Network Activity Evoked by Neocortical Stimulation in Area 36 of the Guinea Pig Perirhinal Cortex

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Biella, Gerardo, Laura Uva, and Marco de Curtis. Network activity evoked by neocortical stimulation in area 36 of the guinea pig perirhinal cortex. J Neurophysiol 86: 164–172, 2001. The perirhinal cortex is a key structure involved in memory consolidation and retrieval. In spite of the extensive anatomical studies that describe the intrinsic and extrinsic associative connections of the perirhinal cortex, the activity generated within such a network has been poorly investigated. We describe here the pattern of synaptic interactions that subtend the responses evoked in area 36 of the perirhinal cortex by neocortical and local stimulation. The experiments were carried out in the in vitro isolated guinea pig brain. The synaptic perirhinal circuit was reconstructed by integrating results obtained during intracellular recordings from layer II–III neurons with simultaneous current source density analysis of laminar profiles performed with 16-channel silicon probes. Both neocortical and local stimulation of area 36 determined a brief monosynaptic excitatory potential in layer II–III neurons, followed by a biphasic synaptic inhibitory potential possibly mediated by a feed-forward inhibitory circuit at sites close to the stimulation electrode and a late excitatory postsynaptic potential (EPSP) that propagated at distance within area 36 along the rhinal sulcus. During a paired-pulse stimulation test, the inhibitory postsynaptic potential (IPSP) and the late EPSP were abolished in the second conditioned response, suggesting that they are generated by poli-synaptic circuits. Current source density analysis of the field responses demonstrated that 1) the monosynaptic activity was generated in layers II–III and 2) the sink associated to the disynaptic responses was localized within the superficial layer of area 36. We conclude that the neocortical input induces a brief monosynaptic excitation in area 36 of the perirhinal cortex, that is curtailed by a prominent inhibition and generates a recurrent excitatory associative response that travels at distance within area 36 itself. The results suggest that the perirhinal cortex network has the potentials to integrate multimodal incoming neocortical information on its way to the hippocampus.

INTRODUCTION

Clinical reports and animal studies demonstrated that the parahippocampal region supports complex cognitive functions, in particular related to memory formation, consolidation, and retrieval (Alvarez and Squire 1994; Amaral 1999; Braak and Braak 1993; Brown and Aggleton 2001; Brown and Xiang 1998; Gaffan and Parker 1996; Suzuki 1996; Young et al. 1997; Zola-Morgan et al. 1989). The two major sub-regions of the parahippocampus, the entorhinal cortex (ERC) and the perirhinal cortex (PRC), do not serve as mere cortical transfer areas of incoming inputs to the hippocampus, but retain integrative properties that are essential for memory processing. It has been demonstrated that selective ablations of the PRC in rats and monkeys affect visual discrimination (Buckley and Gaffan 1997, 1998; Meunier et al. 1993; Myhrer and Wangen 1996; Riches et al. 1991; Suzuki et al. 1993; Wan et al. 1999), associative memory (Murray and Bussey 1999), spatial memory (Liu and Bilkey 1998a,b; Wiig and Bilkey 1994), and odor recognition (Otto and Eichenbaum 1992a; Young et al. 1997) and exacerbated memory impairment induced by hippocampal lesions (Ennaceur and Aggleton 1997; Wiig and Bilkey 1995; Zola-Morgan et al. 1993). In addition, the demonstration that responses of PRC neurons can be enhanced or depressed during repetitive presentation of complex sensory stimuli (Otto and Eichenbaum 1992b; Xiang and Brown 1999; Young et al. 1997; Zhu and Brown 1995) suggested that the PRC is involved in memory representation and in some form of recognition memory (Brown and Xiang 1998; Bussey et al. 1999). The integrative role of the PRC in memory function is presumably sustained by the associative interactions among neurons within the parahippocampal network. The study of the connections within the PRC has been object of extensive anatomical investigations in different mammalian species (Burwell and Amaral 1998a,b; Burwell et al. 1995; Lavenex and Amaral 2000; Lopes da Silva et al. 1990; Van Hoesen and Pandya 1975). According to these works, neocortical inputs from the somatosensory, auditory, and visual uni-polimodal cortical areas project to the PRC (Burwell et al. 1995; Suzuki 1996; Suzuki and Amaral 1994), from where they are transmitted to the hippocampus proper either directly (Naber et al. 1997, 1999; Witter et al. 2000) or via a pathway mediated by the ERC (Burwell and Amaral 1998a; Lopes da Silva et al. 1990; Van Hoesen and Pandya 1975). In spite of the extensive anatomical studies, very little is known about the physiological features of the responses generated within the PRC by either neocortical or intrinsic PRC stimulation (Bilkey 1996; Cho et al. 2000; Zia-kopoulos et al. 1999). In the present study we performed a detailed electrophysiological study of the associative cortico-cortical interactions between neocortex and area 36 in the isolated guinea pig brain maintained in vitro, a preparation that allows a facilitated approach to the intact rhinal area through a direct visual control of the placement of the recording and stimulating electrodes. The present results have been communicated in abstract form (Biella et al. 2000).

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METHODS

After barbiturate anesthesia (sodium pentothal, 20 mg/kg ip) the brain of young adult guinea pigs (150–200 g, 4–6 wk old) was dissected out according to the standard procedure previously described in details (de Curtis et al. 1991, 1994a, 1998). The isolated brain was arterially perfused at 5.5 ml/min with a solution (composition, in mM: 126 NaCl, 3 KCl, 1.2 KH2PO4, 1.3 MgSO4, 2.4 CaCl2, 26 NaHCO3, 15 glucose, and 2.1 HEPES, with 3% dextran M.W. 70.000) oxygenated with a 95% O2-5% CO2 gas mixture (pH 7.3). Experiments were performed at 32°C. Stimuli were delivered via tungsten electrodes arrays formed by two or three electrode pairs vertically separated by 500–1,000 \( \mu \)m (FHC, Bowdoinham, ME). The tungsten electrode pairs were positioned at different depths within the cortical structures. Extracellular recordings were performed with either glass micropipettes (filled with 1 M NaCl) or stainless steel electrodes. Electrodes arrays formed by four to six stainless steel rods separated by 410 \( \mu \)m (provided by FHC) were also utilized for extracellular recordings. Lamellar profiles of evoked activity were performed by averaging four to seven responses recorded with 16-lead silicon probes (single recording sites separated by 50 \( \mu \)m on a single vertical shaft; kindly provided by Jamille Hetke of the Center of Neural Communication and Technology of the University of Michigan, Ann Arbor, MI). The position of the electrodes could be easily and rapidly modified during the experiment under direct visual control with a stereoscopic microscope. The field potentials were amplified (extracellular amplifier by Biomedical Engineering, Thornwood, NY), digitized via an ATMIO-64E3 National Instruments board, and stored on tape (Biologic, Claix, France) for off-line analysis. Intracellular recordings were performed with micropipettes filled with 2 M potassium acetate and 2% biocytine. The intracellular signals were recorded with a Neurodata amplifier (New York, NY). Data acquisition and analysis was performed by using CLAMPVIEW, a software developed in our Department by Gerardo Biella in collaboration with the Italian branch of National Instruments. Current source density analysis (CSD) was performed with a 200-\( \mu \)m differentiation grid on laminar profiles recorded with the 16-channel silicon probes (50-\( \mu \)m inter-electrode spacing) according to the standard procedure previously described (Biella and de Curtis 1995, 2000; Ketchum and Haberly 1993).

Electrolytic lesions performed at the end of the experiments were utilized to mark the position of both the stimulating electrodes and the silicon probes (see METHODS in Biella and de Curtis 2000). When intracellular biocytine injections were performed, brains were processed for biocytine–horseradish peroxidase visualization. After fixation in 4% paraformaldehyde, 75-\( \mu \)m sections were cut by vibratome, and intracellular biocytine was revealed by processing the sections with ABC kit (Vector Laboratories). Sections were counterstained with thionin to identify cortical layers.

RESULTS

Experiments were carried out on 38 isolated guinea pig brains. The borders between the neocortex (NC) and the PRC were identified on coronal sections of the guinea pig brain stained with thionin on the basis of cytoarchitectonic criteria previously described in the rat (Burwell and Amaral 1998b; Insausti et al. 1997). In comparison to NC, area 36 in the PRC shows a broader layer II and a less distinct subdivision in six layers. No recordings were performed in the most caudal part of the rhinal region, named postrhinal cortex.

The general pattern of propagation of associative excitation from the NC to area 36 was investigated in 16 experiments by positioning 2 array of 3 electrodes, each separated by 410 \( \mu \)m, along the rhinal sulcus in area 36 (see schematic drawings in Fig. 1). Stimuli were applied to the temporal NC region (2 mm dorsal to area 36) with pairs of stainless steel electrodes inserted at 100- to 300-\( \mu \)m depth. Area 36 recordings close to the NC stimulation site (electrodes 6 and 5 in Fig. 1A) showed an early negative potential followed by a positive potential (re-
cordings performed at 600-μm depth). At recording sites >800 μm caudal to the stimulus location, an isolated late depth-positive response, not preceded by the negative component, was observed (electrodes 1–4 in Fig. 1A). A similar pattern, with opposite rostro-to-caudal distribution, was observed when the NC stimulating electrode was positioned at the caudal end of the recording electrode array (close to electrode 1; not shown). Stimulation of superficial layers in area 36 itself induced biphasic negative-positive responses just caudal and rostral to the stimulation site (electrodes 3 and 4 in Fig. 1B), whereas late depth-positive potentials were recorded at more remote locations (electrodes 1, 2, 5, and 6 in Fig. 1B). Stimulation of deep NC layers induced either no response or small amplitude potentials in area 36 (n = 8). No consistent local response was induced within area 36 by local stimulation of deep layers (not shown; n = 3).

To evaluate the pattern of local network activation in area 36, CSD analysis of field potential laminar profiles was performed (n = 7) (Biella and de Curtis 1995; de Curtis et al. 1994b). Figure 2A illustrates a typical field potential profile

![Figure 2A](http://jn.physiology.org/)

**FIG. 2.** Perirhinal cortex potentials and currents evoked by NC stimulation. Current source density (CSD) analysis of NC-evoked field potential laminar profiles performed with a 16-channel silicon probe (50-μm inter-lead spacing) positioned in area 36, close (36c) and far (36f) from the stimulating electrode (st. NC in the drawing in A). In the *left column* in A, the laminar field potential profile evoked in area 36c is shown. The relative CSD profile is illustrated in the *right panel*. In B, the contour plot obtained from the CSD traces shows the distribution of current sinks and sources in the cortical layers in the position 36c (see Biella and de Curtis 2000 for details on contour plots). The correlation between the sinks/sources dipoles and the superimposed field responses recorded with the multi-channel silicon probe are shown. The dotted line marks the stimulus artifact. In C, recordings performed in the same experiment and relative CSD contour plot obtained in position 36f are shown. Separation between isocurrent lines 10 mV/mm².
recorded with the 16-channel silicon probe after superficial NC stimulation (FP profile; left column) and the relative extracellular current profile (CSD profile; right column). The distribution in time and space of the extracellular currents induced by NC stimulation are shown in the contour plot in Fig. 2B, where the field potential traces at different depths are superimposed on top of the contour plot. The results demonstrated that at recording sites close to NC stimulation an early current sink located at 250- to 400-μm depth with a 10- to 12-ms delay from the stimulus artifact correlated to the early surface-posi-

FIG. 3. PRC potentials and currents evoked by area 36 stimulation. In the drawing the positions of the stimulating electrode and recording electrodes are schematically represented. In A, the superimposed field traces recorded with the 16-channel silicon probe and the CSD contour plot obtained from area 36c (close to the stimulation site) are illustrated. In B, the same is shown for a recording site in area 36f, 1 mm rostral to the stimulation site. Recordings were performed in the same experiment. Separation between isocurrent lines 10 mV/mm².

FIG. 4. Correlation between intracellular potentials and field responses simultaneously recorded in area 36 after superficial NC stimulation. A: the early component of the field potential (recorded at 500-μm depth) correlates with an excitatory postsynaptic potential (EPSP) in a layer II principal cell in area 36; the late depth-positive potential is associated with the intracellularly recorded slow inhibitory postsynaptic potential (I-IPSP) and a late EPSP marked by the asterisk. The membrane potential reversal of the fast and slow components of the IPSP is also illustrated. The EPSP-IPSP and rebound spike are illustrated with a slower time scale in the inset. B: the amplitudes of the early (e-EPSP) and late EPSPs (l-EPSP) increased with membrane hyperpolarization. Note that the IPSP is depolarizing at resting membrane potential (RMP; horizontal dotted line = –73 mV).
aptic potentials (EPSPs). The first EPSP (e-EPSP) preceded the onset of a biphasic inhibitory postsynaptic potential (IPSP), whereas the second EPSP (l-EPSP, marked by the asterisk) occurred during the onset phase of the IPSP. In the large majority of recorded neurons, the early IPSP was depolarizing at resting membrane potential (average resting membrane potential (RMP) = -69 ± 5.9 mV, mean ± SE; dotted line in the neuron illustrated in Fig. 4). The time course and the membrane potential reversal of the early and late IPSPs (e-IPSP: −64.6 ± 4.3 mV; l-IPSP: −89 ± 2.1 mV; n = 7) were compatible with the activation of GABAa and GABAb receptor-mediated potentials, respectively.

The e-EPSP correlated with both the early depth-negative extracellular potential and the early sink located in layers II–III (Fig. 5). The late depth-positive wave and the sink-source dipole that peaked at 15–20 ms correlated to both the l-EPSP (marked by the asterisk) and the onset of the e-IPSP. The disynaptic nature of the e-IPSP and the l-EPSP was demonstrated by applying a pairing stimulation test with 30- to 50-ms stimulus intervals (Fig. 6; n = 4). The subtraction of the paired response at 40 ms (b) from the response evoked by a single shock (a) demonstrated the abolition of the e-IPSP and the l-EPSP in the conditioned response (trace b-a in Fig. 6). Not a single neuron recorded in layers II and III discharged an action potential before the IPSP on either NC or area 36 stimulation at resting conditions, suggesting that the IPSPs are generated from principal neurons in layer II and III neurons. Of 18 neurons recorded, 6 stained neurons showed either multipolar or pyramidal morphology. Similar intracellular/extracellular correlation patterns were observed following both superficial NC (n = 4) and local area 36 stimulation (n = 18). As illustrated in Fig. 4, stimulation induced two small-amplitude depolarizing potentials separated by approximately 7–8 ms that increased in amplitude on membrane hyperpolarization (Fig. 4B). On the basis of their membrane potential dependence, both potentials were interpreted as excitatory postsynaptic potentials (EPSPs). The first EPSP (e-EPSP) preceded the surface-negative/depth-positive component of the field response. The largest sink at this delay was located in the surface; a sink in deep layers (>500-μm depth) was observed in 3 of 10 CSD profiles. NC-induced active current events were restricted to the superficial 700 μm in area 36. The absence of local sinks or sources below 700-μm depth was verified in four experiments, in which the laminar profile was extended to 1,300 μm either by lowering the 50-μm inter-lead silicon probe or by using 16-channel probes with inter-lead separation of 100 μm. CSD analysis of laminar profiles performed in area 36 at the recording site rostral to the coronal level in which the NC stimulus was delivered (see schematic drawing in Fig. 2A) revealed a superficial sink with a delay >20 ms that correlated with a late field response (Fig. 2C). At recording sites both near and far from the stimulation, field potential components and associated sinks with a long delay from the stimulus artifact were seldom observed (see sinks with a >40-ms delays in the contour plots in Figs. 2, B and C, and 3A). Such responses were not analyzed in detail in the present study and were restricted to the network mechanisms that generate activity in the early 40–50 ms after the stimulus.

Figure 3 illustrates typical profiles evoked in area 36 by stimulation of area 36 itself (n = 12). As for the response to NC stimulation, at cortical sites close to the stimulation electrode (see scheme), a sink at 200- to 300-μm depth followed by a larger and longer-lasting sink-source dipole between 100 and 200 μm was observed (Fig. 3A). The late potential at 15–20 ms observed in area 36 at a distance higher than 800 μm from the coronal level of the stimulating electrode correlated to a superficial current sink that had the same depth location of the late sink in the close recording site (Fig. 3B; probe positioned at 1 mm from the stimulating electrode). No differences in current distribution pattern was observed if the stimulating electrode was positioned either caudal or rostral to the recording electrodes. The results obtained with CSD analysis demonstrated that quite stereotyped responses were generated in area 36 following both NC and local cortical stimulation.

CSD analysis allows to detect laminar-segregated excitatory synaptic potentials and population events but is not ideal to detect inhibitory synaptic potentials and spatially distributed events. Therefore, on the basis of the CSD analysis, only a partial picture of the events induced by NC and local area 36 stimulation can be extrapolated (Ketchum and Haberly 1993; Mitzdorf 1985). To further characterize the network activation pattern in area 36, extracellular laminar profile recordings with the 16-channel probe were coupled to intracellular recordings from principal neurons in layer II and III neurons. Of 18 neurons recorded, 6 stained neurons showed either multipolar or pyramidal morphology. Similar intracellular/extracellular correlation patterns were observed following both superficial NC (n = 4) and local area 36 stimulation (n = 18). As illustrated in Fig. 4, stimulation induced two small-amplitude depolarizing potentials separated by approximately 7–8 ms that increased in amplitude on membrane hyperpolarization (Fig. 4B). On the basis of their membrane potential dependence, both potentials were interpreted as excitatory postsynaptic potentials (EPSPs). The first EPSP (e-EPSP) preceded the onset of a biphasic inhibitory postsynaptic potential (IPSP), whereas the second EPSP (l-EPSP, marked by the asterisk) occurred during the onset phase of the IPSP. In the large majority of recorded neurons, the early IPSP was depolarizing at resting membrane potential (average resting membrane potential (RMP) = -69 ± 5.9 mV, mean ± SE; dotted line in the neuron illustrated in Fig. 4). The time course and the membrane potential reversal of the early and late IPSPs (e-IPSP: −64.6 ± 4.3 mV; l-IPSP: −89 ± 2.1 mV; n = 7) were compatible with the activation of GABAa and GABAb receptor-mediated potentials, respectively.

The e-EPSP correlated with both the early depth-negative extracellular potential and the early sink located in layers II–III (Fig. 5). The late depth-positive wave and the sink-source dipole that peaked at 15–20 ms correlated to both the l-EPSP (marked by the asterisk) and the onset of the e-IPSP. The disynaptic nature of the e-IPSP and the l-EPSP was demonstrated by applying a pairing stimulation test with 30- to 50-ms stimulus intervals (Fig. 6; n = 4). The subtraction of the paired response at 40 ms (b) from the response evoked by a single shock (a) demonstrated the abolition of the e-IPSP and the l-EPSP in the conditioned response (trace b-a in Fig. 6). Not a single neuron recorded in layers II and III discharged an action potential before the IPSP on either NC or area 36 stimulation at resting conditions, suggesting that the IPSPs are generated
by a feed-forward circuit (see DISCUSSION). As illustrated in Fig. 7, action potential could be generated exclusively by stimuli of very low intensity (30–40 μA). Interestingly, such a spike was followed by a very small amplitude IPSP. When the biphasic IPSP appeared by further increasing the stimulus intensity (>50 μA), the spike was abolished and the duration of the e-EPSP was shortened (n = 3).

In five experiments, local stimulation in area 36 at different rostral-caudal levels was delivered to mimic two separate inputs far and close to the recording site (see schematic drawing of the electrode placement in the inset in Fig. 8A). Stimulation of the far site evoked a late EPSP that increased in amplitude and duration by injecting into the cell a depolarizing current (Fig. 8A), whereas stimulation at the electrode positioned close to the intracellular recording site induced a typical EPSP-IPSP sequence (dotted line) that showed a shorter delay than the EPSP induced by far site stimulation. In all the cells recorded far from the stimulation site, no IPSPs were observed (n = 5), even when the membrane reached action potential threshold, as illustrated in Fig. 8A. Paired-pulse test using the stimulation site far from the recording electrode in area 36 neurons induced a complete abolition of the EPSP in the conditioned response (trace b-a in Fig. 8B).

**DISCUSSION**

In the present study, simultaneous, combined evaluation of intracellular potentials and extracellular laminar profiles was utilized to characterize the propagation pattern of synaptic activity in area 36 of the PRC, in response to both NC stimulation and intrinsic stimulation within area 36 itself. The electrophysiological study of associative interactions within the rhinal cortex has been largely facilitated by the ability to perform simultaneous extra/intracellular recordings and CSD analysis of field potential laminar profiles in the in vitro isolated guinea pig brain, a unique preparation to study limbic system physiology (Biella and de Curtis 2000; de Curtis et al. 1994b; Dickson et al. 2001; Paré et al. 1992). We demonstrate that synaptic excitation in area 36 (1) is curtailed by a prominent inhibitory disynaptic activity close to the stimulation site and (2) is effectively propagated at distance within area 36. These results confirm the observation recently reported in guinea pig horizontal PRC slices, which demonstrated the presence of EPSP-IPSP sequences and pure late EPSPs in PRC neurons located, respectively, close to and remote from the NC stimulating electrode (Martina et al. 2001).

In our experiments, similar responses were observed following superficial layer stimulation in the temporal NC and in area 36, which most probably activated the output fibers of layer II–III neurons that terminate in area 36 (Burwell and Amaral 1998a; Burwell et al. 1995; Deacon et al. 1983). NC/intrinsic stimulation of deep layers, which contain cells that participate to a lesser extent to the PRC projection, did not evoke a consistent activation pattern. CSD analysis of extracellular field profiles demonstrated that the associative inputs (both neocortical and intrinsic) induce a monosynaptic sink centered at 250- to 400-μm depth, presumably on the basal and apical dendrites of layer II–III neurons (Faulkner and Brown 1999). The experiments performed with multi-electrode arrays dem-

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**FIG. 6.** Mono- and polysynaptic responses in the intracellular responses evoked by area 36 stimulation in a principal cell of layer II. The recording was performed at resting membrane potential (~75 mV); the e-IPSP is depolarizing. Single stimuli to the NC (a) and paired stimuli at 40-ms (b) and 600-ms (c) intervals. The digital subtraction of the single from the paired responses (b-a and c-a) shows that the l-EPSP and the e-IPSP in the conditioned response are abolished, whereas the monosynaptic e-EPSP is preserved.

**FIG. 7.** Action potential firing in layer II–III cells is observed exclusively at low stimulation intensity. Intracellular recording from a multipolar neuron in layer II following NC stimulation at different intensities (values on the left). All recordings were performed at −60 mV (RMP = −75 mV). Weak stimuli induced an EPSP and a spike. Stronger stimuli determined the appearance of the IPSP, associated with the abolition of the action potential discharge.
The monosynaptic EPSP was followed by a biphasic IPSP. The early IPSP was depolarizing at resting membrane potential and showed a potential reversal compatible with the activation of GABAergic postsynaptic receptors in a cortical structure (Connors et al. 1988; Scharfman and Sarvey 1987). The time course and the reversal potential of the late IPSP was consistent with a GABAergic receptor-mediated response (Connors et al. 1988; Scharfman and Sarvey 1987). The IPSPs were presumably generated by the stimulus-evoked discharge of inhibitory interneurons with a superficial axonal arborization, similar to the neurons described in the layers I–II of the rat PRC (Faulkner and Brown 1999) or in the neighboring entorhinal cortex (Funahashi and Stewart 1998; Jones and Buhl 1993). The pairing test demonstrated that the IPSPs observed in our experiments are polysynaptic and excluded the possibility that they could be due to a monosynaptic activation mediated by the direct stimulation of inhibitory interneurons via extracellular diffusion of the current coupled to the electrical stimulus. Several indications suggest that a feed-forward circuit, and not a feed-back inhibition, mediates the IPSPs recorded in our experiments. First, the IPSP was evoked in the absence of action potential firing by principal layer II-III neurons. Indeed, we showed that these cells generate a spike on synaptic activation at low stimulus intensity exclusively; when the NC/intrinsic stimulus intensity was increased, the neurons cease to fire in coincidence with the appearance of the IPSP. Second, when a single action potential was activated at low stimulus intensity (see Figs. 7 and 8), no clear IPSP was observed, suggesting that recurrent inhibition generated by neuronal firing in this region is possibly weak. Accordingly, the spikes evoked in an area 36 neuron by remote NC stimulation was followed by a small-amplitude, if any, hyperpolarizing potential, whereas stimulation of the same cell with an electrode located close by induced a typical large-amplitude and biphasic IPSP (see Fig. 8). Interestingly, spikes were often generated with a long delay after the onset of the depolarizing EPSP. This observation may be related to the demonstration that PRC layer II–III neurons with variable morphology show typical delayed firing in response to a just supra-threshold intracellular current injection (Faulkner and Brown 1999). Finally, the time-to-onset of the IPSP is faster than the onset of the late EPSP, which is supposedly due to the activation of a recurrent excitatory circuit. These data suggest that feed-forward inhibition mediated through the activation of inhibitory cells by afferent stimulation controls the excitability of area 36 neurons. Moreover, the results of the experiments with remote and close by stimulations suggest that, as for the e-EPSP, the feed-forward IPSPs do not propagate at distance in the rostrocaudal dimension. Therefore we infer that the axonal arborization of area 36 interneurons that generate the feed-forward IPSP is spatially restricted in the rostrocaudal dimension. The presence of a prominent feed-forward activation turned on by strong incoming inputs could account for the recent demonstration of a marked paired-pulse depression of the NC-induced response reported in PRC rat slices in vitro (Ziakopoulos et al. 1999), a mechanism that has been associated with the decrease of PRC neuronal responses to familiar visual stimuli in a recognition memory task (Cho et al. 2000).
The superficial current sink demonstrated by CSD analysis in area 36 at both sites close to and distant from the stimulating electrode correlated to the e-IPSP and the late EPSP. It is likely that, at least at sites close to the stimulation electrode, the inhibitory synaptic events might contribute to the generation of the disynaptic superficial sink, since in our experiments the e-IPSPs were depolarizing at resting membrane potential; an outward current, i.e., an extracellular sink, is expected during the e-IPSP in these conditions. In addition, the presence of a sizable late superficial sink at sites remote from the influence of the feed-forward e-IPSP demonstrated that the late excitatory potential also contributes substantially to the extracellular current. Indeed, a late EPSP was identified in area 36 neurons following both NC and local stimulation in spatial vicinity and at a distance from the stimulation site. The disynaptic nature of both the e-IPSP and the l-EPSP was assessed by performing a pairing test; indeed, the reactivation of such potentials in the conditioned response was prevented when a shunting e-IPSP was induced by the first conditioning stimulus delivered 20–30 ms earlier (Biella et al. 1996). Since the large majority of the neurons in layer II–III did not generate an action potential at stimulus intensities that evoked the e-IPSP, what is the substrate for the generation of the disynaptic l-EPSP? Following our previous assumption that IPSPs do not propagate at distance, the demonstration that layer II–III principal cells discharge an action potential only at low stimulus intensity whereas disynaptic EPSPs can be activated also at high stimulus intensity (in the presence of a pronounced IPSP) lead us to speculate that the disynaptic EPSPs could be due to recurrent excitation mediated by the activation of neurons located at the periphery of the region of influence of the feed-forward inhibitory circuit, that fire an action potential.

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