Enhancement of whole cell calcium currents following transient MCAO

Claus Bruehl, Tobias Neumann-Haefelin, O.W. Witte

Abstract

Cerebral infarctions have been shown to cause widespread changes of neuronal excitability in non-infarcted tissue. Calcium currents are major determinants of neuronal behavior, and pathological modulation of Ca\(^{2+}\)-channels is known to lead to altered excitability states in a variety of paradigms. In the present study we addressed the question to what extent whole cell calcium currents are altered after middle cerebral artery occlusion (MCAO) in both the ipsi- and contralateral sensory cortex. Transient middle cerebral artery occlusion was induced for 1 h in rats using the intraluminal thread model. After 7 or 28 days survival, whole cell patch clamp studies were carried out on freshly isolated neurons of the ipsi- and contralateral sensory cortex, and high voltage activated (HVA) calcium currents were examined. In lesioned animals, we found a significant increase of calcium current amplitude and maximal conductance in the sensory cortex contralateral to the infarcts. This was paralleled by a prominent positive shift of the potential of half-maximal activation (V\(_{50}\)) in these cells. Changes were long-lasting and at least stable for the following 28 days. These alterations were present in animals with lesions of moderate size, but not in those with massive infarction, and only in the cortex contralateral to the lesion. Following cortical infarctions, changes of calcium current properties are selectively observed in neurons contralateral to the lesion. At the behavioral level, compensatory mechanisms involving the unaffected hemisphere may induce this alteration of calcium current properties.

Theme: Disorders of the nervous system
Topic: Ischemia
Keywords: Rat; Ischemia; Whole-cell patch-clamp; Ion currents

1. Introduction

Over the past few years several studies have reported a variety of abnormalities in both peri-infarct and remote brain regions following experimental stroke. These alterations are of interest since they may have an impact on the functional characteristics of the non-infarcted tissue, thereby influencing neurological recovery. They include acute changes such as peri-infarct depolarizations [15] and disturbances of calcium homeostasis [32], as well as long-lasting effects, e.g. inhibition of protein synthesis [24], abnormal transmitter release, apoptosis [4,21,22,29,39] and excitability changes of the neuronal network.

Most recently altered functional properties in remote brain areas have been demonstrated following the induction of ischemic brain lesions. In these studies a reduction of GABAergic inhibition paralleled by increased firing rates were found ipsi- as well as contralateral to the lesion site [3,30,31]. This finding has been coined ‘electrophysiological diachisis’ though it is opposite to what has been postulated by von Monakow in his classical description [35], which describes diachitic phenomena merely as decreases in activity.

Long-term excitability changes have been shown to be associated with both an increase in NMDA-receptor-mediated responses and a reduction of GABAergic inhibition [22], leading to a net increase in neuronal excitability at the single neuron level. Presently, there are no reports available that focussed on whether changes of voltage-gated ion channels contribute to long-term excitability...
changes following focal ischemia [26,27,29,31,39]. Calcium influx into neurons, however, is known to occur during ischemia through both voltage- and ligand-gated Ca\(^{2+}\)-channels, and Ca\(^{2+}\)-overload is believed to trigger intracellular cascades that contribute to neuronal cell death. This pathway is of importance during ischemia and affects mainly the ischemic region itself, rather than remote brain regions. During the subacute to chronic stages, on the other hand, a number of changes occur in remote brain regions that could potentially influence voltage-gated Ca\(^{2+}\)-channels, including inflammatory reactions and free radical formation, which are associated with an upregulation of Interleukine-1 beta (IL1-\beta) [28], tumor necrosis factor alpha (TNF-\alpha) [9,33] and nitric oxide (NO) [5].

In this study we focussed on calcium current characteristics and their possible modulation in peri-infarct and remote brain regions at relatively late time points (1 and 4 weeks) following focal ischemia. To this purpose we used the whole cell voltage patch clamp technique on freshly isolated cortical neurons from animals which were subjected to transient middle cerebral artery occlusion (MCAO).

2. Materials and methods

A total of 21 adult male Wistar SPF strain rats (weighing 270–330 g) were used for the experiments. The animals in the experimental group (n=9), the controls (n=6) and the native group (n=6) were kept on a 12 h-light cycle. All experimental procedures were conducted according to protocols approved by the Governmental Animal Care Committee.

The animals in the experimental group were subjected to transient middle cerebral artery occlusion (MCAO) using the slightly modified intraluminal thread model described by Koizumi et al. [17]. The spontaneously breathing animals were anesthetized with enflurane (1.5%) in a mixture of N\(_2\)O/O\(_2\) (70/30%) and body temperature was kept constant using a heating pad (36.5±0.5°C). The right cervical carotid bifurcation was exposed and both the proximal common carotid artery (CCA) as well as the external carotid artery (ECA; before the origin of the occipital artery) were ligated and a 4-0 silicone-coated filament was introduced into the distal common carotid artery. The filament was then advanced by 16–17.5 mm (origin: carotid bifurcation) into the internal carotid artery (ICA) until a weak resistance was felt. The filament was secured in this position using a tight ligature around the CCA. After 1 h of occlusion, the neurological status of the animals was assessed using the score introduced by Bederson [1], next the intraluminal thread was removed and the CCA was permanently occluded. Reperfusion was not directly assessed, but arterial backflow through the ICA occurred in all animals indicating that no major clots had formed at the tip of the filament. Animals (inclusive the sham group) were then allowed to recover for 1 week (n=12) or 4 weeks (n=3). The sham-procedure consisted of placing sutures around the right CCA and ECA without tightening them (sham group) while another group of animals (native group) received no treatment prior to the electrophysiological measurements.

CA1 pyramidal neurons were isolated enzymatically [16] as described in detail previously [36]. Brains were rapidly removed from the skull and stored for 2 min in ACSF chilled with ice. 400 \(\mu\)m-thick slices from the whole brain were cut with a vibratome (Leica VT1000S) at around 4°C. Tissue pieces (approx. 1 by 2 mm) were cut from the corresponding areas of the sensory cortex (mainly: Par1) of either side of the brain (Fig. 1), or in the case of a total loss of the infarcted brain hemisphere, only from the contralateral sensory cortex. These tissue pieces were incubated for 21 min at 32°C in oxygenated dissociation solution (in mM: NaCl 120, KCl 5, CaCl\(_2\) 1, MgCl\(_2\) 1, PIPES 20, \(\alpha\)-glucose 25; pH:7.0) containing 1 mg/ml protease (Type XIV), they were finally washed several times and kept in protease-free dissociation solution (19°C) until used. A subslice was dispersed in bath solution by trituration through Pasteur pipettes of decreasing diameter and brought into the perfusion chamber shortly before the measurements.

Bath solution contained in all experiments (in mM): NaCl 110, KCl 5, CaCl\(_2\) 5, MgCl\(_2\) 1, 4-AP 5, TEACl 25, HEPES 10, \(\alpha\)-glucose 25, and tetrodotoxin (TTX) 1 \(\mu\)M; pH was set at 7.4. All chemicals were obtained from Sigma (USA) or Merck (Germany).

Only neurons (n=116) with a bright and smooth appearance and no visible organelles were selected for recording. From the various groups of cortical neurons only pyramidal like cells of medium size were used for the measurements. Current amplitudes and capacity were homoge-

Fig. 1. Schematic drawing of the approximate location and size of a moderate infarction. Tissue pieces (2–3 pieces per slice; diameter: 1.5×2 mm) were taken from the dotted area from both sides of the cortex.
neously distributed throughout the measured cells, indicating that there was no dichotomy of membrane properties due to different cell classes. Currents were measured under whole-cell voltage-clamp conditions at room temperature (20–22°C) using patch pipettes of 2–4 MΩ resistance when filled with (in mM): CsF 110, MgCl₂ 2, CaCl₂ 0.5, TEACl 20, EGTA 10, phosphocreatine 20 mM, MgATP 2, NaGTP 0.1, leupeptin 0.1, HEPES 10, and phosphocreatine kinase 50 units/ml; pH was set at 7.3. Run-down phenomena were prevented by the ATP regenerating system, thus the time-dependent decrease in current amplitude never exceeded 5% within the recording period (7–8 min). Calcium currents were recorded with an Axopatch 200 B amplifier (Axon Instruments) and stored on an Atari ST computer (1 kHz sample frequency) using a custom made data acquisition system and software (‘Neuron’ by W.J. Wadman). Holding potential was kept at −80 mV. Series resistance was compensated for more than 90% and any capacitive transient was removed on-line. All current traces were off-line corrected for aspecific linear leak currents (reversal potential 0 mV) specified at holding potential by small voltage-steps (−5 and +5 mV).

From the two calcium current components present in cortical neurons, only high voltage activated currents (HVA) were evoked during this study, while low voltage activated currents (LVA) were not investigated. The high voltage activated calcium currents were activated by 200 ms voltage steps to voltage levels between −70 and +30 mV (Fig. 2), while the steady-state inactivation of the calcium current was determined using a test depolarization to +10 mV following a period of 3 s at potentials between −105 and 0 mV (Fig. 5). For analysis, peak amplitudes of the evoked currents were plotted against the membrane potential to examine voltage dependence of activation. The IV-curve of the current evoked from −70 mV was fitted with a combination of a Boltzmann activation function and the Goldman–Hodgkin–Katz current equation (GHK-fit) (for details see Kortekaas and Wadman [18]):

\[
I(V) = \frac{2 \alpha F P_o [Ca^{2+}]_{in}^{out} - \exp(-\alpha V)}{1 + \exp \left( \frac{V - V_h}{V_c} \right) - 1 \exp(-\alpha V)},
\]

with \( \alpha = \frac{2F}{RT} \)

where; \( P_o \) is the maximal permeability, \( V \) is the membrane potential, \( V_h \) is the potential of half maximal activation, \( V_c \) is proportional to the slope of the curve at \( V_h \), \( T \) is the absolute temperature and \( F/R \) are the Faraday and gas constant resp.

Maximal conductance was calculated using:

\[
g_{\text{max}} = 2 \alpha F P_o [Ca^{2+}]_{out}^{out}
\]

Fig. 2. Raw traces of whole cell calcium currents were evoked by an activation protocol (lower diagram). Neurons were held at a potential of −80 mV for 3 s. And test steps to more positive potentials (increment: 10 mV; range: 70… +30 mV) were applied. A: shows current traces from a neuron that derived from a sham operated animal. B gives current traces from a neuron which was dissected from the contralateral side of the neocortex from an animal with a moderate infarction at P7. Note the larger current amplitude in B.
The kinetics of the calcium current were described with a second order activation and a first order inactivation function (\( n^2 \tanh \)): 

\[
I(T) = I_{\text{max}} \left[ 1 - \exp \left( \frac{T_a - T}{\tau_a} \right) \right]^2 \exp \left( \frac{T_s - T}{\tau_s} \right) 
\]

(2)

where: \( I_{\text{max}} \) is the current amplitude and the voltage dependent activation (\( \tau_a \)) and inactivation (\( \tau_s \)) time constants following a voltage step at time \( t_c \).

The voltage dependence of steady state inactivation can be estimated using the relation between the normalized peak amplitude of the current and the prepulse potential. This relation was well described by a Boltzmann function:

\[
N(V) = \frac{I(V)}{I_{\text{max}}} = \frac{1}{1 + \exp \left( \frac{V - V_h}{V_c} \right)} 
\]

(3)

where: \( N(V) \) is the level of steady state inactivation determined from the current amplitude \( I(V) \) normalized to \( I_{\text{max}} \); \( V \) is the prepulse potential; \( V_h \) is the potential of half maximal inactivation and \( V_c \) is a factor proportional to the slope of the curve at \( V_h \).

Data are given as the mean±standard error of the mean (S.E.M.). Statistical comparisons were made by two-way ANOVA and Bonferroni test. Significance was achieved with \( P<0.05 \).

3. Results

Among six animals that were allowed to recover for 1 week, three animals had neocortical infarcts of moderate size, i.e. at least half of the ipsilateral cortex was still intact. The other three animals each had a large cortical infarct, where only relatively small neocortical regions (close to midline) remained intact. In the long-term group (\( n=3 \)), with a 4 week recovery period, all animals had (by chance) cortical infarcts of moderate size. Infarction of the striatum was evident in all experimental animals. None of the control animals (sham and native group; both \( n=6 \)) showed signs of ischemic damage.

Neurons were measured from both ipsi- and contralateral sensory cortex in the native and the sham group, as well as in those animals with lesions of moderate size, while only neurons from the contralateral sensory cortex were taken from the group with large infarcts. The groups will be referred to as: ipsi/mod (ipsilateral/moderate infarct); cont/mod (contralateral/moderate infarct); cont/sev (contralateral/severe infarct). Neurons measured from animals surviving 4 weeks will be referred to as: ipsi/4wk (ipsilateral/4 weeks survival) and con/4wk (contralateral/4 weeks survival).

Calcium currents from neurons of untreated native animals (\( n=28 \)) had similar current amplitudes (measured at +10 mV) and maximal conductances as neurons derived from sham operated animals (\( n=28 \)). The maximal conductance in the native group was 213±22 nS, which was not significantly different from the value derived from the sham group (182±29 nS). In both groups the calcium currents, evoked by an activation protocol (Fig. 2), had an activation threshold around –40 mV and reached their maximal amplitude between 0 and 10 mV (Fig. 3). The mean current amplitude at 10 mV, as evaluated by a fit with Eq. (1), was –1.3 nA in the native group and –1.2 nA in cells from the sham operated animal group. Also the potential of half-maximal activation (\( V_{\text{h,a}} \)) and the slope (\( V_c \)) at the point \( V_{\text{h,a}} \) were not different among both groups, with \( V_{\text{h,a}}: -1±2 \) mV (sham), 2±1 mV (native) and \( V_c: -7.4±0.3 \) mV in the sham group and –7.5±0.2 mV in the native group. Calcium currents in neurons (\( n=10 \)) from contralateral sensory cortex of infarcted animals (moderate infarct) had a maximal current amplitude (around 15 mV) which by 10% larger (–1.5 vs. –1.3 nA) than that measured in sham operated animals (Fig. 2). The current amplitude at a test potential of 10 mV was similar in both groups (1.4 vs. 1.3 nA). The increase in maximal amplitude was paralleled by a significant increase in maximal conductance (Fig. 4) to 342±54 nS (\( P<0.05 \)). Moreover, the potential of half-maximal activation was shifted by 11 mV (Table 1) in positive direction to 10±2 mV (\( P<0.01 \)), while the slope \( V_c \) was not different (–7.6±0.3) when compared with the sham group (Fig. 3C). Similar changes in calcium current properties were also found in neurons (\( n=7 \)) from animals, which were allowed to recover from surgery for 4 weeks, which implies that these alterations were long-lasting. The maximal amplitude (–1.5 nA) and the maximal conductance (403±82 nS; \( P<0.01 \)) were still larger than in sham operated animals, and the half-maximal potential of activation (\( V_{\text{h,a}} \)) remained almost at the same positive value as in the group of animals with 1 week of recovery (13±4 mV, \( P<0.01 \); when compared with the sham group). Again, the slope \( V_c \) was not altered and had a value of –7.7±0.3 mV.

Calcium currents of neurons from the contralateral hemisphere (\( n=13 \)) in animals with severe infarction, lacking wide parts of the ipsilateral sensory cortex, had current characteristics which equaled much more than those from native or sham operated animals. The maximal conductance was not different (220±50 nS) and the potential of half-maximal activation (2±2 mV) resembled the values of the native or sham group. The same held true for the mean current amplitude measured at 10 mV (1.3 nA) and the slope \( V_c \) at the point of \( V_{\text{h,a}} \) (–8.3±0.4 mV).

Alterations of calcium current characteristics in neurons from the ipsilateral sensory cortex of animals with moderate infarction were only moderate, when compared to neurons from the contralateral side, and showed no significant difference to the sham operated animal group. The mean current amplitude (at 10 mV) and the maximal conductance in the group of neurons (\( n=12 \)) with moderate infarction and a recovery period of 1 week were quite
similar to those in the sham or native group (−1.2 nA and 229±48 nS, resp.). The same held true for the parameters of the activation $V_{h,a}$ and $V_c$. When compared with the sham group, $V_{h,a}$ was slightly more positive in this group (5±2 mV) and the slope $V_c$ at the point of $V_{h,a}$ was in the same range with −7.0±0.2 mV. As described above, neurons from the contralateral hemisphere showed no additional, time-related changes when the groups with 1 week or with 4 weeks recovery were compared. In contrast to this finding neurons from the infarcted brain hemisphere, in animals with 4 week recovery, tended to show smaller maximal conductances and smaller mean current amplitudes, than neurons from animals with 1 week recovery. The mean maximal conductance amounted to 156±29 nS and the mean current amplitude, measured at 10 mV, was lowered to −1.0 nA. The potential of half-maximal activation $V_{h,a}$ was similar to the sham or native group (0±2 mV), but the slope $V_c$ was slightly steeper with a value of −6.7±0.3 mV.

The time constants of activation and inactivation were fitted with Eq. (2) for five different voltages (−20, −10, 0, 10, 20 mV). This evaluation revealed no significant differences among all groups compared. Since time constants at the given voltages did not differ, they were lumped together for each group giving values for the time constant of activation of −1.2 and 70–80 ms for inactivation (for details see Table 1). Only neurons from animals with a severe infarction showed a significantly faster activation (−0.9±0.1 ms; P<0.05) and inactivation (−61±7 ms; n.s.). The steady state inactivation of calcium currents can be evaluated by holding the cell for 3 s at stepwise increased potentials, which are followed by a fixed test-pulse to 10 mV.

When the amplitudes of the evoked calcium currents were normalized to the maximal current amplitude and plotted against the pre-potentials, the resulting current voltage relationship could well be described by Eq. (3). This Boltzmann fit delivers the half-maximal potential of inactivation $V_{h,i}$ and the slope of the Boltzmann curve $V_{c,i}$ at this point. This evaluation showed a highly significant, but unique, increase in the slope of the Boltzmann curve for all groups with infarction (range of $V_{c,i}$: 10.7±0.7 ... 13.8±1.7 mV vs. 9.3±0.3 mV in sham; for detail see Table 1). Furthermore, there was a tendency for the potential of half-maximal inactivation $V_{h,i}$ to be more positive (even in the sham group) than in native animals (range $V_{h,i}$: −30±3 ... −26±3 mV vs. −33±2 mV in native).

4. Discussion

The present study demonstrates two distinct alterations of whole cell calcium current characteristics from neurons in the sensory cortex contralateral to a moderate infarction following transient MCA-occlusion. First, the maximal conductance for calcium ions was enhanced at day 7 in these cells and stayed at this level at least up to day 28 after infarction. Second, the half-maximal potential of
Maximal calcium conductances as were observed within the different animal groups. The conductance was significantly increased (as compared to sham operated animals) only in animals with a moderate infarct.

Calcium currents play a major role in the generation of action potentials, synaptic transmission and in a huge variety of intracellular regulatory mechanisms. Any alteration of size and voltage dependence of calcium currents should therefore be followed by serious consequences for neurons and for the entire neuronal network. Such consequences due to up-regulation of whole cell calcium currents under pathological circumstances have been reported in a chronic model of kindling epileptogenesis in CA1 neurons, which led to spontaneous epileptogenic discharges lasting for several weeks [8,36,37]. On the one hand, an increase in maximal conductance, as observed in our study in neurons from contralateral regions, may account for an increase in excitability of the network, due to increased calcium influx and larger and broader action potentials. Following permanent MCAO, increased field potential amplitudes and enhanced disinhibition within contralateral sensory cortex neurons have recently been reported [30], thus supporting this assumption. On the other hand, a shift in the potential of half-maximal activation of the size observed in this study can diminish the enhancing effects of maximal conductance, since maximal calcium current amplitude is only activated at, more or less, unusually positive potentials. Therefore, one may speculate that these high potentials are not reached during normal neuronal activity. In accordance with this, only mild increases of field potential amplitudes and disinhibitions were observed 7 days after transient MCAO (data not published) with moderate infarctions. The observed current alterations in the contralateral area may therefore be selectively relevant during high frequency and repetitive spiking, and may be a prerequisite for plasticity of the network. An increased ion conductance, as observed in the present study, can be achieved by alterations of the

![Graph showing maximal conductances](image-url)
Fig. 5. Normalized current amplitudes were plotted against the selected pre-potentials (see voltage protocol), giving the Boltzmann relation for the steady state inactivation. No relevant differences in the halfmaximal potential of inactivation ($V_{h,i}$) were found within the animal groups tested. Nevertheless, infarcted animals had a somewhat steeper slope of the curve at the point of $V_{h,i}$.

Gating properties or by the formation of additional channels, thereby increasing the channel density of the neuronal membrane. It has been reported that during the establishment of a kindled focus — thus caused by repeated stimulation, i.e. in an activity-dependent manner — within rat hippocampus, additional calcium channels are formed. This process was associated with an up-regulation of the expression of mRNA encoding for different subunits of those channels [14]. It is conceivable that such a mechanism also accounts for the increase in maximal conductance.

| Table 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Cont/mod        | Cont/4wk        | Cont/sev        | Ipsi/mod        | Ipsi/4wk        | Sham            | Native          |
| Animals        | 3               | 2               | 3               | 3               | 3               | 6               | 6               |
| Cells          | 10              | 7               | 13              | 12              | 13              | 28              | 28              |
| Capacitance [pF] | 8.6±0.6         | 6.4±0.7         | 6.6±0.7         | 9.2±0.6         | 9.3±0.8         | 7.9±0.6         | 6.5±0.4         |
| Impedance [MΩ] | 656±93          | 758±87          | 671±144         | 533±85          | 730±161         | 698±81          | 759±79          |
| Activation     |                |                |                |                |                |                |                |
| $I_{a}$ [nA]   | −1.4            | −1.3            | −1.3            | −1.2            | −1.0            | −1.2            | −1.3            |
| Max. G [nS]    |                |                |                |                |                |                |                |
| $V_{a}$ [mV]   | 342±54*         | 403±82**        | 220±50          | 229±48          | 156±29          | 182±29          | 213±22          |
| $V_{act}$ [mV] | −7.6±0.3        | −7.7±0.3        | −8.3±0.4        | −7.0±0.2        | −6.7±0.3        | −7.4±0.3        | −7.5±0.2        |
| $T_{act}$ [ms] | −1.1±0.1        | −1.4±0.1        | −0.9±0.1*       | −1.3±0.1        | −1.3±0.1        | −1.2±0.1        | −1.3±0.1        |
| Inactivation   |                |                |                |                |                |                |                |
| $V_{h}$ [mV]   | −28±2           | −28±3           | −30±3           | −26±3           | −30±3           | −27±2           | −33±2           |
| $V_{i}$ [mV]   | 11±1            | 14±2**          | 11±1            | 11±1            | 12±1*           | 9±0.3           | 10±0.4          |
| $T_{max}$ [ms] | −76±7           | −70±7           | −61±7           | −75±6           | −77±7           | −81±7           | −80±8           |

Data are given as mean±S.E.M.; with * $P<0.05$ and ** $P<0.01$. 
ance in neurons of the contralateral and not infarcted brain hemisphere. Surprisingly, alterations of current characteristics were strictly dependent on the size of neocortical infarcts. Thus, in neurons from animals with a severe infarction no increase of calcium conductance and no positive shift of the potential of half-maximal activation ($V_{1/2}$) was found. This points towards a mediation of the calcium current changes by behavioral activity of the animals. Animals with moderate infarct size show relatively small behavioral deficits [1], and have the ability to compensate for the deficit of the affected forepaw. Infarcted animals rely primarily on the unaffected ipsilateral forepaw resulting in a preferential use of this limb. This was paralleled by an overgrowth of dendrites of layer V pyramidal neurons in the non-infarcted hemisphere. The sprouting of the dendrites was use-dependent and could be prevented by an immobilization of the unimpaired limb [19]. It is therefore conceivable that the modulation of calcium currents may be initiated by qualitatively similar mechanisms. The signaling cascade responsible for these alterations of calcium currents is not known. The liberation of trophic factors is modulated by activity, and trophic factors have been described to alter neuronal calcium current [2,20]. They are therefore possible candidates which may induce the observed calcium current changes. Rats, suffering from severe infarction, not only have a higher mortality (up to 30%) but show, in contrast, strongly reduced spontaneous activity and clumsy movements (unpublished observation). It is therefore conceivable that the use-dependent modulation, as is to be assumed in animals with a moderate infarction, is absent in animals with severe brain damage due to their reduced mobility. Interestingly, neurons of the ipsilateral infarcted cortex displayed calcium conductances that were close to normal. This finding is in line with a recent study demonstrating reduced depolarization induced calcium accumulations in hippocampal neurons after transient vessel occlusion [7]. This reduction was not accompanied by an enhanced intracellular calcium buffering and was therefore attributed to a reduction in calcium permeability through voltage gated calcium currents. In a recent report concerning the expression of mRNA encoding for the α1- and α2-subunits of GABA<sub>α</sub>-receptors following the induction of a focal cortical lesions [26,27], it was found that a partial translation block of that mRNA occurs. It was speculated that spreading depression or edema formation, both frequently observed after cortical insults, might account for this blockade of gene translation. This assumption is furthermore substantiated by the fact that both phenomena are clearly restricted to the ipsilateral and affected brain hemisphere. Following transient ischemia Connor et al. [7] showed some reduction in the intracellular calcium levels in CA1 neurons, which could be an additional factor that hampers gene translation, since the latter is strongly calcium dependent [11–13,34]. Together with these findings our data suggest that the formation of calcium channels in neurons from the peri-infarct region is prevented by those mechanisms, although an up-regulation of mRNAs encoding for calcium channel subunits may occur within these cells like in contralateral neurons. To prove this assumption further investigations are necessary, using experimental protocols which prevent spreading depression and reduce post-infarct edema formation. The presumably use-dependent increase of the calcium currents in the hemisphere contralateral to the lesion suggests that an enhancement of the use of the affected limb e.g. by physical therapy might favor processes of plasticity and thus increase the recovery from the lesion. It should be noted, however, that a forced use of the affected limb e.g. due to casting of the healthy limb might exert a detrimental effect if started too early following lesion induction [19]. Calcium plays a major role in processes of brain plasticity, and it is conceivable that the alteration of the calcium currents is not only a consequence of adaptation, but may also enhance such processes of brain plasticity [6]. Therefore, the use of substances which modulate calcium currents like calcium antagonists as given to many patients might also impair the brain plasticity. The criteria used in this study discriminating the neurons taken for the measurements surely covers the risk that different neuron classes with different membrane properties are pooled together leading to inaccurate results and false differences between the animal groups compared. Moreover neuronal necrosis occurring within the peri-infarct region [10,23,25] may have induced an unintended pre-selection of cells, with different properties in whole cell calcium currents. Nevertheless, parameters describing the properties of the membrane of the concerned neurons, like capacitance and impedance were uniformly distributed and showed no sign for a dichotomy between the selected neurons. Therefore, it is unlikely that an incorrect selection of neurons may have contributed to the observed differences in calcium current properties. We used freshly isolated neurons, since the experimental conditions can be tightly controlled and currents can be measured without space-clamp problems due to the compact appearance of cells. Nevertheless, the information collected with this configuration is clearly focussed on the single cell level, especially the somatic part of the cells, since most of the synaptic mass has been disrupted by the dissociation process. Despite this limitation the parameters measured under these conditions are a good indication of what will be seen in cells within their natural network environment [8,36,38].

In summary, we have demonstrated significant alteration of calcium current properties within neurons contralateral to a neocortical infarct of moderate size. These alterations were obvious at P7 and remained unchanged until at least P28. Since calcium influx into neurons (especially postsynaptic) is a pre-requisite for plasticity and learning, the increased calcium conductances in neurons from the contralateral area may favor those mechanisms and may enhance functional compensation after infarction.
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