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CHANGES IN IMMEDIATE EARLY GENE EXPRESSION IN THE RAT BRAIN AFTER UNILATERAL LESIONS OF THE HIPPOCAMPUS

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Abstract—Activity of the immediate early genes c-*fos* and *zif*268 was compared across hemispheres in rats with unilateral, excitotoxic lesions of the hippocampus (dentate gyrus and CA fields 1–4). Counts of the protein products of these genes were made shortly after rats performed a test of spatial working memory in the radial-arm maze, a task that is sensitive to bilateral lesions of the hippocampus. Unilateral hippocampal lesions produced evidence of widespread hypoactivity. Significant reductions in immediate early gene counts were observed within all three anterior thalamic nuclei, as well as the entorhinal, perirhinal, and postrhinal cortices, and much of the subicular complex. In contrast, no observable changes were detected in the anterior cingulate, infralimbic or prelimbic cortices, as well as several amygdala nuclei, even though many of these regions receive projections from the subiculum. Instead, the immediate early gene changes were closely linked to sites that are thought to be required for successful task performance, with both immediate early genes giving similar patterns of results. The findings support the notion that the anterior thalamic nuclei, hippocampus, and parahippocampal cortices form the key components of an interdependent neuronal network involved in spatial mnemonic processing. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: hippocampus, Fos, *zif*268, spatial memory, parahippocampal region, anterior thalamus.

Both anatomical and neuropsychological findings point to the functional importance of the hippocampal connections with the medial diencephalon (Delay and Brion, 1969; Gaffan, 1992a; Aggleton and Brown, 1999). Efferents from the subiculum convey hippocampal information to the medial diencephalon, principally via the fornix (Meibach and Siegel, 1977; Aggleton et al., 1986; Amaral and Witter, 1995). These efferents terminate in the mammillary bodies, anterior thalamic nuclei and rostral midline thalamic nuclei, and these same thalamic nuclei may reciprocally influence the hippocampal formation. There is growing evidence that hippocampal–diencephalic interactions are critical for episodic memory in humans and spatial memory in animals (Barbizet, 1963; Gaffan, 1992b; Aggleton and Saunders, 1997; Parker and Gaffan, 1997; Warburton et al., 2000, 2001). Much of this evidence has come from measuring the cognitive and behavioral consequences of damage to particular components of this circuit. The present study forms part of a series of experiments that has mapped the more global consequences of disruption to this circuit by measuring immediate early gene (IEG) expression in rats. Previous studies have reported the effects of lesions in the fornix (Vann et al., 2000c) and anterior thalamic nuclei (Jenkins et al., 2002a,b, 2004), while the current study describes the consequences of hippocampal lesions on IEG expression in other brain areas. By this means it is possible to appreciate the wider impact of these lesions, not only on other parts of the hippocampal–diencephalic circuit but also in other regions implicated in learning and memory.

Immunohistochemical methods were used to measure the activity of two IEGs, c-*fos* and *zif*268. The IEG, c-*fos*, is widely spread throughout the brain and has been used as a marker of neuronal activation (Dragunow and Faull, 1989). There is, in addition, evidence that the c-*fos* gene may have a more specific contribution to learning processes (Herdegen and Leah, 1998; Tischmeyer and Grimm, 1999). Performing the radial-arm maze task leads to increased Fos, the protein product of c-*fos*, in a network of interlinked sites including the anterior thalamic nuclei, hippocampus, subicular complex and retrosplenial cortices (Vann et al., 2000a,b; He et al., 2002). It has also been found that blocking c-*fos* expression in the dorsal hippocampus impairs spatial memory formation in the radial-arm maze (He et al., 2002), so providing more direct evidence for the importance of this IEG. *zif*268 complements c-*fos* in that it is also widely expressed in the brain and, like c-*fos*, has repeatedly been linked to learning and memory (Okuno and Miyashita, 1996; Guzowski et al., 2001; Hall et al., 2001; Jones et al., 2001; Bozon et al., 2002; Davis et al., 2003). While the time course of *zif*268 expression overlaps with c-*fos* (Zangenehpour and Chaudhuri, 2002), these two IEGs are differentially expressed and so do not provide redundant information (Wisden et al., 1990).

In the present study, rats with unilateral hippocampal lesions were trained on a spatial working memory task in the radial-arm maze and Fos levels compared across hemispheres. The advantage of studying unilateral hippocampal lesions is that the rats can still perform the task

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Abbreviations: AD, anterodorsal nucleus; AIp, insular cortex; AM, anteromedial nucleus; AUDp, primary auditory area; AV, anteroventral nucleus; BL, basolateral nucleus; IEG, immediate early gene; La, lateral nucleus; MD, mediodorsal thalamic nucleus; PBS, phosphate-buffered saline; PBST, 0.1 M phosphate-buffered saline containing 0.2% Triton X-100; VISp, primary visual area.

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to high levels of accuracy (Warburton et al., 2001) and within-subject comparisons provide the closest behavioral control. These considerations are relevant as rats with bilateral hippocampal lesions are severely impaired on the radial-arm maze task (Jarrard, 1978; Olton et al., 1978), and so it would not be possible to match their abnormal arm choices with those of a control group also performing the radial arm maze task. It is, however, the case that the intact hemisphere cannot be regarded as normal as it will have lost commissural inputs from the contralateral hippocampus. An additional, smaller group of rats received the same surgical procedure but IEG levels were measured in rats taken straight from the home-cage. Comparisons between the home-cage and radial-arm maze groups made it possible to determine whether the experimental condition had raised IEG levels, as expected (Vann et al., 2000a,b). A raise in IEG levels would help to rule out floor effects as a possible cause of any apparent failures of the hippocampal lesions to alter IEG levels.

EXPERIMENTAL PROCEDURES

Subjects

The main experiment involved 11 male hooded Lister rats (Harlan, Oxon, UK) weighing from 281 g to 342 g. A further four hooded Lister rats (281–334 g) were used to examine baseline levels of Fos and *zif*268 ('home-cage controls'). Approximately 14 days after surgery animals were food deprived to 85% of their free-feeding body weight and maintained at this level throughout the experiment. Water was available *ad libitum*. Animals were housed under diurnal conditions (14-h light/10-h dark) and all testing occurred at a regular time during the light period. Animals were thoroughly habituated to handling before the study began. All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines. All efforts were made to minimize the number of animals used and their suffering.

Apparatus

Testing was carried out in an eight-arm radial maze, which consisted of an octagonal central platform (34 cm diameter) and eight equally spaced radial arms (87 cm long, 10 cm wide). The base of the central platform and the arms were made of wood, while panels of clear Perspex (24 cm high) formed the walls of the arms. At the end of each arm was a food well (2 cm in diameter and 0.5 cm deep). At the start of each arm was a clear Perspex guillotine door (12 cm high) that controlled access in and out of the center area. Each door was attached to a pulley system enabling the experimenter to control access to the arms. All animals were tested in the same rectangular room (295 cm×295 cm×260 cm) that contained salient visual cues, such as high contrast stimuli and geometric shapes on the walls.

Surgery

Rats were anesthetized by i.p. injection of pentobarbitone sodium (Sagatal) at a dose of 60 mg/kg. Animals were then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, USA) and the scalp cut and retracted to expose the skull. A craniotomy was made above the saggital sinus and the dura cut to expose the cortex above the target region.

Unilateral hippocampal lesions were produced by injections of 0.63 M ibotenic acid (Biosearch Technologies, Tujunga, USA), dissolved in phosphate buffered saline (pH 7.4), made via a 1 ml syringe (Hamilton, Bonaduz, Switzerland) and placed into 14 sites within the same hemisphere as described previously (Coutureau et al., 1999; Ward-Robinson et al., 2001). The left and right hemispheres were used as surgical targets in different animals. The stereotaxic coordinates relative to bregma with the incisor bar set at flat skull to the horizontal plane were [AP, LAT, HT (from top of cortex), volume (ml)]: −5.4, ±5.0, −6.1, 0.08; −5.4, ±5.0, −5.3, 0.08; −5.4, ±5.0, −4.5, 0.09; −5.4, ±4.2, −3.9, 0.1; −4.7, ±4.5, −6.5, 0.05; −4.7, ±4.0, −7.2, 0.1; −4.7, ±4.0, −3.5, 0.05; −3.9, ±3.5, −2.7, 0.1; −3.9, ±2.2, −3.0, 0.1; −3.9, ±2.2,−1.8, 0.1; −3.1, ±3.0, −2.7, 0.1; −3.1, ±1.4, −3.0, 0.1; −3.1, ±1.4, −2.1, 0.1; −2.4, ±1.0, −3.0, 0.05.

Each injection was made gradually over a 4 min period, following which the needle was left *in situ* for a further 4 min before being withdrawn. At the completion of all surgeries the skin was sutured and an antibiotic powder (Acramide, Dales Pharmaceuticals, Skipton, UK) was applied. All rats also received a 5 ml s.c. injection of glucose saline.

Behavioral training

Animals in the experimental condition were trained to run in the maze using a standard working memory procedure (Olton et al., 1978). Thus, at the start of a 'run' all eight arms were baited with a single food pellet (45 mg; Noyes Purified Rodent Diet, Lancaster, NH, USA). When the rat returned to the central platform all doors were closed for about 5 s before they were again opened, permitting the animal to make a choice. This continued until all eight arms had been visited. Retrieving all eight pellets constituted a single 'run,' composed of a minimum of eight arm choices. Training consisted of eight sessions. The only noteworthy aspect of the training was that each session consisted of multiple runs in the radial-arm maze, one after the other, so that each session lasted for 30 min. Therefore, after entering all eight arms the animals were removed from the maze while it was rebaited and then returned to the maze to perform a new trial. This was repeated for 30 min. The delay between each trial (2min) was the time it took to rebait all of the arms, and during this period the animals were placed back in their home cage.

Final session: The final (eighth) session was the same as those in training, i.e. 30 min of radial-arm maze testing (approximately five radial-arm maze runs). Immediately before every session, including the final session, each animal was placed in a sound-proof box in a dark, quiet room for 30 min. At the completion of every session, including the final session, each animal was returned to this box for 90 min prior to perfusion. This quiet period was to minimize c-*fos* and *zif*268 activation in the periods before and after the radial-arm maze task. In contrast, the home-cage control animals were taken straight from their cage and then perfused.

Immunohistochemistry

Ninety minutes after completing the final radial-arm maze session rats were deeply anesthetized with pentobarbitone sodium (1 mg/kg) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M PBS. The brains were removed and postfixed in 4% paraformaldehyde for 4 h and then transferred to 30% sucrose overnight. Coronal sections were cut at 40 μm on a freezing microtome. Two series of sections were collected in 0.1 M PBS containing 0.2% Triton X-100 (PBST). A peroxidase block was then carried out where the sections were transferred to 0.3% hydrogen peroxide in PBST for 10 min to inhibit endogenous peroxidase and then washed several times with PBST. Sections were incubated in PBST containing Fos rabbit polyclonal antibody (1:5000; Ab-5, Oncogene Science, UK) or *zif*268 (antibody 1:3000; Egr-1 (C-19), Santa Cruz Biotechnology, USA) for 48 h at 4 °C with periodic rotation. Sections were then washed with PBST and incubated in biotinylated goat anti-

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