Afferent Connections of the Hippocampus in the Rat

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Summary: The afferent connections of the hippocampus in the rat were examined by means of a retrograde axonal transport method using horseradish peroxidase (HRP). The results were as follows: During injections of HRP in the dorsal or ventrolateral hippocampus, labeled areas did not show any difference, except for the medial septal nucleus. Numerous labeled cells were consistently found in the ipsilateral medial septal nucleus, the diagonal band nucleus, the entorhinal cortex, the locus coeruleus, the dorsal raphe nucleus, the median raphe nucleus, the posterior hypothalamic nucleus, the supramammillary area, the supraoptic area and, bilaterally, the lateral hypothalamic area. When injections were made into the dorsal hippocampus, HRP-labeled cells of the medial septal nucleus were observed in medial aspect, but injections made into the ventrolateral hippocampus revealed HRP-labeled cells of the medial septal nucleus in the lateral aspect. After injections into the hippocampus, the supraoptic area was labeled, as first reported.

The hippocampus is an important component of the limbic system, its function being related not only to the action of internal organs, but also to aspects such as emotion, study and memory. Also, as the principles of acupuncture analgesia have been extensively studied, it has been determined recently that hippocampal functions are closely connected with this type of treatment (Tang Cimei et al., 1979 and Chen Xianggui et al., 1979). The connections of the hippocampus have been also received extensive study in recent years. For example, Segal et al. (1974) and Sakanaka et al. (1980) successively studied afferents of the hippocampus in the rat using a method of retrograde transport for horseradish peroxidase. However, the results were not consistent. In order to provide morphological information on hippocampal functions, particularly its action during acupuncture analgesia, we have again examined hippocampal afferent pathways using retrograde transport of HRP.

Materials and Methods

Twenty-six adult male and female rats (180–200 g) were used. The animals were anesthetized with 10% chloral hydrate injected into the abdominal cavity, then 0.2 μl of 50% HRP (Type VI, Sigma Chemical Co., R.Z = 3.10) in saline was injected stereotaxically into the dorsal or ventral hippocampus through a glass pipette with a tip diameter of 70–80 μm attached to a 1-μl microsyringe, over an injection time of 25–30 minutes. 24–48 hours after injection, the rats were anesthetized with 10% chloral hydrate and perfused through the heart with 100 ml of physiological saline followed by about 300 ml of a fixative
containing 2% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed, kept in fixative at 4°C for 8–10 hours and then rinsed in 0.1 M phosphate buffer containing 5% sucrose at 4°C for 8–10 hours. Frozen serial sections 40 μm thick were cut in the coronal plane. The greater part of these sections were treated according to the method described by Walberg et al. (1976), while the sections from five other cases were divided into two parts, one of which was treated on the basis of the method of Edwards et al. (1979), while the other was treated with the Mesulam’s method (1978). All sections were mounted on glass slides before finally being examined microscopically.

Results and Discussion

The results of the three treatment methods were identical. After HRP was injected into the rat hippocampus, many HRP-labeled cells were observed in the septal nuclei, the entorhinal cortex, the locus coeruleus, the raphe nuclei and some nuclei of the hypothalamus.

The septal nuclei: Septa fibers projecting into the hippocampus have been reported by many authors (Raisman et al., 1965, Mosko et al., 1976, Siegel et al., 1971 and Ibata et al., 1971), but there are differences of opinion about the nuclei from which they originate. Our result is identical to those of Raisman et al., (1965) and Segal et al., (1974), in that both medial septal and diagonal band nucleus fibers were seen to project into the hippocampus (Figs. 1 and 2). When HRP was injected into the dorsal hippocampus, HRP-labeled cells appeared in the medial aspect of the septal nucleus, whereas HRP injected into the ventrolateral hippocampus produced HRP-labeled cells which appeared in the lateral aspect of the septal nucleus. Thus, the medial aspect of the medial septal nucleus innervates the dorsal area of the hippocampus, while its lateral aspect innervates the ventrolateral hippocampus.

The entorhinal cortex: Cajal (1955), Raisman et al. (1965) and Steward et al. (1976) found evidence to support the fact that the entorhinal cortex is one of the most important fibre sources of the hippocampus, and confirmed that there is one perforant path between the entorhinal cortex and the hippocampus. This result of the present study corresponds to that obtained by the above authors. In this case, when HRP was injected into the hippocampus, some HRP-labeled cells were clearly observed in the ipsilateral entorhinal cortex (Figs. 3 and 4), providing further proof that the perforant path arises from the entorhinal cortex. Steward et al. (1976) suggested, however, that cells of layer III in the entorhinal areas projected into the contralateral hippocampus, while Cajal et al. (1955) claimed that there is one cross-perforant path between the entorhinal cortex and the hippocampus, although these were not confirmed in our experiment.

Hypothalamus: Segal et al. (1974) demonstrated that fibres of the supramammillary region project into the hippocampus; Sakanaka et al. (1980) found evidence that the posterior hypothalamic nucleus, the lateral hypothalamic nucleus and dorsomedial hypothalamic nucleus project into the hippocampus, in addition to those from the supramammillary region. We observed HRP-labeled cells not only in the lateral hypothalamic area (Fig. 5), the posterior hypothalamic nucleus, and the supramammillary region (Fig. 6), but also a few HRP-labeled cells in the supraoptic area (Fig. 7). This was the first evidence produced showing that the supraoptic area innervates the hippocampus.
The raphe nuclei and the locus coeruleus: From a series of degeneration studies and HRP studies, it has been found that the fibres of the locus coeruleus, and the dorsal and median raphe nuclei terminate in the ipsilateral hippocampus (Reinoso-Suarez et al., 1973, Pasquier et al., 1976, Azmititia, 1981, and Kinoshita, 1978). This conclusion was also demonstrated in the present study. Reinoso-Suarez and his co-workers reported that the ventral hippocampus receives fibres from the centralis superior nucleus, but that the dorsal hippocampus receives fibres from the dorsalis raphe and the locus coeruleus nucleus. In this study, after injections into either the dorsal or ventral hippocampus, no differences were observed in the above areas. As regards the raphe nuclei, our result was consistent with that of Segal and Azmititia, in that the dorsal and medial raphe nuclei project into the hippocampus (Figs. 8 and 9). As regards observations on the locus coeruleus, this was consistent with Kinoshita et al. (1978) and Pickel et al. (1974) in that the ipsilateral locus coeruleus projects into the hippocampus (Fig. 10).

The present study demonstrated that the hippocampus receives fibres from the dorsal and median raphe nuclei, as well as areas such as the posterior hypothalamic nucleus, the supramammary region, and the suprachiasmatic area. Some previous research (Lin Baocheng et al., 1979, Wu Dingzong et al., 1979, Zhu Lin et al. 1979 and Zhang Jiaju et al., 1979) has demonstrated that the above-mentioned areas have a relationship with acupuncture analgesia. The hippocampus receives so many fibres related to acupuncture analgesia that it is not difficult to suppose that the hippocampus plays an important role in this type of treatment.

Recently, it has been thought that the dorsal raphe nucleus and the centralis superior nucleus make 5-HT which promotes the action of acupuncture analgesia (Pasquier, 1976 and Han Jisheng, 1971), while the locus coeruleus can produce the NA (noradrenaline) which antagonizes acupuncture analgesia (Han Jisheng 1971). However, some articles have reported that 5-HT nerve fibres of the hippocampus come mainly from the raphe nuclei, whereas NA (noradrenaline) nerve fibres of the hippocampus originate from the locus coeruleus nucleus (Ungerstedt, 1971) and Chu N. S., et al., 1974). Yu Guangdi et al. (1979) have demonstrated in lip-acupuncture analgesia experiments that some antagonistic action between the locus coeruleus and the dorsal raphe nucleus is involved in the mechanism of acupuncture. Since fibres of both the dorsal raphe nucleus and the locus coeruleus project into the hippocampus, it is probable that the hippocampus is one of the centres regulating pain in the central nervous system. Hippocampal function therefore requires further investigation.

References

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PLATES
Explanation of Figures

Plate I

HRP labeled neurons following HRP injection into the hippocampus in the rat.

Fig. 1. Neurons in the medial septal nucleus x 320.

Fig. 2. Neurons in the diagonal band nucleus x 160.

Fig. 3. Neurons in the entorhinal cortex x 100.

Fig. 4. Neurons in the entorhinal cortex x 100.

Fig. 5. Neurons in the lateral hypothalamus x 300.

Fig. 6. Neurons in the supramammillary region x 300.
Plate II

Fig. 7. Neurons in the supraoptic area × 200.

Fig. 8. Neurons in the media raphe nucleus × 640.

Fig. 9. Neurons in the dorsal raphe nucleus × 320.

Fig. 10. Neurons in the locus coeruleus × 200.