ordinary room light (condition 2, lower circles). In control trials, the arena remained in its usual position (0°); in experimental trials, it was rotated by ±90° before the animal had left the nest exit. For further explanations see figure 2.

induced to walk actively along a circular path of three full turns of 360° each before collecting food items, but fail to do so after active rotations of five full turns (n = 4). 1b) The hamsters compensate passive rotations incurred during the collection of food on a centrally located platform up to one or two full turns, but they are no longer able to do so when the rotations reach three turns (n = 3). Thus, both active and passive rotations are compensated up to different limits, the range of possible compensations being larger for active displacements, which involve not only vestibular, but all categories of idiothetic information. 2) Another series of experiments involved misleading the animals as to the time and place of departure. This was achieved by transferring them from the nest exit to the food source in a narrow transportation tube. Under these conditions, 10 subjects invariably returned to the arena's periphery by inverting the direction in which they had left the transportation tube at the food source by 180°, most probably because they made a confusion between the tube and the nest exit (a narrow channel in natural conditions).

These complementary findings confirm that under IR light the homing behaviour of our subjects is based exclusively on path integration. They also illustrate the cumulative effect of errors on the integration of path-dependent information as long there is no possibility of applying appropriate corrections provided by stable cues from the environment. Thus, the dusk and night active golden hamster may well navigate over limited distances by path integration, but uses peripheral visual information as soon as it becomes available.

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Intraocular 6-hydroxydopamine prevents the persistent estrus induced by continuous light

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Summary. Following the intraocular injection of 6-hydroxydopamine, which can destroy the retinal dopaminergic neurons, female rats showed a normal estrous cycle in LD 12:12 but not a persistent estrus in continuous light.
Key words. 6-Hydroxydopamine; intraocular administration; continuous light; persistent estrus; female rat.

In adult mammals, the retina is the only route for reception of light information[1,2]. Dopamine (DA) is the principal catecholamine in a specific subpopulation of amacrine cells localized within the innermost part of the inner nuclear layer[3]. Since there is no dopamine-containing axon in the optic nerves[4], all dopaminergic neurons are intraretinal. The DA-accumulating amacrine cells synapse with other amacrine cells which connect the bipolar and ganglion cells[5,6]; some light information may be mediated by DA acting as a retinal neurotransmitter[7].

In continuous light (LL), many circadian rhythms in nocturnal mammals initially show a free-running rhythm followed by a loss of apparent rhythmicity, a state known as 'periodicity fade-out'[8,9]. Since the rat's estrous cycle is based on the circadian rhythm[10], exposure to LL leads first to a free-running rhythm and then a fading out of the estrous cycle[9,11]. The latter condition is eventually characterized by persistent estrus.

Intraocularly administered 6-hydroxydopamine (6-OHDA) can destroy the retinal dopaminergic neurons; this can be confirmed by ultrastructural[12,13] and histofluorescence[13] methods. In this study, we used 6-OHDA to investigate whether the retinal dopaminergic neurons are involved in the induction of persistent estrus in LL.

Material and methods. All animals used were female Wistar strain rats provided with food and water ad libitum. 6-Hydroxydopamine (6-OHDA, Sigma) was dissolved in 0.9% saline containing 1 mg/ml of ascorbic acid. 6-OHDA was injected into the bilatral vitreous bodies under ether anesthesia with a Hamilton microsyringe (50 µg/10 µl). Some animals received 10 µl of saline containing 1 mg/ml of ascorbic acid under the same conditions. Vaginal smears were taken at random times (09.00–17.00 h) to avoid providing a nonphotic 24 h time signal. Persistent estrus was defined as the condition where vaginal cornification was found on more than 7 consecutive days.

Experiment 1. 30 rats, 3–4 months of age, were kept for 20 days in LD 12:12 (lights on at 06.00 h) and then exposed to LL (an average of 400 lux on the floor of their cages) for 20 days. The injection of 6-OHDA (n = 10) and saline (n = 8), respectively, were performed at the beginning of LL (09.00 h). Following the injection, vaginal smears were taken for 20 consecutive LL days. Intact rats (n = 12) were used as controls. For judging the photoreceptive ability of rats treated with 6-OHDA, the period of the free-running rhythm in LL (400 lux) was analyzed[8]. Five rats from each injected and control group