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Stratum radiatum in CA2 of the hippocampus is a unique target of the perforant path in human and nonhuman primates

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Abstract

The perforant path connects two key components of the medial temporal memory system, the entorhinal cortex and hippocampus. Entorhinal layer II projects densely to the outer portion of the molecular layer of the dentate gyrus and the stratum lacunosum-moleculare of CA2 and CA3 of the hippocampus. The present study for the first time reports that the stratum radiatum of CA2 but not of CA3 in the human brains receives “extra”, unique and dense projections from the layer II using an antibody against an abnormally phosphorylated tau (AT8), which stains both degenerating neurons and their processes including axon terminals in aging and early Alzheimer’s disease. This finding is also confirmed in CA2 of monkey hippocampus by means of anterograde tracer injections in the entorhinal cortex. Thus the present results indicate that CA2 in human and nonhuman primates receives stronger activation from the PP than CA3 does. Our finding also suggests that the SR of CA2 is a major converging zone of the projections from the perforant path and Schaffer collaterals. Thus, CA2 may play a unique role in hippocampal memory networks.

Keywords

entorhinal cortex; dentate gyrus; CA3; abnormally phosphorylated tau; anterograde tracing; axon degeneration

The entorhinal cortex (EC) and hippocampus are key components of the medial temporal memory system. The EC receives and processes information from wide-spread polysensory and limbic cortical areas and sends its major outputs to hippocampus via the “perforant path” (PP, Van Hoesen, 1982; Witter, 2007). The PP degeneration was found to be associated with memory impairment in preclinical and clinical Alzheimer’s disease (AD) and other disorders with dementia (Hyman et al, 1986, 1988, Thal et al, 2000; Takeda et al, 2007). Generally the PP comprises entorhinal layer II projections to the DG, CA2 and CA3, and entorhinal layer III projections to CA1 and subiculum (Witter, 2007).

Antibody against an abnormally phosphorylated tau (AT8) stains the soma, dendritic processes and axonal terminals of affected (degenerating) neurons in human EC in normal

aging and early AD (Braak et al, 1994; Thal et al, 2000). The use of this stain as a marker demonstrated the existence of the PP terminals in the DG (Thal et al, 2000). However, it is not known whether the PP terminals also exist in CA2-3 in human. The present study examines this issue using the same marker. Our results show that the PP projections terminate in the stratum lacunosum-moleculare (SLM) of CA2-3. Importantly, we found an “extra” and unique PP termination in the stratum radiatum (SR) of CA2. This finding is further confirmed in monkey CA2 using PP tract tracing.

EXPERIMENTAL PROCEDURES

1. Immunohistochemistry (IHC) for human specimens

Human specimens, from both normal aged and AD individuals, were donated to the Deeded Body Program at the University of Iowa. Procurement of autopsy tissue was obtained in accordance with University of Iowa human study guidelines. Only those brains (six cases) were included, in which the outer two-thirds of molecular layer (ML) of the DG contains AT8 labeled fine punctate-like structures (presumed axon terminals). The temporal lobes from the selected specimens were dissected out, blocked and processed according to the procedures described recently (Ding et al, 2009). Briefly, after immersion fixation in 4% paraformaldehyde (48–72 hours at 4°C) and cryoprotection in 30% sucrose (36 hours at 4°C) a series of 50-µm-thick coronal sections were cut. Two sets of 1-in-10 sections were respectively stained with thionin, and AT8 (1:1,000, Innogenetics, Ghent, Belgium). In addition, some selected AT8 stained sections were re-stained with IHC for neuronal nuclear antigen (NeuN, 1:1,500, Chemicon, Temecula, CA) to identify the hippocampal lamina more clearly.

2. Anterograde tracing of PP in monkey brains

To compare the terminal zone of the PP projections from entorhinal layer II in human and nonhuman primates, we injected ³H-labeled amino acids to the EC of four adult Macaca monkeys (for details, see table 1). The procedures for surgery and tissue processing are the same as described previously (Morecraft and Van Hoesen, 1998). All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by The University Committee on Animal Resources.

RESULTS

1. AT8 labeled PP terminals in DG, CA2 and CA3 in human brains

AT8+ axonal terminals in the DG, CA2 and CA3 of the hippocampus displayed fine punctate-like structures with diffuse distribution (Fig. 1A–D). In the DG, AT8+ axonal terminals were observed in the outer two-thirds of the ML (OML) while the inner portion of the ML showed no or few AT8+ axon terminals (Fig. 1A). In both CA3 and CA2, dense AT8+ axonal terminals were found in full thickness of the SLM (Fig. 1A–C). Surprisingly, AT8+ axon terminals were observed to extend from the SLM into the superficial portion of the SR only in CA2. This has not been previously reported. Moreover, the terminal zone in the SR of CA2 displayed a crescent-like shape with clear stripe-like terminal arrangement within it (Fig. 1A–C). Finally, it also should be mentioned that a few AT8+ axon terminals

were seen in the SLM of CA1 and in the ML of the subiculum while a moderate number of AT8+ axon fibers were found traveling in the deeper portion of them. These axon fibers were characterized by segment-like appearance (Fig.1E) rather than fine puncture-like one.

We also examined the density and laminar distribution of AT8+ neurons in the EC for probable source of the AT8+ axon terminals. Most of AT8+ neurons were observed in layer II (Fig. 1D, F) with a few in the superficial layer III (close to layer II) and layer V. Few AT8+ neurons were detected in layers III and VI. These results strongly suggest that entorhinal layer II neurons are the major source of the axon terminals in the OML of the DG, in the SLM of CA3 and in the SLM and SR of CA2.

2. Anterogradely labeled PP terminals in the DG, CA2 and CA3 in monkeys

To investigate whether the SR of CA2 in monkeys, like that in human, is a target region of the PP from entorhinal layer II, we examined PP terminals in the DG, CA2 and CA3 of four monkeys following injections of the tritiated amino acids in the EC (table 1). In addition to confirmation of previous findings (Witter et al, 1989, Witter and Amaral, 1991), we consistently found a protruding of the PP terminal zone from the SLM into the SR of CA2 when the injections involved layer II of the EC (table 1; Fig.1G). The PP terminal zone in the SR of CA2 (Fig. 2) displayed a similar crescent shape to that in human.

DISCUSSION

1. Origins and terminal zone of human PP: comparison with monkeys and other animals

In addition to the PP terminals in the OML of the DG (Hyman et al, 1988; Thal et al, 2000), we observed AT8+ PP terminals in the SLM of CA2 and CA3 as expected. Surprisingly we also observed AT8+ PP terminals in the SR of CA2. The existence of AT8+ neurons preferentially in layer II of the EC matches the existence of AT8+ PP terminals in the terminal zone of entorhinal layer II neurons based on the results from the monkeys (Witter et al, 1989; Witter and Amaral, 1991). This result suggests that entorhinal layer II neurons origin mostly the PP terminal zone in the DG, CA2 and CA3 in human hippocampus. This conclusion is consistent with the results from other animals such as rat and cat that entorhinal layer II neurons are the major contributors to the PP terminal zone in the DG, CA2 and CA3 (Steward and Scoville, 1976; Ruth et al, 1982, 1988; Witter and Groenewegen, 1984; Dolorfo and Amaral, 1998; Witter, 2007). Interestingly, in the mouse, entorhinal layer II neurons were reported to origin the PP terminals in the DG but not those in CA2/3 (van Groen et al, 2003). The present study did not reveal a significant number of AT8+ axon terminals in the SLM of CA1 and in the ML of the subiculum although a significant number of AT8+ bypassing fibers were found in these regions. Consistent with this, only a few of AT8+ neurons were detected in entorhinal layer III, which supplies most of the PP target zone in CA1 and the subiculum based on tracing studies in experimental animals including monkeys (see Witter, 2007). Therefore, in general, the present results indicate that the origins and terminal zones of human PP are comparable with other species, especially with monkeys.

2. Targets of the PP terminal zone in the SR of CA2

The most important finding of the present study is the PP innervation of the SR in CA2 in both human and monkeys. This “extra” crescent-shaped axon terminal zone in the SR was formed by protruding of the PP terminals from the SLM into the superficial half of the SR. Previous Golgi staining and intracellular labeling studies showed that CA2 pyramidal neurons gave off apical dendritic branches in both the SR and SLM (Lorente de No, 1934; Bartesaghi and Ravasi, 1999; Lauer and Senitz, 2006; Mercer et al, 2007). Therefore, the most probable target of the PP terminals in the SR is the middle portion of the dendrites of pyramidal neurons in CA2. The distal portion of the dendrites should be the target of the PP terminals in the SLM, which is also originated from entorhinal layer II. The crescent appearance of the PP terminal zone in the SR of CA2 likely suggests that these terminals target only the dendrites of CA2 pyramidal neurons, but not the invading dendrites of the pyramidal neurons in adjoining CA1 and CA3 (see Fig.2). In addition, the stripe-like arrangement of PP terminals in the SR may indicate that these PP terminals densely target the primary dendritic branches, which tended to display vertical arrangements (Lorente de No, 1934; Bartesaghi and Ravasi, 1999; Lauer and Senitz, 2006; Mercer et al, 2007).

The second possible target of the PP terminals in the SR of CA2 is the dendrites and/or cell bodies of interneurons located within this terminal zone. For example, both basket and bistratified neurons in the stratum pyramidale (SP) of rat CA2 extend their dendrites into the superficial SR (Mercer et al, 2007) and thus may be the targets of the PP terminals in the SR. Innervation of dendrites and cell bodies of interneurons by the PP terminals originated from entorhinal layer III has been reported in the SLM of rat CA1 (Kajiwarra et al, 2008). Further studies are needed to clarify whether or what types of interneurons are innervated by the PP terminals in the SR of CA2.

3. Functional consideration of the PP innervation in CA2

The classic concept that the PP terminals from entorhinal layer II targets the OML of the DG and the SLM of CA2 and CA3 implies that the PP terminals target only the distal portions of the dendrites of the principal neurons and some interneurons and their dendrites within these terminal zones. The present finding of “extra” PP terminals in the SR of CA2 indicates that the PP terminals also target more proximal portion of the dendrites of the pyramidal neurons, making CA2 a unique region in hippocampus. In comparison with CA3, which receives PP terminals only in its distal dendritic region, CA2 should thus receive much stronger activation from the PP since it receives PP terminals in both its distal and middle dendritic regions. In classic trisynaptic circuits, the EC presumably excites the DG granular cells, which then activates CA3 pyramidal neurons via mossy fibers; CA3 finally activates pyramidal neurons in CA1. Thus, CA2 were largely ignored. However, recent physiological studies of guinea pigs have showed evidence that CA2 may play a crucial role in hippocampal networks (Bartesaghi and Gessi, 2004; Bartesaghi et al, 2006). Specifically, activation of the EC by synaptically elicited PP volleys stimulates two parallel systems (the EC-DG-CA3 and EC-CA2-CA1 systems) and CA1 can be activated by CA2 independent from CA3. They also reported that CA2 neurons were more easily activated than CA3 neurons. Anatomical basis for these findings is not clear and it is not known if some “extra” PP terminals exist in the superficial SR of CA2 in lower animals such as guinea pigs.

However, the present finding that CA2 neurons receive “extra” PP innervation may provide an alternative explanation for its physiological behaviors, which should also exist in human and monkeys. Finally, since whole thickness of the SR in CA3 and CA2 receive dense projections from Schaffer collaterals originated from CA3 pyramidal neurons (Rosene and Van Hoesen, 1977; Kondo et al, 2009), the present finding also strongly suggests that the PP and Schaffer collateral projections converge in the superficial SR of CA2 and this convergence may play important roles in hippocampal memory networks, especially in human and nonhuman primates.

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Abbreviations

AD	Alzheimer’s disease
AT8	abnormally phosphorylated tau
CA1-4	subfields 1-4 of the hippocampus
DG	dentate gyrus
EC	entorhinal cortex
IHC	immunohistochemistry
ML	molecular layer
NeuN	neuronal nuclear antigen
OML	outer portion of the ML
PP	perforant path
SLM	stratum lacunosum-meloculare
SR	stratum radiatum
SP	stratum pyramidale

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Fig. 1.

(a–c) AT8 and NeuN double-stained sections from the anterior (a) and posterior (b, c – higher power) portions of the human hippocampus show laminar distribution of AT8 + axon terminal zones in the outer portion of the molecular layer (OML) of the dentate gyrus (DG), the stratum lacunosum-moleculare (SLM) of CA2 and CA3, and the stratum radiatum (SR) of CA2. Note the crescent-shaped terminal zone (stars) in the superficial SR of CA2. The arrow in (a) indicates the thick OML of the DG. (d) A low-power image shows the dense AT8 + axon terminal zone in the hippocampus and the dense AT8 + neuronal zone in layer

II of the entorhinal cortex (EC) in humans. A higher-power view of layer II is shown in (f). (e) A higher-power image shows AT8 + fibers of passage in the SLM of CA1. (f) A higher-power image shows AT8 + neurons in layer II of the EC. (g) A dark-bright field image shows an example of ³H-amino acid-labeled perforant path terminal zone in the superficial SR (star) of CA2 in monkeys. Dotted lines indicate the borders between SR, SLM, and OML. Bars: 500 mm in (a), (b); 200 mm in (c), (f), (g); 3 mm in (d); 100 mm in (e). CA1–4, subfields 1–4 of the hippocampus. SP, stratum pyramidale.

Fig. 2.

A schematic drawing showing the probable targets of the presumed perforant path terminal zone (dotted region) in the stratum radiatum (SR) of CA2 and in the stratum lacunosum-moleculare (SLM) of CA2–3. The targets in the SR of CA2 are proposed to be the middle portions of the dendrites emitted from the pyramidal neurons in CA2 rather than the invading dendrites (dashed portions) from the neurons in adjoining CA1 and CA3 based on the crescent shape of the terminal zone. SP, stratum pyramidale.

Table 1

Summary of injection sites and results from anterograde PP tracing in monkeys

Case number	³ H-AA injection sites in entorhinal cortex	Terminal density ^a	
		OML (DG),	
		SR (CA2)	SLM (CA2–3)
M1 (<i>Macaca Mulatta</i>)	Ei (layers I–VI)	++	+++
M2 (<i>Macaca Fascicularis</i>)	Ecl (layers I–VI + white matter)	++	+++
M3 (<i>Macaca Mulatta</i>)	Ec (layers I–IV)	+++	+++
M4 (<i>Macaca Mulatta</i>)	Er (layers V–VI + amygdala)	–	+

AA, amino acids; DG, dentate gyrus; OML, outer molecular layer; PP, perforant path; SLM, stratum lacunosum-meloculare; SR, stratum radiatum.

^a +, low; ++, moderate; +++, high; –, negative; Ec, Ei, Ecl, and Er, subareas of the entorhinal cortex following Witter *et al.* [9].