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Anatomical and electrophysiological evidence for a direct projection from Ammon's horn to the medial prefrontal cortex in the rat

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Summary. Following microinjection of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) into the medial prefrontal cortex (defined as the neocortical area innervated by the thalamic mediodorsal nucleus) labelled cells were observed in the pyramidal layer of the CA1 field of Ammon's horn. Observations made using antidromic stimulation confirmed these results, and revealed the slow conduction velocity of the fibres of the hippocampal cells innervating the prefrontal cortex. Taken together, these data provide evidence for a direct projection of CA1 cells to the medial prefrontal cortex.

Key words: Prefrontal cortex – Ammon's horn – CA1 – WGA-HRP – Antidromic stimulation method – Rat

Introduction

In non primate species, the prefrontal cortex has been defined as the neocortical areas receiving projections from the mediodorsal nucleus of the thalamus (Leonard 1969; Markowitsch et al. 1978; Guldin et al. 1981). In the rat, these neocortical areas correspond to the rostral quarter of the dorsal bank of the rhinal sulcus and the rostral half of the medial hemispheric wall (Beckstead 1976; Krettek and Price 1977; Divac et al. 1978). This latter part, designated as the medial prefrontal cortex, can be divided further into three areas: medial precentral, anterior cingulate and prelimbic (Fig. 1) (Krettek and Price 1977). The infralimbic area, which lies ventrally to the prelimbic area, is also considered as a part of the prefrontal cortex by some authors (Divac et al. 1978; Sarter and Markowitsch 1983) but not by others (Krettek and Price 1977; Beckstead 1979). The infralimbic area has been reported to receive a direct projection from field CA1 of Ammon's horn (Swanson 1981). This study was undertaken to determine whether the medial prefrontal cortex (as defined above) also receives a direct projection from the CA1 field of Ammon's horn. For this purpose, two techniques were used, retrograde labelling with Wheat Germ Agglutinin – Horseradish Peroxidase (WGA-HRP) (Gonatas et al. 1979) and antidromic stimulation.

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Methods

Retrograde labelling

Four male Sprague-Dawley rats, anaesthetised with ketamine (80 mg/kg i.p.) received a unilateral injection of WGA-HRP into the medial prefrontal cortex. WGA-HRP (4%) dissolved in saline (pH 7.5) was applied iontophoretically through a glass micropipette (3.5 MΩ impedance) by passing a positive direct current for 6 min (6 μA, pulsed at 1 min-on/1 min-off). After 48 h, the animals were re-anaesthetised with ketamine and perfused intracardiacally with 0.9% NaCl (150 ml), followed by a solution containing 4% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4; 300 ml) and finally with a 10% sucrose solution in 0.1 M phosphate buffer solution (pH 7.4; 250 ml). The brains were removed and placed in 10% sucrose-phosphate buffer at 4° C until sectioned. Frozen sections (50 μm) were collected in phosphate buffer solution 0.1 M (pH 7.4) and exposed to tetramethylbenzidine according to the procedure of Mesulam (1978). These sections were mounted on gelatin chrome-coated slides, cleared, cover-slipped, and examined under dark- and bright-field illumination. The pattern of retrogradely-labelled cells was reconstructed accurately from the frontal sections using a camera lucida.

Electrophysiological approach

Electrophysiological experiments were performed on five rats anaesthetised with ketamine (80 mg/jg, i.p.). Additional injections (80 mg/kg i.m.) were made to maintain a stable level of anaesthesia. The animals were fixed in a conventional stereotaxic

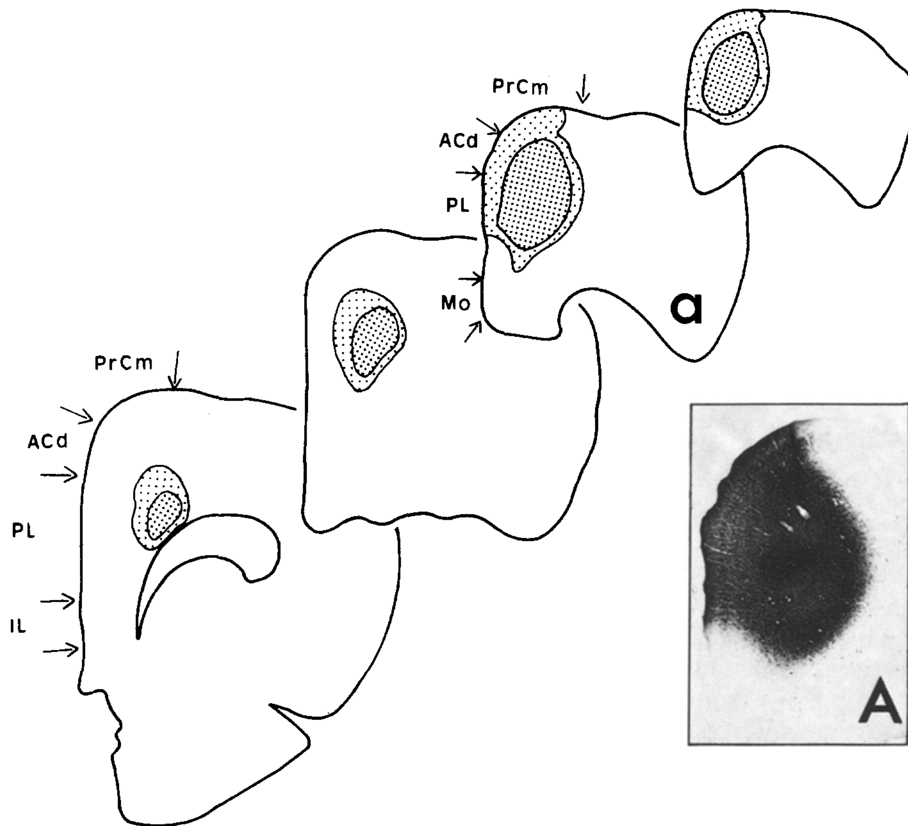

Fig. 1. Camera lucida drawings of the location of the injection site in the medial prefrontal cortex. WGA-HRP spread over the medial precentral (PrCm), anterior cingulate (Acd) and prelimbic (PL) areas but not into the medial orbital (MO) or infralimbic (IL) areas. Right to left: rostral to caudal sections; distance between sections: 500 μm. A: Bright-field photomicrograph of the frontal section represented in a

Horsley Clarke apparatus. Body temperature was monitored by a rectal thermometer and kept between 37° and 38° C.

A bipolar coaxial stimulating electrode (tip-barrel distance 300 μm) was positioned in the medial prefrontal cortex at the following coordinates: A: 10500 μm; L: 0.8 mm; H: +2.5 mm according to the Atlas of König and Klippel (1967). The stimulation consisted of square-wave pulses of 0.6 ms duration and 50 to 200 μA intensity. The activity of single hippocampal neurones on the ipsilateral side was recorded extracellularly with glass micropipettes (impedance 5–10 MΩ) filled with 4% pontamine sky blue dissolved in a 0.4 M NaCl solution. The antidromicity of action potentials was assessed according to three criteria: fixed latency, ability to follow high frequency stimulation and collision with spontaneous spikes. The refractory period of the fibres was measured using a classical double shock test.

At the end of each experiment the tip of the stimulating electrode was marked by a deposit of iron (15 μA anodal 15 s) which was subsequently revealed by a ferri-ferrocyanide reaction. The tip of the recording electrode was marked by iontophoretic injection of pontamine sky blue (8 μA cathodal – 20 min). Animals were perfused through the left ventricle with a solution of 10% formalin. The blue dye was localised on serial sections (100 μm) stained with cresyl violet.

Results

Following unilateral injection of WGA-HRP into the medial prefrontal cortex, the enzyme spread over the medial precentral, anterior cingulate and prelimbic areas but not into the infralimbic area (Fig. 1).

In the ipsilateral hippocampal formation, labelled cells were found only in the temporal part of the CA1 field of Ammon's horn and in the dorsal and ventral prosubiculum, the transition zone between CA1 and the subiculum (Meibach and Siegel 1977). As shown with the camera lucida drawings (Fig. 2), a rostrocaudal gradient of labelling was observed in the temporal part of the hippocampus (anterior 1.9–1.0), a greater number of cells being located in the most caudal part of the CA1 field. No labelled cells were found in other parts of the ipsilateral hippocampal formation (i.e. Dentate Gyrus, CA4, CA3) nor in the contralateral formation.

Most of the labelled cells in the CA1 field were located in the pyramidal layer. Some of them were almost completely filled with the enzyme and exhibited the classical shape of pyramidal neurones, i.e. a single apical dendrite leaving in the direction of the stratum radiatum (Fig. 2). The stratum radiatum itself contained only a few labelled cells. Although cells in the stratum radiatum are primarily interneurones which innervate the pyramidal layer, some pyramidal cells are also found in the stratum radiatum (Swanson et al. 1981). Those cells in the stratum radiatum that were filled completely with WGA-HRP did appear indeed to be pyramidal cells. However, a possible transynaptic transport of WGA-

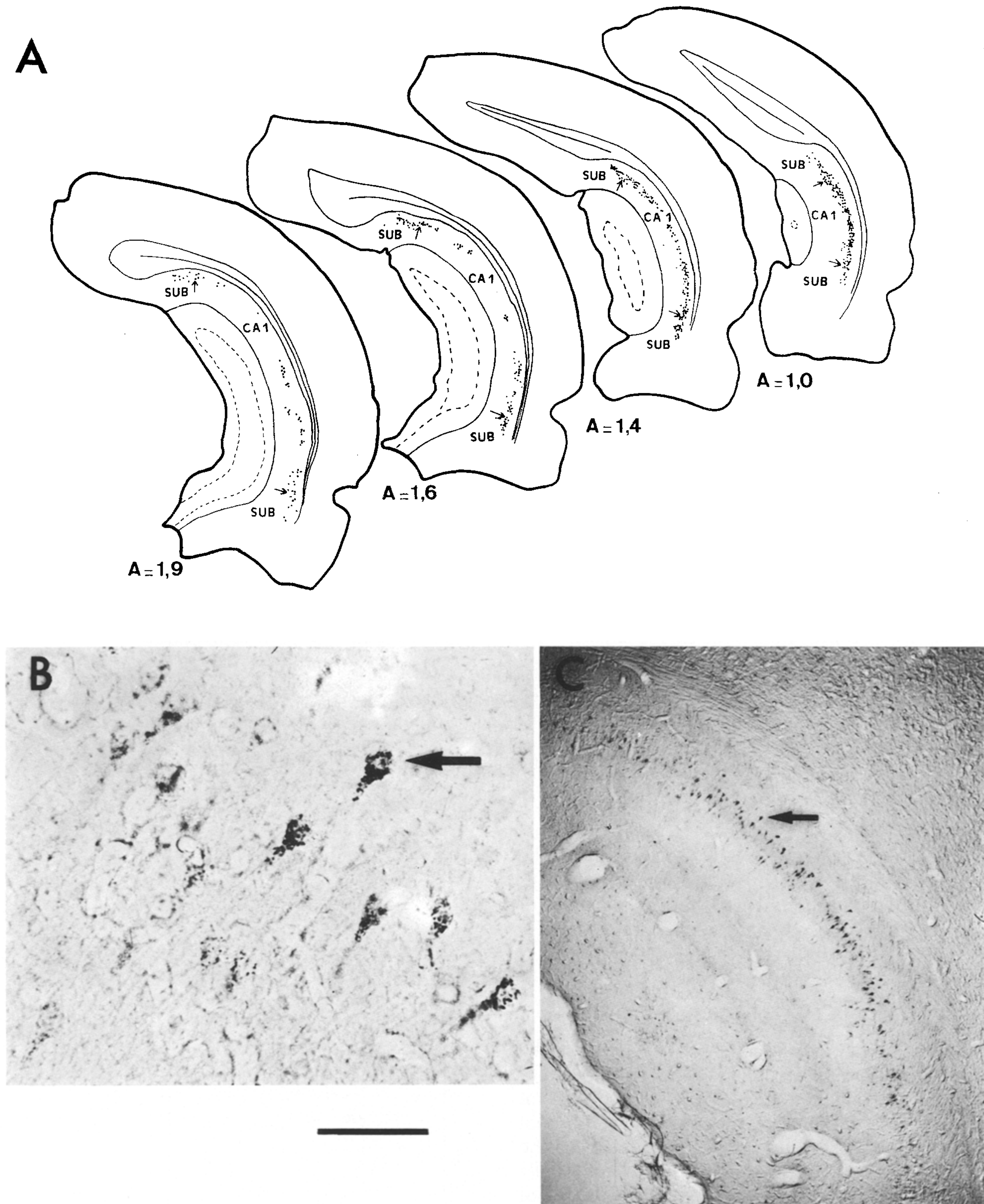

Fig. 2. A–C. Distribution of retrogradely labelled neurones in Ammon's horn following injection of WGA-HRP in the medial prefrontal cortex (injection site shown in Fig. 1). **A** Each retrogradely labelled neuron is marked by a black dot. Labelled cells are located mainly in the pyramidal layer of CA_1 and extend to the dorsal and ventral transition zones between CA_1 and the subiculum (SUB). Note the antero-posterior gradient of labelled cells. Number of labelled cells in each section: 1.9 : 65; 1.6 : 95; 1.4 : 150; 1.0 : 144. **B, C** Bright-field photomicrographs of a frontal section through the temporal pole of Ammon's horn in which labelled pyramidal cells are to be seen. The arrows in **B, C** indicate the same labelled pyramidal cell at different magnifications. Scale bar = 100 μm in **B** and 1 mm in **C**

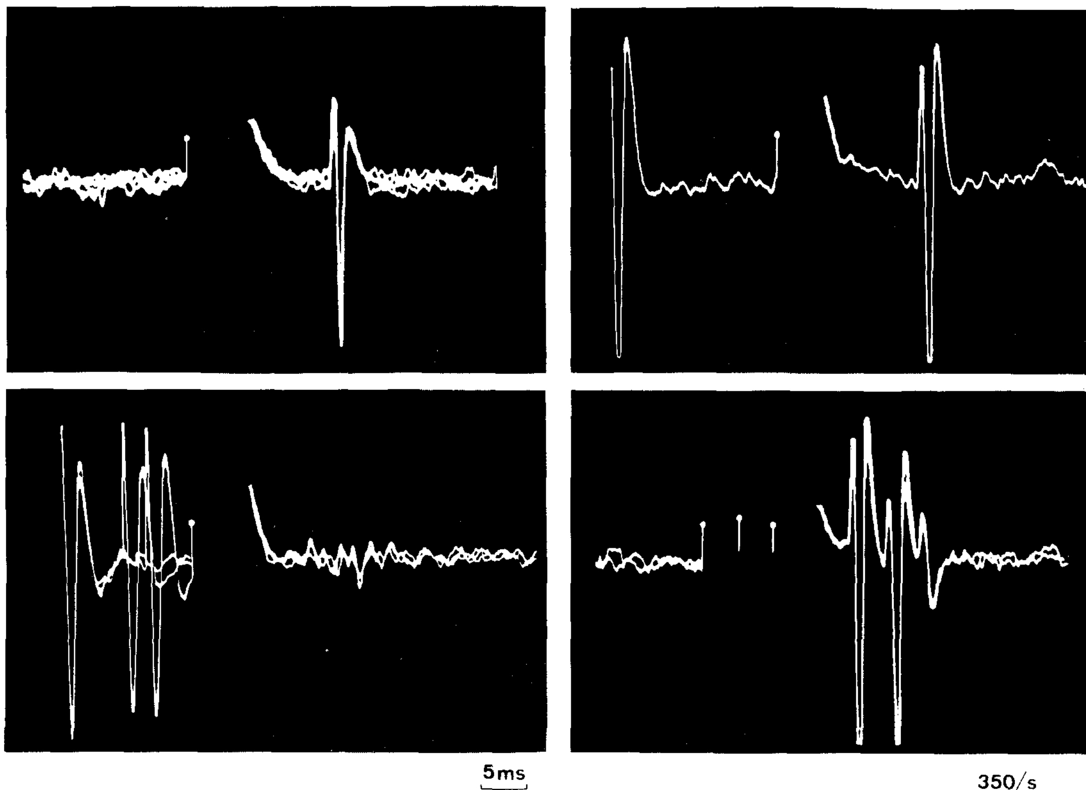

Fig. 3. Characterisation of an antidromic response evoked in a CA_1 cell by stimulation of the medial prefrontal cortex. First column, top to bottom: fixed latency of evoked spikes (lat = 15 ms); collision with spontaneous spikes. Second column, top to bottom: recovery of the antidromic spike when the delay between the spontaneous spike and the stimulation is greater than the sum of the latency of the antidromic response and the refractory period (RP = 2.6 ms); response to high frequency stimulation (350/s)

HRP into some rather weakly labelled cells cannot be excluded. Labelled cells in the transition zones between the subiculum and the CA1 field of Ammon's horn also seemed to be pyramidal cells.

Electrophysiological analysis

To rule out the possibility that WGA-HRP might be transported transynaptically (Sawchenko and Gerfen 1985), antidromic stimulation was used to confirm that the projection of the CA1 field to the prefrontal cortex was direct. Neurones were recorded in the right hippocampal formation. Their firing was usually low (less than 1 spike/s) and consisted frequently of doublets. This low firing may be attributed to ketamine anaesthesia, since this anaesthetic agent has been shown to inhibit the firing rate of hippocampal pyramidal neurones (Bichford et al. 1981). Among the 240 cells tested 85 cells were activated antidromically by stimulation of the ipsilateral medial

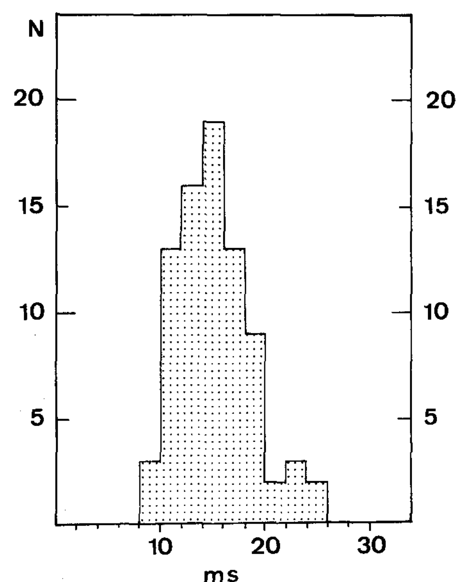

Fig. 4. Distribution of the latencies of antidromic spikes recorded in CA_1 stimulation of the medial prefrontal cortex

prefrontal cortex (Fig. 3). These cells were located in the CA1 field of Ammon's horn. During the high frequency test, the last spike usually was reduced to the initial segment, revealing that a cell and not a fiber was being recorded. The mean latency of the antidromic spikes was 15.6 ± 3.6 ms (Fig. 4) leading to an estimated conduction velocity of 0.6 m/s. Using a classical double shock test the refractory period was found to be 2.3 ± 0.4 ms.

Discussion

Due to the use of WGA-HRP retrograde tracing and antidromic activation, the present study has allowed the demonstration of a projection from a population of pyramidal cells in the temporal part of the CA1 field of Ammon's horn to the medial prefrontal cortex in the rat.

The first report concerning a projection from the CA1 field of Ammon's horn to the medial-rostral pole of the frontal cortex indicated that this projection was limited to the infralimbic area (Swanson 1981). However, in our experiments the possibility that the labelling observed in the hippocampus may be due to retrograde transport of WGA-HRP from the infralimbic area can be excluded, since the halo of the WGA-HRP injection was centered more dorsally in the medial prefrontal cortex. Finally, the majority of labelling observed is likely to be due to uptake of WGA-HRP by terminals only. Uptake of WGA-HRP by injured fibers of passage should not have been significant with this method of injection (diameter of tip of injection pipette = 10 μm).

Our anatomical data demonstrate the presence of numerous labelled cells preferentially located in the pyramidal layer of CA1 but also extending into the transition zones with the subiculum (both ventrally and dorsally). They extend previous preliminary observations made by other workers who mentioned the occurrence of labelled cells in Ammon's horn following injection of either HRP or WGA-HRP made respectively into the medial precentral area and into the prelimbic area (Donoghue and Parham 1983; Wilhite and Teyler 1985). The existence of a direct projection from the temporal part of the CA1 field of Ammon's horn to the medial prefrontal cortex was confirmed further by the ability to record antidromic spikes in cells of the CA1 field after stimulation of the medial prefrontal cortex. Furthermore, it should be noted that the efferent fibres of CA1 which project to the medial prefrontal cortex have a slow conduction velocity (0.6 m/s).

It should be noted that the projection of the CA1 field of Ammon's horn to the medial prefrontal cortex is strictly unilateral, as has also been observed for the other described cortical (infralimbic area, entorhinal cortex, subiculum) or subcortical (lateral septum, medial nucleus accumbens) efferents of the CA1 area (Swanson and Cowan 1977). Of particular interest is the finding that the medial prefrontal cortex, as well as these other cortical and subcortical areas innervated by the temporal field of CA1, receive a mesencephalic dopaminergic innervation (see for review Björklund and Lindvall 1984). Finally, it should be added that the dopaminergic innervation of the hippocampal formation which also originates in the ventral mesencephalic tegmentum is located mainly in both the subiculum and the temporal part of the CA1 field (Verney et al. 1985). Electrophysiological experiments are thus in progress to determine whether mesencephalic dopaminergic neurones may influence CA1 pyramidal cells projecting to the prefrontal cortex as well as their cortical target cells.

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