The effects of intra-subicular ibotenate on resistance to extinction after continuous or partial reinforcement

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Summary. Intracerebral injections of ibotenate were used to produce, in rats, extensive cell loss in the subiculum. These rats and sham-operated controls were trained to run in a straight alley for food reward delivered on a continuous (CR) or partial (PR) reinforcement schedule. In controls PR training gave rise to the well-known partial reinforcement extinction effect (PREE), i.e., greater resistance to extinction than that observed in CR-trained animals. Previous experiments have shown that large aspiration lesions of the hippocampal formation eliminate the PREE; and that ibotenate-induced lesions of the subicular region plus either the hippocampus or the entorhinal cortex disrupt it. In contrast to these previous results, the PREE was unaltered in the present experiment by damage largely restricted to the subiculum. This lesion caused only relatively small changes in running speeds during acquisition. Thus the critical region(s) of damage within the hippocampal formation for disruption of the PREE remains uncertain.

Key words: Subiculum - Partial reinforcement - Extinction effect - Ibotenate

Introduction

If rats are trained to run in a straight alley for food reward and the running response is subsequently extinguished (by omission of food from the goalbox), resistance to extinction is a function of the reinforcement schedule used during acquisition of the response. If acquisition is on a continuous reinforcement (CR) schedule (i.e., every response rewarded) extinction occurs more rapidly than if acquisition is on a partial reinforcement (PR) schedule (i.e., reward on only some, randomly chosen, trials). This increase in resistance to extinction produced by PR schedules is known as the 'partial reinforcement extinction effect' (PREE). The PREE is an extremely robust phenomenon, both in the alley and in other experimental paradigms. It is, however, highly susceptible to damage to the septo-hippocampal system (SHS), being markedly attenuated or abolished by electrolytic lesions to the entire anterior septal area (Henke 1974, 1977) or its lateral portion (Feldon and Gray 1979a, b), aspiration lesions of the hippocampal formation (Rawlins et al. 1980) or complete section of the fornix-fimbria (Feldon et al. 1985).

It is not clear, however, whether this pattern of results can be reproduced using the more recently developed selective neuronal cytotoxins, such as ibotenate (Köhler and Schwarcz 1983; Jarrard 1986), which destroy cell bodies in the area of injection but spare fibres of passage. Aspiration lesions of the hippocampal formation eliminate the PREE by both increasing resistance to extinction in CR-trained animals and decreasing resistance to extinction in PR-trained animals (Rawlins et al. 1980), as do electrolytic lesions of the entire anterior septal area (Henke 1974, 1977) and fornix-fimbria section (Feldon et al. 1985). But injections of ibotenate into the hippocampal formation failed to reproduce this complete pattern of change (Jarrard et al. 1986). Instead, in animals that had sustained extensive ibotenate-induced cell loss in the dentate gyrus and hippocampus proper, Jarrard et al. (1986) observed increased resistance to extinction after both CR and PR training, the PREE being preserved. In the same experiments, however, ibotenate-induced cell loss in the subicular area did reduce the PREE, increasing resistance to extinction in CR-trained rats and decreasing it in the PR-training condition, though
only in the goal section of the alley. The critical region for this effect remains in doubt, however, since the subicular lesions were always accompanied by additional damage either to the entorhinal area or to the hippocampus proper and dentate gyrus.

In the present study, therefore, we re-examined the effects of intra-subicular ibotenate on the PREE, attempting to minimise damage to extra-subicular neurones, while otherwise repeating the essential features of our previous experiments (Jarrard et al. 1986; Rawlins et al. 1980).

**Methods**

**Surgical and anatomical**

The procedures were similar to those described previously (Jarrard et al. 1986). Naive male Sprague-Dawley rats, weighing 250-300 g, were anaesthetised with a mixture of chloral hydrate and sodium pentobarbital and placed in a Kopf stereotaxic instrument. An incision was made in the scalp and holes were drilled to allow microinjections of ibotenic acid (Sigma) dissolved in phosphate buffered saline (final pH 7.4) at a concentration of 10 mg/ml. Injections were made with a 2-~1 Hamilton syringe mounted on the stereotaxic frame and held with a Kopf 5000 Microinjector. The stereotaxic coordinates used are given in Table 1. The animals were randomly allocated to lesion or control condition. In the former group injections of 0.1 ~1 were made manually over 1 rain at each site and the syringe left in place for an additional 1 min to prevent spread up the needle track. Controls were subjected to the same surgical procedure except that the needle was only lowered through the cortex at the appropriate site with no injection.

Following behavioural testing, all lesioned animals were perfused with physiological saline and formalin. The brains were embedded in paraffin and cut horizontally on a microtome at 10 mm; every twentieth section was stained with cresyl violet for cell bodies. The analysis of histological results was done blind to behavioural condition or results, and resulted in the exclusion of 5 lesioned animals with insufficient subicular damage or too much damage to other structures. Final group sizes were therefore: lesion CR, 8; lesion PR, 9; control CR, 8; control PR, 9.

**Behavioural**

The 1.7-m alley was essentially as described by Jarrard et al. (1986), but made of aluminium and equipped with a solenoid-operated startbox door and a guillotine goalbox door. The reward (4.45-mg pellets) was placed manually in a foodcup in the goalbox. Three photobeams, using visible light and located 14 and 105 cm from the startbox door and above the foodcup, permitted measurement of start, run and goal times to the nearest 0.01 s.

Animals were run daily in squads of 4 (one from each experimental group), resulting in an inter-trial interval of 3-5 min. Extinction, which followed immediately after acquisition, lasted 6 days and was identical to acquisition except that no trials were rewarded. Goalbox confinement time on nonrewarded trials was 30 s. Animals were considered extinguished when they had two consecutive trial times of 100 s; they were no longer run and assigned 100 s for each section in remaining trials.

A reciprocal (speed) transformation was used to produce data suitable for parametric analyses of variance for the effects of reinforcement schedule, lesion and days, separately for start, run and goal speeds during acquisition and extinction.

**Results**

**Anatomical**

Reconstructions of the smallest and the largest accepted lesions are presented in Fig. 1. Figure 2 shows the areas of smallest and largest cell loss at each of four horizontal levels for all of the rats with accepted lesions.

All of the rats retained for behavioural analysis had bilateral regions of cell loss in the subiculum; however, the extent of cell loss was variable between animals. As Figs. 1 and 2 indicate, there was some sparing of this region, particularly at dorsal levels in four rats with smaller lesions; whereas six rats with larger lesions had extensive subicular damage which also included damage to hippocampal cell fields, especially at the mid and ventral levels. In a further three animals, extensive subicular loss was accompanied by some damage to the deeper layers of entorhinal cortex. There was virtually no damage to the pre- or para-subiculum in any animal.

**Behavioural**

Figure 3 shows the course of acquisition and extinction for each alley section.

There was a tendency for subicular lesions to reduce speeds during acquisition, especially in the start section, in the PR condition, and later in