Induction of a Calcium-dependent Long-term Enhancement of Excitability in the Rat Olfactory Bulb

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Abstract

We have investigated whether a transient increase in extracellular calcium concentration is able to induce long-term modification of neuronal excitability in the olfactory bulb. High-calcium artificial cerebrospinal fluid containing picrotoxin (Ca-PTX solution) was applied locally near the mitral cell layer through a push–pull device for 10 min in anaesthetized rats. Changes in the neuronal excitability were monitored through electrically-evoked field potentials. Application of the Ca-PTX solution induced a rapid increase in the granule cell response amplitude, whereas mitral/tufted cells response amplitude increased more progressively and reached its maximum within a few hours. The increase in mitral/tufted cells response and granule cells response reached between 30 and 100% in experiments which lasted for 4–8 h. Pre-application of amino-phosphonovalerate (NMDA receptor blocker) potently reduced both short- and long-term enhancement produced by the Ca-PTX solution. Neither application of the high calcium solution alone nor the picrotoxin solution alone induced long-term changes. The results point out the possible importance of Ca^{2+} and NMDA receptors in persistent forms of olfactory bulb plasticity. Relevance of this phenomenon in normal olfactory bulb physiology remains to be examined. **Chem. Senses 21: 159–168, 1996.**

Introduction

Several studies have clearly shown that the mammalian main olfactory bulb (OB) undergoes long-term functional and anatomical changes during olfactory learning (Freeman and Schneider, 1982; Leon, 1987). Others have suggested that the OB may be integral to the circuit for olfactory information storage (Brennan *et al.*, 1990; Lévy *et al.*, 1990; Kendrick *et al.*, 1992; Mouly *et al.*, 1993). Yet, it is still unknown which of the cellular characteristics is responsible for such modifications. One can imagine that the underlying mechanism is a long-term synaptic potentiation which has been revealed in different forms in several other areas of the mammalian brain (Racine *et al.*, 1983; Squire, 1986; Artola and Singer, 1987; Rasmusson and Dykes, 1988; Stripling *et al.*, 1988; Iriki *et al.*, 1989). In all these cases LTP or LTP-like phenomena were shown to be induced by repetitive high frequency stimulation (Kirkwood *et al.*, 1993). The high-frequency stimulation is, in fact, responsible for depolarizing the cell membrane potential allowing unblocking of NMDA receptors by Mg^{2+}. This results in an increase of Ca^{2+} influx and build-up of high

Ca^{2+} concentration. The Ca^{2+}, entering the cell, triggers a chain of biochemical reactions leading to the expression of LTP (Bliss and Collingridge, 1993). However, no such phenomenon has ever been described in the OB, even though OB cytoarchitecture (Shepherd, 1972), biochemistry (Halasz and Shepherd, 1983) and electrophysiology (Mori, 1987) are relatively well characterized. Electrical stimulation paradigms turned out to be inefficient to induce promptly long-term modifications of the bulbar response (unpublished data). Long-term enhancement in OB responsiveness following electrical paradigm was obtained only in awake rats receiving trains of high-frequency stimulation applied in the granule cell layer of the OB for at least three daily sessions (Stripling *et al.*, 1991). In this case, the component of evoked field potential (EFP) which showed a long-term enhancement had a latency of 30–40 ms from the electrical shock. This indicates that the enhancement likely resulted from the potentiation of excitatory feed-back from the piriform cortex. The ineffectiveness of electrical stimulation to induce rapid changes (i.e. within minutes) on early components of EFPs conceivably resides in the particular connectivity of the OB and the dynamics of its synapses. In fact, the inhibitory interneurons, granule cells (Gr) outnumber the relay neurons, mitral and tufted (M/T) cells, by about 200 to 1 (Shepherd, 1972). In addition, M/T cells possess very long secondary dendrites reaching up to 500–800 μm within the external plexiform layer (EPL) and thus receive numerous inhibitory inputs from Gr cells. Typically, the electrical activation of M/T cells through stimulation of the lateral olfactory tract (LOT) or the olfactory nerve (ON) leads to the excitation of a very large population of Gr (through dendrodendritic synapses) which, in turn, exert a strong inhibitory feedback for tens to hundreds of ms (Shepherd, 1972; Mori, 1987). Consequently, during tetanic stimulation, pulses which fall within the inhibition period fail to evoke an action potential in the M/T cells. For example, during tetanic stimulation of the LOT at 27 Hz one of every two pulses fails to evoke an antidromic action potential (Mori *et al.*, 1977).

The present experiment was designed to test whether a long-term change in the neuronal excitability can be triggered in the OB by direct application of Ca^{2+}. This procedure was found to be very efficient in the hippocampus both *in vitro* (Turner *et al.*, 1982) and *in vivo* (Bliss *et al.*, 1984). The reduction of the overall inhibition was also shown to be either facilitating or necessary for inducing LTP in the hippocampus and the neocortex (Wingström and Gustaffson, 1983; Artola and Singer, 1987; Buzsaki *et al.*, 1987; Chavez-Noriega *et al.*, 1989). Electrophysiological response to electrical stimuli was assessed by recording evoked field potentials (EFPs). OB EFPs present the advantages that (a) they reflect the response of both populations of neurons, M/T cells and Gr cells; and (b) they are very stable over time in the absence of any treatment (Mouly *et al.*, 1995). A large body of experimental data and theoretical considerations explains the generation of the early components (Rall and Shepherd, 1968; Mori, 1987). The two early components obtained following antidromic stimulation reflect M/T cell somata and dendrites depolarization (component I), and then Gr cell synaptic activation (component II). With orthodromic stimulation, the two early components reflect synaptic excitation in the glomerular layer (component I) and Gr cell synaptic activation (component II). The feedback inhibition can also be assessed using twin-shock stimulation. EFPs also proved to be useful to test the effect of local pharmacological treatments in the OB (Jacobson *et al.*, 1986, 1990; Elaagouby *et al.*, 1991).

Materials and methods

Material and procedure

The experimental set-up and procedure were described previously in Elaagouby *et al.*, (1991). Male Wistar rats (200–300 g) were anaesthetized by i.p. injection of 60 mg/kg of pentobarbital which was supplemented every hour by 20 mg/kg. The anaesthetized animal was placed in a stereotaxic apparatus. Two windows were drilled into the skull, one over the OB and the other over either the LOT or the ON. The dura was carefully removed using a hypodermic needle. A stimulating electrode (400 μm OD tungsten concentric bipolar electrode, Rhodes Instruments) was implanted according to stereotaxic co-ordinates (Paxinos and Watson, 1982), either into the ON or into the LOT. Two monopolar recording electrodes (80 μm diameter, 15 kΩ maximum impedance at 1 kHz) were glued to the outer cannula (800 μm diameter) of the concentric push–pull cannula. Electrode tips protruded from the inner cannula tip by 100 and 800 μm for the proximal and distal electrodes, respectively. Recording electrodes were Teflon-coated except at the tip. The device was lowered into the OB and placed such that the proximal electrode was in the mitral cell layer. Typically, this was indicated by the inversion of the polarity of most EFPs' components (Figure 1). In this layer the multi-unit activity (of mitral cells) reached its maximal amplitude. In this configuration the tip of the distal electrode was located in the granule cell layer which shows low multi-

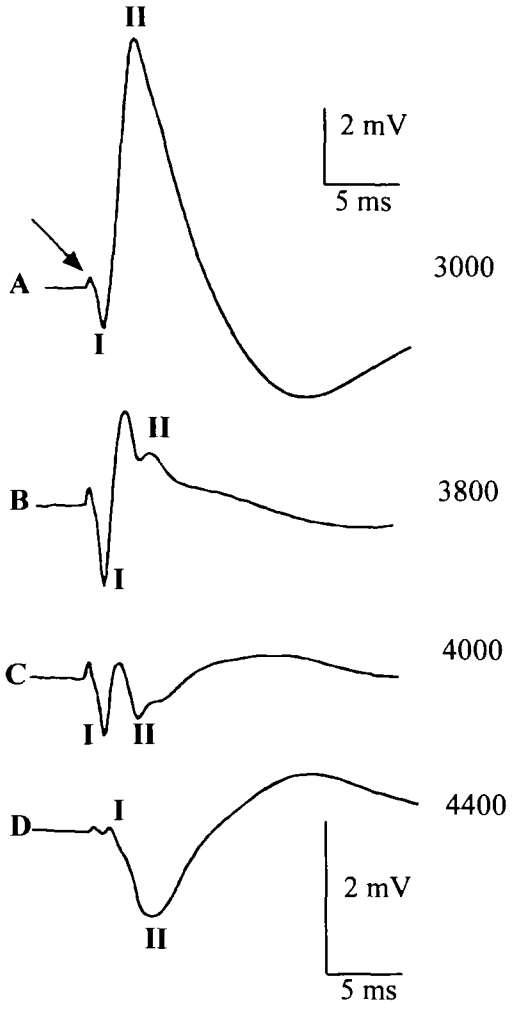

Figure 1 Depth profile of averaged antidromic EFPs obtained in the ventral region of the olfactory bulb. Depth of each recording is indicated at the right from 3000 to 4400 μm. Trace in B shows an additional component between component I and II ascribed to dendritic EPSP which can be detected only at this depth where component II presents its lowest amplitude. As shown in Figures 2, 5 and 6, measures of component I and II were taken from EFP as recorded from granule cell layer as in A. Note inversion of polarity of both components I and II from B to D. Similar reversal was obtained with orthodromic stimulation. Arrowhead points to stimulus artifact. A, top scale bars; B, C and D bottom scale bars

unit activity and a large positive component II of the EFP. The data presented in this paper are those collected from the distal electrode.

Stimulation was delivered once every 10 s. A stimulation consisted of either a single pulse (100 μs square pulse) or a twin-shock consisting of two single pulses delivered 30 ms apart for LOT stimulation and 80 ms for ON stimulation. Stimulus intensity was about 200 μA (four times the threshold intensity). The activity was band-pass filtered (1–3000 Hz). Each EFP was digitized by an A/D converter (Bakker Electronics BE485) interfaced with a PC-computer. Ten successive recordings were averaged and the mean and the variance were stored on disk for subsequent analysis. EEG activity was low-pass filtered (cut-off frequency: 200 Hz) and digitized at 200 sample/s.

The artificial cerebrospinal fluid contained (in mM) 118 NaCl, 4.7 KCl, 1 Na_2HPO_4, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 24 $NaHCO_3$ and 10 glucose. After oxygenation for 20 min this solution had a pH of 7.4. The Ca^{2+}-enriched solution contained 10 mM of $CaCl_2$. Neuro-active drugs were dissolved in artificial cerebrospinal fluid a few minutes prior to use. These included picrotoxin (GABA-A receptor blocker), and DL-2-amino-5-phosphonovaleric acid (APV, NMDA receptor blocker) (purchased from Sigma).

After positioning the push–pull device in the OB, the artificial cerebrospinal fluid was introduced at the lowest flow rate. Perfusion was controlled by a low-flow-rate peristaltic pump. The flow rate was then increased progressively to 20 μl/min while monitoring the EFPs. If changes in the amplitude of EFPs due to mechanical stress exceeded 5% of the signal, the experiment was discarded. Perfusion was then maintained throughout the experiment at a constant flow rate.

Each EFP value is the average of 10 single recordings. After an equilibration period of 45 min, three EFPs samples were taken with a 10-min interval between each sampling. The average of these three values was considered the control value. The values from those recordings were used as a control to normalize the subsequent recordings. The perfusion was then switched for 10 min to the high Ca^{2+} solution. In some experiments the solution also contained 400 μM of picrotoxin (Ca-PTX solution); in others, perfusion was switched for 10–15 min to artificial cerebrospinal fluid containing 500 μM of APV before the application of the Ca-PTX solution. EFPs recordings were made at the end of each treatment and then every 30 min for 3–8 h.

Data analysis

For each recording, the mean amplitudes of components I and II of both conditioning and test EFPs were measured and expressed as a percentage of control values. As stated in the results (see next section), the changes observed in different animals covered a wide range. We found a linear relationship between the initial amplitude of EFP component II (in mV) and the percentage change induced by Ca^{2+}. Therefore, in order to represent averages of values from different experiments the data were scaled using the parameters of the straight line describing the best fit of the

data from all experiments (Figure 4). Analysis of variance (ANOVA1) was used to test the evolution of the amplitude value as a function of time, and the two-tail analysis of variance (ANOVA2) was used to test the difference between two different protocols. Paired student *t*-test was used to compare values at different time points.

Results

Induction of long-term changes

Following 10-min applications of the Ca-PTX solution, the following effects were observed (Figure 2). With antidromic stimulation (n = 7), component II amplitude started to increase shortly after the infusion. The increase usually continued for 5–10 min after switching back to normal artificial cerebrospinal fluid, peaking at around 20 min. After a slight reduction, the amplitude remained well above control until the end of the experiment. Amplitude of component I showed a clear-cut increase, but not until 1 h after the end of the application of the Ca-PTX solution. On average, this value tended to increase slightly until the end of the experiment (Figure 3). With orthodromic stimulation (n = 5), granular cell response (component II) was also enhanced for several hours (Figures 2 and 3). As seen in Figure 2, the duration of component II was also enhanced, but this was not a systematic observation. Importantly, amplitude of component I which reflects excitatory intra-glomerular transmission was unchanged.

Figure 3 shows the average time course of M/T and Gr response to antidromic (n = 7) and orthodromic (n = 5) EFPs. Granule cell response amplitude (component II) increased rapidly during and after the application period, reaching an average increase (at 10 min) of 51% for LOT-EFPs and 11% for ON-EFPs. However, individual values from different experiments ranged from 27 to 98% for LOT-EFPs and 7 to 100% for ON-EFPs. The wide range of the average increase likely reflects differences in the thickness of OB layers at different sites. After the first 30 min, the amplitude of Gr response (component II) started to decrease. It stabilized after approximately 2 h in the range of 36–48% for LOT-EFP and 25–30% for ON-EFPs.

M/T response increased slowly and almost continuously for 2 h and then stabilized. The average increase was in a range of 35–45% for LOT-EFP and 20–25% for ON-EFP. ANOVA1 showed that the changes occurring in the first 2 h were significant ($P < 0.001$). The paired student *t*-test showed that the changes are highly significant starting at 30 min ($P < 0.001$). In four out of seven LOT stimulation experiments, the experiment lasted 8 h. In those cases, a further increase occurred between 4 and 5 h (not shown)

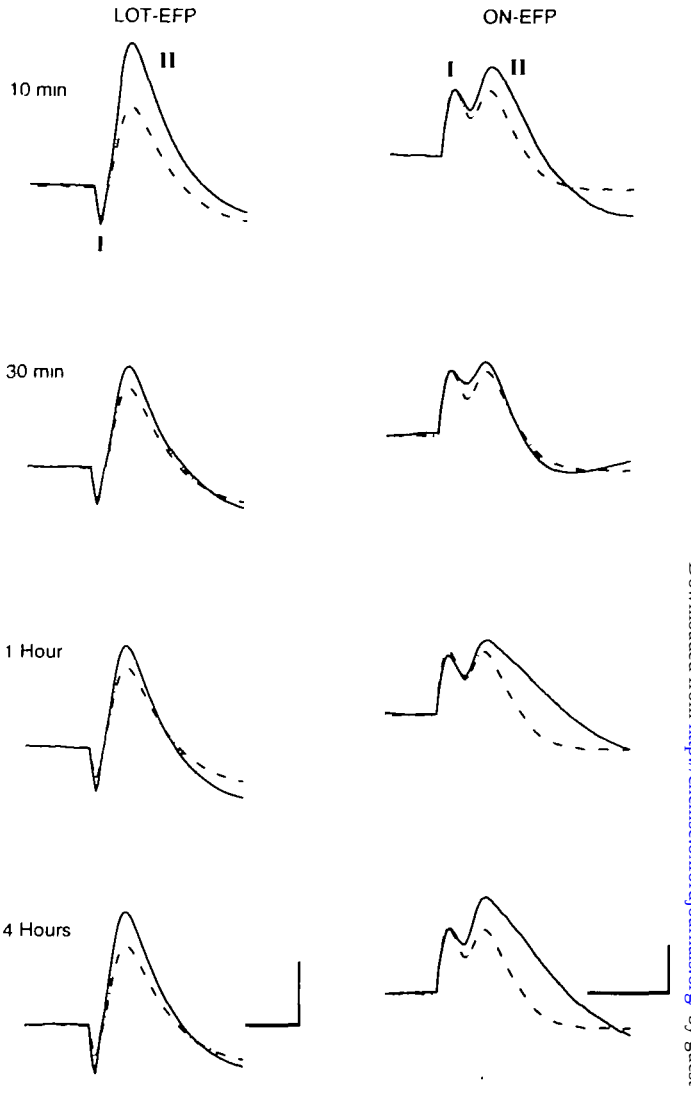

Figure 2 Examples of the evolution of EFPs recorded in the granule cell layer following perfusion for 10 min with the Ca-PTX solution (Ca^{2+} 10 mM; PTX 400 μM) near the mitral cell layer through the push–pull device. Each trace is an average of 10 consecutive recordings. The control is shown as a dash-and-dot line trace. Traces in solid lines are, from top to bottom, recordings at 10 min, 30 min, 1 h and 4 h. The plots in the left and right columns represent examples of LOT-EFP and ON-EFP, respectively. The stimulation artifact was removed by hand for clarity. For LOT-EFP note that changes in component I can be seen only after 1 h, while the massive enhancement in component II did not return to the control value. For ON-EFP note the stability of component I together with the persistent increase in amplitude and duration of component II. Application for 10 min, of the high Ca^{2+} solution without PTX produced the effect shown at the time point 10 min, but the effect vanished within 1 h. PTX alone did not affect EFPs. Scale bars represent on the left 5 ms horizontal, vertical 1 mV; on the right, horizontal 10 ms, vertical 0.5 mV. Roman numbers I and II refer to EFP components.

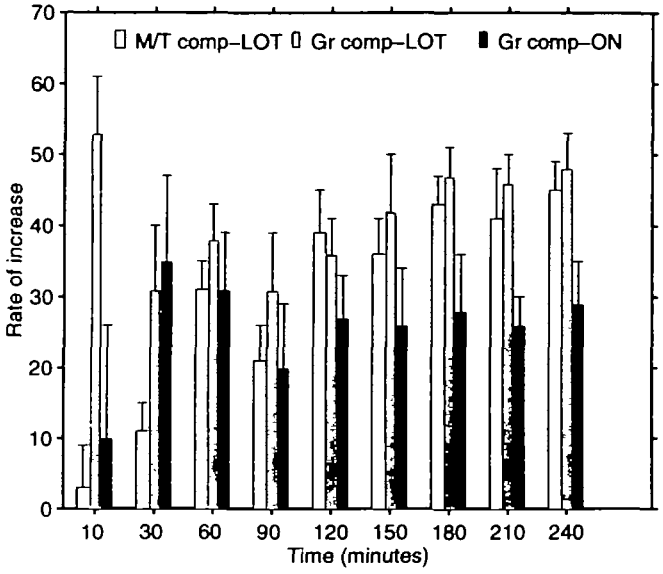

Figure 3 Average time course of rate of increase of EFP components following application for 10 min of the Ca-PTX solution The bars represent values for antidromically EFP (n = 7) for component I (M/T comp-LOT) (white bars) and component II (Gr comp-LOT) (gray bars) as well as for orthodromically EFP (n = 5) for component II (Gr comp-ON) (black bars). Since amplitude of component I of ON-EFP remained very stable for the whole duration of the experiment, it was omitted for clarity

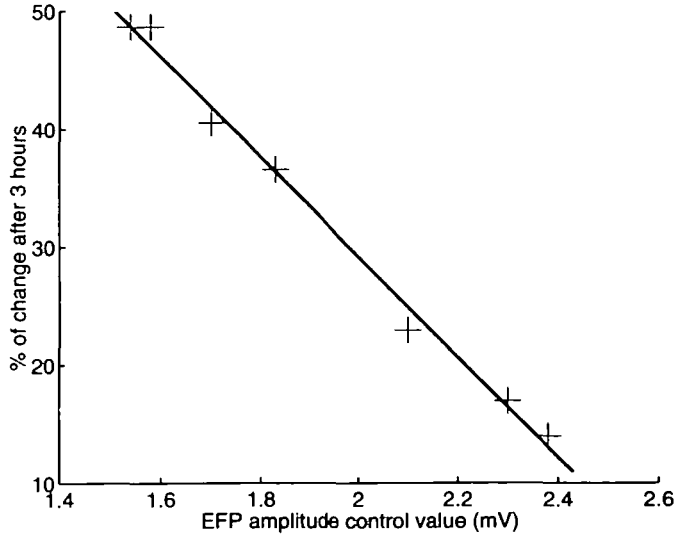

Figure 4 Inverse relationship between the increase rate in component II of LOT-EFP at the time point 3 h and its initial amplitude before application of the Ca-PTX solution. Each data point is from a single experiment The straight line representing the best fit has a = −42.47 and b = 114.14. (r = −0.92, P < 0.001).

reaching an average rate of increase at 6 h of 90 and 96% for M/T and Gr response, respectively.

There was a large variability in the rate of increase between different experiments. However, there was an inverse linear relationship between the increase rate and the initial value of the EFPs' amplitude, although this is true only for time points starting after the first hour following the application of the Ca-PTX solution. This relation shows that the treatment had a relatively larger effect when the initial amplitude of EFP was relatively low (Figure 4).

The twin-shock stimulation shows important changes in the amplitude of the test volley (Figure 5). Soon after the end of the application of the Ca-PTX solution, the amplitude of both components of the test-EFP was severely depressed. The average ratio of component II test/conditioning volley fell from 0.81 to 0.19 at the time point 10 min. (P < 0.001). The importance of the early decrease of the test volley was correlated to the increase of the conditioning volley (n = 7 antidromic stimulation, r = −0.95). At the time point 30 min, the test volley recovered promptly with an average ratio slightly over control (0.92, NS). At the time point 4 h, the average ratio (0.84) was very close to the control value (0.83). Thus, the evolution of the ratio showed that the strength of feed-back inhibition was enhanced soon after the application of the Ca-PTX solution, but later returned to control values.

The observed effects were obtained under continuous perfusion with artificial cerebrospinal fluid and were induced by transient introduction of a high Ca^{2+} solution containing picrotoxin. The impact of each of these parameters was examined separately. The effect of prolonged perfusion at the same flow rate and OB cell layer as those of the present study was carefully tested in a recent study (Mouly *et al.*, 1995). During a 2-h continuous application, variation in component I and component II amplitude and variation in the ratio of the conditioning/test volley never exceeded 5% when compared to baseline level. Similarly, in the present study, variations in components amplitude obtained from the three samples taken 10 min apart to establish the control value were in the range of 4–5%. Thus, under continuous perfusion, EFP characteristics were very stable.

A 10-min infusion of the high Ca^{2+} solution, without picrotoxin, produced no detectable effect on component I and a reversible increase in component II amplitude (LOT-EFP, n = 4). Typically, component II amplitude started to increase shortly after the 10-min infusion of the high Ca^{2+} solution. The increase usually continued for 5–10 min after switching back to artificial cerebrospinal fluid and peaked at +42% at the time point 10 min (P < 0.001). This effect was likely due to amplification of synaptic transmission in the presence of high extracellular Ca^{2+} concentration. The amplitude of component II did not statistically differ (+4%) from control at time point 1 h and remained stable until the

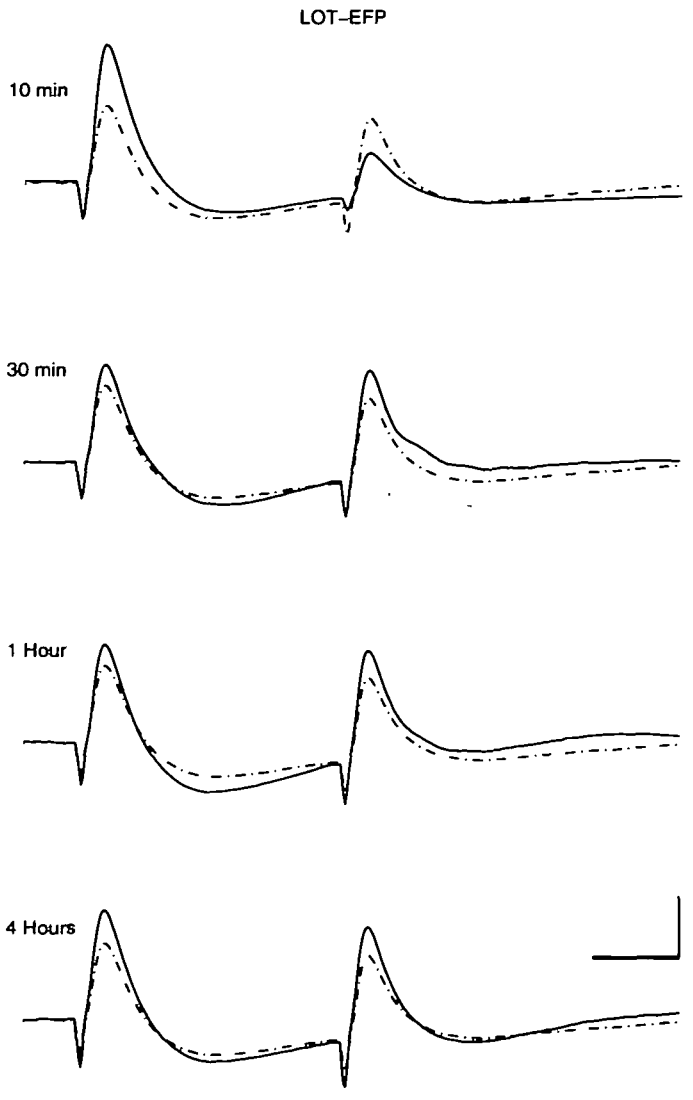

Figure 5 Typical time course of LOT-EFP induced by paired-pulse stimulation (200 μA, four times above threshold) with 30 ms interval following application of the Ca-PTX solution. Each pair-pulse was delivered every 10 s (n = 10). The dash-and-dot line is the control before application of the Ca-PTX solution and solid line represents from top to bottom plot EFPs recorded at 10 min, 30 min, 1 h and 4 h. after the treatment. Note the persistent increase in the amplitude of the conditioning response, the early decrease of test response followed by its progressive recovery. From time point 30 min, the ratio of the amplitude of component II of the test/conditioning volley did not differ when compared to the control value. Scale bar: horizontal, 10 ms; vertical, 1 mV.

end of the experiment. This effect was contrary to when high Ca^{2+} was co-applied with picrotoxin. Thus, perfusion for 10 min with high Ca^{2+} solution without picrotoxin produced no long-term effect. This finding also pointed out the fact that an excess of Ca^{2+} can be washed out, and that high Ca^{2+} application did not provoke a Ca^{2+} accumulation which could have been responsible for long-lasting changes in EFP characteristics.

Application of picrotoxin alone (400 μM) for 10 min produced no significant effect in LOT-EFP neither on component I nor on component II amplitude at any time points (n = 4). However, as previously reported (Elaagouby and Gervais, 1992) this treatment induced spontaneous synchronous discharges in the local field potentials for about 1 h. Fast Fourier transform analysis showed an enrichment of the frequency band around 25 Hz. However, no detailed study of the spectral changes was carried out. In six experiments lower doses of picrotoxin (<400 μM) were applied. In this case neither spontaneous local field potentials nor long-term changes of the EFPs developed. Thus, picrotoxin alone induced no change in EFP amplitude except transient development of spontaneous discharges in the local field potential recorded in the granular cell layer.

In summary, long-term changes in EFP amplitude observed following application of the Ca-PTX solution were not reproduced by application of either Ca^{2+} alone or picrotoxin alone. In addition, variations in the characteristics of EFP observed under continuous perfusion with artificial cerebrospinal fluid were very small when compared to those obtained following the Ca-PTX solution.

Effect of NMDA receptor antagonist

Autoradiographic studies show a high density of NMDA receptors in the OB, particularly in the external plexiform layer (Sakurai *et al.*, 1991). In this region, the density is 33% of that in the CA1 region of the hippocampus, the area of maximal NMDA receptor density in the rat brain. Interestingly, several forms of synaptic plasticity depend on the activation of NMDA receptors (Artola and Singer, 1987; Kauer *et al.*, 1988). The involvement of NMDA receptors in the long-term effect of Ca^{2+} was studied by blocking these receptors with APV (500 μM), either prior to or during the application of Ca-PTX solution. As illustrated in Figure 6b, in the presence of APV the increase in component II was small (+15%, n = 5, $P > 0.5$). This is in contrast to the early large increase induced following application of the Ca-PTX solution (+ 52%, n = 7, $P < 0.001$). At the time point 60 min, average values of component I and II did not differ significantly from the control values. For ON-EFP (n = 4) , APV also largely reduced the effect of the Ca-PTX solution. In fact, at the time point 10 (Figure 6c) and 30 min (Figure 6d) component I and II did not vary significantly when compared to control values (in each case, $P > 0.5$).

Finally, the effect of APV alone was tested in a set of experiments. This study involved five rats with LOT-

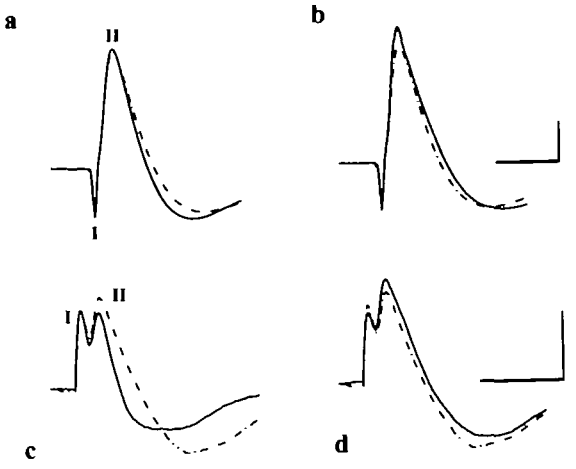

Figure 6 Typical effects of transient application of APV (500 μM) on EFP amplitude in absence and in presence of the Ca-PTX solution. **(a)** LOT-EFP, 10 min after its application, APV alone did not affect components I and II. **(b)** LOT-EFP, 10 min following application of the Ca-PTX solution containing APV. In the presence of APV component II of LOT-EFP increase by 5% only, while this value reached in average +52% following push–pull infusion of the Ca-PTX solution; **(c)** ON-EFP, 10 min after its application, APV alone induced a decrease in component II amplitude but this effect vanished within 30 min **(d)** ON-EFP, 10 min following application of the Ca-PTX solution containing APV Again, the presence of APV counteracted the effect produced by the Ca-PTX solution Dash and dot line is the control EFP recorded under perfusion with artificial cerebrospinal fluid, and solid line is the EFP recorded after the treatment. Scale bars: (a and b): horizontal 5 ms, vertical 1 mV, (c and d), horizontal 10 ms, vertical 0.5 mV.

EFPs and three rats with ON-EFP. APV alone (500 μM for 10 min) produced no significant changes in the amplitude of EFP in single pulse stimulation (Figure 6a). With paired-pulse stimulation, the response to test volley increased transiently. For example, with LOT stimulation, at the time point 30-min component I and II of the test volley were increased by 21 and 20%, respectively (in each case, $P < 0.001$). This resulted in an increase in the ratio conditioning/test volley. Importantly, this effect vanished within 60 min after the end of APV application. This result reproduced findings reported by Jacobson *et al.* (1986, 1990).

Discussion

Specificity of the action of the calcium-picrotoxin solution

Since long-term changes in EFP amplitude were observed following the application of a high Ca^{2+} solution containing picrotoxin and under continuous push–pull perfusion one has to consider the effect of each parameter independently. First, as reported in previous studies (Elaagouby *et al.*, 1991; Mouly *et al.*, 1995), flow rates were chosen so that the mechanical stress of a continuous perfusion with artificial cerebrospinal fluid did not induce variations in EFP amplitude over time larger than 5%. Similarly, in the present experiment, all data were collected in animals showing spontaneous variations in EFP amplitude of no more than 5% during the hour which preceded the treatment. It is thus very unlikely that changes in the range of 20–40 % observed following the 10 min application of the Ca-PTX solution were due to the sole perfusion. A second issue is the dosage used. Drug doses were chosen so that each agent could be washed out within 60 min without producing any irreversible effect when applied alone. Indeed, the results showed no significant changes in EFP amplitude 1 h following application of the high Ca^{2+} solution alone or picrotoxin alone. Thus, long-term changes could be attributed to the combination of the Ca^{2+} and picrotoxin effects. However, it remains to be investigated whether Ca^{2+} is necessary only as a current carrier or as an inductor of specific metabolic events.

In the present study, the concentration of Ca^{2+} in the high-Ca^{2+} solution was 10 mM. This concentration is higher than the one used to induce LTP in hippocampal slices (4 mM during 7–10 min; Turner *et al.*, 1982; Reymann *et al.*, 1986). However, the same concentration was used *in vivo* with push–pull technique to induce LTP (Bliss *et al.*, 1984). The higher concentration required *in vivo* is due to the low rate of exchange between the perfusing medium and the tissue. Indeed, pre-experiments revealed that this rate of exchange was only 10%. Moreover, the 800 μm distance separating the infusion site from the recording site is likely to contribute to a further reduction in extracellular Ca^{2+} concentration near the recording electrode. Another important point is related to the measurement of component I of antidromic EFP which could be contaminated by stimulation artifact. This is unlikely because (a) stimulation artifact occurred 2 ms before component I; (b) typical depth profiles were routinely observed showing inversion in component I polarity near the mitral cell layer, while the polarity of stimulation artifact remained unchanged; and (c) the artifact amplitude remained stable while component I amplitude varied over time according to the treatment.

Interpretation of the results and possible mechanisms

The effects observed soon after the application of the Ca-PTX solution (time point 10 min) were an early increase in component II amplitude of the conditioning volley, and an enhanced feed-back inhibition. Both effects are likely a

consequence of increased neurotransmitter release in the presence of a high extracellular Ca^{2+} concentration which amplified the reciprocal synaptic transmission. This situation is equivalent to increasing the stimulation current intensity, which leads to an increase of the conditioning EFP and a decrease of the test EFP. In the absence of picrotoxin, high Ca-induced changes returned to control values within 1 h. Thus, the changes induced by the co-application of Ca^{2+} and PTX are considered to be long-lasting after 1 h from the application (e.g. after complete wash-out of exogenous Ca^{2+} excess). Long-term modifications were expressed as a 30–40% increase in M/T cells and Gr cells response to antidromic stimulation both for conditioning and test volley. One hour after the treatment, the ratio of the test/conditioning volley did not significantly differ from the control value.

Orthodromic stimulation showed no changes in the strength of olfactory nerve to M/T cells transmission. Therefore, it is likely that the long-term effect took place in the deep layers where the infusion was done. The M/T-Gr interactions in deep layers are a good candidate for such changes. Finally, since long-term changes were abolished by the presence of APV, NMDA receptors seem to play a key role in the long-lasting effect of Ca^{2+}. As mentioned above, this also argues against a possible effect due solely to accumulated extracellular Ca^{2+}. In addition, this shows that it is unlikely that the Ca^{2+} effect would have resulted from diffusion to other brain structures. Indeed, the high buffering capacity of the tissue is able to rapidly reduce Ca^{2+} concentration. Thus, Ca^{2+} cannot diffuse in significant concentrations to piriform cortex where long-term changes were shown to be induced following electrical stimulation of the OB (Stripling *et al.*, 1991).

The mechanisms underlying the long-term changes in excitability we have observed are still unclear. An enhancement of M/T responsiveness can arise from changes in both intrinsic membrane properties and in synaptic interactions with interneurons. Clearly, further experiments are required to resolve this issue. However, several lines of evidence suggest that Ca^{2+} affects M/T cells activity by acting on their secondary dendrites. Intracellular recordings from the turtle OB have revealed a Ca^{2+}-dependent bursting activity ascribed to self-excitation (Nicoll and Jahr, 1982), while stimulation of ON evokes a slow excitatory component due to long-lasting depolarization. This is suspected to be mediated by Ca^{2+} conductances with slow kinetics in the secondary dendrites measured both with electrical (Nowycky *et al.*, 1981; Mori, 1987) and optical signals (Cinelli and Salzberg, 1990). Recently, Ca^{2+} imaging in single M/T cell in the salamander OB clearly showed that ON electrical stimulation or odor stimulation give rise to long-lasting Ca^{2+} activity in secondary dendrites (Cinelli, 1995). In our case, Ca^{2+} entry in M/T secondary dendrites mainly following activation of NMDA receptors could have enhanced output cells activity and/or could have triggered metabolic events leading to long-term changes. Several lines of evidence show that activation of NMDA receptors in the external plexiform layer produce rapid changes in M/T and Gr cell activities (Sakurai *et al.*, 1991; Trombley and Shepherd, 1993; Garcia *et al.*, 1995). Alternatively, the time course of the enhanced bulbar EFP response in the h following treatment supports the hypothesis of metabolic events. The time course in the enhancement of bulbar EFPs amplitude was similar to the one observed in hippocampal slices following high Ca^{2+} (Turner *et al.*, 1982; Reymann *et al.*, 1986) and in piriform cortex following theta burst stimulation of M/T cells axons (Jung *et al.*, 1990)

In the present study, the application of the GABA-A receptor blocker picrotoxin did not affect EFP amplitude, but its presence was required for the expression of Ca-induced long-term changes. This is similar to the induction of LTP in neocortex, where GABAergic blockade is necessary (Artola and Singer, 1987). However, this is in contrast with induction of LTP in the hippocampus where GABAergic blockade is only facilitating. The most prominent feature of GABAergic blockade in the OB was the enhancement in the synchronization in the Gr cells activity. This was manifested as large spontaneous field potential deflections occuring cyclically at the breathing rhythm. Thus, picrotoxin may not have played a role in the induction of long-term effects by blocking the Gr cell to M/T cell inhibition, but rather by reducing reciprocal Gr-to-Gr inhibition (Wellis and Kauer, 1994). Synchronous Gr cells activity should produce a synchronous feed-back inhibition on output cells, thus setting bursting discharge in a large population of M/T cells which may be responsible for Ca^{2+} entry into M/T cells dendrites. The importance of mutual inhibition in the setting of coherent oscillatory activity has been recently demonstrated in hippocampal slices (Whittington *et al.*, 1995), while in the salamander OB, addition of bicuculline enhances the magnitude, the duration and the spatial spread of Ca^{2+} flow into M/T cell dendrites (Cinelli, 1995). Finally, one can not rule out the possibility of a persistent reduction of the level of inhibition in the long-term effect described in the present study.

In conclusion, we report evidence for the existence of a Ca^{2+}-triggered and NMDA-dependent long-term enhance-

ment in OB neuronal excitability. Whether a similar phenomenon also plays a key role in important changes in M/T cells responsiveness to odors following learning (Keverne, 1995) remains to be investigated. Furthermore, elucidation of underlying mechanisms requires extensive studies.

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