**Report**

**Lurcher Mice Exhibit Potentiation of GABA<sub>A</sub>-Receptor–Mediated Conductance in Cerebellar Nuclei Neurons in Close Temporal Relationship to Purkinje Cell Death**

C. Linnemann,* F. Sultan,* C. M. Pedroarena, C. Schwarz, and P. Thier

*Department of Cognitive Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany*

Submitted 21 February 2003; accepted in final form 2 October 2003

**INTRODUCTION**

The GABA<sub>A</sub>ergic Purkinje cell (PC) projection onto the deep cerebellar nuclei (DCN) undergoes a degeneration in the spontaneous, semidominant mouse mutation Lurcher (Phillips 1960). Homozygous mutants (Lc/Lc) die shortly after birth. Heterozygous mice (Lc+/Lc) become ataxic as a result of an apoptotic death of cerebellar PCs during postnatal development (Caddy and Biscoe 1979; Wetts and Herrup 1982). In Lc+/+, the first signs of PC degeneration are observed at postnatal day (p) 8 (Dumesnil-Bouzé and Sotelo 1992) and, by p26, about 90% of PCs have disappeared (Caddy and Biscoe 1979). On the other hand, the majority of DCN neurons survive, although deprived of their inhibitory PC input (Heckroth 1994; Sultan et al. 2002). In view of the almost total disruption of cerebellocortical signal processing, the ataxia in Lc+/+ is surprisingly mild and some degree of sensorimotor learning is even retained (Lalonde et al. 1996). The ataxia, however, is substantially aggravated by lesioning the DCN (Caston et al. 1995). Hence, the DCN seem to play a beneficial role for the motor performance of Lc+/+. We asked whether there are any cellular adaptations in the DCN of Lc+/+ allowing them to compensate for the functional consequences of the massive denervation. To this end, we investigated the properties of inhibitory postsynaptic currents (IPSCs) recorded in DCN of mutants in comparison to wild-type (WT) mice during postnatal development before and after the onset of the PC degeneration.

**METHODS**

**Animals and molecular genotyping**

Heterozygous B6CBAcA-<sup>ALc</sup> (Jackson Laboratories, Bar Harbor, ME) were mated with B6CBA (Charles River, Sulzfeld, Germany) mice. B6CBA mice were also used as WT controls. Animals were kept and used in experiments according to the institutional and national animal care guidelines (also conforming with National Institutes of Health guidelines on animal care). Animals from the Lurcher progeny not showing the characteristic Lc+ phenotype (ataxic gait and tendency to fall) were genotyped. The genomic DNA was isolated (DNeasy Tissue Kit, Qiagen, Hilden, Germany). Primers (MWG-Biotech, Ebersberg, Germany) 5′-TAAAAGCATATTGGTGTGTG-3′ or 5′-GCACTGAAATGTTATGACTTCAG-3′ and 5′-CAGCATTTGTCCAGTTGAGAC-3′ (Zuo et al. 1997) were used in PCR to amplify the region of interest, including a guanine to adenine transition at nucleotide position 1960 in exon B of the mouse δ2 glutamate receptor gene. The PCR product was purified (CONCERT Rapid PCR Purification System, Gibco BRL, Rockville, MD) and sequenced employing the fluorescent dideoxynucleotide technology and evaluated using Lasergene software (DNastar, Madison, WI).

**Electrophysiology and data analysis**

The mice were deeply anesthetized by application of ketamine (150 mg/kg ip). In some cases, the mice were intracardially perfused with ice-cold modified artificial cerebrospinal fluid (ACSF) for 2 min before further preparation. The modified ACSF contained (in mM) 126 sucrose, 2.5 KCl, 1.3 Na<sub>H</sub><sub>P</sub>O<sub>4</sub>, 3 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 0.1 CaCl<sub>2</sub>, and 20 D-glucose and was bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub>. The brains were removed and put in the ice-cold modified ACSF. The cerebellum was isolated and cut into two parasagittal halves. Parasagittal slices of 250 to 275 μm thickness were obtained using a sagittal slicer of page charges. The article must therefore be hereby marked ‘‘advertisement’’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*L. Linnemann and F. Sultan contributed equally to this study.

Address for reprint requests and other correspondence: C. Linnemann, Department of Cognitive Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen, Auf der Morgenstelle 15, 72076 Tübingen, Germany (E-mail: Christoph.Linnemann@uni-tuebingen.de).

**The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.**
vibratome (VT1000s, Leica, Nussloch, Germany) and transferred into modified ACSF at room temperature. The modified ACSF was replaced within the next 90 min with ACSF containing (in mM) 125 NaCl, 2.5 KCl, 1.3 NaH₂PO₄, 2 MgCl₂, 26 NaHCO₃, 2 CaCl₂, and 20 D-glucose, oxygenated with 95% O₂ – 5% CO₂ at room temperature. Experiments were performed with this ACSF to which the selective non-N-methyl-D-aspartate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione [DNQX, 20–25 μM, for recordings of spontaneous IPSCs (sIPSCs)], and in some recordings also TTX [1 μM, to detect miniature IPSCs (mIPSCs)] and/or the GABA_A antagonist bicuculline (10 μM, Tocris, Bristol, UK), were added.

Whole-cell voltage-clamp recordings of the DCN were conducted with patch pipettes that had a resistance of 2.0–6.0 MΩ when filled with a solution containing (in mM) 124 CsCl, 10 K⁺-HEPES, 5 EGTA, 4.6 MgCl₂, 4 K⁺ ATP, 0.4 Na⁺ GTP, 0.1 CaCl₂, and 5 QX-314 (Tocris), adjusted to pH 7.3 with CsOH, thus obtaining chloride-symmetrical experimental conditions. The patch procedure was visualized using a motorized (Luigs & Neumann, Ratingen, Germany) microscope (Axioscope, Zeiss, Göttingen, Germany) with water immersion objective (×40, Zeiss numerical aperture 0.75), infrared illumination, Normaski optics and infrared sensitive CCD camera (Newvicon C2400—07-C, Hamamatsu, Japan). The recordings were performed with an EPC-7 or EPC-8 amplifier (Heka, Lambrecht, Germany). The data were sampled at a rate of 20 kHz and subsequently low-pass filtered (cutoff frequency 1 kHz) (Spike2, CED, Cambridge, UK). Membrane resistances for the recordings used for quantitative analysis were 494.8 ± 379.4 MΩ (mean ± SD, throughout the paper) and the mean ratio serial resistance/membrane resistance was 0.078. The membrane and serial resistances did not differ significantly between genotypes. Neurons were voltage clamped at ~70.4 ± 4.4 mV. Data were not quantitatively analyzed if under these conditions the serial resistance increased more than 25% of the initial value or if the membrane resistance of the recorded neuron was below 150 MΩ.

Analysis of the postsynaptic currents was performed with the MiniAnalysis program (Synaptosoft, Decatur, GA). For the analysis of the synaptic waveforms only synaptic events were evaluated that were not interrupted by other synaptic events. On average, 145 ± 190 events (n = 92 recordings) were evaluated. Average synaptic conductance (g_syn) values were calculated as g_syn = I/mean/(V_h – E), where I/mean is the mean peak amplitude of the IPSCs, V_h is the holding potential, and E is the measured equilibrium potential for the IPSCs of the respective recording. Decay time mentioned in this paper is the time it takes the current values to decay to 1/e of the peak values of the IPSC amplitudes. Peak-scaled nonstationary fluctuation analysis was performed using the respective module of MiniAnalysis, version 3.0.
5.6.29 (adapted from the original algorithm by Traynelis et al. 1993). Those IPSCs that were interrupted by other events and did not return to baseline within the chosen time window were discarded. The recording sections used for the nonstationary fluctuation analysis showed no statistically significant correlation between IPSC amplitude and time, decay time and amplitude, or IPSC amplitude and half-width. The unitary current ($i$) was estimated by fitting the equation $\sigma^2(t) = \frac{I(t) - I(t)}{N} + \sigma^2_{\text{basal}}$, where $\sigma^2$ represents the variance at given time $t$ of the mIPSC, $I$ the current at given time $t$, and $\sigma^2_{\text{basal}}$ the variance in baseline noise measured before the peak. The algorithm forces the probability of channel opening at the peak of the mIPSC to approach unity (1.0), consequently $N$ represents the average number of channels open at peak ($N_{\text{open,peak}}$). Fitting was done after subtracting the baseline variance and without including the offset. The unitary conductance values ($\gamma$) were calculated as $\gamma = \frac{i(V_h - E)}{N_{\text{open,peak}}}$. 

Statistically significant differences and correlations were tested with the Student’s $t$-test and Pearson product moment correlation test for normally distributed data, otherwise with the Mann–Whitney test and Spearman rank order correlation test (italicized $P$ values throughout the paper). Statistical significance was assumed for $P < 0.05$.

**RESULTS AND DISCUSSION**

Voltage-clamp recordings, performed from neurons in the DCN of mutant mice (p3–21, $n = 41$) as well as from control animals (p4–28, $n = 51$) consistently revealed dense patterns of IPSCs. These IPSCs could be blocked regularly in both $Lc^+/+$ and WT (not shown, $n = 7$ recordings) by application of 10 $\mu$M bicuculline, indicating that they were GABAergic.

In those experiments in which mutants were studied well after the onset of the PC degeneration, i.e., 14 or more postnatal days, the $g_{\text{syn}}$ values in $Lc^+/+$ were found enlarged compared with those in WT (for sIPSCs: $1.38 \pm 0.50$ nS ($n = 12$, $Lc^+/+$) versus $0.66 \pm 0.28$ nS ($n = 14$, WT), $P \leq 0.001$; for mIPSCs: $1.23 \pm 0.51$ nS ($n = 10$, $Lc^+/+$) versus $0.57 \pm 0.21$ nS ($n = 19$, WT), $P \leq 0.001$).

We next asked whether the conductance changes appear in parallel to PC axonal degeneration or follow a different time course. Figure 1 shows recordings of sIPSCs and mIPSCs from the DCN of $Lc^+/+$ and WT at different postnatal days. An
increase of $g_{\text{syn}}$ is obvious in the recordings obtained at p14 in the mutants, but not in the WT. The $g_{\text{syn}}$ in animals 13 or fewer postnatal days did not show any statistically significant difference between $Lc/+\leq$p13 versus $0.62 \pm 0.32$ nS ($n = 14$, WT $\leq$p13), $P = 0.95$]. The respective values for recordings of mIPSCs were $0.50 \pm 0.39$ nS ($n = 3$, $Lc/+\leq$p13) versus $0.39 \pm 0.16$ nS ($n = 4$, WT $\leq$p13), $P = 0.62$. At p14, $g_{\text{syn}}$ in $Lc/+$ showed an increase while those in WT stayed on the level found in younger animals. Hence the change in conductance occurs only after the onset of PC degeneration. Figure 2, A and B summarize the findings. The $g_{\text{syn}}$ are plotted as a function of the postnatal age of the recorded neuron. An analysis of amplitude distribution revealed that this increase in conductance was due to a decrease in the number of small events accompanied by an increase in the number of large events. There was no obvious indication for the appearance of a new population of IPSCs in $Lc/+\leq$ compared with WT 14 postnatal days or older (Fig. 2C).

We analyzed various parameters defining the properties of IPSCs known to undergo typical developmental changes in other inhibitory GABAergic systems. Figure 3 illustrates the developmental courses of the 10–90% rise time and the decay time for IPSCs in $Lc/+\leq$ and WT. The 10–90% rise time as well as the decay time of the sIPSCs showed a significant decrease in both $Lc/+\leq$ and WT (10–90% rise time: for $Lc/+\leq$ $r = -0.64$, $P = 0.004$, $n = 18$; for WT $r = -0.75$, $P < 0.001$, $n = 20$; decay time: for $Lc/+\leq$ $r = -0.52$, $P = 0.026$, $n = 18$; for WT $r = -0.57$, $P = 0.009$, $n = 20$). These developmental changes reflect a speeding up of GABAergic IPSCs in both $Lc/+\leq$ and WT. The results can be brought very well into line with studies of GABAergic synapses in other parts of the mammalian CNS where developmental changes in the kinetics with unchanged IPSC amplitudes are reported and correlated with changes in the GABAA receptor subunit composition (Dunning et al. 1999; Hollrigel and Soltesz 1997; Vicini et al. 2001). Importantly, the maturation of the synaptic transmission in $Lc/+\leq$ seems to follow a normal pattern with the exception of a step-like increase in $g_{\text{syn}}$ in the mutant at p14. The data are consistent with a functional synapse with an enhanced inhibitory potency per afferent terminal at this point in time. One might speculate that intrinsic fibers/interneurons are involved in these compensatory mechanisms. Sultan et al. (2002) found indications for an increase in number and size of non-PC GABAergic synapses in $Lc/+\leq$, most probably corresponding to interneurons.

An analysis of the frequencies of the IPSCs revealed no differences between mutants and WT, either for animals younger than p14 [for sIPSCs: $2.0 \pm 1.8$ Hz ($n = 16$, $Lc/+\leq$) versus $1.8 \pm 3.6$ Hz ($n = 14$, WT), $P = 0.119$; for mIPSCs:
The synaptic potentiation occurs in close temporal relationship to the PC degeneration, which starts at p8 showing first signs of PC anomaly, progresses with first cell deaths at p12 (Dumesnil-Bouze and Sotelo 1992), and leads to the death of about 90% of PCs at p26 (Caddy and Bisceo 1979). Therefore the synaptic potentiation at p14 in the mutants may well be the consequence of the degeneration of the DCN’s only GABAergic afferents rather than a direct, primary element of Lc/+ pathology.

Electrophysiological in vitro recordings from the DCN are possible in a limited postnatal time window only. The high myelinization of the structure in older animals complicates the isolation of viable neurons and the formation of a stable patch configuration and—in our hands—renders such recordings virtually impossible in older animals. Due to this limited postnatal time window it is difficult to issue a statement about the electrophysiological properties in older animals. It is therefore not possible at this point to decide if our present data reflect a transient or permanent mechanism of adaptation.

GABAergic synapses on DCN neurons have been shown to undergo long-term potentiation (LTP) in response to an electrical pulse train to their afferents in the white matter or after sole intracellular depolarization (Aizenman et al. 1998; Ouardouz and Sastry 2000). This form of LTP is accompanied by increases in mIPSC frequency without changes in their amplitude but stronger response to extracellularly applied GABA agonists, a finding that could be explained by the activation of previously “silent” synapses (Ouardouz and Sastry 2000). While the potentiation of GABAergic IPSCs in Lc/+ as described here might be taken as a form of LTP, it must employ a mechanism different from the one described earlier (Ouardouz and Sastry 2000) because amplitudes of mIPSC are increased. Similar types of LTP, based on changes in N_{open,peak} without alterations in the γ values, have been observed in other GABAergic synapses, e.g., after kindling of hippocampal inhibitory synapses (Otis et al. 1994; Nusser et al. 1998) or after depriving activity at neocortical synapses (Kilman et al. 2002).

An important implication of the present study is therefore that forms of LTP may not only be involved in learning and memory, but may also be important for adaptive processes following brain damage or degeneration (Mittmann and Eysel 2001; Wilson et al. 1979) and that they might even have an adaptive value, compensating for the loss of the extrinsic inhibition.

**ACKNOWLEDGMENTS**

The sequencing procedures were performed by the sequencing service facility of the Center for Interdisciplinary Clinical Research Tübingen. We thank U. Großehnig and B. Baumann for technical assistance and Drs. M. Möck and V. Gauck of our laboratory and Dr. B. Wissinger of the Molecular Genetics Laboratory, University Eye Hospital Tübingen for discussion, advice, and support.
GABAergic TRANSMISSION IN LURCHER CEREBELLUM

This work was supported by the German Science Foundation (SFB 430-C6), the Center for Interdisciplinary Clinical Research, Tübingen (IZKF IC1), and the Hermann and Lilly Schilling Foundation.

REFERENCES


