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POST-SURGICAL INTERVAL AND LESION LOCATION WITHIN THE LIMBIC THALAMUS DETERMINE EXTENT OF RETROSPLENIAL CORTEX IMMEDIATE-EARLY GENE HYPOACTIVITY

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Abstract—Four experiments examined the disruptive effects of selective lesions in limbic thalamic nuclei on retrosplenial cortex function, as characterized by striking changes in immediate-early gene activity. Major goals were to test the specificity of these retrosplenial changes, to define better their time course, and to assess the spread of retrosplenial dysfunction with time post-surgery. Experiment 1 examined the activity of two immediate-early genes (c-Fos, Zif268) in the retrosplenial cortex after unilateral anterior thalamic nuclei lesions (1, 2, or 8 weeks post-surgery). Marked immediate-early gene hypoactivity in the hemisphere ipsilateral to the thalamic lesion was consistent across these different post-surgical intervals and, hence, across different rat strains. Concurrent processing of brain tissues from rats either 4 weeks or 1 year after anterior thalamic lesions (Experiments 2 and 3) enabled direct comparisons across very different survival times. The results confirmed that over time the immediate-early gene disruption expanded from the superficial laminae to the deep laminae of granular b cortex and to the dysgranular subregion, indicative of more global disruptions to retrosplenial cortex with extended survival. Associated, subtle changes to cell morphometry (size and sphericity) were found in the retrosplenial cortex. In contrast, unilateral lesions in the adjacent laterodorsal thalamic nucleus (Experiment 4) did not significantly alter retrosplenial cortex c-Fos activity, so highlighting the anatomical specificity of the anterior thalamic lesion effects. These findings not only indicate that the impact of anterior thalamic lesions on cognition could be enhanced by retrosplenial cortex dysfunction but they also show that the effects could increase with longer post-insult survival.

Key words: thalamic, cingulate cortex, diencephalic amnesia, diaschisis, c-Fos, Zif268.

The anterior thalamic nuclei and the retrosplenial cortex are reciprocally connected (Vogt et al., 1981; van Groen et al., 1993) and conjointly support memory (Sutherland and Hoesing, 1993). Damage to these regions induces similar learning deficits in rats. For example, deficits in allocentric spatial memory and object-in-place discrimination are a shared feature of both anterior thalamic nuclei lesions (Aggleton et al., 1996; Sziklas and Petrides, 1999; Warburton et al., 2000; Wilton et al., 2001; Mair et al., 2003) and retrosplenial cortex lesions (Vann and Aggleton, 2002; Parron and Save, 2004; van Groen et al., 2004; Cain et al., 2006). The present interest in the interactions between these two areas stems from the ways in which they might jointly contribute to neurological disorders characterized by memory deficits. An example is that pathology in both regions is associated with human amnesic syndromes (Valenstein et al., 1987; Clarke et al., 1994; Reed et al., 1999, 2003; Aupée et al., 2001; Maguire, 2001ab; Aggleton and Brown, 2006; Svoboda et al., 2006). Likewise, in Alzheimer's disease where initial pathology commences in the entorhinal cortex and the anterior thalamic nuclei (Braak and Braak, 1991a,b), it is the retrosplenial cortex that typically shows the first and most extreme metabolic decreases (Minoshima et al., 1997; Nestor et al., 2003; Buckner, 2004; Liang et al., 2008). A possible explanation is that anterior thalamic pathology is a principal cause of changes to retrosplenial cortex function (Clarke et al., 1994; Jenkins et al., 2004). This explanation fits with initial findings from studies with rats showing that anterior thalamic, but not entorhinal cortex, lesions markedly disrupt retrosplenial cortex immediate-early gene (IEG) activity (Jenkins et al., 2004; Albasser et al., 2007).

The principal goals of the present study were to understand better the time course of retrosplenial changes following distal thalamic damage and to identify the specificity of the impact of anterior thalamic damage upon retrosplenial activity. In addition, new quantitative analyses were applied to the numbers and morphometry of retrosplenial cortex cells to determine whether the indirect lesion-induced changes in this region are truly covert. The overall goal was to help determine how retrosplenial cortex dysfunction might add to the cognitive deficits seen after pathology in the limbic thalamus.

Previous studies have reported that large thalamic lesions in rats, involving many nuclei, decrease levels of metabolic enzymes and various presynaptic receptors in granular retrosplenial cortex (van Groen et al., 1993). More selective anterior thalamic nuclei lesions are sufficient to induce marked changes in the expression of the protein products of the IEGs c-*fos* and *zif268* (Jenkins et al., 2002b, 2004). Thus, 8 weeks after anterior thalamic nuclei lesions, as many as 90% of the cells in the superficial laminae of the granular retrosplenial cortex appear to stop

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Abbreviations: ANOVA, analysis of variance; IEG, immediate-early gene; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; PBST, 0.1 M phosphate-buffered saline containing 0.2% Triton X-100; PFA, paraformaldehyde; Rgb, granular b retrosplenial cortex.

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producing c-Fos (Jenkins et al., 2004). As there is no evidence of cell death in retrosplenial cortex following anterior thalamic lesions (Vogt et al., 1981; Jenkins et al., 2004) these striking change may reflect "covert pathology," i.e. a functional lesion where there is no overt pathology (Aggleton, 2008). Unlike the traditional view of diaschisis (Baron et al., 1992; Luauté and Luauté, 2005), the retrosplenial changes may be permanent (Clarke et al., 1994; Jenkins et al., 2004). Furthermore, the dysfunctions within the retrosplenial cortex following distal thalamic lesions include a loss of long-term depression (LTD) (Garden et al., in press), a change reflecting an intrinsic loss of plasticity from within the cortex, and not merely the concurrent loss of afferent information from the anterior thalamic nuclei.

The present study, therefore, sought to characterize more precisely the nature of the retrosplenial cortex IEG hypoactivity in response to distal thalamic damage. A key issue was testing the specificity of this effect by measuring the consequences of damage to the laterodorsal thalamic nucleus, which is often seen as an additional anterior thalamic nucleus. Two IEG protein products (c-Fos and Zif268) were used as markers of the status of the retrosplenial cortex. c-*fos* And *zif268* are activity-dependent transcription factors induced by a variety of stimuli and conditions, including those associated with learning, memory, and neuronal plasticity (Herrera and Robertson, 1996; Herdegen and Leah, 1998; Hughes et al., 1999; Tischmeyer and Grimm, 1999; Kasahara et al., 2001; Fleischmann et al., 2003; Lindecke et al., 2006). The potential value of these IEGs as markers of retrosplenial cortex function is reinforced by the finding that the loss of both of these IEGs reported after anterior thalamic lesions (Jenkins et al., 2004) is associated with much more widespread alterations in the retrosplenial transcriptome, most notably in the activity of genes associated with energy and plasticity (Poirier et al., 2008).

Both the anterior thalamic nuclei (van Groen et al., 1993; Tomitaka et al., 2000; Wang et al., 2001) and the laterodorsal thalamic nucleus (van Groen and Wyss, 2003; Miyashita and Rockland, 2007) have dense, direct projections to the retrosplenial cortex that remain ipsilateral. For this reason, unilateral lesions were made to these target thalamic sites in Experiments 1, 3 and 4. For all of these experiments, granular b retrosplenial cortex (Rgb) was the principal region of interest for two reasons. First, Rgb receives dense inputs from the anterior thalamic nuclei (Vogt et al., 1981; van Groen et al., 1993). Indeed, Rgb appears to receive more widespread inputs from the anterior thalamic nuclei than any of the other retrosplenial subregions (Van Groen and Wyss, 2003). Second, the most prominent retrosplenial IEG changes have been seen in granular, not dysgranular, retrosplenial cortex after anterior thalamic lesions (Jenkins et al., 2004). Dysgranular retrosplenial cortex was, however, examined in Experiments 2–4 for comparison purposes and also because this subregion receives dense inputs from the laterodorsal thalamic nucleus (Experiment 4). Thus, projections to the retrosplenial cortex from the laterodorsal thalamic are thought to terminate in all retrosplenial subregions, preferentially in Rdg (Sripanidkulchai and Wyss, 1986; van Groen and Wyss, 1992a,b). In contrast, the anteromedial thalamic nucleus projects only strongly to Rdg, opposite to the anterodorsal and anteroventral thalamic nuclei, which project strongly to Rgb, with additional weak projections from the latter to Rdg (Sripanidkulchai and Wyss, 1986; van Groen and Wyss, 1992a,b, 1995, 2003; Shibata, 1993).

Crucial issues addressed in the present study concerned the generality of the IEG effect across rat strains (Experiment 1), whether the extent and distribution of IEG hypoactivity changes with different post-surgical survival times (Experiments 1–3), whether this IEG hypoactivity extends to the dysgranular retrosplenial cortex (Experiments 2–4), and whether these distal lesion effects are specific to the anterior thalamic nuclei among the limbic thalamus (Experiment 4). Other advances included more detailed analyses of the status of Nissl-stained cells and of c-Fos-positive cells following different survival times after anterior thalamic lesions. The goal was to uncover whether the signaling dysfunction reflected by the IEG changes is purely "covert" or whether it also includes overt, morphological effects.

EXPERIMENT 1: TIME COURSE OF EFFECTS OF UNILATERAL ANTERIOR THALAMIC LESIONS ON LAMINAR Rgb c-Fos AND Zif268

In Experiment 1, the Lister hooded strain was used, instead of the dark agouti strain which had been used in all previous studies of this IEG relationship (Jenkins et al., 2002a,b, 2004; Poirier et al., 2008). This feature made it possible to test the generality of these effects across rat strains. The time points for tissue sampling were 1, 2, 4, and 8 weeks after the surgeries, the latter being close to the earliest time point (9–11 weeks) previously tested for IEG (c-Fos and Zif268) activity (Jenkins et al., 2004).

EXPERIMENTAL PROCEDURES

Subjects

The subjects were 25 male pigmented rats (Lister hooded strain, Harlan, Bicester, Oxon, UK) weighing between 250 and 300 g, and 2–3 months old, at the time of surgery. The rats were housed in pairs under diurnal light conditions (14-h light/10-h dark) and after their arrival a period of at least a week was allowed before the rats received surgery. The rats were given unrestricted access to food and water in their home cages. The rats were randomly assigned to four groups of different post-operative recovery periods: 1-week, $n=6$; 2-week, $n=7$; 4-week, $n=6$; 8-week, $n=6$. All experiments conformed with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, and their design sought to minimize the number of animals and their suffering.

Surgical and behavioral protocols

The rats were first anesthetized with an i.p. injection of pentobarbitone sodium (Sagatal, Rhône Mérieux, 80 mg/kg), and then placed in a stereotaxic frame (Kopf Instruments, CA, USA) using