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# 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 $m^2$ (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

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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.

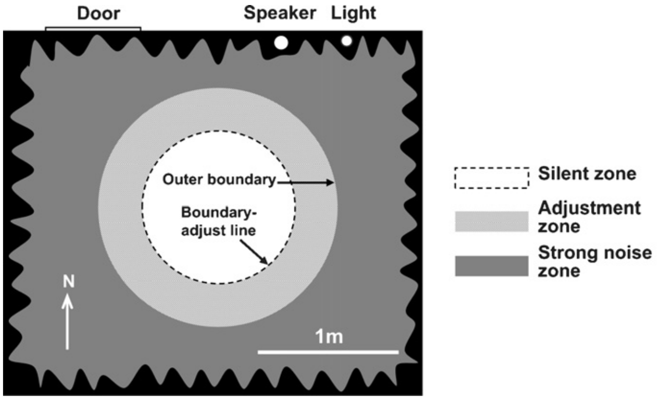

Fig. 1. Experimental room showing initial location of sound barriers. The local box was always located in the centre of the silent zone but is omitted for clarity. Outer, dark grey area shows strong white noise zone and inner, light grey circle shows gentle white noise zone. Wavy lines at edge depict black floor-to-ceiling curtains. Line at bottom right of figure shows scale of room.

(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

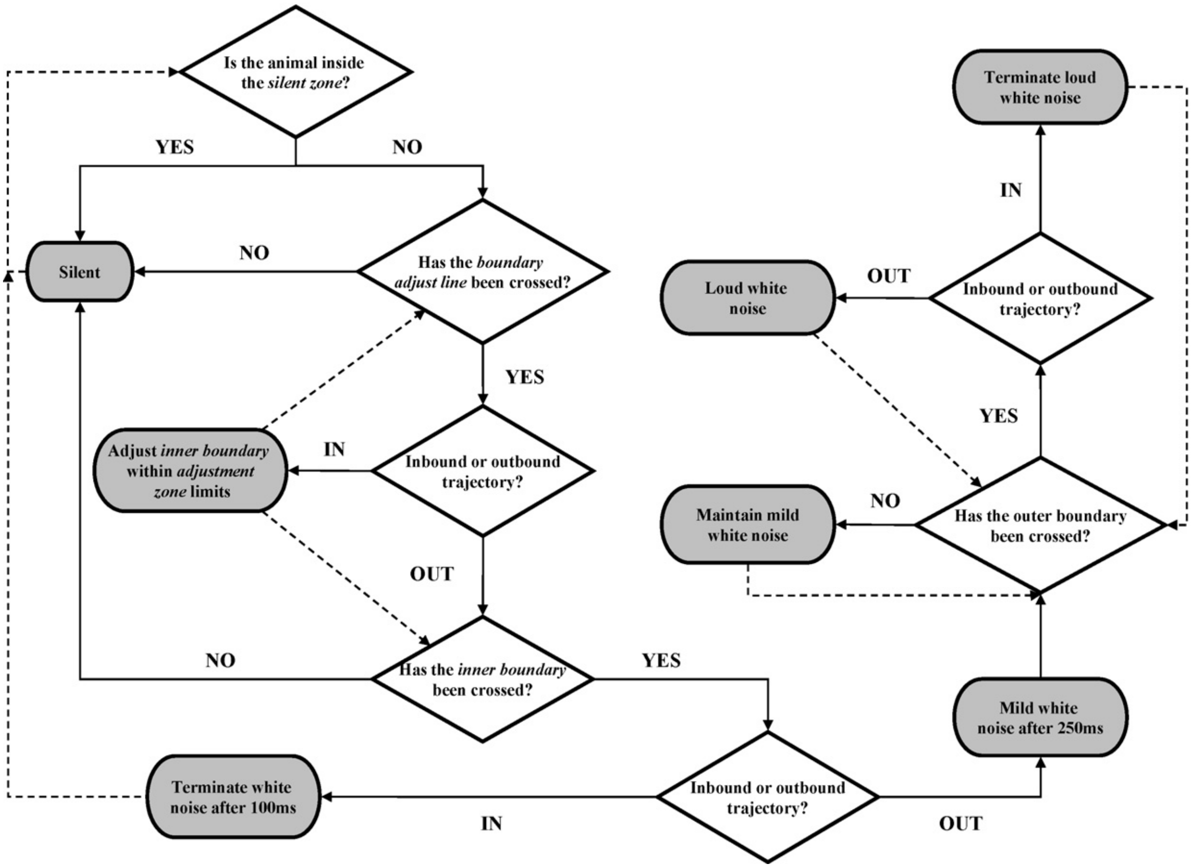

Fig. 2. Decision flowchart detailing the spatial reinforcement algorithm. Decision points are contained in diamonds and outcomes in colored, rounded boxes.