Developmental changes in the expression of low-voltage-activated Ca^{2+} channels in rat visual cortical neurones

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1. The functional properties of low-voltage-activated (LVA) Ca^{2+} channels were studied in pyramidal neurones from different rat visual cortical layers in order to investigate changes in their properties during early postnatal development. Ca^{2+} currents were recorded in brain slices using the whole-cell patch-clamp technique in rats from three age groups: 2, 3 and 12 days old (postnatal day (P) 2, P3 and P12).

2. It was demonstrated that LVA Ca^{2+} currents are present in neurones from superficial (I–II) and deep (V–VI) visual cortex layers of P2 and P3 rats. No LVA Ca^{2+} currents were observed in neurones from the middle (III–IV) layers of these rats. The LVA Ca^{2+} currents observed in P2 and P3 neurones from both superficial and deep layers could be completely blocked by nifedipine (100 μM) and were insensitive to Ni^{2+} (25 μM).

3. The density of LVA Ca^{2+} currents decreased rapidly during the early stages of postnatal development, while the density of high-voltage-activated (HVA) Ca^{2+} currents progressively increased up to the twelfth postnatal day. No LVA Ca^{2+} currents were found in P12 neurones from any of the layers. Only HVA Ca^{2+} currents with high sensitivity to F$^-$ applied through the patch pipette were observed.

4. The kinetics of LVA Ca^{2+} currents could be well approximated by the m^2h Hodgkin–Huxley equation with an inactivation time constant of 24 ± 6 ms. The steady-state inactivation curve fitted by a Boltzmann function had the following parameters: membrane potential at half-inactivation, $-86 \cdot 9$ mV; steepness coefficient, $3 \cdot 4$ mV.

5. It is concluded that, in visual cortical neurones, LVA Ca^{2+} channels are expressed only in the neurones of deep and superficial layers over a short period during the earliest postnatal stages. These channels are nifedipine sensitive and similar in functional properties to those in the laterodorsal (LD) thalamic nucleus. However, the cortical neurones do not express another ('slow') type of LVA Ca^{2+} channel, which is permanently present in LD thalamic neurones after the second postnatal week, indicating that the developmental time course of cortical and thalamic cells is different.

The functional properties of nerve cells in different parts of the nervous system are determined by the specificity of their morphological structure as well as by the composition of their voltage- and receptor-operated ion channels. The expression of channel proteins is extremely variable in different neurones and highly dependent on the developmental stage of the animal (Thompson & Wong, 1991; Fedulova, Kostyuk & Veselovsky, 1994). Some of these proteins appear only for a short period, being responsible for triggering the differentiation of neuronal precursors and the establishment and maturation of neuronal connections, while others form a constant basis for the activity of neuronal networks. Analysis of the expression and functioning of voltage-operated Ca^{2+} channels (VOCCs) is especially important for the understanding of these

processes, as Ca^{2+} ions are known to play a critical role in the formation of the nervous system (Al-Mohanna, Cave & Bolsover, 1992; Amato, Al-Mohanna & Bolsover, 1996), and to be the main messengers in synaptic transmission at all neuronal junctions. Of especial interest in this respect is the presence of low-voltage-activated (LVA) Ca^{2+} channels, which can be activated at voltages close to the membrane resting potential provided that their steady-state inactivation is removed by some preceding hyperpolarizing influences, and which can therefore easily generate the spontaneous Ca^{2+} transients necessary for morphogenesis. At present, data on the development of expression of such channels in different neurones are extremely variable. Thus, during artificially induced differentiation in neuronal tumour cell lines, only LVA Ca^{2+} channels appear at the

start of differentiation, and then decrease as other types of ion channels are expressed (Veselovsky & Fomina, 1986). In rat and mouse dorsal root ganglion neurones LVA (T-type) Ca^{2+} channels show a peak of expression just around the time of birth, while later on their density starts to decline until their complete disappearance in most neurones beyond the first few weeks (Fedulova, Kostyuk & Veselovsky, 1986; Fedulova *et al.* 1994). In cultured *Xenopus* sensory neurones they are expressed only during the first 20–40 h of culture (Barish, 1991). The same is true for neurones cultured from adult or embryonic rat and guinea-pig hippocampal pyramidal neurones (O'Dell & Alger, 1991; Thompson & Wong, 1991) and rat neostriatum (Bargas, Surmeier & Kitai, 1991). In contrast to this, however, LVA Ca^{2+} channels are the predominant type of VOCC in certain thalamic and hypothalamic neurones and are a constant feature of the excitability mechanism, playing an important role in the organization of slow rhythmic activity (Akaike, Kostyuk & Osipchuk, 1989; Huguenard & Prince, 1992). The developmental appearance of such channels is quite complicated. Thus, the population of LVA Ca^{2+} channels in neurones of the rat laterodorsal (LD) thalamic nucleus was found to be homogeneous in kinetic and pharmacological properties only during the first postnatal week. The corresponding Ca^{2+} currents demonstrated fast inactivation kinetics with a monoexponential time course (~30 ms) and high sensitivity to nifedipine ($K_d = 2.6 \ \mu$M). However, from the second postnatal week onwards, a more slowly inactivating component appeared in LVA Ca^{2+} currents from the same neurones, which showed insensitivity to nifedipine but high sensitivity to Ni^{2+} (Tarasenko, Kostyuk, Eremin & Isaev, 1997). The two types of LVA Ca^{2+} channel are probably associated with different neuronal functions, as the appearance of the 'slow' channels (with an inactivation time constant of ~60 ms) coincided with development of the dendritic tree in the corresponding cells.

These findings stimulated us to analyse in more detail the kinetic, pharmacological and developmental properties of LVA Ca^{2+} channels in neocortical neurones closely connected functionally to the neuronal activity of associative thalamic nuclei. The presence of LVA Ca^{2+} currents has been reported in pyramidal neurones in slices from the rat visual cortex (Franz, Galvan & Constanti, 1986; Sutor & Zieglgansberger, 1987) and in pyramidal neurones acutely isolated from the rat sensorimotor cortex (Sayer, Schwindt & Crill, 1990). However, the properties of these channels have not as yet been fully characterized. The cellular heterogeneity of the cortex and the difficulty of identifying specific cell types has substantially limited the study of characteristics and developmental changes. This complexity is evident from a recent study of pyramidal neurones from guinea-pig medial frontal cortex, which found that only cells located lower than 500 μm from the pial surface (approximately layers V–VI) were able to generate LVA Ca^{2+} currents and low-threshold Ca^{2+} spikes (LTS) (De la Pena & Geijo-Barrientos, 1996).

In the present study we attempted to investigate this problem further by using neurones from different layers of the rat visual cortex during the first 12 days of postnatal development.

METHODS

Slice preparation

Brain slices were obtained from Wistar rats of three age groups: 2, 3 and 12 days old (postnatal day (P) 2, P3 and P12, the day of birth being designated as P1). The procedure to obtain brain slices was similar to that previously described by Tarasenko *et al.* (1997). Animals were anaesthetized with ether and decapitated only after observation of deep sleep. Incubation time in the recovery solution depended on the age of the animal: P2 and P12 slices were incubated for 30 and 60 min, respectively. Slices were 300 μm thick.

Patch-clamp recordings

Whole-cell patch-clamp recordings were performed as reported previously (Tarasenko *et al.* 1997). Patch pipettes were pulled from thick-walled molybdenum glass capillaries, and had 3–6 MΩ resistance for P2 and P3 cortical neurones and 2–4 MΩ resistance for P12 neurones. The inner diameters of the pipettes varied from 1 to 3.5 μm depending on the size of the cell studied.

Taking into account variations in membrane surface properties and cell morphology in the different cortical layers and postnatal developmental stages, we controlled both the membrane capacitance and input resistance of the neurones. The membrane capacitance was calculated by subtracting the pipette electrode capacitance from the whole-cell capacitance, which in turn was estimated from the integral of the corresponding transient current induced from a holding potential of −80 mV by 10 mV hyperpolarizing pulses after rupture of the cell membrane. Generally, these transients had fast monoexponential time decay and could therefore be treated as a single monoexponential unit (Kay & Wong, 1987) and accepted for analysis. In addition, only those cells that completely satisfied all the criteria of voltage-clamp control formulated in our previous work (Tarasenko *et al.* 1997) were included for further study. We could not compensate the series resistance (R_s) but determined it from the amplitude or time constant of the capacitive current after rupture of the membrane. As the amplitude of Ca^{2+} currents did not exceed 200 pA and R_s was no more than 15 MΩ, the error in voltage clamp was less than 3 mV.

Input resistance was evaluated by dividing the voltage step by the leakage current. After rupture of the cell membrane the leakage currents were compensated by using an additional electrical circuit throughout the recording session. The compensation procedure was considered as satisfactory if the current trace elicited at the onset of a 10 mV hyperpolarizing command potential from the holding potential coincided with the zero line.

For observation of the upper surface of the slice we used an upright microscope (Ergoval; Carl Zeiss) with a × 3.2 Semiplan objective lens fitted with a calibrated eyepiece. Visual cortex (areas 17 and 18) was identified according to the rat brain stereotaxic atlas (Paxinos & Watson, 1982); a × 20 Leitz Wetzlar objective lens was used to identify the type of cortical cell and the particular cortical layer. The cell surface was thoroughly cleaned before seal formation by saline streamed from a neighbouring pipette. Ca^{2+} currents were isolated by blocking Na^+ and K^+ channels and by replacing intracellular K^+ with Cs^+ (see Solutions and Drugs, below). Currents were recorded at room temperature (19–22 °C) using an analogue of

the List EPC-5 patch-clamp amplifier at an output cut-off frequency of 1 kHz (−3 dB, 8-pole active Bessel filter). Ca^{2+} currents were elicited by a series of increasing depolarizing steps every 5–7 s, digitally sampled at 8 μs intervals, stored and analysed with an IBM PC-compatible computer. The current amplitude was measured as the difference between peak inward current and zero current. Other specific protocols are described in the text or figure legends. Graphing and curve fitting was performed by SigmaPlot 5.0 (Jandel Scientific GmbH, Erkath, Germany). Time constants and current kinetics were described by fitting with a Hodgkin–Huxley model, using methods identical to those described earlier (Coulter, Huguenard & Prince, 1989). Results are given as means ± s.e.m. and the number of observations is indicated in parentheses.

Solutions and drugs

The solution for slice cutting and recovery contained (mM): NaCl, 125; KCl, 2·5; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 24; NaH_2PO_4, 1·25; glucose, 25. To maintain the solution at pH 7·4 it was continuously bubbled with 95% O_2–5% CO_2.

The extracellular recording solution contained (mM): choline chloride, 113; TEA-Cl, 27; Tris-Cl, 20; $CaCl_2$, 2; $MgCl_2$, 0·5 and tetrodotoxin (TTX), 1 μM; pH adjusted to 7·4 with Tris-OH.

The pipette solution contained (mM): CsCl, 130; EGTA, 10; TrisCl, 10; $MgCl_2$, 5; $CaCl_2$, 1; pH adjusted to 7·2 with CsOH.

The presence of TEA-Cl in the extracellular solution and CsCl in the pipette solution enabled us to block potassium channels completely during recording. To block sodium channels, TTX was added to the extracellular solution and Na^+ was replaced with choline chloride.

To study the pharmacological properties of LVA Ca^{2+} channels, the following substances were used: nifedipine (Sigma), made as a 50 mM stock solution in dimethyl sulphoxide; and Ni^{2+}, dissolved in extracellular recording solution to the final concentrations indicated in Results. Ni^{2+} was added to the extracellular solution as $NiCl_2$. All drugs were applied by a fast-application technique through a pressure-ejecting micropipette (tip diameter, 40–50 μm).

RESULTS

Passive properties of visual cortical neurones

The results presented were obtained in neurones located in different layers below the external surface of the cortex. As described in Methods, the membrane capacitance was evaluated as the integral of the capacitive transient obtained in response to hyperpolarizing command pulses of 10 mV from a holding potential of −80 mV. In P2 rat neurones from deep layers the membrane capacitance was 23 ± 4 pF ($n = 8$). However, in P3 and P12 rats the population of cortical neurones was observed to be heterogeneous. Two groups of cells were identified. The neurones of the first group appeared to be in the process of increasing their dendritic tree, as their cell capacitances were 36 ± 5 pF ($n = 10$) and 76 ± 12 pF ($n = 12$) for ages P3 and P12, respectively (Fig. 1). The neurones of the second group did not appear to be undergoing changes in their cellular membrane surfaces as their capacitances were 24 ± 4 pF ($n = 9$) and 24 ± 6 pF ($n = 7$) for P3 and P12 cells, respectively; these cells probably corresponded to non-pyramidal neurones and were not included in any further analysis.

The situation was different for the neurones of layers I–II. We did not find any substantial developmental changes in the membrane capacitance of these neurones. Membrane capacitance was 52 ± 11 pF ($n = 15$), regardless of postnatal stage.

The decay in the capacitive transients of the cells (measured after rupturing the cell membrane) could be fitted by a single exponential curve. The time constant of decay (τ) varied, depending on the age group, from 140 μs to 1·2 ms. The series resistance determined from the capacitive transients did not exceed 15 MΩ for those neurones tested.
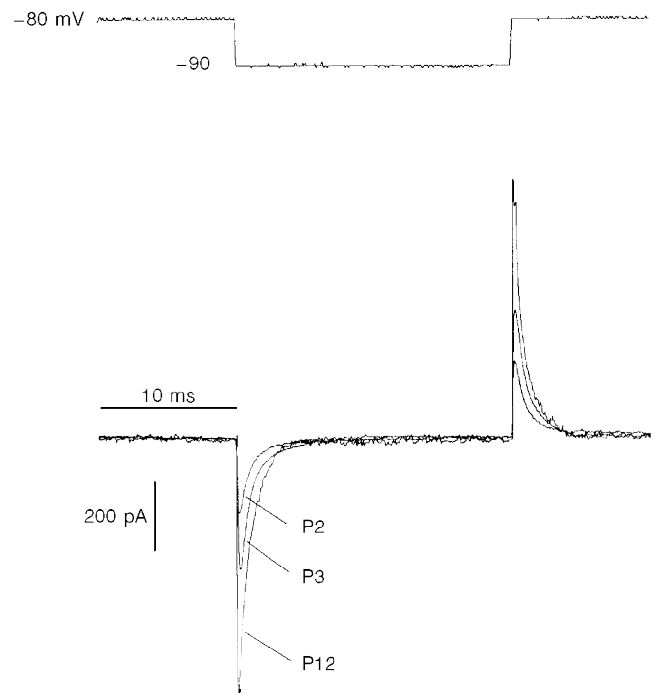
**Figure 1. Passive properties of visual cortical pyramidal neurones**

Developmental changes in capacitance of pyramidal neurones from deep layers of the visual cortex. The transient capacitive current was evoked by a 10 mV hyperpolarizing command potential from a holding potential of −80 mV. Constant leakage current for the neurones shown here was 5, 10 and 12 pA for P2, P3 and P12, respectively. The calculated values of membrane capacitance were 29, 46 and 68 pF for P2, P3 and P12, respectively. The decay of the capacitive current was fitted by a single exponential curve with time constants of 0·52, 0·64 and 0·41 ms for P2, P3 and P12 neurones, respectively.

No age-related differences in leakage currents were observed. As a rule, the leakage current was ~20 pA and no greater than 27 pA. The mean input resistance of tested cells was 500 MΩ.

Developmental changes in Ca²⁺ conductance in cortical neurones from different layers

Typical records of Ca²⁺ currents in P2 and P3 neurones from deep layers are shown in Fig. 2. Generally, in P2 neurones, predominantly transient Ca²⁺ currents evoked by step depolarization to −55 mV from a holding potential of

−95 mV could be observed. A sustained current may also have been revealed at depolarization potentials more positive than −45 mV but its mean value was significantly less than that of the transient current (Fig. 2A). The current–voltage (*I–V*) relationship had two maxima at about −50 and −15 mV, indicating the presence of both LVA and high-voltage-activated (HVA) Ca²⁺ currents (Fig. 2B). The amplitude of Ca²⁺ current induced by step depolarization to −50 mV was 38 ± 6 pA (*n* = 8). In P3 neurones under the same conditions, it was also possible to observe the LVA Ca²⁺ current (Fig. 2C). The *I–V* curve
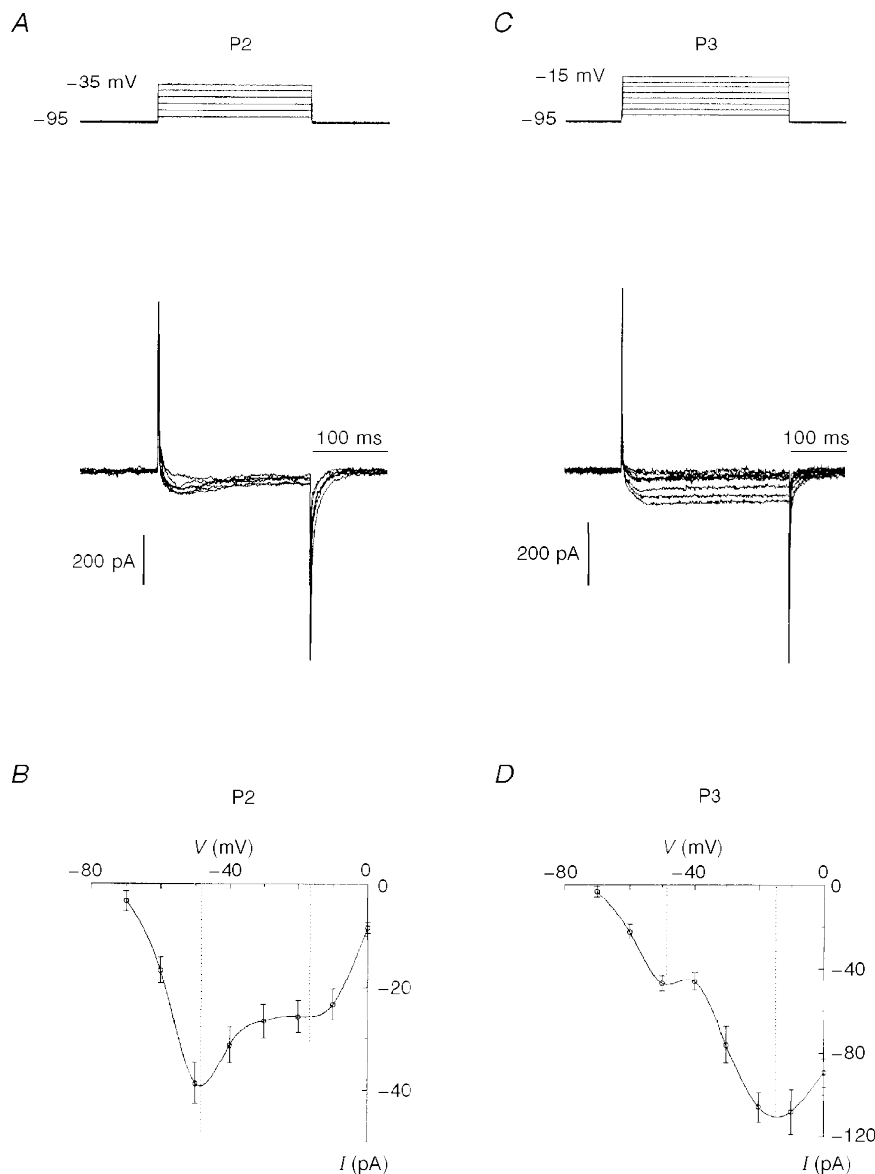
Figure 2. Changes in whole-cell Ca²⁺ current during the earliest stages of postnatal development

A and *C*, depolarizing voltage steps from a holding potential of −95 mV for P2 (*A*) and P3 (*C*) neurones from layers V–VI evoked the superposition of transient LVA Ca²⁺ current and slowly inactivating HVA Ca²⁺ currents, the latter of which was predominant in P3 (*C*) neurones. The voltage protocol for each panel is shown at the top. *B* and *D*, *I–V* relationships of peak currents for neurones of the two age groups are presented beneath the current traces. The *I–V* relationships for neurones of both P2 and P3 age groups have 2 peaks, at about −50 and −15 mV, corresponding to LVA and HVA Ca²⁺ currents, respectively. For construction of the *I–V* curves data were averaged using 5 cells for P2 and 8 cells for P3 neurones.

again had two characteristic peaks around −50 and −15 mV (Fig. 2*D*). However, the amplitude of HVA Ca²⁺ current was 108 ± 12 pA, and it became predominant. There were no LVA Ca²⁺ currents in P12 neurones over a depolarization range between −75 and −45 mV (Fig. 3*A*) and the *I–V* curve had only one peak around −15 mV. The maximum amplitude of HVA Ca²⁺ current was 738 ± 64 pA (*n* = 7). To verify that P12 neurones expressed only HVA Ca²⁺ channels, 5 μM F⁻ was added to the pipette solution to block HVA Ca²⁺ currents from the inside (Fig. 3*B*), a method previously used in isolated thalamic neurones (Bertollini, Biella, Wanke, Avanzini & De Curtis, 1994). As LVA Ca²⁺ current persisted in the presence of intracellular F⁻ (Carbone & Lux, 1987; Fraser & MacVicar, 1991), the *I–V* relationship could be expected to have two peaks if LVA

Ca²⁺ currents were present. The blocking effect of F⁻ was 90 ± 5% (*n* = 12) and in the presence of this anion the *I–V* curve had only one peak at about −15 mV (Fig. 3*C*).

As it turned out, the expression of LVA Ca²⁺ channels was not a property common to all visual cortical neurones in the earliest stages of postnatal development. There were no LVA Ca²⁺ currents in neurones from the middle layer (III–IV) on either the second or twelfth postnatal days. However, the developmental changes in neurones from the superficial layer (I–II) had exactly the same time course as those of neurones in the deeper layer (V–VI). LVA Ca²⁺ currents were present in P2 and P3 neurones, but only HVA Ca²⁺ currents were recorded in P12 neurones. To clarify the question as to whether the neurones from deep
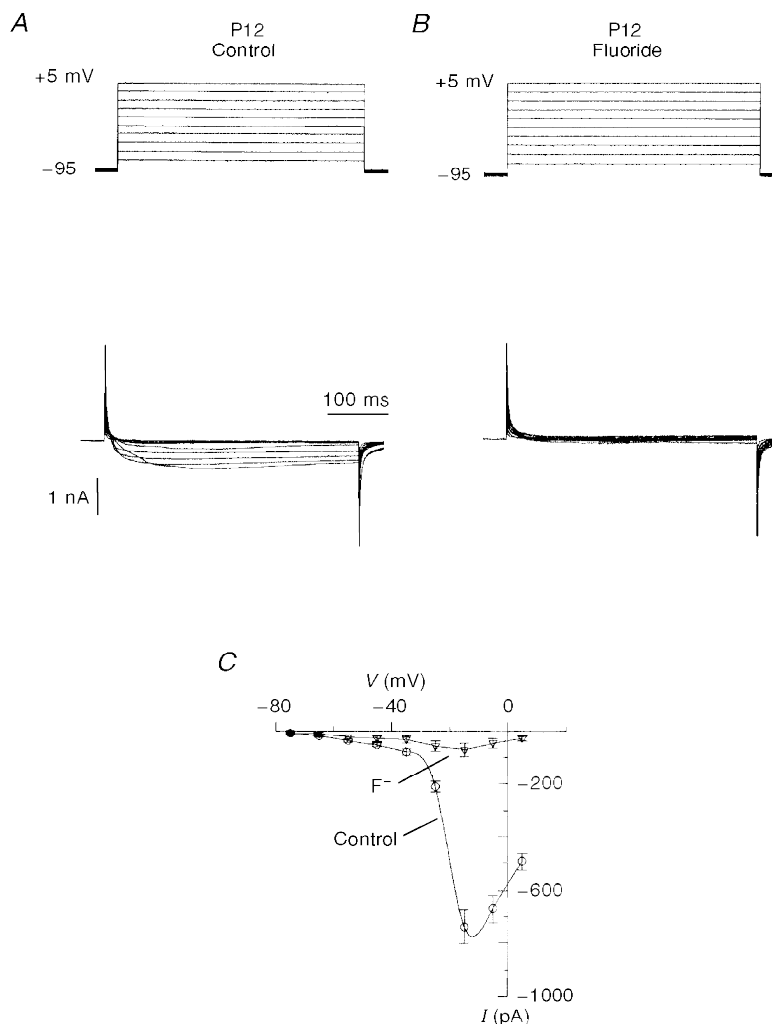
Figure 3. Ca²⁺ currents in P12 neurones

A, typical records of Ca²⁺ current in P12 neurones obtained by membrane depolarization from −95 mV in 10 mV steps. There were no transient Ca²⁺ currents in the region from −70 to −40 mV. The threshold for activation of Ca²⁺ currents was about −45 mV. The voltage step protocol is shown at the top. *B*, Ca²⁺ currents in the presence of 5 μM F⁻ after a short period (5 min) of intracellular perfusion. Fluoride ions were added to verify whether the LVA Ca²⁺ current is present in P12 neurones and hidden by the larger HVA Ca²⁺ current. In general, in the presence of F⁻ ions, 90 ± 5% of the Ca²⁺ current was blocked and no LVA current was found. *C*, *I–V* relationships of peak currents registered in the absence and presence of F⁻ have only one peak at about −15 mV, corresponding to HVA Ca²⁺ current.

and superficial layers expressed the same LVA Ca^{2+} channels, a special pharmacological and kinetic study of LVA Ca^{2+} currents in neurones from different layers was undertaken.

Pharmacological properties of LVA Ca^{2+} current

As we have shown previously (Tarasenko *et al.* 1997), LD thalamic neurones can express two types of LVA Ca^{2+} channels with different sensitivities to nifedipine and Ni^{2+}. In the present study we found that neurones from both deep and superficial layers had LVA Ca^{2+} currents with high sensitivity to nifedipine (Fig. 4*A*). The nifedipine-sensitive component was obtained as the difference between the control current and the current obtained after blocking by nifedipine (100 μM). The amplitude of nifedipine-sensitive current in P2 deep neurones (Fig. 4*B*) was larger (22 ± 4 pA, $n = 6$) than that in P3 neurones (12 ± 4, $n = 8$) The same postnatal changes in amplitude of LVA Ca^{2+} currents were observed in neurones from the superficial layer. To determine whether any other component was present in the LVA Ca^{2+} current, Ni^{2+} at 25 μM was applied to the neurones tested. No changes were observed in the control Ca^{2+} current in either deep ($n = 5$) or superficial neurones ($n = 7$).

Steady-state inactivation of the LVA Ca^{2+} current

Taking into account the specificity of steady-state inactivation of the slow LVA Ca^{2+} current component in LD neurones (half-inactivation membrane potential ($V_{0.5}$), $\sim$−100 mV; Tarasenko *et al.* 1997), in the next part of our study a special protocol for recording Ca^{2+} currents was

used. A membrane depolarization to −50 mV followed a hyperpolarization prepulse in the range −90 to −120 mV of 500 ms duration. There was no difference in the amplitude of LVA Ca^{2+} currents between neurones from the two (superficial and deep) layers, demonstrating the presence of the same type of LVA channel. The recordings of LVA Ca^{2+} currents made using this protocol are shown in Fig. 5*A*. The inactivation time constant for LVA Ca^{2+} current was the same using this protocol as when using the normal protocol (see above), at 23 ± 4 ms ($n = 4$). This fact led us to conclude that visual cortical neurones do not express slowly inactivating LVA Ca^{2+} channels after the second postnatal week, although they are permanently present in LD thalamic neurones (Tarasenko *et al.* 1997). The normalized peak amplitudes of the evoked currents could be fitted by a Boltzmann function (Fig. 5*B*). The $V_{0.5}$ of −86·9 mV and slope factor (k) of 3·4 mV were similar to those described in LD thalamic neurones for the 'fast' component ($I_{T,f}$) of the LVA Ca^{2+} current.

Kinetic properties of the LVA Ca^{2+} current

The LVA Ca^{2+} current induced by membrane depolarization to −60 mV from a prepulse potential of −95 mV showed monoexponential decay and declined completely in about 100 ms in both P2 and P3 neurones. Analysis of the activation and inactivation time courses of this current indicated that it could be well fitted in terms of the m^2h Hodgkin–Huxley equation, as has already been shown for the kinetics of other types of Ca^{2+} currents (Kostyuk, Krishtal, Pidoplichko & Shakhovalov, 1979; Kay & Wong,
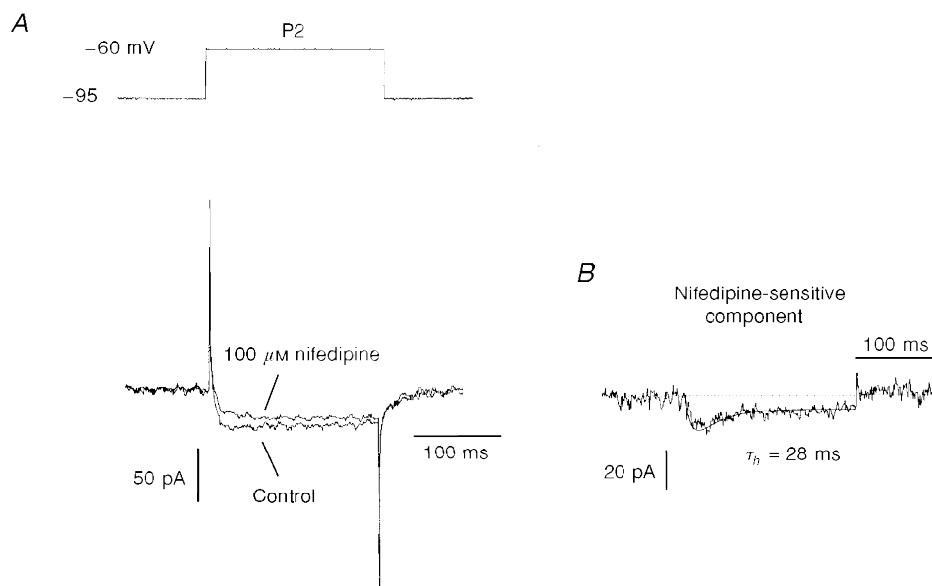
Figure 4. Blocking effect of nifedipine on LVA Ca^{2+} current in P2 visual cortical neurones

A, LVA Ca^{2+} current recorded in P2 neurones before (Control) and after blocking by 100 μM nifedipine. The current was obtained by 35 mV step depolarization from a holding potential of −95 mV (top). *B*, the nifedipine-sensitive component was obtained by subtracting LVA Ca^{2+} current after blocking by 100 μM nifedipine from the control LVA Ca^{2+} current. It was fitted in terms of the m^2h model (continuous line) with the inactivation time constant (τ_h) indicated.

1987). The equation used for fitting the LVA Ca²⁺ current (I_{Ca}) was:

$$I_{Ca} = A[1 - \exp(-t/\tau_m)]^2[\exp(-t/\tau_h) + I_\infty/A], \qquad (1)$$

where t is time, τ_m and τ_h are the activation and inactivation time constants, respectively, I_∞ is a sustained Ca²⁺ current, and A is an amplitude scaling factor. The fitting parameters for the LVA Ca²⁺ current in P2 neurones (Fig. 6A) were $\tau_m = 3\cdot6 \pm 0\cdot5$ ms and $\tau_h = 24 \pm 6$ ms ($n = 15$). No differences were observed between the LVA Ca²⁺ current kinetics of P3 and P2 neurones. For P3 neurones the time constants were $\tau_m = 4\cdot2 \pm 0\cdot6$ ms and $\tau_h = 26 \pm 5$ ms ($n = 14$) for activation and inactivation, respectively (Fig. 6B). Since the pharmacological sensitivity and kinetics of the LVA current in cells from both age groups did not show significant differences ($P > 0\cdot1$), we concluded that these neurones express nifedipine-sensitive LVA Ca²⁺ channels similar to 'fast' channels expressed in P12 LD neurones.

Postnatal changes in densities of Ca²⁺ current

According to our estimation, the current density for LVA currents in P2 neurones was $1\cdot65 \pm 0\cdot17$ pA pF⁻¹ ($n = 6$) and for HVA currents it was $1\cdot08 \pm 0\cdot13$ pA pF⁻¹ ($n = 8$); current density did not depend upon whether the neurone was situated in the deep or the superficial layer. However, already by the third postnatal day (P3), the relative proportion of LVA to HVA Ca²⁺ currents had substantially changed. The density of LVA Ca²⁺ current ($1\cdot34 \pm 0\cdot12$ pA pF⁻¹, $n = 6$) in P3 neurones became smaller compared with that of neurones of the first age group, P1 ($P < 0\cdot01$). On the other hand, the density of slowly inactivating HVA Ca²⁺ current in P3 neurones became significantly greater than in P2 neurones and reached $3\cdot08 \pm 0\cdot31$ pA pF⁻¹ ($n = 6$). By the twelfth postnatal day the density of HVA Ca²⁺ current had increased considerably compared with P2 and P3 neurones, reaching $9\cdot7 \pm 0\cdot7$ pA pF⁻¹ ($n = 12$).
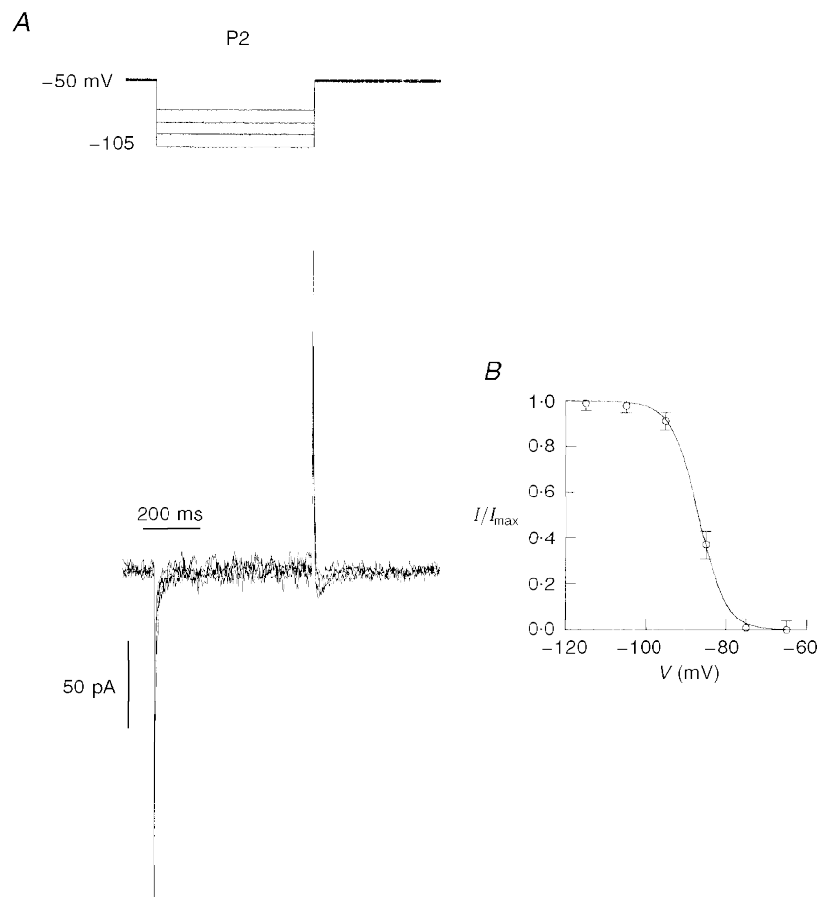
Figure 5. Steady-state inactivation of LVA Ca²⁺ current in P2 neurones

A, voltage dependence of steady-state inactivation of the nifedipine-sensitive LVA Ca²⁺ current. The voltage protocol is shown at the top. The membrane was depolarized to −50 mV from different holding potentials between −105 and −75 mV. The duration of the prepulse impulse was 500 ms. *B*, the inactivation curve fitted by a Boltzmann equation ($I/I_{max} = [1 + \exp(V - V_{0\cdot5}/k)]^{-1}$, where I is the current amplitude, I_{max} is the maximum current amplitude, V is the voltage, $V_{0\cdot5}$ is the half-inactivation voltage and k is the slope factor) with $V_{0\cdot5} = -86\cdot9$ mV and $k = 3\cdot4$ mV ($n = 7$).

DISCUSSION

Using the whole-cell patch-clamp technique combined with the direct visualization of neurones during recording in brain slice preparations, we studied the early postnatal development of the electrophysiological properties of pyramidal and non-pyramidal neurones of rat visual cortex. Special attention has been paid to LVA Ca^{2+} currents because of the multiple roles these currents play in the functioning of different neurones (short-lasting currents that trigger neuronal morphogenesis and continuous currents that are responsible for special forms of neuronal activity) as well as the recently established existence of several kinetically and pharmacologically different subtypes of the corresponding LVA Ca^{2+} channels (Tarasenko *et al.* 1997).

The present study has demonstrated that LVA Ca^{2+} channels are expressed in the visual cortex only in pyramidal neurones of deep (V–VI) and superficial (I–II) layers for a very short period during the earliest stages of postnatal development. HVA Ca^{2+} channels are expressed in these neurones at this developmental time at a much lower density. Obviously, the LVA channels immediately after birth are the main gates for intracellular Ca^{2+} entry. These channels are, by their kinetic and pharmacological properties, analogous to those previously described by us as 'fast' LVA
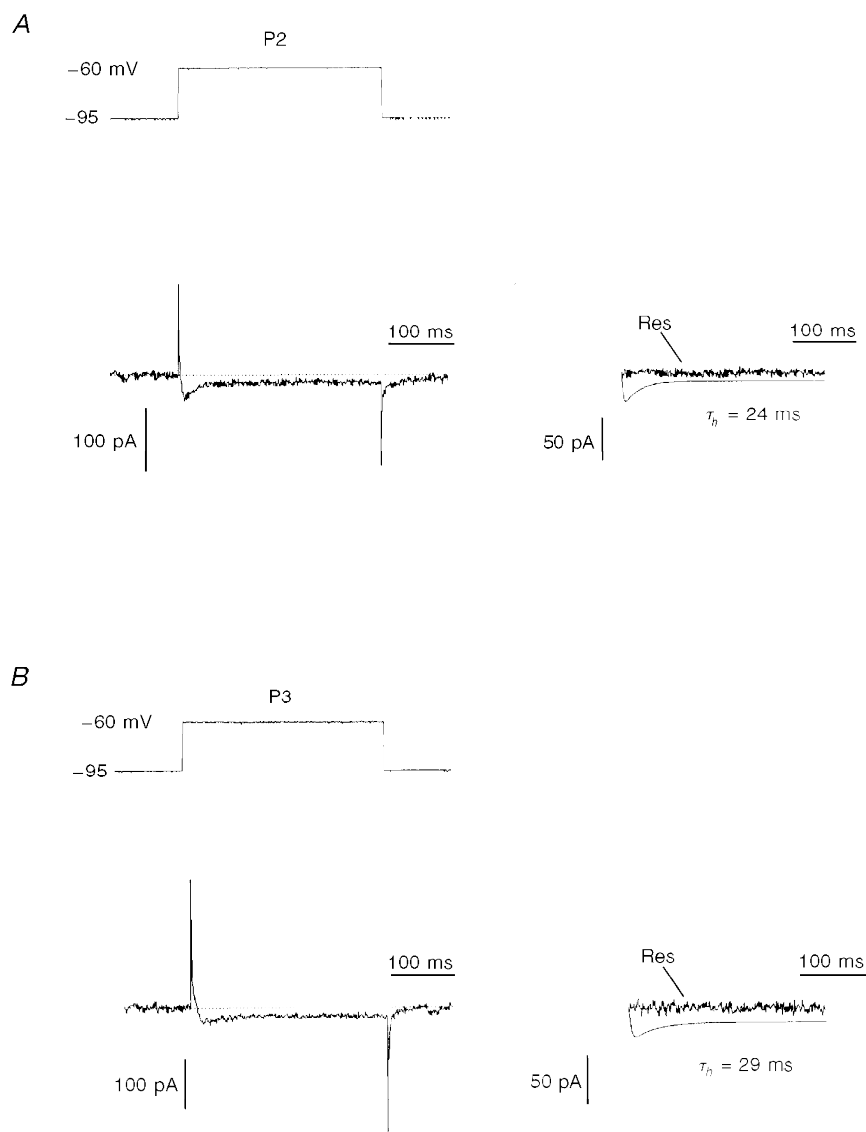
Figure 6. Inactivation kinetics of LVA Ca^{2+} currents in P2 and P3 visual cortical neurones

A and *B* (left), a transient LVA Ca^{2+} current in P2 (*A*) and P3 (*B*) deep layer neurones activated by depolarization to −60 mV from a prepulse potential of −95 mV. *A* and *B* (right), the current traces were fitted by computer simulation using the m^2h equation with the inactivation time constants (τ_h) indicated. The residue (Res) current was obtained by subtraction of the simulated current from the LVA Ca^{2+} current (control).

Ca^{2+} channels in the LD thalamic neurones, indicating that in both cases the function of these channels may be similar, i.e. triggering neuronal differentiation and establishing neuronal connections. However, the time characteristics of the expression of these channels in neocortical and thalamic neurones are substantially different. In cortical neurones, by as early as the third postnatal day the density of LVA currents decreased while that of HVA currents increased. At the twelfth day after birth we could not observe any LVA Ca^{2+} current in neurones from either layers I–II and V–VI; in thalamic neurones at this developmental time the 'fast' component was still well expressed (Tarasenko *et al.* 1997). One may speculate that the development of thalamic projections is a much more time-consuming process and demands the longer-lasting triggering action of intracellular Ca^{2+} transients provided by the activity of LVA Ca^{2+} channels. During neocortical development this process may go faster. In this respect it should be mentioned that LVA Ca^{2+} channels disappeared simultaneously in the superficial and deep cortical layer neurones; during neocortical development the interaction between neurones of layer I–II (Cajal-Retzius cells) and layer V–VI is extremely important for the correct positioning of migrating neurones and their maturation (Ogawa *et al.* 1995).

In the middle layer (II–III) neurones, only HVA Ca^{2+} currents were found even on days P2 and P3. One day later, the pyramidal neurones from both the deep and superficial layers also started to express a substantial number of HVA Ca^{2+} channels. It has been shown previously that in guinea-pig medial frontal cortex only pyramidal neurones are able to generate low-threshold Ca^{2+} spikes; they were rarely observed in layers II–III and frequently in layers V–VI (De la Pena & Geijo-Barrientos, 1996). This may indicate that neurones in the middle layers differentiate very early on and achieve maturity even before birth, while those in the superficial and deep layers still have immature membrane properties during the postnatal period and continue to develop over the first two postnatal weeks (Zhou & Hablitz, 1996).

In contrast to neocortical neurones, neurones of certain thalamic and hypothalamic structures maintain a high density of LVA Ca^{2+} channels in the adult state (Akaike *et al.* 1989; Huguenard & Prince, 1992). Obviously, besides contributing in some way to neuronal differentiation and the establishment of synaptic contacts, these channels are important for generating specific types of neuronal activity in the form of membrane potential oscillations and repetitive firing, enabling thalamic neurones to act as pacemaker elements in thalamocortical circuits (Dossi, Nuñez & Steriade, 1992; Huguenard & Prince, 1992; Kang & Kitai, 1993; Hutcheon, Miura, Yarom & Puil, 1994). The observed differences clearly indicate a leading role for subcortical structures in the generation and maintenance of the corresponding rhythms. The Ca^{2+} channels responsible for such activity evidently form a special subtype of LVA

channel, differing from the transient subtype in inactivation kinetics, location and pharmacology. As has already been stated, in LD thalamic neurones these channels seem to be located mainly on dendrites; in accord with this location, the thalamocortical rhythmic activity in rats starts just after maturation of the dendritic tree of the thalamic neurones (Muller, Misgeld & Swandulla, 1992; Karst, Joels & Wadman, 1993).

The pharmacological specificity of the permanent LVA Ca^{2+} channels in thalamic neurones merits special attention. A more extensive evaluation of possible specific antagonists to this type of channel may produce drugs that could attenuate paroxysmal thalamocortical activity without affecting the normal course of postnatal development of the corresponding structures.

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