

## Research Report

# Interference of chronically ingested copper in long-term potentiation (LTP) of rat hippocampus

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### Abstract

The objective of our study was to find the evidence of copper interaction in LTP, motivated by copper involvement in neurodegenerative illness, like Parkinson, Alzheimer and Amyotrophic Lateral Sclerosis, and we initiated the study of this element in the LTP. For this purpose we used hippocampus slices of rats chronically consuming copper dissolved in water (CuDR;  $n = 26$ ) and non-copper-consuming rats (CR;  $n = 20$ ). The CuDR rats received 8–10 mg/day during 20–25 days. Electrophysiological tests showed absence of LTP in CuDR slices, contrary to CR slices. The stimulus–response test applied before and after LTP showed significant increases of synaptic potential in the CR group. This did not occur in the CuDR group, except for the initial values, which probably seem associated to an early action of copper. The paired-pulse (PP) test, applied to CR and CuDR prior to tetanic stimulation, showed a significant reduction in PP, for the 20-, 30- and 50-ms intervals in CuDR. At the end of the experiments, copper concentration was 54.2 times higher in CuDR slices, compared to the concentration present in CR slices. Our results show that copper reduces synaptic sensibility and also the facilitation capability. These effects represent a significant disturbance in the plasticity phenomenon associated with learning and memory.

*Theme:* Synaptic transmission in hippocampus

*Topic:* Long-term potentiation

*Keywords:* Chronic copper ingest; Hippocampal slice; LTP

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## 1. Introduction

In 1912, Kinnier Wilson described in his monography “progressive lenticular degeneration”, a hereditary-recessive degenerative pathology, involving primarily the liver and the nervous system and which invariably lead to death. It was only in 1984 that copper was identified as the toxic element of Wilson’s disease, when a failure in biliary secretion of the metal caused an accumulation of this metal in liver and brain, particularly in the lenticular nucleus of the basal ganglion [6,26,27].

In 1962, Menkes described in two infants a sex-linked hereditary-recessive pathology, with developmental retar-

dation and brain and cerebellar degeneration that rapidly evolved to decerebration and death [22]. More recently, Menkes’s disease has been associated to copper, due in this case to an alteration in the absorption of this metal. Thus, both the excessive accumulation of copper, Wilson’s disease and also the absence of copper in the newborn infant will impact preferably the central nervous system, with an obvious neurological and psychiatric expression. At present, the pathological role of copper in Alzheimer, Parkinson and amyotrophic lateral sclerosis [2,32] is being discussed.

From a different perspective, it has been considered that copper is an essential nutrient for the development of plants and animals because it is an integral part of several proteins, and also necessary cofactor for many enzymes. The action of copper is extremely useful in the performance of

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cytochrome *c* oxidase, superoxide dismutase, metallothionein, dopamine beta hydroxylase, tyrosinase, lysyl oxidase, clotting Factor V and Factor VIII [18]. Additionally, copper levels have been estimated at about 100  $\mu\text{M}$  in the cortical synaptic clefts [13,15].

On the other hand, we have shown that hippocampus slices with 1, 10 and 100  $\mu\text{M}$  of copper decrease significantly field excitatory post-synaptic potential (fEPSP) of CA1 produced by stimulation of Schaffer's collaterals, an independent effect of concentration [16]. With the aim to progress in the study of copper action at synaptic level, we studied *in vitro* its effect on long-term potentiation (LTP) in the CA1 area of rat hippocampus. We showed that once LTP was established, it disappeared when a 10- $\mu\text{M}$  copper sulfate solution was added to the bath. However, after a continuous flushing of the slice during 20 min, LTP reappears but with hyperexcitability characteristics [17].

The aim of the present paper was to study the excitability and plasticity of CA1 neurons in hippocampal slices of rats that are chronic consumers of copper dissolved in water, compared to slices from non-copper-consumer rats. For that purpose we used *in vitro* electrophysiological tests of synaptic stimulus response, LTP and paired pulses (PP).

## 2. Materials and methods

All indications prescribed by IBRO concerning laboratory animal care were followed, as well as the standards established by Universidad de Chile.

Experiments were carried out in 46 rats weighting between 180 and 250 g. One of the groups ( $n = 26$ ) was placed in isolated cages with food and water with copper sulfate *ad libitum*. Each of these rats drank 8–12 mg/day of copper during 20–25 days. The weight of the animals, as well as locomotor and rearing behavior, was controlled daily. The same procedure was carried out with the control group of rats ( $n = 20$ ), except that this group drank tap water. Prior to the surgical procedure, the animals were anesthetized with halothane and decapitated with a Stoelting decapitator. This procedure was performed after all reflexes to puncturing the back legs had disappeared. The entire brain, rapidly removed and continuously moistened with cold Krebs–Ringer (Ks–R), was briefly immersed in cold Ks–R.

The hippocampus was isolated and removed from the hemi-brain, slicing it in coronal slices of 400  $\mu$ , and using for this procedure a McIlwain tissue chopper (Mickle Laboratory Engineering, Surrey, UK). Afterwards slices were immersed for 1 h in an incubation chamber and then transferred to a recording chamber under continuous perfusion with lukewarm Ks–R ( $31 \pm 0.1^\circ\text{C}$ ). Krebs–Ringer solution (in mM) consisted of 124 NaCl, 5 KCl, 1.25  $\text{MgSO}_4 + 7 \text{H}_2\text{O}$ , 2  $\text{CaCl}_2$ ,  $\text{NaHCO}_3$  and 10 glucose

at pH 7.4, previously bubbled with carbogen (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ). The perfusion system allows a continuous flow of 2–3 ml/min.

### 2.1. *In vitro* neuronal recording

Hippocampal slices were stimulated through bipolar tungsten electrodes, applied to Schaffer's collaterals, using a DG2 Digitimer Trigger Generator connected to a DS2A Isolated Stimulator (<http://www.digitimer.com>). Single stimuli of 0.05–20 V and 0.030 ms in duration were applied. The stimulating electrodes were located near the CA1 pyramidal cells. At the beginning of the experimental tests, we used stimulation frequencies of 0.7–30 s with intensities twice the threshold. In this condition we recorded field excitatory post-synaptic potentials (fEPSP) and on certain occasions we found simultaneously population spike responses [8]. Recordings were carried out using glass micropipettes pulled in an M.I. (Model 63-15) Micropipette Puller. Micropipettes contained Ks–R as a conductor with an impedance of 1.5  $\text{M}\Omega$ . These microelectrodes were positioned in CA1 by means of visual observation assisted by a binocular loupe (Carl Zeiss). A hydraulic micro-manipulator allowed us to move downwards with the micropipette all across the slice thickness. In order to obtain neuronal activity, we used a (Bio-Logic VF-180) amplifier, displaying its content in an oscilloscope (Hitachi VC-6020). This information was stored in a tape recorder (SONY. DTC.59 E.S.) for a deferred analysis. The analysis consisted in an analog-to-digital data conversion by a Cambridge Electronic Device (CED), and afterwards a treatment of digitalized data by means of an average program (SIGAVG) on a Pentium 4 microcomputer.

Each slice that showed appropriate fEPSP responses, associated with the indicated stimulus values, was used for different electrophysiological tests. The first test was designed to determine response parameters for each slice, using an input/output (I/O) protocol. In this case, the slice was stimulated at a range of voltages (usually between 0.2 and 5 V), starting at the threshold voltage to elicit fEPSP, and then the stimulus strength was increased in steps of 0.5 V, starting with the threshold voltage for the beginning of fEPSP, and then the stimulation intensity was incremented in steps of 0.5 V (stimulation frequency of one pulse every 30 s), until the magnitude of fEPSP reached its maximum. Only those slices that showed a maximum fEPSP amplitude  $>5$  mV were used in this study, and we also discarded any slice presenting multiple population spikes in response to synaptic activation. For the development of the paired-pulse (PP) protocol, the stimulus voltage was adjusted for each slice, so that the fEPSP be approximately equal to 50% of the maximum fEPSP amplitude. The paired-pulse protocol was used for intervals of 10, 20, 30, 50, 100, 200 and 300 ms (ten trials of each pulse interval were averaged; with stimulation frequencies of 1 every 60–90 s). Slices that

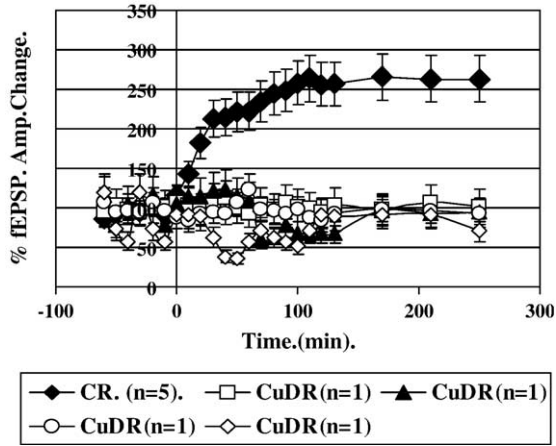


Fig. 1. The effects of chronic Cu<sup>++</sup> on long-term potentiation (LTP) in Schaffer' collaterals CA1 system. The graphic shows the percentage mean variation in fEPSP amplitude slope in five slices ( $n = 5$ ), obtained from tap water drinker rats (CR), during (a) control periods (60 min) and (b) LTP period (120 min after TS). At the same time, the graphic shows the mean percentage variation in fEPSP amplitude slope in four single slices ( $n = 4$ ), coming from copper drinker rats (CuDR). Ordinate: Mean percentage increase in fEPSP amplitude (% Mean fEPSP.Amp.). Abscissa: time in minutes: (a) 60 min, before the tetanic stimulation (TS) recording samples ( $n = 10$ ), each 10 min by slice; (b) 120 min after TS recording samples ( $n = 10$ ), each 10 min by slice. The amplitude and  $\pm$  SD have been indicated. Observe that % mean fEPSP amplitude increase significantly in slices obtained from tap water drinker rat after TS, then LTP is present. On the contrary, the LTP was not present in any slice coming from copper drinker rats.

did show PP (i.e., the second fEPSP larger than the first at 10-ms pulse intervals) were discarded based on abnormal response conditions.

In all protocols we measured between the peak of the first rising component and the peak of deflection drop in fEPSP and calculated the fEPSP slope. This measurement modality was also used in the test applied to generate long-term potentiation (LTP), as well during the control period (60 min) as after in the tetanic stimulation (TS) 250–300 min. During the control period (CP), 10 fEPSP measurements were taken every 10 min, obtained with the stimulation threshold. Similar measurements were carried out after the TS. The TS consists in four trains of 5 Hz, which last 20 s, with a 10-s interval. The intensity of stimulations used was identical to the one indicated for the control period.

2.2. Measurement of copper quantity in hippocampal slices using the microprobe (Cameca No. 4)

The microprobe is an electronic microanalyzer capable of measuring quantities of chemical elements from boron to uranium in different mineral materials or in fixed organic tissues. In essence, the system emits a beam of electrons, 2  $\mu$ m in diameter and with enough energy to remove the electrons of the “K” layer, which is characteristic of the considered chemical element. The removed electrons emit their own X-ray radiation in quantity according to the

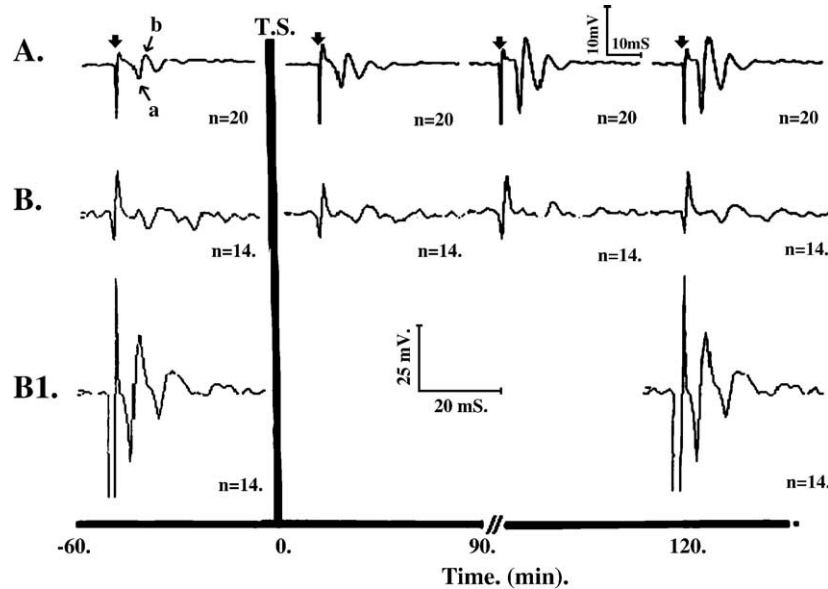


Fig. 2. fEPSP was preserved in slices coming from CuDR. (A) In slices obtained from tap water drinker rats (CR). Mean fEPSP record of twenty responses ( $n = 20$ ) to threshold stimulus before the tetanic stimulus (TS) and three successive mean fEPSP records taken after TS during 120 min. Observe the increase of mean fEPSP after TS and the development of LTP. Gross arrows mark the threshold stimulus. Thin arrows mark fEPSP magnitude. (B) In slices coming from copper drinker rats (CuDR). Mean fEPSP record of fourteen response ( $n = 14$ ) to threshold stimulus before TS, and three successive mean fEPSP records taken after TS, during 120 min. There is no LTP. (B1) In the same panel B slices, it shows mean fEPSP records using double threshold stimulus before TS ( $n = 14$ ). The same was employed 120 min after TS. Observe that mean fEPSP shows similar amplitude without significant changes compared with control record. Abscissa: time before TS (-60 min). TS (0 min). Time after TS (120 min). Calibration (Cal.) for panels A and B: 100 mV, 10 ms. Calibration for panel B1, 25 mV, 20 ms.

atomic element studied. Counters immersed in a chamber with argon/methane gas detected the X-ray dispersion. These counters have a calibrated setting for copper and other elements.

The slices obtained from rats with copper and from control rats were immersed in 10% formaldehyde during 48 h. Each slice was then located in a small plastic base. First, we used the microprobe to measure and calibrate the quantities of copper in a standard sample. The small plastic base was then taken to the microprobe chamber and copper measurements were recorded in different areas of the slices in order to obtain average values. The copper quantities in the slice appear after establishing comparison relationships with the standard sample. For this comparison we used the software program “Phi Rho Z” of the Montpellier University. This program corrects parameters such as absorption, fluorescence and atomic number [30]. In order to compare our data from the electrophysiological tests, like those from copper measurement in the slices, we used the statistical method Student’s *t* test.  $P < 0.05$  was considered significant.

### 3. Results

#### 3.1. LTP in slices obtained from CR and CuDR

Hippocampal slices from CR, which were drinking only tap water, showed a progressive development of LTP with significant and consistent amplitude at 150 min after TS. These results were not seen in CuDR recorded in the same conditions as CR. Fig. 1 shows the averages of fEPSP in 5 different slices during the control period of 60 min prior to TS. During this period, the percentage of change in fEPSP between CuDR and CR does not show statistically significant changes. Samples ( $n = 10$ ) were taken every 10 min, keeping the threshold stimulation constant. After TS, the percentage of change in the average amplitude of fEPSP in CR slices showed a significant and gradual increase. On the contrary, averages of fEPSP in CuDR slices did not show the LTP phenomenon. Fig. 2 shows several fEPSP at different time periods, before and after TS, in CR and in CuDR slices. Clearly, CuDR slices do not show changes in fEPSP averages after TS. It is worthwhile to emphasize that the amplitude of fEPSP in CuDR slices does not change when stimulations twice the threshold are applied during the control period (60 min) and 120 min post-TS (Fig. 2B1). In consequence, copper has eliminated the plastic phenomenon of LTP, maintaining the synaptic functioning and indicating absence of damage.

#### 3.2. Sensibility in CR and CuDR slices

As indicated previously, the CuDR slices do not show LTP. It is possible that copper interferes at the synaptic level, modifying its sensibility to the stimulus–response test. To

test this possibility, we stimulated the slice with threshold stimuli so as to generate fEPSP, and then the intensity of the stimulus was increased in consecutive steps until the average response of maximal fEPSP was attained, as described in Materials and methods. This procedure was applied to both CR and CuDR slices, before TS and also after 250 min of post-tetanic stimulation.

In CR slices with LTP, the average percentage of fEPSP amplitude increased progressively with the intensity of the stimulus before TS. A similar phenomenon was noticed after TS. Nevertheless, the averages of fEPSP grow during this period progressively and significantly more than in the pre-TS period (Fig. 3A). This result indicates that LTP increases significantly the synaptic sensibility. We found a

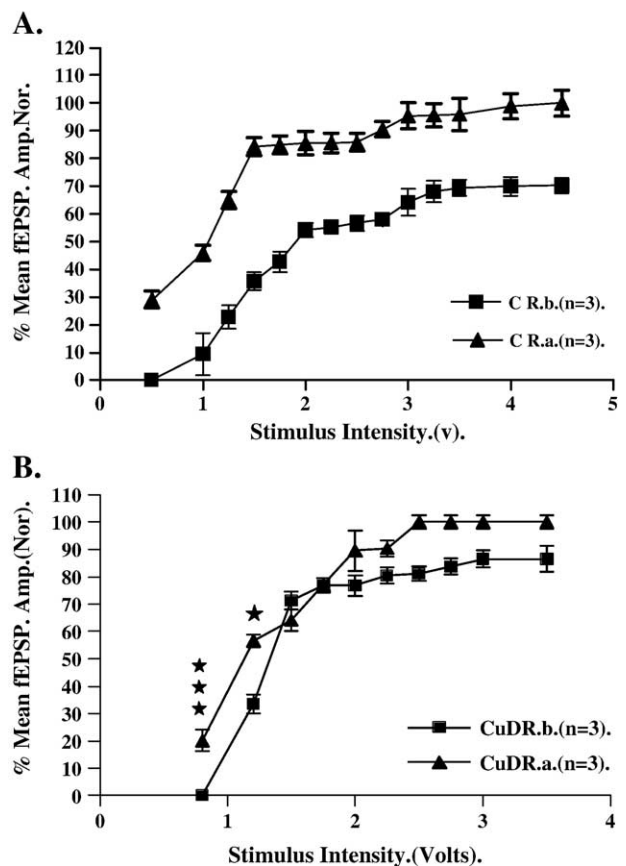


Fig. 3. (A) Change sensibility mass after TS in slices coming from tap water drinker rats (CR). Mean fEPSP show gradual and successive grow associated to stimulus–response test previous to TS in slices ( $n = 3$ ) obtained from tap water drinker rats. The same stimulus–response test applied after TS showed a significant increase in mean fEPSP amplitude in whole the successive amplitude value,  $P < 0.001$ , compared with previous periods. Observe that the last values series were taken after TS (250 min) during LTP emergence. (B) Changes in sensibility after TS in slices coming from copper drinker rats (CuDR). Mean fEPSP shows a gradual and successive increase associated to stimulus–response test before TS in slices ( $n = 3$ ) coming from CuDR. The same stimulus–response test applied after TS showed significant changes only for 0.8 and 1.25 V stimulus intensity.  $P < 0.001$  and  $P < 0.05$ , respectively. Observe that the last values series were taken after TS (250 min), without LTP. Ordinate: % Mean fEPSP.Amp.Norm.  $\pm$  SEM. Abscissa: stimulus intensity (V).

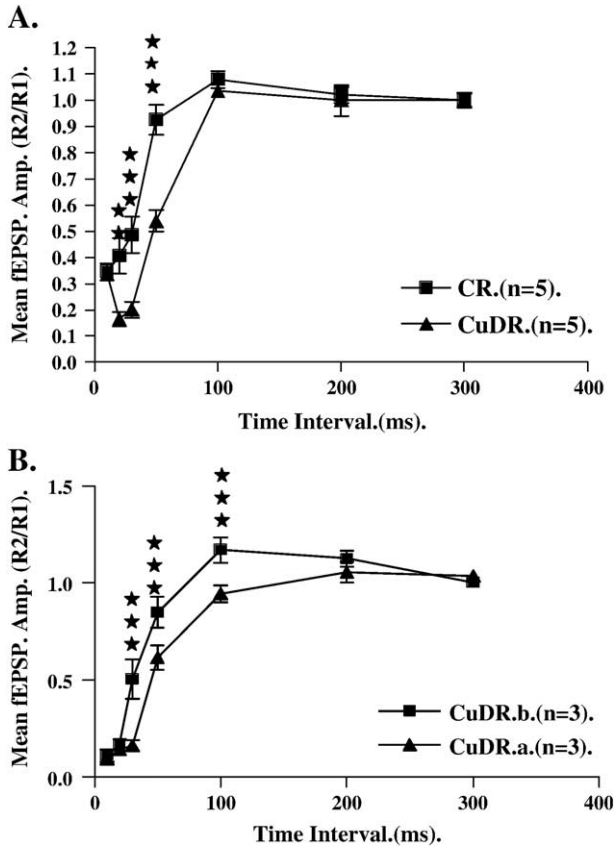


Fig. 4. (A) Decrease of paired pulse (PP) before the TS in slices ( $n = 3$ ) coming from CuDR compared with slices ( $n = 3$ ) coming from tap water drinker rats (CR). Mean fEPSP amplitude (R2/R1) responses. PP were significantly decreased for stimulus interval (20, 30 and 50 ms) in slices obtained from CuDR, compared with PP, in CR value slices ( $P < 0.01$ ,  $<0.001$  and  $<0.001$ , respectively). (B) Decreased PP after TS in slices ( $n = 3$ ) obtained from CuDR. Mean fEPSP amplitude (R2/R1) responses. PP were decreased after TS for stimulus interval 30, 50 and 100 ms,  $P < 0.001$ ,  $<0.001$  and  $<0.001$ , respectively, compared with PP generated before TS in slices coming from CuDR. Observe that these slices did not show LTP. Ordinate: Mean fEPSP.Amp (R2/R1).Norm.  $\pm$  SEM. Abscissa: time interval (ms).

different result in CuDR slices, using the same technique described. The average percentage of fEPSP amplitude showed in this case an increment correlative to the increment in stimulus intensity during the control period. After 250 min of TS, the stimulus-response test showed a correlative change similar to the one observed in pre-TS period. Only at the beginning of the test, a pair of average sample series presented significant differences. In fact, with stimulus intensities of 0.8 V, the average of fEPSP was 0.0 mV,  $n = 30$ , before TS and  $16.7 \pm 1.8$  mV,  $n = 30$ , after 250

min of TS, with a  $t = 15$ ,  $P < 0.001$ . A similar phenomenon happened with the stimulus intensity of 1.2 V, the average of fEPSP was  $16.7 \pm 1.8$  mV,  $n = 30$ , before TS, and  $56.5 \pm 3.9$ ,  $n = 30$ , after 250 min of TS, with a  $t = 2.8$   $P < 0.02$ . The comparative analysis of the remaining values did not show significant differences; in consequence, the synaptic sensibility in general did not vary in the entire range with TS (Fig. 3B).

### 3.3. Assessment of paired pulses (PP) in CR and CuDR slices

The PP test has been generally used in hippocampal excitability studies, both in physiological and pathophysiological situations. According to our results, the average amplitude values of fEPSP for the quotient R2/R1 at intervals between paired stimuli of 20, 30, 50, 100, 200 and 300 ms (R1 = conditioned fEPSP response; R2 = fEPSP test response) showed in CR and in CuDR an increment in paired-pulse facilitation, associated with an increment at the intervals between stimuli (Fig. 4A). Nevertheless, we found a significant reduction of facilitation in CuDR slices compared with CR. This reduction occurred at 20, 30 and 50 ms intervals (Table 1).

### 3.4. Assessment of PP in CR before and after TS

The PP test applied to CR slides ( $n = 3$ ), and performed 250 min after TS, showed only two significant changes compared with the pre-TS period. These changes occur at the 30- and 50-ms intervals. At 30 ms, quotient R2/R1 was  $0.1 \pm 0.01$  mV,  $n = 30$ , before TS and  $0.2 \pm 0.03$  mV,  $n = 30$ , after TS, indicating a significant increment  $t = 2.8$ ,  $P < 0.05$ , for the said interval. On the other hand, at the 50-ms interval the quotient R2/R1 was  $0.7 \pm 0.03$  mV,  $n = 30$ , before TS and  $0.4 \pm 0.04$ ,  $n = 30$ , after TS, showing a significant decrease  $t = 5.7$ ,  $P < 0.0003$ . In consequence, TS generates alternating changes in CR slices only at the shorter intervals between stimuli.

### 3.5. Assessment of PP in CuDR before and after TS

The PP test applied to CuDR slices ( $n = 3$ ) and performed 250 min after TS showed a significant decrease in its values of the R2/R1 quotient for the intervals between 30-, 50- and 100-ms stimuli, compared with the values of this quotient found before TS (Table 2). In consequence, the effect of copper on the cycle of neuronal synaptic

Table 1  
The fEPSP R2/R1 before TS, using the PP test in CR and CuDR slices

	Interval, 20 ms	Interval, 30 ms	Interval, 50 ms
CR (fEPSP.R2/R1), before	$0.5 \pm 0.07$	$0.5 \pm 0.07$	$0.9 \pm 0.06$ (mV)
CuDR (fEPSP.R2/R1), before	$0.2 \pm 0.03$	$0.2 \pm 0.03$	$0.5 \pm 0.04$ (mV)
	$t = 3.2$ , $P < 0.002$	$t = 3.7$ , $P < 0.0005$	$t = 5.8$ , $P < 0.0001$

For each interval,  $n = 30$ .

Table 2  
The fEPSP, R2/R1 before and after TS, using the PP test in CuDR slices

	Interval, 30 ms	Interval, 50 ms	Interval, 100 ms
CuDR (fEPSP.R2/R1), before	0.5 ± 0.10	0.9 ± 0.08	1.2 ± 0.06 (mV)
CuDR (fEPSP.R2/R1), after	0.2 ± 0.03	0.6 ± 0.06	0.9 ± 0.04 (mV)
	$t = 4.0, P < 0.0004$	$t = 2.7, P < 0.011$	$t = 2.8, P < 0.008$

For each interval,  $n = 30$ .

excitability has been confirmed, when copper was chronically ingested by drinking (Fig. 4B).

### 3.6. Copper concentrations in hippocampus

Copper amounts in hippocampus were measured with a microprobe in CR ( $n = 10$ ) and CuDR ( $n = 10$ ) slices. Average copper concentration was  $122.6 \times 10^{-14}$  SEM  $\pm$  0.84 g,  $n = 30$ , in CuDR slices; in CR slices de average concentration was  $2.26 \times 10^{-14}$  SEM  $\pm$  0.16 g,  $n = 30$ . CuDR slices showed a pronounced and significant copper concentration,  $t = 25.51, P < 0.001$ . This concentration is 54.2 times greater than the concentration found in the CR slices. Simple observation of the behavior of CuDR for 20–25 days, when measuring its weight, did not show visible changes compared with RC.

## 4. Discussion

In previous experiments performed with hippocampal slices we showed that after obtaining the LTP in CA1 by stimulation of Schaffer's collaterals, a perfusion of a 10- $\mu$ M copper sulfate solution in the bath suppressed LTP. Nevertheless, after a continuous flushing with Ks–R during 40 min, the record of LTP reappeared but now with epileptic characteristics [17]. In the same sense, a previous paper reported that the hippocampal slices perfused with copper sulfate at the time of applying TS suppressed LTP, interfering completely with its development [10]. With this information, we decided to study LTP in slices obtained from the hippocampus of rats that have consumed copper sulfate in drinking water (see Materials and methods). These slices did not show any evidence of LTP 170 min after TS application, in continuous records and monitoring.

The disappearance of LTP in CuDR slices showed other characteristics in comparison with the electrophysiological tests performed. For example, the absence of a fEPSP increment after TS was shown in the response–stimulus test, an effect not seen in CR. In PP, the average values of CuDR slices showed significant decreases compared with those observed in CR slices. This decrease appears in the intervals between stimuli of 20, 30, 50 and 100 ms, showing furthermore a clear diminution of the synaptic facilitation. This result is a valuable indicator explaining the absence of LTP. On the other hand, the CR slices submitted to the same test show alternate facilitation changes with significant increases of PP averages for the 20-ms interval after TS and

a significant reduction for the average values for the 50-ms interval. These changes in PP may have their origin in an adaptive synaptic effect, which occurs parallel to the LTP development in CR slices. In the same sense it might explain, on the one hand, the reduction of the post-synaptic response due to acutely perfused copper [16], as well as the effect of chronic exposure shown with the response–stimulus test, where the significant changes in sensibility only represent two points in CuDR slices.

The absence of LTP in CuDR slices showed other issues in comparison with the electrophysiological tests performed. For example, the stimulus–response test showed the absence of a fEPSP increment after TS, an effect not seen in CR. The persistence of fEPSP in both conditions, but especially in CuDR, shows that the synaptic function persisted active even when LTP was not present in CuDR slices. This result seems to indicate that the post-synaptic element did not “recognize” the TS stimulus used to elicit LTP. In summary, copper suppresses LTP, maintaining the function of synaptic traffic.

On the other hand, there is a copious literature emphasizing the role of NMDA and AMPA receptors in LTP generation [3,14,21,25]. It is therefore logical to suppose that copper could block one or both receptors, interfering for example in some of the subunits of the NMDA receptor [31]. It is widely accepted that copper may react avidly with amine, thiolate and carboxylic groups, which are present in macromolecules [4]. The subunit NR2 of the NMDA receptor is responsible for the pharmacological and kinetic interaction [19,31]. In the same sense, recently it has been discovered that the subunit NR1/NR2A has a dominant contribution in the genesis of LTP with respect to the subunit NR1/NR2B [12]. We think that the interaction of copper with these subunits might remove flexibility from the NMDA receptor, compared with the activity generated by glutamate. Nevertheless, a difference would still persist between the possible action of copper on the glutamate receptors and the antagonists of this neurotransmitter. The metal not only blocks the generation of LTP, as we have shown, but annuls it after having been produced, a fact that does not happen with glutamate antagonists, which only block its development [21,25].

Thus, it is possible that inside the CA1 neurons other probable targets for copper may exist. The study of copper concentration in CuDR showed a marked cuprosis compared with CR slices, and therefore it is possible to suppose that the chronic metal accumulation may generate several modifications, both structural and functional, altering

furthermore the synthesis or liberation of neurotransmitters non-directly involved in LTP, but nevertheless participating additionally in our results.

Much information is also available on the active involvement of protein CaMKII in the long-term control of LTP [14,20,28], and it is also known that CaMKII inhibitors or the genetic elimination of any of its subunits suppress its capability to maintain LTP [21]. It is possible that the copper chronically present in the rat may interfere in the molecular cascade of the mechanism bound to maintain LTP in rats.

The development of the facilitation reduction in CuDR slices has been correlated with an increase in glutamate liberation, as has been suggested in PP studies made in slices with different ways of facilitation [1,5,7,9,11,24,29]. In our case, the chronic copper might have caused an increment in glutamate as a compensating mechanism associated with the loss of LTP and plasticity, but maintaining the synaptic traffic, favoring the functional continuity, with a strong interaction with AMPA receptors; these receptors being recognized as active participants in plasticity phenomena [23].

In summary, according to our experimental results, CuDR slices show a loss of LTP and a reduced capability of facilitation and sensibility. These effects may represent the interference of copper with NMDA receptors, which participate in synaptogenesis, learning and memory; results that should be kept in mind when the participation of copper in pathologies like Alzheimer, Parkinson and amyotrophic lateral sclerosis are being discussed.

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## References

- [1] M. Andreasen, J.J. Hablitz, Paired-pulse facilitation in the dentate gyrus: a path-clamp study in the rat hippocampus in vitro, *J. Neurophysiol.* 72 (1994) 326–336.
- [2] A.I. Bush, The metallobiology of Alzheimer's disease, *Trends Neurosci.* 26 (2003) 207–214.
- [3] G.L. Collingridge, S.J. Kehl, H. McLennan, Excitatory amino acids in synaptic transmission in the Schaffer collateral–commissural pathway of the rat hippocampus, *J. Physiol. (London)* 334 (1983) 33–46.
- [4] R.J. Cousins, Metal elements and gene expression, *Annu. Rev. Nutr.* 14 (1994) 449–469.
- [5] R. Creager, T. Dunwiddie, G. Lynch, Paired-pulse and frequency facilitation in the CA1 region of the in vitro rat hippocampus, *J. Physiol. (London)* 229 (1980) 409–424.
- [6] D.M. Danks, Disorders of copper transport, in: C.R. Scriver, A.L. Beaudet, W.M. Sly, D. Valle (Eds.), *The Metabolic and Molecular Basis of Inherited Disease*, 7th. ed., Mc. Graw-Hill, New York, 1995, pp. 2211–2235.
- [7] D. Debanne, N.C. Guerineau, B.H. Gähwiler, S.M. Thompson, Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation effects subsequent release, *J. Physiol. (London)* 491 (1996) 163–176.
- [8] R. Dingleline, *Brain Slices*, Plenum Press, New York, 1983.
- [9] L.E. Dobrunz, C.F. Stevens, Heterogeneity of release probability, facilitation, and depletion at central synapses, *Neuron* 18 (1997) 995–1008.
- [10] N. Doroulee, Y. Yanovsky, H. Haas, Suppression of long-term potentiation in hippocampal slices by copper, *Hippocampus* 7 (1997) 666–669.
- [11] T.V. Dunwiddie, H.L. Haas, Adenosine increase synaptic facilitation in the in vitro rat hippocampus: evidence for a presynaptic site of action, *J. Physiol. (London)* 369 (1985) 365–377.
- [12] K. Erreger, Sh.M. Dravid, T.G. Banke, D.J.A. Wyllie, S.F. Traynelis, Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles, *J. Physiol.* 563 (2005) 345–358.
- [13] D.E. Hartter, A. Bernea, Evidence for release of copper in the brain: depolarization-induced release of newly taken-up 67 copper, *Synapse* 2 (1988) 412–415.
- [14] E.R. Kandel, T.M. Schwartz, T.M. Jessell, *Neurociencia y Conducta*. Chap. 36, Prentice Hall, Madrid, 1997, pp. 730–738.
- [15] J. Kardos, I. Kovacs, F. Hajos, M. Kalman, M. Simonyi, Nerve ending from rat brain tissue release copper upon depolarization. A possible role in regulating neuronal excitability, *Neurosci. Lett.* 103 (1989) 139–144.
- [16] J. Leiva, M. Palestini, M. Tetas, J. López, Copper sensitivity in dorsal hippocampus slices, *Arch. Ital. Biol.* 138 (2000) 175–184.
- [17] J. Leiva, M. Palestini, P. Gaete, Copper interaction on the long-term potentiation, *Arch. Ital. Biol.* 141 (2003) 149–155.
- [18] M.C. Linder, M. Hazegh-Azam, *Biochemistry and molecular biology*, *Am. J. Clin. Nutr.* 63 (1996) 797S–811S.
- [19] S.A. Lipton, Y.B. Choi, H. Takahashi, D. Zhang, W. Li, A. Godzik, L. Bankston, Cysteine regulation of protein function—As exemplified by NMDA-receptor modulation, *Trends Neurosci.* 25 (2002) 474–480.
- [20] R.C. Malenka, R.A. Nicoll, Long-term potentiation—A decade of progress? *Science* 285 (1999) 1870–1874.
- [21] S.J. Martin, P.D. Grimwood, R.G.M. Morris, Synaptic plasticity and memory: an evaluation of the hypothesis, *Annu. Rev. Neurosci.* 23 (2000) 649–777.
- [22] J.L. Menkes, M. Alter, G.K. Steiggleder, D.R. Weakly, J.H. Sung, A sex-linked recessive disorder with retardation of growth, peculiar air, and focal cerebral and cerebellar degeneration, *Pediatrics* 29 (1962) 764–779.
- [23] J.M. Montgomery, D.V. Madison, Discrete synaptic states define a major mechanism of synapse plasticity, *Trends Neurosci.* 27 (2004) 747–750.
- [24] T.E. Nelson, C.L. Ur, D.L. Gruol, Chronic intermittent ethanol exposure alters CA1 synaptic transmission in rat hippocampal slices, *Neuroscience* 94 (1999) 431–442.
- [25] P. Revest, A. Longstaff, *Molecular Neuroscience*, Springer-Verlag, Inc., New York, 1998, pp. 151–190.
- [26] I.H. Scheinberg, I. Sternlieb, *Wilson's Disease*, Saunders, Philadelphia, 1984.
- [27] I.H. Scheinberg, I. Sternlieb, Wilson disease and idiopathic copper toxicosis, *Am. J. Clin. Nutr.* 63 (1996) 842S–845S.
- [28] T.J. Teyler, P. DiScenna, Long-term potentiation, *Annu. Rev. Neurosci.* 10 (1987) 131–161.
- [29] D.J.A. Tshida Manabe, W.D.J. Perkel, R.A. Nicoll, Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus, *J. Neurophysiol.* 70 (1993) 1451–1459.
- [30] A.J. Tousimis, L. Marton, *Electron Probe Microanalysis*, Academic Press, New York, 1969, pp. 5–43.
- [31] B. Van Zundert, A. Yoshii, M. Constantine-Paton, Receptor compartmentalization and trafficking at glutamate synapses: a developmental proposal, *Trends Neurosci.* 27 (2004) 428–436.
- [32] D.J. Waggoner, T.B. Bartnikas, J.D. Gitlin, The role of copper in neurodegenerative disease, *Neurobiol. Dis.* 6 (1999) 221–230.