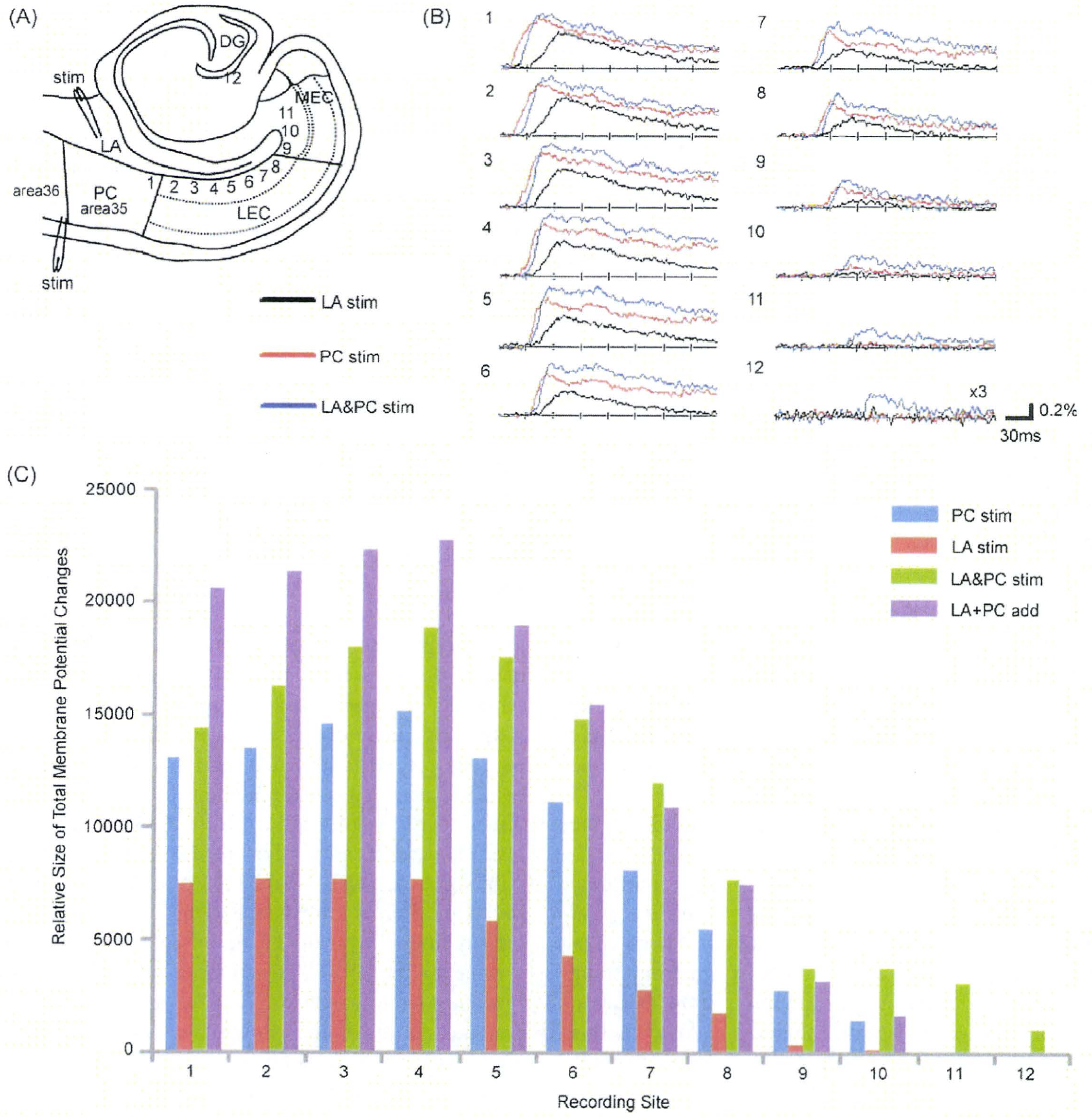

Fig. 4. Comparison of total membrane potential changes in various sites of the deep layer of rhinal cortices and the dentate gyrus caused by PC or LA stimulation, or co-stimulation of LA and PC. (A) Arrangement of stimulating electrodes and the sites (1–12) from which optical signals in (B) were collected. Each optical signal was measured from an area of 300 μm^2. (B) Optical signal traces obtained from the sites of 1–12 shown in (A). In each group of record (1–12), the optical signal trace of red was obtained by PC stimulation, black by LA stimulation and blue by co-stimulation of LA and PC. In 12, signals were shown by 3-fold magnification. (C) Relative sizes of total membrane potential changes. Number of column (1–12) represents the site of recording in (A). In each column, applied stimulations were represented with different colors. PC stimulation; blue, LA stimulation; red, co-stimulation of LA and PC; green, and the mathematical sum of the size of PC stimulation and LA stimulation; purple.

4. Discussion

The parahippocampal cortex, comprised of the perirhinal, postrhinal and entorhinal cortices, is the gateway to the hippocampus. It is critically involved in complex functions, such as memory, object recognition, sensory representation and spatial orientation (Suzuki and Eichenbaum, 2000; Murray and Richmond, 2001; Witter and Wouterlood, 2002). A number of sensory modalities most likely converge in the rat perirhinal cortex. The neural pathway from the perirhinal cortex to the entorhinal cortex is considered one of the main paths into the entorhinal–hippocampal network (Burwell and Amaral, 1998; Burwell, 2000; Burwell and Witter, 2002; Witter and Amaral, 2004). In horizontal slices of the rat brain near the rhinal sulcus, the neural circuit connecting the PC, EC, hippocampal formation and amygdala is preserved (Iijima et al., 1994, 1996; von Bohlen und Halbach and Albrecht, 2002; Kajiwara et al., 2003). Using such preparations, in a previous study we reported on the potential associative function of deep layers of perirhinal area 35 with respect to sensory and motivational stimuli and the influence of this association on the perirhinal–entorhinal–hippocampal neurocircuit (Kajiwara et al., 2003). In the present study, we conducted similar experiments with horizontal slices dissected out just ventral to the rhinal sulcus,

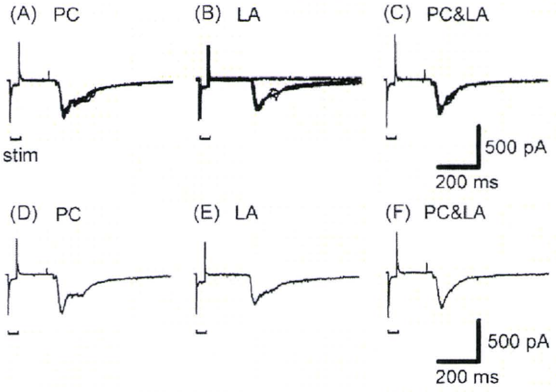

Fig. 5. Recordings of EPSCs from a neuron in layer V of LEC. Upper panel represent the typical EPSCs obtained from the same cell when the stimulation was applied to the superficial layer of PC (A), LA (B), and both LA and PC (C). Ten consecutive recordings are superimposed in (A)–(C), respectively. In the lower panel, a typical EPSC was selected from the EPSCs shown in the upper panel. In every experiment, the superficial layer of area 35 was stimulated.

as to maintain the circuitry between area 35 and the entorhinal cortex. This was achieved in 12/29 (41%) of the slices. In all these slices we found that the neural excitation caused either in the superficial layer of area 35 or in LA was successfully conducted to the dentate gyrus of the hippocampus and generated action potentials. We further observed that the transfer of neural activity from the superficial layers of area 35 or LA to the dentate gyrus, is mediated by the circuits in the deep layers V/VI of the entorhinal cortex.

Increasing attention is being paid to the role of the entorhinal cortex as a dynamic processor of information both entering and leaving the hippocampus. Evidence accumulated recently suggests that the EC and the perirhinal cortex, far from being passive relay stations, actively gate impulse traffic between neocortex and hippocampus, because they are endowed with a powerful intrinsic inhibitory system. It is proposed that the cross-talk between the PC and EC is not organized to unrestrictedly transfer information, but to select relevant inputs (de Curtis and Paré, 2004; Woodhall et al., 2005). The critical question is how the inhibitory control of perirhino–entorhinal communication is regulated, and how it influences the reciprocal information transfer between the neocortex and the hippocampus. The extrinsic excitatory inputs might overcome the local inhibition by causing a sufficient depolarization of projection cells. Another likely possibility is that they activate a subgroup of GABA interneurons that inhibit other GABA cells (Pelletier et al., 2005; Pinto et al., 2006), comparable to what has been demonstrated in the hippocampus, where calretinin-positive hippocampal interneurons only contact other interneurons (Gulyás and Freund, 1996). As a result, excitatory afferents to these local circuit cells would produce a disinhibition of principal projection cells.

In 7 slices out of 29 (24%), single stimulations in either PC or LA did not result in an eventual activation of the dentate gyrus, whereas we did observe the associative effect of the two stimulations. In all cases the site of stimulation turned out to be in area 36 and LA thus replicating our previous results (Kajiwara et al., 2003). The facilitation of spread of neural activity by two coincident inputs suggest the interaction of the two input pathways. We examined with VSD imaging where in the circuit this interaction between the two pathways is mediated. We hypothesized that if the cortical and amygdala pathways are absolutely independent, the sum of the size of neural event associated with only PC stimulation and that associated with only LA stimulation would be almost equal to the size of neural event associated with the co-stimulation of PC and LA. Our results show this is not the case. That is, a non-linearity is seen over all of the columns. Interestingly, in the deep layer of LEC, the size of neural event associated with the co-stimulation of PC and LA exceeds the sum of the size of neural event associated with only PC stimulation and that associated with only LA stimulation. This means that the association of the two different inputs strengthened the overall excitation in the more medial portions of LEC and even more so in MEC. These findings were taken to indicate that the integration of sensory and emotional inputs is most probably mediated by the circuitry in the deep layer of LEC. Our patch-clamp recordings in the deep layers of LEC are in line with this hypothesis. We showed that there are the cells in the deep layer of LEC that respond to both cortical and amygdala stimulation. Although we made patch-clamp recording on neurons whose shape was apparently pyramidal, we did not identify the class of neurons precisely. The distribution and classification of such cells are now under examination. In view of the fact that we aimed for pyramidal cells, it is quite likely that we included at least a sizable proportion of layer V projection neurons. Although it is well established that EC provides the major source of afferent information to the hippocampus primarily via the superficial layer of EC, via projections from layer II to the dentate gyrus, and CA3 and from layer III to CA1 (Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976; Tamamaki and Nojyo, 1993; Witter, 1993; Witter et al., 1988, 2000), an additional pathway connects the deep layer of the EC (layer V) to the dentate gyrus. The relevance of this connection, although described in both anatomical and electrophysiological studies (Köhler, 1985; Deller et al., 1996; Dugladze et al., 2001; Gloveli et al., 2001), remains obscure. The propagation of neural activity into the dentate gyrus by way of the deep layer of EC as well as the potential for integration of two input pathways in these deep layers as observed in this study indicates the functional relevance of layer V.

It is important to point out that the organization and thus the potential mechanism underlying the observed integration may not be that simple, since in the entorhinal cortex horizontal connections and vertical connections exist both in the superficial layer (layers I–III) and in the deep layer (layers V–VI), such that cells in the superficial and deep layers are functionally interconnected (Köhler, 1986; Lingenhöhl and Finch, 1991; Burwell and Amaral, 1998; Dolorfo and Amaral, 1998; Dugladze et al., 2001; Gloveli et al., 2001; van Haeften et al., 2003). However, the present findings indicate that activation in deep layers of the entorhinal cortex alone is sufficient to activate the dentate gyrus. We therefore suggest that our current functional concept of the entorhinal cortex as having layers II and III as input layers and layer V as the main output layer is in need for revision.

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