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INPUT–OUTPUT RELATIONS IN THE ENTORHINAL CORTEX–DENTATE–HIPPOCAMPAL SYSTEM: EVIDENCE FOR A NON-LINEAR TRANSFER OF SIGNALS

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Abstract—In the current study we analyzed the input–output relations in the entorhinal–dentate–hippocampal system, a major network involved in long-term memory. In anesthetized guinea pigs, the system was driven by activation of perforant path neurons in the entorhinal cortex (ENT), via presubicular fibers directly stimulated in the dorsal psalterium. Perforant path neuron discharge activated in parallel the dentate gyrus (DG) and hippocampal field CA2. Whereas the output from the DG activated hippocampal field CA3, the output from the sole field CA2 was sufficient for activation of field CA1. Signals from field CA3 operated in concert with CA2, likely contributing to discharge field CA1. These findings indicate the existence of two in parallel disynaptic systems: an ENT–CA2–CA1 and an ENT–DG–CA3 system. The convergence of the latter with the former gives origin the classical trisynaptic circuit, the ENT–DG–CA3–CA1 system. The input–output relations between the population excitatory postsynaptic potentials (pEPSP) evoked in the DG, CA3, CA2 and CA1 and the population spike (PS) evoked in the structure upstream (the input) were described by smooth sigmoid curves. In contrast, the input–output relations of the PS versus the pEPSP within each structure were described by steep sigmoid curves. The net input–output functions of the DG (ENT–DG system), field CA2 (ENT–CA2 system), field CA3 (ENT–DG–CA3 system) and field CA1 (ENT–CA2–CA1&ENT–DG–CA3–CA1 system) were described by sigmoid curves. While the DG and field CA2 exhibited steep sigmoids, fields CA3 and CA1 had less steep sigmoid functions. The present study demonstrates that all structures downstream to the ENT operate according to sigmoid input–output functions, characterized by specific parameters. These different behaviors may contribute to different memory processes. We additionally demonstrate that field CA1 can be activated by field CA2, independently from field CA3. This functional dissociation between CA3 and CA1 may subserve specific roles of each field in memory encoding/retrieval.

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Abbreviations: CA1, hippocampal field CA1; CA2, hippocampal field CA2; CA3, hippocampal field CA3; DG, dentate gyrus; ENT, entorhinal cortex; pEPSP, population excitatory postsynaptic potentials; PS, population spike; PSD, dorsal psalterium.

Key words: hippocampal region, *in vivo*, field potentials, neuronal networks, memory.

A body of evidence has clearly established that the medial temporal lobe plays a key role in long-term declarative memory functions. The medial temporal lobe memory system is formed by the hippocampal formation (dentate gyrus (DG) and hippocampus proper), subiculum, presubiculum, parasubiculum, entorhinal cortex (ENT), perirhinal cortex and parahippocampal cortex (Van Hoesen, 1982; Amaral and Witter, 1995; Burwell et al., 1995). Within the networks of the medial temporal lobe, the entorhinal–hippocampal connections emerge as a crucial element for processing of neocortical signals. The ENT is connected with various unimodal and polymodal association areas (Burwell et al., 1995; McIntyre et al., 1996; Burwell and Amaral, 1998; Lavenex and Amaral, 2000; Lavenex et al., 2002) and gives origin to a heavy projection to the hippocampal formation, the perforant pathway. Through this projection the neocortex can ultimately be connected with the hippocampal formation (Amaral and Witter, 1995). The hippocampal formation heavily projects to the ENT (Amaral and Witter, 1995), which, in turn, gives origin to efferent projections reciprocating the cortical input (Suzuki and Amaral, 1994; Burwell et al., 1995). Signal processing along the entorhinal–hippocampal–entorhinal loop is thought to be essential for the formation of long term memories and the return projection to the neocortex is thought to enable the formation of long term memory stores (Squire, 1992).

The pattern of signal processing by each of the different elements forming the trisynaptic circuit (perforant pathway–DG–hippocampal field CA3 (CA3)–hippocampal field CA1 (CA1)) has been clarified by studies in which the effects of direct stimulation of the input to each structure have been analyzed (Andersen et al., 1971; Andersen, 1975). These studies have demonstrated that each structure of the loop is coupled to the structure upstream by excitatory connections, the stimulation of which may lead to massive downstream neuron discharge. Numerous studies at the level of single neurons or neuron populations, in addition, have cleared the cellular mechanism whereby synaptic currents lead to neuron discharge. This type of analysis, at the level of single elements of the loop, in conjunction with the unidirectional connections linking the elements forming the trisynaptic circuit, has led to the notion that signals coming from the ENT are processed en cascade by the whole system. Based on this type of necessarily fragmentary approaches, several models have been

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constructed describing the manner by which processing of signals by this hippocampal network may contribute to memory encoding/retrieval. Physiological studies by Deadwyler and coworkers (Deadwyler et al., 1975, 1979; Deadwyler and Hampson, 1997, 1999) and by Lopes Da Silva and coworkers (Trabka et al., 1989; Naber et al., 2000; Kloosterman et al., 2003, 2004) have provided valuable evidence of how information is encoded by ensembles of hippocampal neurons. Relatively few other groups, however, have investigated the loop as a whole, providing demonstration of how signals are sequentially processed along the entire series of stations forming this loop. To construct reliable models of operation of this memory network, it is essential to establish the manner by which signals from the ENT are transferred across the whole in series elements of the system. In our laboratory we have investigated the entire loop, in electrophysiological experiments in which the loop was driven by signals coming from the ENT (Bartesaghi et al., 1988, 1989). These experiments demonstrated that the trisynaptic circuit may be very "permeable," so that even a submaximum input travels across all its stations. Moreover, we demonstrated that signals from the hippocampus, and relayed by the subiculum, return to the ENT, closing this loop. Thus, once the ENT is sufficiently activated, all structures of the system are sequentially fired, which leads to a reentrant input to the ENT.

In a previous investigation, using the same experimental model mentioned above, we analyzed the input–output relations in the ENT and DG and obtained evidence that, while the entorhinal response to the presubicular volley increased in an almost linear manner, entorhinal signals were processed in the DG in a somewhat all-or-none manner (Bartesaghi et al., 1995), suggesting that the DG may act as a gate that hinders the transfer of entorhinal signals, unless these have reached a critical level. It is not known whether the hippocampal structures downstream of the DG behave in a similar manner and to what extent the coupling between functionally linked stations may transform their final output. The aim of the current study was to reconstruct input–output relations for the whole series of stations of the entorhinal–dentate hippocampal system. The obtained data, may help clear the pattern by which the input from the ENT is processed along this system and, thus, outline a comprehensive picture of the signal flow through networks that play a crucial role within the medial temporal lobe memory systems.

EXPERIMENTAL PROCEDURES

Animals

The experimental subjects were 32 female albino guinea pigs of the Brescia strain (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy). The experiments were carried out after receiving approval by the Committee on Ethics in Animal Experimentation of Bologna University and governmental approval, in compliance with the Italian guidelines for care and use of laboratory animals (Italian Legislative Decree 116/92; in accordance with the European Community Council Directive 86/609/EEC on animal welfare). All efforts were made to minimize animal suffering and to reduce the number of animals used.

General procedures

The animals were weighed, anesthetized with sodium thiopental (45 mg/kg, i.p.; Farmotal, Pharmacia, Milan, Italy), paralyzed with pancuronium bromide (0.2 mg/kg, i.m.; Pavulon, Organon Italia, Rome, Italy), artificially ventilated and mounted in a stereotaxic apparatus. The head was stereotaxically oriented (Luparello, 1967). Supplementary doses of the anesthetic agent were administered during the experiment to maintain anesthesia at a level at which 4–10/s spindle waves were present in the EEG recorded from the frontal area. Pressure and wound points were infiltrated with 2% procaine solution. In order to avoid the swimming or athetoid movements that may be exhibited by the guinea pig even at a deep plane of anesthesia, the animals were paralyzed (see above) and artificially ventilated. Body temperature was maintained at 37–38 °C. The hippocampal formation was exposed bilaterally and maintained in a pool of warm mineral oil. The dorsal hippocampal commissure, the ventral hippocampal commissure and the interhippocampal commissural plane were transected to prevent the interhippocampal transfer of signals.

Stimulation

Electrical stimuli (square-wave pulses 3–40 V; 0.05 ms duration; 0.1/s frequency) were delivered to the dorsal hippocampal commissure (dorsal psalterium, PSD) through bipolar Epoxylite-coated tungsten microelectrodes (interpolar distance 0.2–0.3 mm, resistance in the brain 0.4–0.9 Mohm; Epoxylite Corp., Anaheim, CA, USA), with the inter-pole axis parallel to the PSD long axis. The electrodes were placed on the PSD ipsilaterally to the recording side, 1.5–2.5 mm lateral to the midline and close to the PSD caudal border.

Recording

Extracellular field potentials were monopolarly recorded by Epoxylite-coated tungsten microelectrodes (0.5–5.0 Mohm at 1000 Hz). Simultaneous stereotaxic recordings were made from layer II or II/III of the ENT, upper blade of the DG, and hippocampal fields CA3, CA2 and CA1. A screw in the skull served as reference electrode.

Data acquisition

A hardware/software Transputer-based system was used for the automatic and programmable control of stimulation, data-acquisition, and on-line visualization of the evoked potentials. The acquisition was made at 10–20 kHz per channel.

Placement of the electrodes

The recording electrode aimed at the dorsal ENT was oriented at 45° with respect to the vertical plane and placed at the lateral stereotaxic plane L 6.0–6.5. The electrode was lowered to layer II or II/III, based on the pattern of the evoked potentials (Bartesaghi et al., 1988). The recording electrode aimed at the upper blade of the dorsal DG was placed at the lateral planes L 2.0–5.0, approximately midway along the rostro-caudal extent of the upper blade, and lowered to the granule cell layer. The recording electrodes aimed at fields CA3, CA2, and CA1 were placed at the lateral planes L 2.5–5.0 and lowered to the pyramidal layer. The laminar location of the recording electrodes was recognized based on electrophysiological criteria (see first section of Results). In some experiments simultaneous recordings were performed from the synaptic site in the DG (stratum moleculare) and the source site in the DG (granular layer) and from the synaptic site in field CA2 (stratum lacunosum), field CA3 (stratum lucidum) and field CA1 (stratum radiatum) and from the source site in fields CA2, CA3 and CA1 (pyramidal layer), respectively (see first section of Results). In all structures, the magnitude of the positive wave recorded from the source site closely reflected the magnitude of the negative