The fuzzy-boundary arena—A method for constraining an animal’s range in spatial experiments without using walls

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Abstract

A method is described for confining an animal within an experimenter-defined area without the use of physical boundaries. The area of exploration is constrained by the presence of an aversive noise, triggered whenever the animal steps across a computer-controlled boundary. The radius of the invisible boundary is constantly reset so that the boundary becomes “fuzzy” and the animal cannot use it as a spatial localizing cue. The effectiveness of this technique is demonstrated both with behavioural data confirming reliable confinement, and also recordings of hippocampal place cells made from rats exploring the arena. The place cell data reveal that indeed, the cells did not appear to be controlled by the fuzzy boundary, in contrast with the strong control normally exerted by fixed boundaries. This technique is thus promising for studies of spatial behaviour in which the strong influence of walls needs to be removed in order to allow the study of more subtle processes such as landmark use and path integration.

Keywords: Spatial cognition; Exploration; Foraging; Spatial reinforcement; Virtual cues; Place cells; Hippocampus; Rat

1. Introduction

The open field maze is one of the most widely used environments for investigating spatial behaviour in both rats and mice (Hall, 1936a,b; see Choleris et al., 2001; Walsh and Cummins, 1976 for reviews). Although the details can change, the maze is typically a bounded arena, often lacking any intra-maze landmarks or objects. Physical and practical limitations constrain the available area the animal can explore; there are only a handful of examples where this is larger than 1 m\textsuperscript{2} (Blanchard et al., 2001; Hafting et al., 2005; Zorner et al., 2003). Exploration is usually constrained by either raising the maze above floor level or by bounding it with walls.

Because physical boundaries such as walls and edges provide a strong spatial cue (and indeed, the geometry of an environment can be used by mammals to orient their exploratory behaviour (Cheng, 2005; Cheng and Newcombe, 2005), their presence limits the range of questions that can be asked about the influence on behaviour of other cues such as extended surfaces, landmarks, surface topography and path integration. This limitation is problematic given the current interest of spatial cognition researchers in the relative influence of boundaries vs. landmarks (Cheng and Gallistel, 2005; Graham et al., 2006) and path integration (Etienne and Jeffery, 2004). This report describes a method that allows an animal to be confined to a restricted area of the laboratory without the need for physical boundaries, allowing an uncontaminated investigation of the effects of other kinds of spatial cue.

The use of sound as a negative reinforcer in a place preference task has been reported previously to guide navigation to a focal location (Kentros et al., 2004). The success of this procedure encouraged us to consider the possibility that a similar technique might be used to keep an animal out of a given area and keep it confined to a region in the centre of a room. More importantly, we wanted to do this using a technique that would prevent the noise barrier from becoming, itself, a spatial cue, so that influences on spatial cognition could be assessed independently of the strong contribution that walls are known to make.

Here, we report the development of an experimental paradigm that allows computer-controlled constraint of the possible regions an animal can explore. The area of exploration is limited by the onset of an aversive noise triggered whenever the
animal steps across an imaginary line. A particular feature of this arrangement is that the spatial position of the barrier varies continually so that the animal cannot use it as a constant spatial cue. As we show, the method works well and allows animals to forage freely within the quiet zone, while strongly constraining their range. An example of how this method can be used is provided in the form of a single-unit recording study of place-responsive neurons in the hippocampus of freely behaving rats. The data suggest that, as intended, the variable ‘virtual’ boundary does not appear to act as a fixed spatial cue, thus making it useful for studying intra-maze cues in isolation.

2. The fuzzy-boundary arena

The configuration of the fuzzy-boundary arena is as follows. We define several regions within the arena (Fig. 1); the algorithm for activation/deactivation of the various sound zones is given in Fig. 2. The sizes of all regions can be defined experimentally: the ones used in the current experiment are given after the description of each zone.

1. A silent zone, within which the sound is never activated and the animals can forage undisturbed. The radius of this region is fixed by the experimenter. The radius of the silent zone in the current experiment was 75 cm.

2. An adjustment zone, a region outside the silent zone within which is located a variably positioned imaginary boundary (see below), the crossing of which will trigger a gentle (80 dB) white noise (on outbound trajectories) or switch it off (on inbound ones). This boundary is reset frequently so that any point within the adjustment zone will sometimes lie on the quiet side of the boundary and sometimes on the noisy side. Radius of adjustment zone: 50 cm.

3. At any given moment, there will be, somewhere within the adjustment zone, the boundary described above – called the
inner boundary – defining the point at which the gentle noise will switch on or off. The exact location of the inner boundary varies throughout a trial, being reset when the animal crosses a line called the boundary-adjust line (see below). Thus, the point at which the noise will come on is unpredictable to the animal. To increase this uncertainty even further (though this is probably not necessary), we can introduce a temporal delay of up to 250 ms between triggering of the noise and its actually coming on. Radius of the inner boundary: 100 ± 25 cm.

(4) The boundary-adjust line, the fixed imaginary line separating the silent zone from the adjustment zone. When the animal crosses this line on an outbound trajectory (towards the centre of the arena) this triggers a resetting of the inner boundary so that on the next outbound path, the noise will occur at a different radius. Crossing this line on an outbound trajectory has no effect, other than to carry the animal into the adjustment zone, where there is now some non-zero probability of gentle (80 dB) white noise occurring. Radius of boundary-adjust line: 75 cm.

(5) An outer boundary, separating the adjustment zone from the strong noise zone. This boundary is fixed at an experimenter-defined distance because in practice, animals so rarely cross the outer boundary that it does not seem necessary to introduce positional uncertainty. However, a positional and temporal delay could be introduced here, too, if desired. If animals do cross this boundary then a strong white noise, of ~95–100 dB, is triggered, being switched off if the animal crosses the boundary again on its return towards the centre. Radius of outer boundary: 125 cm.

3. Materials and methods

To test the effectiveness of the fuzzy noise boundary in constraining animals without acting as a fixed spatial cue, we first restrained animals within an enclosed square region of the environment in the centre of the room, and then removed walls to break open the square and allow the animal to escape into the surrounding area. Normally, animals would exhibit thigmotaxis and make their way to the walls of the room, where they would spend most of their time. As we show, however, with the noise boundary present the animals remained in the centre, well constrained by the imaginary boundary.

One purpose of this procedure was to monitor the activity of hippocampal place cells, whose spatially localized activity is determined strongly by local walls (O’Keefe and Burgess, 1996). For this reason, we did not use a no-noise comparison procedure to show the effectiveness of the fuzzy boundary, because we wished to prevent the animals from contacting the room’s walls at all. Instead, we show that there is a sudden transition in occupancy density between the silent zone and the gentle-noise zones, indicating the effectiveness of the fuzzy boundary in confining the animals. In addition, we report some of the place field data, to show that place fields did not form along the fuzzy boundary, and it thus does not seem to act like a real, physical barrier. Some of the place field data have been reported previously; see Barry et al. (2006). They are presented here for illustrative purposes, to support our contention that the fuzzy boundary was not a stable spatial cue.

3.1. Subjects

Data were collected from four adult male Lister hooded rats (250–400 g) obtained from the UCL’s Biological Services facility. Following surgery they were housed singly with restricted access to food and ad libitum access to water. All procedures were approved by the Home Office, subject to the restrictions and provisions contained in the Animals (Scientific Procedures) Act of 1986.

3.2. Experimental room

The main recording room was 6.3 m × 5.3 m with black floor-to-ceiling curtains attached to all walls such that the walls behind the curtains were not visible (see Fig. 1). The video camera used to capture the position of the rat was located in the centre of the ceiling. The loudspeaker was held in a pocket sewn into the curtains so that it was held between the curtain and the wall. The data acquisition system and computer were located in an adjacent room, so as to avoid providing a spatial cue to the animals.

The floor of the main recording room was lined with a set of black A1-sized sheets of polypropylene. In order to attenuate the influence of local cues the sheets were regularly wiped with ethanol and randomly shuffled between each trial. A weak directional cue was provided in the form of a low-powered 5 W bulb situated 1.5 m above ground level in the middle of the North wall.

3.3. Local environment

A box (referred to as the local environment) was located within the main recording room. This consisted of four transparent Perspex panels supported at their ends by four opaque polypropylene pillars, each 60 cm tall with a radius of 6 cm. The panels fitted into slots on the pillars, which were weighted to give the whole apparatus rigidity. Running along the length of each panel and circumference of each pillar (approximately 5 cm above the bottom edge) was a strip of “glow-in-the-dark” paint approximately 3 cm wide. This consisted of 40% (by volume) Ultra Blue “Glow-in-the-dark” phosphorescent powder (Glow Inc., MD, USA) that was mixed with Ronseal polyurethane matt finish paint (Ronseal, Sheffield, UK). Its purpose was to help the experimenter locate the box/components and also to provide a weak source of local illumination for the rat. The local environment was designed to facilitate easy assembly/disassembly so that any combination of walls/pillars could be used. When a wall was removed from the local environment the imaginary centre was used to line up the remaining components.

3.4. Recording procedure

Full details of the surgical procedure can be found in Hayman et al. (2003). All animals were surgically implanted with movable microelectrodes in order to record multiple neurons. The
animals were given at least 1 week to recover following surgery, following which screening began for place cells. Once place cells were identified, recording trials were run as follows.

At the start of each recording session the rat was brought into the main recording room in an opaque transport box. In near-darkness (to prevent the walls of the room being seen) the rat was removed from the box and attached to the headstage. It was then replaced in the transport box while any changes were made to the stimulus configuration, then removed from the box and placed in the local environment, aligned with and next to the North wall, facing East.

During the trial rice was dropped within and (if walls were removed) around, the local environment to encourage the rat to forage. When the trial had finished the rat was picked up from wherever it was in the room and placed back into the transport box while the next trial was set up. While the rat was in the transport box the experimental room was cleaned by wiping the floor tiles with ethanol and the polypropylene sheets were shuffled around. The walls and pillars of the local environment were also wiped with ethanol and randomized.

In the first wall removal manipulation only a single wall was removed; this was hidden behind the curtains lining the walls of the experimental room. In the second and subsequent wall removal manipulations a wall and the adjoining pillar was removed (also hidden behind the curtains). Following completion of a wall/pillar removal manipulation the next trial was begun. At the end of the recording session, when the rat was still in the transport box, the lights were turned off, the rat was removed from the transport box, unplugged from the headstage, placed back in the transport box and removed from the main recording room. When three or less walls were present the trial duration varied from 8 to 12 min; with all four walls present the trial duration was always 4 min. In the analysis of path data presented below only data from trials with three or less walls was included so the occupancy data was not biased towards the middle of the recording arena. The number of sessions recorded per day varied from four (three walls → two walls → one wall → single pillar) to eight (three walls → two walls → one wall → single pillar × 5). Data from 20 trials in total was collected over four sessions, one rat per session.

The position of the animal was monitored via a small infrared LED assembly on the headstage assembly that connected to the movable microelectrode device. The LED assembly was tracked by a video camera located in the ceiling at the centre point of the room. The position of the LED (roughly 2 cm above the rat’s head) was sampled at 50 Hz and captured and converted into two camera coordinates. Whenever the tracked LED’s exceeded the coordinate parameters of the fuzzy noise boundaries defined above, the relevant sound stimulus was triggered via the recording system (Dacq, Axona Ltd., St. Albans, UK).

Fig. 3. Paths from a single session for all types of wall removal trial. (A) Three walled trial with the East wall absent, (B) the East and South walls are removed, (C) the North wall remains and (D) the North-East pillar is left. The inner blue circle represents the minimum radius at which the mild white noise would sound (equivalent here to the boundary adjust line) and the outer red circle shows the outer boundary (beyond which strong white noise always sounds). Initiation of the mild white sound barrier was randomly variable between the two circles following the animas re-entry to the quiet zone (i.e., within the blue circle). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
3.5. Data analysis

Data was collected using Dacq (Axona Ltd., St. Albans, UK) and analyzed using Tint (Axona Ltd., St. Albans, UK) and Matlab (The Mathworks, Natick, MA, USA). In brief, the recording environment was divided into a 64 × 64 grid and the amount of time the animal spent in each bin of the grid was calculated and output to file. The occupancy maps for each trial \((n = 20)\) were summed together to provide a unified occupancy map and the recording area was divided into several concentric zones. For simplicity only three zones were used; a silent zone (where sound never occurred), a gentle zone (where there was some non-zero probability of mild white noise occurring) and a strong zone (where loud white noise always occurred). The occupancy in each zone was normalized by dividing the time spent in that zone by the area the zone circumscribed. These data were then entered into a one-way ANOVA.

4. Results

As soon as the first wall was removed from the local environment the rats had access to the rest of the experimental room. Following a wall removal, rats left the region bounded by the remaining walls and explored the area immediately outside the local box. The effect of the white noise boundary can be seen in Fig. 3. Upon encountering the “fuzzy” barrier rats rapidly returned to the vicinity of the local box in order to turn the white noise off. Visual inspection of the paths of the rats shows that the barrier was very effective in preventing them from coming into contact with the experimental room walls.

The summed occupancy map can be seen in Fig. 4; it can be seen that the vast majority of the animals’ time was spent in the silent area in the middle of the recording environment. This was further confirmed by comparing the amount of time spent in each of the three zones (silent, gentle and strong) using a one-way ANOVA \((F_{(2,57)} = 312.2, \ p < 0.0001;\) see Supplementary Figure 1 for the paths of all trials included in the analysis). Multiple comparisons revealed that the silent zone was significantly different from both the gentle and strong zones. The gentle and strong zones were not significantly different from one another.

Further confirmation that the boundary is indeed “fuzzy” is provided by the single-unit data. Place cells are known to respond strongly to extended surfaces (see below); as can be seen from Fig. 5, when the local environment consisted of all four walls place cells abutted and aligned the long axis of their firing field with one of the walls. When the walls of the local environment were sequentially removed the activity of the place cells became more dispersed, suggesting that the fixed walls acted as important spatial cues for these cells. Importantly, observation of place cell activity in the region of the fuzzy boundary showed that the place fields did not abut the boundary. This suggests that unlike the fixed walls, the fuzzy boundary did not serve as a reliable indicator of location.

5. Discussion

This article described a method that allows an animal to be constrained in its exploration of a large arena without the need for physical barriers such as walls. Using a variable “fuzzy” barrier, in which crossing of an imaginary, variable boundary triggered an aversive noise, the path of rats was reliably constrained within experimenter-defined limits. The possibility of the white noise barrier functioning as fixed spatial cue was foiled by the positional uncertainty introduced into the noise onset. Place cell recordings revealed that whereas the cells normally use fixed boundaries as a strong localizing cue (O’Keefe and Burgess, 1996; Barry et al., 2006), they did not seem to use the fuzzy boundary in this way. The implication is that, as intended, the fuzzy boundary did not act like a fixed boundary in the spatial representation. Thus, the fuzzy-boundary arena appears to be a useful method of isolating the effects of subtle spatial cues such as landmarks and path integration.

The place cell studies were conducted because single neuron recording can provide insights into the nature of a cognitive representation that cannot be obtained by simply observing behaviour. We found that in the presence of fixed boundaries the activity of place cells was well localized, whereas when these boundaries were removed the firing of the cells became more dispersed (Fig. 5). Observation of the activity of the cells at the edge of the arena was additionally revealing. Place cells respond strongly to the presence of extended surfaces (Hartley et al., 2000; Muller and Kubie, 1987; Rivard et al., 2004), with the long axis of the place field usually aligned to the barrier. If the fuzzy barrier acted as a real barrier in this regard, we should frequently have seen place fields apposed against the fuzzy boundary, but we almost never did. This suggests that the fuzzy sound barrier did not serve as a reliable indicator of location. A possible counter-argument is that perhaps the fields did not form against the barrier because it was too large. However,
Fig. 5. Effect of wall removal on four place cells. The activity of the cells is shown as a contour plot in a similar way as for the occupancy analysis in Fig. 4 (high activity = hot colors, low activity = cold colors). Manipulation to the local environment is shown at the top (four walls, three walls remaining, etc.). Cell number is shown on the left. Note that the activity of the cells is tightly localized when all four walls are present, and tends to be close to the walls. As walls are removed the firing becomes less well localized. Thus, walls appear to be strong spatial cues.

recordings in equally large spaces defined by hard boundaries do find place fields apposed to the walls (Hafting et al., 2005; A. Fenton, personal communication), so the size of the environment does not appear to be an issue. Rather, it seems more likely that we were successful in making the barrier hard to define in spatial co-ordinates.

Under what circumstances would such a fuzzy-boundary arena be useful? The most obvious application lies in spatial studies investigating the use of environmental cues such as landmarks, where it is desirable to test these cues in isolation (as in the landmark study of Collett et al., 1986). A problem with landmark studies is that there is a strong interaction with the boundaries of the environment, and in many cases, the boundaries seem to override the landmarks. In a classic study, Cheng (1986) found that arena geometry dominated over local cues in guiding navigation in a food localization task. Subsequently, Biegler and Morris (1993) found that landmarks could only be learned as predictors of food location if they remained stable within the frame of reference defined by the boundaries of the environment, and Hayward et al. (2003) found that the influence of landmarks was overridden by the shape of the enclosure in a watermaze task. Collectively, these studies suggest that the geometry of the arena boundaries provides a powerful spatial cue that may override the influence of other cues, making these hard to study.

One non-boundary spatial cue of particular interest is path integration, in which self-motion cues are used to update positional estimates in the absence of static sensory information (see Etienne and Jeffery, 2004 for review). Path integration is particularly sensitive to “resetting” by environmental cues. Because the fuzzy boundary in our experiment did not have a fixed location, it probably could not be used to reset the path integrator.
in a way that would allow a coherent updating of position; this is supported by our observation that place cells, which can use path integration as a localizing cue (Gothard et al., 1996), did not form stable fields in the arena (Barry et al., 2006). This would make our method a useful tool to study path integration in isolation. For example, it could be used to estimate the natural rate of drift of the path integrator (which is unknown at present, since exploring animals frequently come into contact with arena walls), and also the nature of the cues that can be used to reset it.

Of particular interest is the possibility that the fuzzy-boundary arena could be used to study the neurophysiological basis of path integration. Evidence suggests that one component of the path integrator may reside in the entorhinal cortex, in the form of the ‘grid cells’ that have recently been discovered there (Fyhn et al., 2004; Hafting et al., 2005). Grid cells are of enormous interest because their activity peaks arise in a regularly spaced array that may form the underlying metric of the spatial representation (Jeffery and Burgess, 2006). Understanding how the grids attain their particular spacing is a current high priority, but this enterprise is likely to be thwarted by the recent finding that boundaries play an important role in resetting grids (Barry et al., 2007) and thus contaminate any estimates of how intrinsic grid spacing is controlled. The fuzzy-boundary arena could be used, therefore, to study factors that control grid spacing independent of the environmental boundaries.

We have presented a new method of constraining an animal’s exploration without the use of fixed boundaries, so that spatial cognition may be studied in the absence of the strong effect known to be exerted by proximal boundaries. This method is successful in constraining the animals, as shown by our behavioral data, and also appears successful in preventing the arena boundary from acting as a spatial cue, as shown by our place cell recordings. We believe this method could be of great utility in studies of spatial cognition where the influence of fixed boundaries would confound the study of the cues of interest.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2007.08.014.

**References**
