Differential activation of the rat hippocampus and perirhinal cortex by novel visual stimuli and a novel environment

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Received 18 March 1997; received in revised form 30 May 1997; accepted 9 June 1997

Abstract

Two groups of rats were shown individual novel visual objects. One group had been familiarised to the environmental context within which the objects were shown, the other experienced the situation for the first time. The activation of neurones in perirhinal cortex and the hippocampal formation was determined by counts of nuclei stained for products of the immediate early gene c-fos. The ratio of counts in the hippocampal formation to that in perirhinal cortex was compared for the two groups: the ratio was significantly higher (4.2:1) in the group experiencing the environment for the first time. Thus, whereas perirhinal neurones are activated by novel rather than familiar objects, hippocampal neurones are preferentially activated by a novel rather than a familiar environment.

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Keywords: Learning; Memory; Recognition; Priming; Visual; Immediate early genes; c-Fos; Amnesia; Rhinal cortex; Hippocampus; Rat

Research into the neural systems underpinning recognition memory has led to a vigorous debate concerning the contribution of the hippocampus compared to other temporal lobe regions. In particular, studies of neuronal responses [3,12,17,18] and of selective brain lesions [6,8,11,12,14] have suggested the need for a radical reappraisal of which regions are necessary for establishing whether or not visual stimuli have been encountered previously; such information is essential for recognition memory. Here we report differential activation of hippocampal neurones by novel stimuli and novel environments.

Studies of neuronal responses have indicated that many neurones in the perirhinal cortex of monkeys and rats respond more strongly when shown individual novel objects than they do when shown individual familiar objects [3,12,17,18]. Contrastingly, relatively few hippocampal neurones are more strongly responsive to novel than familiar objects [3,17,18]. However, hippocampal neurones have been found to be responsive to features of different spatial environments [5,7,9,15]. In agreement with such electrophysiological findings, lesions of perirhinal cortex produce major deficits in the performance of tasks that require the discrimination of the relative familiarity of stimuli, such as delayed non-matching to sample, whereas hippocampal lesions do not [1,6,8,11,12,14]. In contradistinction, lesions of the hippocampal formation cause problems with the performance of tasks requiring the use of spatial information [6,8,10,15,16].

It is possible to determine the activation of neurones by immunohistochemically staining for the products of immediate early genes such as c-fos [13]. Use of c-fos products as a marker for neuronal activity has an advantage over recording studies of rapidly allowing determination of the activation of whole populations of neurones [19]. It has an advantage over lesion studies of allowing regions of the brain involved in processing a particular type of information to be determined in the intact brain. Accordingly, determination of the activation of immediate early genes can provide a powerful new tool for investigating the neuroanatomical substrates of mnemonic processing. This technique has been used in the preliminary experiments reported below to determine the activation of neurones in perirhinal cortex and the hippocampal formation by novel stimuli or a novel environment.

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Individual rats (male, 180 g, pigmented, DA strain, Ban-tin and Kingman Ltd., UK) were exposed to 30 novel objects that the rats had never encountered previously. The objects differed from each other in shape, size and individual features. Methods followed those detailed by Zhu et al. [19]. Briefly, each rat was placed in a viewing box (45 × 30 × 35 cm) with opaque sides except for a perspex front wall. The rat was trained to hold its head in a hole in the perspex, in front of which was a half-silvered mirror. When lights were turned on in a box behind the mirror an object became visible to the rat; at other times the mirror reflected a black background. The object was illuminated only when the correct positioning of the rat’s head was detected by an infrared beam whose signal was fed to the computer controlling the experiment. After the object had been illuminated for 2 s a drop of juice was delivered through a tube accessible only when the rat's head was in position in the hole. The distance from the object to the rat’s eyes was 30 cm. For at least 3 h before and for 2 h after exposure to the novel objects the rat was kept in the dark in its home cage. Two hours after exposure to the objects the rat was deeply anaesthetised with chloral hydrate (0.6 g/kg), transcardially perfused, and the brain removed.

The brains of two groups of rats were histologically pro-cessed following the critical test during which each rat was successively shown the novel objects. One group of rats (n = 8) had been exposed to the apparatus on 11 previous occasions over 6 days, the last of these previous occasions being 3 h before the critical test. During these previous exposures to the apparatus this group of rats had seen other sets of objects, including other novel objects. The purpose of this procedure, to make one set of objects highly familiar to the rats, is incidental to the present report.) The other group of rats (n = 4) had not previously been exposed to the apparatus or shown objects. The showing of the 30 novel objects during the critical test took 5–6 min for each of the experienced rats and 10–15 min for each of the naive rats. Sections (25 μm) were taken including the hippocam-pal formation and perirhinal cortex in both hemispheres. The sections were immunohistochemically processed using the avidin–biotin complex immunoperoxidase method for visualising Fos [13], as detailed by Zhu et al. [19]. The primary antibody was a c-fos specific rabbit poly-clonal raised against the N-terminal region of rat c-fos peptide (from Dr D. Hancock, Biochemistry of the Cell Nucleus Laboratory, Imperial Cancer Research Institute, London, UK); the secondary antibody was goat anti-rabbit (Vectas-tain, Vector Laboratories). Counts of the number of stained nuclei within 1.02 × 0.74 mm rectangles (sampling frames) were made in both the hippocampal formation (including the dentate gyrus and subfields CA1 and CA3 of the hippo-campus) and perirhinal cortex on the same sections using an image analysing computer (Seescan plc, Cambridge) [19].

Counts of stained nuclei in perirhinal cortex were high (>100 per sampling frame) for both groups of rats exposed to novel objects: the mean number of stained nuclei was non-significantly lower for the rats experiencing the spatial environment for the first time than that for those rats experi-encing the situation for the twelfth time (see Fig. 1). In contrast, counts in the hippocampal formation were low (<20 per sampling frame) in all subfields in the group of rats that had been exposed to the situation previously, but were increased in the rats experiencing the apparatus for the first time. Indeed, the ratio of the number of stained nuclei in the hippocampal formation to the number in perirhinal cor-tex was significantly (independent t-test, t = 3.36, df = 10, P < 0.01) higher (by a factor of 4.2) in the rats for which the situation was novel than for those for which it was familiar (Fig. 1).

This relative increase in hippocampal counts may have
been caused by any of the factors that differed between the two groups, including the rats’ behaviour and emotional state. However, hippocampal counts were differentially increased relative to perirhinal counts so that the increase is not non-specific with respect to brain region. All the rats had been previously handled in their home cage and both groups of rats were shown 30 novel objects.

The findings show that relatively few neurones in the hippocampal formation were activated by the sight of individual novel objects in a familiar environment: more hippocampal neurones were activated when rats were shown the novel objects in a new spatial environment. The difference in hippocampal activation has thus to be ascribed to the novelty of the environment and the rat’s reaction to it. Further experiments are now required to determine the aspects of the novel environment or of the interaction between the novel environment and the novel objects that are responsible for the difference in hippocampal activation. In contrast, similar numbers of neurones were activated in perirhinal cortex whether novel objects were viewed in a novel or a familiar environment. Activation of perirhinal cortical neurones by novel objects shown in a familiar environment has been reported previously by Zhu et al. [19]. In that study [19] it was also shown that more perirhinal neurones were activated by novel than by familiar objects. These findings therefore add to those of electrophysiological and ablation experiments suggesting a double dissociation between the functions of the hippocampus and perirhinal cortex: the hippocampus being concerned with learning about spatial, configural or contextual relationships between stimuli, while perirhinal cortex is concerned with the relative familiarity of and how recent was occurrence of the novel environment.

We thank Dr D Hancock, Biochemistry of the Cell Nucleus Laboratory, Imperial Cancer Research Institute for supplying antibodies, Ms L. Ni and Mr A Griffiths for technical assistance, and the MRC and BBSRC for financial support.


