EFFECTS OF THE NOVELTY OR FAMILIARITY OF VISUAL STIMULI ON THE EXPRESSION OF THE IMMEDIATE EARLY GENE c-fos IN RAT BRAIN

X. O. ZHU,† M. W. BROWN,‡‡ B. J. McCABE‡ and J. P. AGGLETON§

†Department of Anatomy, University of Bristol, University Walk, Bristol BS8 1TD, U.K.
‡Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, U.K.
§Department of Psychology, University of Wales, Cardiff CF1 3YG, U.K.

Abstract—To investigate substrates of recognition memory, the cellular expression of Fos protein in rat brain has been studied after groups of rats were either shown sets of novel or highly familiar objects, or were exposed to the same pattern of illumination without objects being shown. Counts of stained nuclei were made in eight brain regions, where information about novel or familiar visual stimuli is likely to be processed or stored. The counts were relatively high in occipital visual association cortex and area TE of temporal cortex, intermediate in perirhinal cortex, entorhinal cortex, anterior cingulate cortex and the diagonal band of Broca, and low in the hippocampal formation and mediodorsal nucleus of the thalamus. The number of Fos-stained cells was significantly higher for the rats shown novel objects than for those shown familiar objects in perirhinal cortex, area TE, occipital cortex and anterior cingulate cortex.

Arguments are advanced that these differences in counts indicate areas involved in the processing and/or storage of information about the novelty or familiarity of visual stimuli, information important to recognition memory.

Key words: learning, memory, recognition, visual cortex, rhinal cortex, hippocampus, prefrontal cortex, thalamus.

Recognition memory requires judgments concerning the prior occurrence of stimuli. One basis for such judgments is the relative familiarity of stimuli: i.e. is a stimulus novel, having never been seen before, or is it familiar, possibly having been seen many times previously? Damage to the medial temporal lobe or medial diencephalon of humans results in an amnesia that includes impairment of recognition memory.11,21,30 However, there is currently an active debate concerning the relative contributions of different structures within these regions to the loss of recognition memory. It is now possible to study the involvement of different brain regions in recognition memory using a new technique based on the activation of immediate early genes (IEGs).

IEGs were identified as a result of research into signal transduction cascades. The IEGs are proto-oncogenes whose basal transcription is low but which can be induced rapidly and transiently by a variety of second messenger molecules. IEG expression is induced by cellular activation produced by, for example, neurotransmitters and neurotrophic growth factors. Their encoded proteins function as intermediary transcription factors that control the expression of late response genes.18,19 These protein products may be readily visualized, allowing detection of activated cells. By this means, mapping of IEG induction has been used, for example, to identify the locations of cells involved in neurophysiological responses to sensory and electrical stimulation.12,18

The present experiment is a first attempt to use the expression of one of these IEGs, c-fos, to investigate the involvement of cells in different brain regions in processes related to recognition memory. To this end we have measured the expression of c-fos-encoded protein induced by showing rats stimuli, complex three-dimensional objects, that varied in their relative familiarity. The areas chosen for study included those that either ablation or electrophysiological evidence suggested might be involved in recognition memory. Thus, the hippocampal formation (hippocampus plus dentate gyrus: HIPP) and entorhinal cortex (ENT) were included because of the long-held hypothesis that these are the most important structures for such memory.25 Adjacent cortical areas within the temporal lobe, the perirhinal cortex (PRH) and area TE (TE), were sampled because of evidence from recent recording and ablation experiments in monkeys and rats that these areas rather than the HIPP are those...
crucial for judgments concerning the prior occurrence of stimuli.\textsuperscript{6,9,13,14,19,23,37} Visual association areas found in the occipital cortex (OCCIP) were included both because of electrophysiological evidence for their involvement and because they provide afferents to the other regions.\textsuperscript{5,15} Within the diencephalon there is evidence from both recording and ablation studies of the importance of the mediodorsal nucleus (MD) of the thalamus.\textsuperscript{2,6} Additionally, ablation findings suggest a role for the medial prefrontal cortex (anterior cingulate gyrus: ACG) and there is recording evidence for the involvement of the diagonal band of Broca (DBB).\textsuperscript{13}

**Experimental Procedures**

**Subjects, apparatus and visual stimuli**

The subjects were 30 experimentally naive, male pigmented rats of the DA strain (Bantin and Kingman, Hull, U.K.), weighing 150 g at the beginning of the experiments. Animals were maintained on a 12:12 h light:dark cycle. Behavioural training and testing was conducted during the dark phase of the cycle. Rats were taken through the experiment in blocks of six, each block of rats being kept together in one cage. Data for the first three rats (one rat from each of the three groups) were rejected because of the faintness of the staining.

The behavioural apparatus comprised the following: rat box, object box, one-way mirror, lighting system and water delivery system. Rats were trained in the rat box (45 cm × 30 cm × 35 cm). The front wall of the rat box was made of perspex; the other sides and the floor were made of opaque plastic. An observing hole 3 cm in diameter was made 6 cm above the floor in the middle of the front wall. A water tube was located outside the hole and in front of a one-way mirror (20 × 18 cm). The mirror was placed 15 cm from the perspex at an angle of 60° from the horizontal plane. The mirror therefore reflected the black roof of the chamber that contained the apparatus. Behind this mirror was the object box into which objects were introduced one at a time. Each object was placed centrally in the box; about 30 cm from and 30° above the rat’s eyes when its muzzle was in the observing hole. Lights were installed in the box to illuminate the displayed object from each side, the light bulbs being positioned so that they could not be seen by the rat. The sides and back of the object box were covered with black cloth, so that the displayed object appeared against a black background. The illumination was 22 lux where the rat was and 88 lux in the area where the objects were displayed. An object became visible to the rat when, and only when, the lights were turned on behind the one-way mirror; in the case of human observers the sudden illumination caused visual fixation of the object.

Visual stimuli were 120 different three-dimensional objects (toys, junk items, etc.) varying in size, shape, colour and texture. The greatest dimension of each object lay between 3 and 15 cm. The objects were organized into four sets of 30. Each set was made up of objects of roughly the same range of sizes, colours, textures and variations in complexity. For each block of rats, one of the sets of objects was used as the “familiar set”; this was shown to the rats repeatedly during training. The three other sets were of novel objects, each set being shown only once. The orientation of each familiar stimulus was kept the same each time it was presented. One of these three sets of novel objects was used on the test day: this is termed the “novel set”. The particular sets of objects used as the novel and familiar sets on the training day were rotated across the blocks of rats.

**Behavioural procedure and analysis**

The behavioural procedure comprised three stages: pre-training, training and testing. During all this period water was made available to the rats for 20 min at the end of training each day. During the pretraining stage, each rat learned to poke its muzzle through the observing hole in the perspex and maintain that position for 3-4 s. When positioned in the hole, the rat’s muzzle interrupted an infra-red beam. Interruption of the beam was followed by a randomly variable 1-2 s delay, after which the object in the box was illuminated for 2 s. When the lights went out the rat was rewarded with a drop of diluted blackcurrant juice. A computer (Vigen 486DX2) was used to initiate the trial, detect the presence of the muzzle, and control the onset and offset of lights and juice delivery. The rat’s behaviour was video monitored and recorded for behavioural analysis.

After learning to keep its muzzle in the observing hole, each rat entered the training procedure. The training was carried out over five days. Objects were shown on three occasions each day: twice in the morning and once in the afternoon. One set of 30 objects was shown on each occasion. One object was presented on each trial. Each trial took 3-4 s; intertrial intervals were 4-5 s. There was no break between the two presentations in the morning. The interval between the end of the morning session and the start of the afternoon session was 3 h. On each day each rat was shown one set of objects, the familiar set (e.g. set A), either twice (days 2, 4 and 5) or three times (days 1 and 3). Thus, this set of objects (in this case set A) became very familiar to the rats. On days 2 and 5 (when rats saw the familiar set only twice), a set of novel objects was shown in the afternoon session. On the afternoon of day 4 rats were given 30 trials, during which the lights were illuminated for the same time as previously and with similar interstimulus and intertrial intervals, but without any objects being displayed (light-only condition). The purpose of introducing novel objects and light-only trials was to accustom the rats to these conditions.

In the morning of the test day (day 6), rats were shown the familiar set of objects twice, as on each of the previous training days. Three hours later, the block of six rats was randomly divided into three groups (two rats in each). Each group received a different treatment: two rats were shown the familiar set of objects, two were shown the novel set of objects (which had never been seen before), and two experienced 30 light-only trials without any objects being displayed. Rats were kept in the dark both before and after testing.

**Immunohistochemical procedure**

Two hours\textsuperscript{18} after the end of the test, each rat was anaesthetized deeply with chloral hydrate (60 mg/ml, 1 ml/100 g) and transcardially perfused with 50-100 ml 0.1 M phosphate buffer (PB; pH 7.4) and 250-300 ml fixative containing 4% paraformaldehyde in 0.1 M PB. The brain was removed and immersed in 0.1 M PB containing 4% paraformaldehyde for 12 h and then in 30% sucrose for 12 h for cryoprotection. The brain was frozen in dry ice and coronal sections (25 μm) were cut at −17 °C in a cryostat. Fos antisemur (kindly provided by Dr D. Hancock, Biochemistry of the Cell Nucleus Laboratory, Imperial Cancer Research Institute) was used to detect Fos protein. The avidin–biotin complex (ABC, Vector Laboratories) immunoperoxidase method was used to visualize Fos immunoreactivity. Briefly, the floating sections were washed in 0.1 M phosphate-buffered saline containing 0.2% X-100 (PBST; pH 7.4) and incubated with 0.3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. The sections were then washed and incubated at 4°C in Fos primary antiserum (diluted 1:2000 with PBST) for 48 h. The antiserum was rabbit polyclonal antibody directed against the N-terminal region of the rat c-fos peptide, and
has been tested as c-fos specific. Sections were washed in PBST and incubated in biotinylated goat anti-rabbit secondary antibody (Vectastain, diluted 1:200 with PBST) and 1.5% normal horse serum for 2 h. They were further washed and incubated in PBST containing avidin–biotinylated horseradish peroxidase complex (Vector Laboratories Elite Kit). After washes in PBST, the sections were rinsed again in 0.05 M Tris buffer (pH 7.4). The reaction was visualized in Tris buffer containing 0.025% diaminobenzidine and 0.1% hydrogen peroxide. The reaction was terminated by washing sections in cold phosphate-buffered saline. The sections were mounted on chrome-alum-coated slides, dehydrated through a graded series of alcohols and finally coverslipped for microscopic observation.

**Quantifying Fos-stained nuclei and statistical analysis**

All processing and immunohistochemical procedures were conducted without the group to which the brain belonged being known to the experimenter. One pair of sections at three different levels (approximately +0.5, −3.0 and −5.5 mm relative to bregma) was taken from each rat. The first pair of sections contained the ACG and DBB. The second pair contained the MD. The third pair contained the OCCIP, TE, PRH, ENT and HIPP. Divisions between brain regions followed Paxinos and Watson. Each of the ACG, DBB, MD, OCCIP, TE, ENT and HIPP two frames were sampled; in the PRH three frames were sampled. The size of a frame was 1.02 × 0.74 mm and the frames were positioned as shown in Fig. 1. For each region each frame was positioned and oriented in the same way in every section and then subjected to automated counting by an experimenter who did not know to which group the brain belonged. Thus, from each rat 34 frames were sampled from the eight brain regions. The counting of c-fos-stained nuclei was conducted using a Zeiss Universal microscope having a stabilized light source power supply. Images (512 × 512 pixels) were captured at 256 grey level resolution using a Solitaire 512 television-based image analysing computer (Seescan plc, Cambridge, U.K.). The computer divided transmitted light intensity by incident light intensity; the intensity of the resultant image was a function of optical density. Stained nuclei within the sampling frame were counted using the COUNT=DARK command (Seescan plc): the computer counted a nucleus if one or more of the pixels representing the nucleus was > 30 grey levels darker than the darkest pixel surrounding the image of the nucleus. This threshold corresponded to a difference in optical density > 0.1 unit. Since all sections in any one replication of the experiment were processed identically and simultaneously on the same slide, counts were not biased in favour of any experimental group. For statistical analysis, the data were logarithmically transformed to improve the normality of the distribution. For analysis of differences produced by the different stimulus types, the transformed data were then normalized to remove variability between blocks and between regions by dividing each number (transformed count) by the mean value for the corresponding region and block. The normalized data were subjected to an analysis of variance with the factors stimulus type, region, block and frame. All tests used a significance level of 0.05 and were two-tailed.

**RESULTS**

**Anatomical variations in counts**

The expression of Fos protein was quantified by counting the number of stained nuclei (see Fig. 2) in fixed areas (frames) in each sampled region. The counts varied greatly from region to region (analysis of log-transformed counts: \( F = 79.31; \) d.f. = 7,701; \( P < 0.001 \)). The density of Fos stained cells was high in the visual association areas (OCCIP (mean = 143 nuclei/frame) and TE (mean = 135 nuclei/frame), intermediate in the ACG (mean = 102 nuclei/frame), PRH (mean = 97 nuclei/frame), DBB (mean = 90 nuclei/frame) and ENT (mean = 63 nuclei/frame), and low in the HIPP (mean = 39 nuclei/frame) and MD (mean = 32 nuclei/frame).

There were also qualitative differences between the areas in the distribution of stained nuclei by cortical layer. In the OCCIP and TE, the density of stained nuclei was highest in layers III and IV, and lowest in layers I and V. In the PRH, nuclei were scattered throughout the cortex, without an obviously greater concentration in any particular layer. In the ENT, most of the stained nuclei were in layers II and III. In the ACG, the density of stained nuclei was greater in layers I-IV than in layers V and VI. Thus layer V had few stained nuclei in any of the cortical regions.
Fig. 2. Photomicrograph of nuclei stained for c-fos within the TE. Scale bar = 50 μm.

sampled; the other pyramidal layer, layer III, always had more.

Effect of stimulus type on counts

Different groups of rats were shown novel or familiar objects or were exposed to the same pattern of illumination without objects being shown. The number of Fos-stained nuclei varied between these groups shown different types of stimuli. An analysis of variance on the normalized counts revealed a significant interaction between the factors stimulus type and region (stimulus type × region: F = 3.12; d.f. = 14,701; P < 0.001). Thus, the effect on Fos expression of the type of stimuli seen by the rat was not the same in all the brain regions sampled (see Fig. 3). Separate analyses of variance were therefore performed on the data from each brain region.

A significant effect of stimulus type was found in the OCCIP, TE, PRH, ACG and MD. Further analysis revealed that in each of these regions, except the MD, the expression of Fos protein was significantly higher in the group shown novel objects than in that shown familiar objects (P < 0.01 in each case). In the MD the counts were significantly higher in the familiar than the light-only group, but the novel and familiar groups did not differ significantly (see Fig. 3).

The regions could be grouped into three sets by the pattern of Fos expression for the three stimulus types: (i) the frontal regions, the DBB and ACG; (ii) the posterior regions, the OCCIP, TE, PRH and ENT; and (iii) the MD and HIPP. In set (i), the frontal regions, Fos expression for the novel group was significantly greater than either the light-only group or the familiar group. In the MD and HIPP, set (iii), Fos expression was higher for the familiar than or the novel group, although the difference did not reach significance. These regional groupings were established by the following statistical analyses. Analyses of variance on the normalized counts revealed no significant interaction between the factors region and stimulus type for data from the ACG and DBB; or for data from the OCCIP, TE, PRH and ENT; or for data from the MD and HIPP: the absence of interaction indicated that the pattern of expression of Fos protein between the three stimulus types was similar for each of the regions within each of the three sets. However, there was a significant interaction between region and stimulus type when data from the frontal regions, set (i), were compared with those from the posterior regions, set (ii) (region × stimulus type: F = 1.88; d.f. = 10,540; P < 0.05), and when data from the posterior regions, set (ii), were compared with those from set (iii), the HIPP and MD (region × stimulus type: F = 4.38, d.f. = 10,539, P < 0.001), and when data from the frontal regions, set (i), were compared with those from the HIPP and MD (region × stimulus type: F = 3.44, d.f. = 6,323, P < 0.005). The interaction between sets (i) and (ii) arose because Fos expression was higher in the light-only group than in the familiar object group in the OCCIP, TE, PRH and ENT, but not in the DBB and ACG. The interactions involving set (iii), the HIPP and MD, arose because Fos expression was higher in the familiar than the novel group in these two regions, while the reverse was the case in the other areas.

Behaviour

No clear differences between the behaviour of the three groups were observed. Details of the rats' behaviours during the test session are given in Table 1. There were no significant differences between
the groups in the total time taken for the test session (though the total time taken for the test was shorter for the light-only group than for the other two groups), in the mean time the rats spent grooming or in locomotion during the session, or in the (small) number of trials for which there was a failure to maintain head position.

**DISCUSSION**

The results establish two main findings: (i) that presentation of complex visual stimuli produces considerable variation in the expression of Fos in different brain areas, and (ii) that this expression is strongly influenced by the relative familiarity of the presented visual stimuli. There are good grounds for believing that Fos expression is indicative of a change in neuronal activity. Hence the findings demonstrate that neurons within the rat brain discriminate the relative familiarity of complex objects.

**Differences in Fos expression for novel and familiar stimuli**

The relative familiarity of the stimuli affected the expression of Fos in the PRH, TE, OCCIP and ACG, with counts being significantly higher for the novel stimuli in each region. These results both confirm and extend the findings of electrophysiological experiments. They are consistent with work describing the presence of neurons signalling information about the prior occurrence of visual stimuli in the TE and PRH in the rat and monkey. The consistency extends

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**Table 1. Behavioural observations for rats shown the three types of stimuli**

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Novel</th>
<th>Familiar</th>
<th>Light only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total duration of test (min)</td>
<td>5.6</td>
<td>3.5-9</td>
<td>4.6</td>
</tr>
<tr>
<td>Head turning (no.)</td>
<td>5.1</td>
<td>3-8</td>
<td>3.0</td>
</tr>
<tr>
<td>Wandering off (no.)</td>
<td>3.6</td>
<td>1-5</td>
<td>2.3</td>
</tr>
<tr>
<td>Grooming (min)</td>
<td>0.22</td>
<td>0-1</td>
<td>0.38</td>
</tr>
</tbody>
</table>

None of the measures differed significantly between the groups.
to the direction of change. In the TE and PRH, Fos expression is consistently higher for novel than familiar objects. Correspondingly, in these regions most differentially responsive neurons respond more strongly to the first than to subsequent appearances of stimuli. Such differentially responsive neurons, with the majority responding more strongly to the first than to subsequent appearances of stimuli, have also been found in the OCCIP and ENT in the rat. Paralleling this, Fos expression was higher for the novel than the familiar group in both the OCCIP and ENT, although the difference did not reach significance in the ENT. In contrast, in the HIPP the majority of differentially responsive neurons in the rat respond more to familiar than to novel objects. Again, in accord with the electrophysiological findings, there was greater expression of Fos for familiar than for novel stimuli in the HIPP, although the difference did not quite reach significance. Neuronal responses to stimuli differing in familiarity have yet to be investigated in the ACG and DBB in the rat, though a small number of familiarity-related responses have been reported in the monkey DBB. The potential importance of such neuronal activity to recognition memory is demonstrated by the impairment in the performance of tasks requiring judgements concerning the prior occurrence of stimuli following lesions involving the PRH in the rat and monkey, and following lesions involving the ACG in the monkey.

The general concordance of the present findings with those of recording experiments provides a good reason for concluding that the observed differences in Fos expression are related to the processing of information concerning the prior occurrence of stimuli. One area of apparent inconsistency is that no difference in Fos staining between the novel and familiar groups was found in the MD. There is both ablation and electrophysiological evidence that the MD forms a part of the recognition memory system. However, the reported proportion of "recognition-related" neurons in the MD was low, as was the number of stained cells in the present experiment. Moreover, in the present experiment the rats did not need to alter their behaviour on the basis of the relative familiarity of the stimuli: it may be that such a behavioural contingency is necessary for the activation of a measurable sample of MD neurons.

The differences in Fos expression for the novel and familiar groups can only have arisen from the difference in the rats’ prior experience of the visual stimuli, i.e. they must have a mnemonic basis rather than being artefactual. The difference in Fos expression between stimulus types is not due to the particular stimuli used in the novel set because the same stimuli were used as the familiar set for other blocks of rats, the design being counterbalanced in this respect across blocks. Further, presentation of the different stimuli was the same for each set. Moreover, differences between the groups could not be due to changes in the motivational state of the rats produced by water deprivation or juice reward: these were equivalent in all rats. Indeed, neither training nor rewards were related to the novelty or familiarity of the objects. Thus, the differences in expression must be endogenous rather than induced by procedural learning. No olfactory cues were available for the rats to distinguish the sets: the stimuli were displayed in a separate compartment out of reach of the rats and behind a one-way mirror. Further, all tissue processing and measurements were performed "blind". Therefore, the differences in expression must have arisen from the difference in the relative familiarity of the stimuli and/or from the fact that the familiar but not the novel set had been seen in the recent past. Neurons sensitive to both types of information have been described in the rat.

The experiment does not establish that all the observed changes in Fos expression are direct rather than indirect consequences of the difference in stimulus familiarity. The differences in staining between the groups could arise indirectly from changes in the emotional or motivational state of the rat, or in its behaviour, consequent upon its detection of the difference in the relative familiarity of the stimuli. However, gross changes in motor behaviour cannot readily explain the differences in expression: video monitoring indicated that head position and drinking behaviour did not differ between the three groups. Moreover, measures of grooming, locomotor behaviour and the total time needed for the test did not differ significantly between the groups. Each object was displayed at the centre of the object box and at the same distance from the rat’s eyes when its head was in the viewing position—conditions designed to minimize differences in eye movements from trial to trial. Other possible sources of difference between the groups arise from alterations in either emotional or motivational states or in alertness or attention produced by exposure to the different stimulus conditions. The rats had experienced all test conditions before the test day to minimize differential reactions to seeing the different types of stimuli (or no stimuli at all). Never the less, it remains possible that, for example, the general level of arousal or attentiveness may have been higher for novel than for familiar stimuli. However, the differences in Fos expression between the groups cannot be attributed to nonspecific results of such differences in arousal or attentiveness, since the relative levels of Fos expression in the different groups were not the same in all brain regions. Thus, although indirect effects cannot be excluded, any such effects produced neither observable changes in the rats’ behaviour nor global changes in expression within the brain.

The results do not establish the order in which the different regions process signals concerning the relative familiarity of stimuli. Thus, in particular, it is not known whether changes in the TE or OCCIP (visual association cortex) are dependent
on signals fed back from the PRH, or whether changes in the PRH are dependent on signals fed forward from the TE or OCCIP. Changes dependent on judgment of the prior occurrence of stimuli require their identification (i.e. at least their discrimination at a neuronal level): given the complexity of the stimuli used in the experiment, it is unlikely that the necessary discrimination between stimuli is achieved at early stages of the visual pathway. Accordingly, the changes in the OCCIP may be dependent on changes in the TE, rather than vice versa. In support of this view, the earliest discovered differences in neuronal activity between first and repeat presentations of stimuli (i.e. the shortest differential latencies) were in the TE. 36

**Regional differences in Fos expression**

Irrespective of the type of stimuli seen by a rat, there were regional differences in the total counts of stained nuclei and also in the layers in which these nuclei were found. Relatively high nuclear counts were found in the OCCIP and TE, with lower levels in the PRH, ENT, ACG and DBB; only a small proportion of neurons in the HIPP and MD were stained. High proportions of visually responsive neurons are found in the OCCIP, TE and PRH when rats are shown objects. 37 Thus it is plausible that much of the measured staining in these areas was due to the visual stimulation given. Additional support for this conclusion is provided by the differential staining according to the types of visual stimuli presented. Moreover, on the test day, procedures were designed to minimize the activation of neurons by non-visual stimuli. The rats were kept in the dark in a quiet room, undisturbed except when they were removed to be shown objects, or to be anaesthetized and perfused. Even so, the probability remains that some neurons will have been activated by non-visual stimuli (for example by the rats' general behaviours, such as placing its muzzle in the viewing position or drinking).

In recording experiments many visually responsive neurons are also encountered in the HIPP, where very few cells stained for Fos. 37 Given the close anatomical connections of the hippocampal formation with other, more strongly staining regions, 25,34 the small number of hippocampal neurons expressing Fos requires explanation. One possibility is that hippocampal neurons are activated, but that they do not express Fos detectable by the antibodies used in these experiments. However, while low levels of Fos staining in the HIPP have been reported previously, 27,35 in other experiments Fos has been detected in rat hippocampal neurons following long-term potentiation 5 and in mice after performance of a spatial discrimination task. 10 Thus, a second possibility is that hippocampal neurons are little activated by the discrimination required in the present experiment. In fact, a low level of activation would be in agreement with electrophysiological and behavioural evidence suggesting a relatively minor role for the HIPP in discriminating the familiarity or recency of occurrence of objects. 14,20,23 This second possibility requires that the differential signals from the PRH and ENT do not gain access to the HIPP, even though there are stained cells in ENT layer II, a layer containing neurons that project to the dentate gyrus. 25,31

**Fos expression in the control group**

There was a relatively high level of Fos expression in the light-only group. Some of the Fos expression may have been due to cells activated other than by visual stimulation while the lights were on (e.g. those activated by the rats’ general behaviour in the task). Nevertheless, activation of visual cortical neurons might be expected in the light-only condition. The stimulus was not a brief, diffuse flash, but the illumination of the object box without the presence of an object: although the box was painted black, its internal corners and edges were readily visible. The staining in the light-only group relative to that for the group shown familiar objects varied between regions. In two regions, the TE and PRH, Fos expression was significantly higher for the light-only group than for the familiar object group. Thus, in these two areas more neurons were activated in the absence of objects than when familiar objects were shown. As the effect was not seen in all areas, it is not readily explicable by differences in non-specific factors such as the general level of arousal of the rats in the two groups. There are at least two more specific explanations possible for the difference in staining. Firstly, rats may have moved their eyes more searching for an object when there was no object present (in the light-only condition) than when there was a familiar object on which to focus. In monkeys there is evidence for increased activity of neurons in relation to saccadic eye movements in these regions. 24 Such an explanation does not readily account for the difference in Fos expression for novel and familiar objects in these areas. The rat’s gaze might be expected to be even less likely to wander from the object when it is novel than when it is familiar: by this argument activation should be even less for the novel object—the reverse is true. Secondly, changes in visual selective attention may account for the difference. Responses to non-attended stimuli are reduced in the TE in monkeys when their attention is focused on a stimulus. 16 If the same effect occurs in rats, then neurons are likely to be more easily activated by background features in the light-only condition than when an attended object is present. (In comparison to the familiar or novel conditions, the effect will be offset by the number of neurons activated by the objects themselves.) Again, although this effect may explain the higher level of expression in the light-only than the familiar group in the TE and PRH, it cannot account for the difference in activation between the novel and familiar group because it would predict the reverse result (greater activation for the familiar than
the novel objects) to that observed (greater activation for the novel than the familiar objects).

**CONCLUSIONS**

In summary, expression of Fos protein indicates that many neurons in the ENTR, PRH, TE, OCCIP, ACG and DBB are activated when rats are shown complex objects under the conditions of the present experiment. Within the PRH, TE, OCCIP and ACG, the expression of Fos protein is consistently higher when an object is novel than when it is familiar to the rat, i.e. these regions demonstrate differential activation of cells on the basis of stimulus familiarity. The results provide further evidence that neurons within these regions are involved in processing information of potential importance to recognition memory.

**Acknowledgements**—We are grateful to Dr D. Hancock, Biochemistry of the Cell Nucleus Laboratory, Imperial Cancer Research Institute, for supplying antibodies, to Dr S. Tolchard for advice on immunohistochemistry, to Ms A. Goodall and Mr A. Griffith for technical assistance, and to the MRC and BBSRC for financial support.

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(Accepted 11 July 1995)