Synaptic Plasticity of the Interpositiorubral Pathway Functionally Related to Forelimb Flexion Movements

MARC PANANCEAU, LUCIE RISPAL-PADEL, AND EL MEHDI MEFTAH
Centre de Recherche Cerveau et Cognition, Centre National de la Recherche Scientifique, Faculté de Médecine de Toulouse Rangueil, 31062 Toulouse, France

SUMMARY AND CONCLUSIONS

1. Some connections from the afferents to the magnocellular red nucleus (RNm), like the corticorubral synapses, have plastic properties that are thought to contribute to long-term changes such as functional readaptation, motor learning, and the establishment of conditioned responses. Because previous studies have focused on corticorubral synaptic reorganization after these events, we attempted to investigate cerebellorubral connections in intact adult cats during associative conditioning by pairing electrical stimulation of interpositus nucleus [the conditional stimulus (CS)] with electrical stimulation of the forelimb [the unconditional stimulus (UCS)]. A large increase in the amplitude of the forelimb flexion (conditioned response) induced by the CS was observed after several days of paired CS-UCS presentations.

2. For this purpose, both behavioral and electrophysiological methods were used to correlate synaptic plasticity with changes in the motor response. The somatotopically organized sensorimotor network functionally related to the control of the elbow joint movements was studied in awake adult cats. This circuit was defined on the basis of sites at which elbow flexions could be evoked both as a CS and a UCS. The CS was applied in the cerebellar interpositus nucleus (IN) site and the UCS was given to the skin on the dorsum of the distal part of the forepaw. Daily classical conditioning consisted of repetitive pairings of CS and UCS with an interstimulus interval (ISI) of 100 ms.

3. The transmission efficacy resulting from the conditioning was tested in various targets of the cerebellar efferent pathway, including the RNm. Electrophysiological responses evoked in these relay structures by the CS and the forelimb angular deviations were simultaneously recorded throughout each daily conditioning session. The surface areas of the rubral responses to CS and the percentage response rate, the angular deviation (amplitude), and the latency of the motor responses were systematically measured throughout the conditioning procedure. Test sessions were also performed before and after each period of conditioning. Quantification and statistical analysis were carried out to determine whether changes observed in interpositiorubral synaptic transmission and in the motor responses evoked by the CS were correlated.

4. Daily repetition of paired CS and UCS according to a predefined and fixed temporal schedule led to an increase in the response rate and amplitude of the forelimb flexions. A procedure with repeated presentation of CS preceded by UCS was used to produce extinction of the enhanced motor responses. The associative nature of these changes was confirmed by the fact that the CS given alone for 11 days in a control condition failed to produce any modification of the motor response.

5. The changes in the flexion movements were accompanied by a nearly parallel increase of the amplitude of the "postsynaptic field potentials" evoked in the RNm by the CS (IN stimulation). Changes in the transmission efficacy of the interpositiorubral synapses stayed stable even after several days of interruption and remained constant up the extinction period. Changes affecting both the motor and the central responses were significantly correlated, suggesting that modifications in the interpositiorubral transmission efficacy might be one of the plastic correlates of forelimb flexion conditioning.

6. Examination of the neuronal excitability within either the IN or the RNm or in the spinal cord failed to show any evidence of facilitation suggesting that the increases in the postsynaptic rubral field potential were attributable to a plasticity of the interpositiorubral connections. The long-lasting duration of the increase of cerebellorubral synaptic transmission suggests that structural changes were induced by conditioning in the intact animal. These changes could either take part in the refinement of the pathways involved in the newly learned movements or mediate the adaptation of the motor command in response to the external constraints. Thus, the "activity-dependent" synaptic plasticity could be one of the neural correlates of behavioral adaptation processes, but it could also indicate the RNm participation in some aspects of motor learning.

INTRODUCTION

A crossed descending pathway that controls movements of the limb (Kuypers 1964) and face (Courville 1968) originates from the magnocellular red nucleus (RNm). This nucleus receives inputs from three main sources that are extremely important for motor control: the cerebral cortex, the cerebellum (see reviews by Allen and Tsukahara 1974; Keifer and Houk 1994; Massion 1967), and the spinal cord (Padel et al. 1988). The afferent and efferent connections of the RNm follow a somatotopic organization between regions related to similar parts of the body (Eccles et al. 1975; Fanardjian and Manvelian 1978; Ghez 1975; Gibson et al. 1987, Mewes and Cheney 1994, Pompeiano and Brodal 1957; Rinvik and Walberg 1963; Van Kan et al. 1994; Vinay and Padel 1990). The characteristics of the rubral connections suggest that the RNm could play an important role in the execution and the rapid regulation of movements (Allen and Tsukahara 1974; Padel 1993). However, the red nucleus also seems to be involved in long-term plastic events such as the processes that underlie functional motor readaptation following lesions of the CNS (Murakami et al. 1982; Nakamura et al. 1974; Tsukahara et al. 1974, 1975) or the peripheral nervous system (Murakami et al. 1984, Tsukahara and Fujito 1976; Tsukahara et al. 1982). It might also be involved in the mechanisms subserving functional changes that occur in certain phases of motor learning (Jenkins et al. 1994; Seitz et al. 1990).

According to Tsukahara (1978), the plasticity of corticorubral connections underlies central postlesional reorganization. It might also be involved in processes that ensure the
execution of new motor acts as they become more automatic in the final phase of motor learning (Ito 1984). Moreover, during classical conditioning, the corticorubral plasticity is thought to ensure the recovery of the motor responses to electrical stimulation of the motor cortex after pyramidal tract section (Ito and Oda 1994; Murakami et al. 1988; Oda et al. 1988; Tsukahara 1981).

The plasticity of the cerebellorubral connections has been the source of some controversy. Although Tsukahara and coworkers (Tsukahara et al. 1981) did not observe any changes in the synapses after classical conditioning that influenced plasticity of the corticorubral connections, this type of event could have occurred in the cerebellorubral pathway during early developmental stages. The formation of uncrossed cerebellorubral projections has been observed after unilateral lesion of the cerebellar nuclei (Lim and Leong 1975, Tsukahara and Fujito 1981). Moreover, in the adult, reorganization of the cerebellar synaptic contacts expressed by the broadening of the ‘‘initial receptor field’’ toward a greater proportion of the red nucleus occurred on the rubral neurons after cortical hemispherectomy (Olmstead et al. 1983).

Because the role of the cerebellar systems has often been implicated in processes linked to adaptation (Ito et al. 1982), motor learning (Ito 1984; Jenkins et al. 1994), and the acquisition of conditioned reflexes (Glickstein 1992; McCormick et al. 1981; Yeo et al. 1985), it is reasonable to wonder whether the neuronal elements of the cerebellorubral pathway also undergo modifications during these events. Such plasticity would suggest a role in the establishment of the conditioned motor response command for the red nucleus. Alternatively, if the properties of the cerebellorubral connections remain constant, then their role is limited to the transmission of the readjusted motor command.

The present experiment was designed to determine whether the cerebellorubral connections are, like the corticorubral connections, susceptible to synaptic modification during changes in motor behavior caused by classical conditioning. Moreover, both behavioral and electrophysiological methods were used to determine whether a causal relationship can be established between changes in the efficacy of the cerebellorubral synapses and the modifications produced in motor responses by conditioning.

METHODS

Four chronically prepared cats were used to study the plasticity of the CNS during classical conditioning. A simplified sensorimotor schema involving the interpositoipinocerebellar loop related to forelimb flexion during conditioning is shown in Fig. 1, A and B. One semimicroelectrode was implanted in a site of the right interpositus nucleus (IN) controlling forelimb flexion movements. A similar electrode, implanted in the RNm, which relays the contralateral cerebellar outputs to the spinal cord, enabled us to analyze the monosynaptic responses produced in this structure by IN stimulation. In addition, to ensure that the conditions of this experiment were the same as those used in the study of the cerebello-thalamocortical circuits (CTC) (Meftah and Rispal-Padel 1992), electrodes were also implanted in the contralateral ventrolateral thalamic nucleus (VL) and in the primary motor cortex (MI). A stimulating electrode was placed on the skin of the right forelimb at a location where excitation evoked elbow flexion (Hagbarth 1952). Sensory information arising directly from the electrical stimulus as well as from the evoked movement would reach many structures including the cerebellum, the red nucleus, and the motor cortex.

After a postoperative recovery period, the sensorimotor system underwent a classical conditioning procedure. The efferent interpositus circuits controlling the forelimb movements were activated by a weak intracerebral tetanic stimulation, which was used as a conditional stimulus (CS). One hundred milliseconds later, this stimulation was followed by stimulation of the dorsal part of the right forelimb, which evoked a forelimb flexor reflex. The intensity of stimulation was adjusted to the minimum intensity needed to evoke responses in all trials. In this way, the second stimulus was taken to be an unconditional stimulus (UCS) (Fig. 1C). The effects of the CS-UCS association both on the motor responses (forelimb flexion) evoked by the CS and on the neuronal response of the interpositoipinal link were studied concomitantly.

Because the methods have already been described in detail in a previous paper (Rispal-Padel and Meftah 1992), only a brief version will be given here.

Surgical preparation

Cats were initially sedated with an intramuscular injection of ketamine (10 mg/kg), and were then maintained under general deep anesthesia throughout surgery with an intravenous injection of α-chloralose (60 mg/kg). An oral cannulation of the trachea was performed to enable artificial ventilation of the cats when necessary. Rectal temperature, heart rate, electrocardiogram, and expired CO2 were monitored throughout the surgery. The surgical procedures were carried out with the use of strictly sterile techniques. The cats underwent antibiotic treatment for 3 days immediately after surgery and the skin wounds were treated with a neomycin gel and a local analgesic. The eyes and ears of the animals were also treated with an antibiotic and an analgesic cream.

Implantation of the electrodes

During the surgery, several semimicroelectrodes made of electrolytically sharpened nickel-chrome wire, insulated except for the 100 μm tip, were inserted into the successive relay structures of the efferent cerebellar pathways (Fig. 1B). In the cortical areas, the primary sensory area (SI) and the primary motor area (MI), pairs of transcortical electrodes were inserted through trephine holes and then fixed with dental cement to the bone of the skull and soldered to a microconnector. Each pair of transcortical electrodes was guided normally to the cortical surface by a micromanipulator. The deeper tip was implanted 2 mm in the depth of the cortex and the other was inserted in the cortical surface just below the dura. The pairs of MI transcortical electrodes were placed into the back, shoulder, and elbow motor representation zones (Nieoullon and Rispal-Padel 1976). The elbow representation zone in the motor cortex was carefully determined by moving the electrode tip over the cortical surface to find the place from where the largest responses were evoked by stimulating the skin over the dorsum of the contralateral forelimb. Then the electrode was moved for stimulation and its position was adjusted to the point from which microstimulation induced a forelimb flexion with the lowest current intensity.

Monopolar electrodes were implanted into the IN of the cerebellum, the RNm, the VL, and the rubrospinal tract. To implant the electrode in the IN elbow representation region, we performed trajectories through the cerebellar nucleus, according to stereotaxic coordinates (Berman 1968), while looking for units with receptive fields located on the ipsilateral forelimb skin. When large responses were recorded, the electrode was used for stimulation (single 0.1-

ms square pulses) and its final position was determined as the
Conditioning procedure

Test procedure

FIG. 1. Experimental setup. A: cats were suspended in a textile hammock with the right forepaw slightly raised from the floor. A potentiometer was placed on the elbow joint to measure the right forearm flexion movements (Mvt.). CS, location of electrode used for applying the conditioning cerebellar stimulation; UCS, forelimb cutaneous stimulation site; Rec., microconnector on which the recording electrodes were connected. B: diagram of the simplified sensorimotor neuronal network that was subjected to an associative conditioning procedure. The motor part of the circuit is drawn in a solid line between the interpositus nucleus of the cerebellum (Cb.IN) and the flexor muscles (F.M.). RNm, magnocellular red nucleus; VL, ventrolateral thalamic nucleus; MI, primary motor cortex; Sp.Mn., spinal motoneurons. C: stimulation sequence for the conditioning paradigm. Top 2 traces: illustration of the interstimulus interval (ISI, 100 ms) between the conditional stimulus (CS) (train of 5 shocks) and the unconditional stimulus (UCS) (train of 5 shocks). Bottom 6 traces: each line represents the pairing of CS and UCS in 1 trial. Trials were separated from each other by a 30-s delay. Five CS-UCS pairs given successively followed by a CS applied alone corresponds to 1 series. Dailly, the cats were submitted to 25 series. D: example of stimulation test carried out regularly to examine the efficacy of the conditioning procedures. Ordinate: amplitude of the motor responses evoked by increasing the intensity of the stimulus (abscissa) applied in the brain centers. T, threshold stimulation; for this cat it was equal to 60 μA. The same kinds of tests were performed to examine the efficacy of the transmission in each link of the cerebellocortical and cerebellorubral pathways.

Histological controls

At the end of the experiments, the cats were killed so we could check the electrode positions histologically. The cats were first sedated with ketamine so we could insert a needle into the saphenous vein for the intravenous injection of pentobarbital sodium anesthetic. When the animals were deeply anesthetized, 50 μA of direct anodal current was passed for 20 s through each stimulating electrode for electrolytic deposit at the position of the electrode tip of traces of the iron present in the nickel-chrome alloy, which were revealed by Prussian blue reaction. A solution of 10% Formalin containing 0.3% potassium hexacyanoferrate was then perfused through the carotid artery. The brain was subsequently removed and kept in the same solution for several days.

Serial parasagittal sections of the cerebellum and the anterior part of the cortex were performed and frontal sections of the diencephalic and mesencephalic regions were prepared. The electrode trajectories were identified and the stimulation sites were located by detection of the spots of iron particles deposited by the anodal current.
Pretraining sessions

Every day before undergoing the surgery, the cats were brought into the training room for 0.5–1.5 h/day to accustom them to the training setup. Five days after surgery, these pretraining sessions were repeated. The connecting wires linking the microconnectors and the apparatus were put in place to determine the relevant parameters of the CS and the UCS. These parameters were then kept rigorously constant throughout the subsequent conditioning sessions. The CS consisted of five 0.2-ms square pulses delivered to the IN at a frequency of 500 Hz. The CS intensity selected for the conditioning stimulus amounted to 70% of the threshold required to produce a movement of 0.5–1° (Fig. 1D), and was between 45 and 60 μA (depending on the threshold value determined with each cat). The UCS applied to the forelimb was also a train of five square pulses with a duration of 0.5 ms and a frequency of 300 Hz. The UCS current was the lowest intensity that evoked a flexion in 100% of the trials, corresponding to currents of between 0.5 and 1 mA.

Training procedure

Every day during the acquisition period, the animals were given 125 trials of paired CS and UCS and 25 presentations of the CS alone, evenly distributed over the daily session (i.e., once every 5 paired trials). The interstimulus (CS-UCS) interval was fixed at 100 ms and the intertrial interval at 30 s. The acquisition period was considered complete when the responses to the CS alone reached a stable level of 100% for three consecutive days. Conditioning continued for ~7–10 more days to ensure the consolidation of the learned association. The extinction procedures consisted of either running the reverse stimulus sequence (i.e., on 2 cats, UCS was applied before the CS, with an interstimulus interval of 900 ms) or interrupting the CS UCS procedure for >1 mo (1 cat). To determine whether or not the conditioning effects were labile or of the long-lasting type, the paired sessions were stopped after either the acquisition or the consolidation period.

The associative character of the conditioning effects was also checked by application of the CS alone for ≥10 successive daily sessions on naive cats before conditioning. Because the temporal relationships between the CS and the UCS were then abolished, the extinction procedures served as additional tests to check the associative character of the conditioning.

Data acquisition

The motor responses were recorded with the use of a potentiometer attached to the animal’s elbow: the fixed portion was attached with a velcro band to a support on the hummock and the mobile one to the distal portion of the forearm. The recording of the angular displacement was amplified, digitized at 20 kHz, and stored in a computer. The device was regularly calibrated; its maximum resolution was 0.2°.

The central electrophysiological responses to the cerebellar stimulation were recorded concomitantly at all the relay sites (NRm, VL, MI and SI) in two distinct conditions. The first situation consisted of the associative conditioning sessions, pairing the IN CS stimulation (5 square pulses at 500 Hz), delivered every 30 s, with the peripheral UCS applied to the forelimb skin. In the second situation, responses to IN stimulation were recorded to examine the neuronal excitability and the synaptic transmission in each relay site of the efferent cerebellar pathways. The IN stimulation parameters were different from those described above. The tests were performed with the use of only a single shock per trial, to activate the IN neurons; their frequency of repetition was 1 Hz.

The responses in the rubral (NRm) and thalamic sites (VL) relaying the cerebellar inputs were recorded by means of monopolar electrodes. The cortical responses to stimulation of cerebellar and thalamic sites were recorded with the use of pairs of electrodes with one of the tips implanted in the superficial layers (I or II) of the cortex and the other in a deeper layer (III or IV). Differential amplifiers recorded the electrical activity elicited between the two electrodes. After amplification, all these analog signals were digitized at 20 kHz and stored. Analysis was carried out first on the averages of traces of responses to the CS, given alone (25) or of 40 successive responses recorded during conditioning sessions and 20 successive responses obtained in other conditions during the test sessions. Second, the responses were quantified to seek a relationship between changes affecting motor and central responses, and also to establish the time course of these changes evoked during the conditioning procedures. The field potential surfaces were measured by integration of the mean curve of each wave in relation to the baseline level. This corresponds to the mean computed level of the background activity recorded for 5 ms before the stimulus stimulus at the origin of the response.

Data analysis during the training procedures

Every day, under constant conditions, the motor, rubral, thalamic, and cortical responses evoked by the CS were recorded simultaneously. At each daily session, the motor effects of the conditioning procedure were quantified as the percentage of trials that yielded motor response (positive trials) of 25 trials when the CS was given alone. The mean amplitude of the angular deviation of the elbow joint was calculated from the motor responses of the positive trials. The effects of the conditioning on the thalamic and cortical responses evoked by the 25 CSs given alone were also evaluated. In addition, the traces of ~40 successive daily responses evoked in each group by the CS were averaged. The responses were compared qualitatively and quantitatively. Statistical analyses were carried out to establish what relationships exist between the motor and central effects. All average values are given as means ± SE, and for the comparison of means the statistical significance was evaluated with the use of a two-tailed Student’s t-test.

Data analysis during the control tests

The reactivity of the efferent cerebellar pathways was tested systematically before and after each critical period of the conditioning (acquisition, consolidation, extinction) and the data were subsequently compared to evaluate the effects of each procedure. The test sessions were performed under conditions different from those under which the conditioning sessions were conducted. During the tests, the physical isolation of the animal, which had to be maintained throughout the conditioning sessions, was suspended and the electrode used to apply the UCS was removed.

Throughout the conditioning procedures, the cerebellorubral, cerebellothalamic, and thalamocortical circuits were systematically tested in similar conditions (Fig. 1D). Rubral, thalamic, and cortical responses evoked by a constant interpositus stimulation (1 pulse, 0.2 ms, 50 μA) were recorded. The surfaces of 20 successive responses recorded at each brain site, during a single test session, were measured after averaging for the quantitative analysis.

Antidromic activation of the RNm neurons was also carried out and the responses were studied to check the neuronal excitability level of the RNm.

Results

The electrophysiological responses evoked by the CS in the various relays of the cerebellothalamic and cerebellorubrospinal pathways were analyzed throughout the conditioning process. Statistical tests were then used to de-
termine whether a relationship existed between the variations in the conditioned motor responses and the cerebellorubral field potentials.

Effects of conditioning on the motor responses evoked by the CS and by the UCS

In this experiment, the location of the stimulation electrodes was chosen so that both the CS and UCS caused a forearm flexion, thus situating the work in the same conditions as our previous studies (Meftah and Rispal-Padel 1994; Rispal-Padel and Meftah 1992). The intensity of the CS applied to the IN of the cerebellum was maintained constant throughout the entire conditioning period and was set in such a way as to be clearly underthreshold on the 1st day, when it produced a flexion with a mean amplitude of 0.85° (Figs. 2Aa and 3B) that appeared in only 11% of the trials (Fig. 3A). The intensity of the UCS applied to the skin of the forearm was defined as being the lowest current giving 100% of responses that were, from the outset, of large amplitude: on average 7.56° (Figs. 2Ab and 3C). However, they still had the ability to increase, as indicated by the magnitude of the standard errors and the fact that with a stimulation intensity twice as high as that of the UCS, i.e., of ~1.5 mA, the amplitude of movement reached 152% of that induced by the UCS.

For 10 days or so, conditioning produced a steady increase of the amplitude and of the probability of the appearance of the motor responses evoked by the CS (Figs. 2B and 3A). The latencies, however, hardly changed: they were on average 53.5 ± 10.5 (SE) ms before and 47.4 ± 6.7 ms (n = 50) after the conditioning. The percentage of motor responses produced by the CS alone in the group of four conditioned animals that received paired CS and UCS stimulations rose from a mean of 13.5 ± 2.9% on the 1st day to 100% between the 11th and the 16th days (Fig. 3A). These values were significantly different (t = 66.69, df = 6, P < 0.001). The situation was similar concerning the mean amplitude of the motor responses (positive), which was 0.56 ± 0.26° on the 1st day of conditioning (Fig. 3B) and 3.36 ± 0.16° on the 20th day (t = 20.50, df = 6, P < 0.001). During the extinction (on 2 cats, with UCS-CS 900-ms procedure) a decreasing of the changes produced during the acquisition period was observed (Fig. 3, A and B). However, at the end of extinction, the parameters of motor responses remained greater than on the 1st day of conditioning. The difference was 45 ± 15% for the percentage and 32 ± 6% for the amplitude. For two cats (control animals) among the four cats used in the present experimental series, the CS-UCS association was delivered after a period of 11 days during which the CS was given alone and was followed by 1 mo of rest. The data collected during the CS-UCS paradigm applied afterward did not differ from those collected on the two other animals, which only received the CS UCS association. In the two control animals, during the first period when they received the CS alone, the percentage of positive responses remained practically unchanged: the value for the 1st day of testing was 16 ± 0.2% (Fig. 3B) and that for the 11th day was 21.2 ± 2.1% (Fig. 3A). The mean amplitude was 0.85 ± 0.48° on the 1st day and 1.08 ± 0.54° on the 11th day (Fig. 3B). These two amplitudes did not significantly differ (t = 0.25, df = 2, P > 0.05). It should be noted that the initial amplitudes for the group of control animals and the group of conditioned animals were 0.95 ± 0.48° and 0.56 ± 0.2°, respectively, and that this difference was not significant (t = 1.04, df = 4, P > 0.05).

The characteristics of the motor responses evoked by UCS did not undergo any modifications (Fig. 3C). Their mean amplitude was initially 7.84 ± 0.54°, and at the end of the conditioning period it was 7.75 ± 1.35°. Similarly, the mean latency was 36.16 ± 6.36 ms (n = 134) before and 36.49 ± 6.58 ms (n = 140) after conditioning.

Characteristics of the responses evoked in the VL, the NRm, and the MI by IN and cutaneous stimulations

The CS evoked a short-latency compound potential in the VL and in the NRm responses. The two successive compo
Components of the responses corresponded to the incoming cerebellar volley and to the monosynaptic potential generated by the neurons in the two nuclei, respectively (Fig. 4C, RNm and VL). The waveform recorded in the RNm in cat KAS after stimulation of the IN consisted of an initial positive deflection followed by two negative components. The initial positive-negative deflection corresponded to the presynaptic response: the negative peak had a latency of 0.50 ms from the beginning of the stimulus artifact (Figs. 4C, RNm, and 5C). The second negative deflection followed the first negativity by 0.57 ms, which corresponds to one synaptic delay. These data were representative of those collected from other cats (Figs. 4C, RNm, and 7Ba). In the back representation region of the motor cortex, stimulation of the IN did not produce any responses, as shown by the recording derived from a pair of transcortical electrodes (Fig. 4, B and C, Mlb). In the shoulder control subdivision (Mlb), a small negative wave appeared, but the wave was much larger in the forelimb motor subdivision (Mlc). This confirms that the latter actually was at the origin of a control circuit for

**FIG. 3.** Effects of the associative conditioning procedure on the motor responses. Ordinates in A: percentage of positive responses (flexion > 0.2°) to the 25 CS applied alone during each daily session. Ordinates in B and C: averaged angular deviation of the forelimb flexions. Abscissas: time (days). Plotted values: mean values of the responses. Bars: SDs. The responses were obtained in 4 cats during the acquisition and in 2 cats during the extinction period. A: evolution of the percentage of the motor responses obtained when the CS was applied alone, either in the series of paired CS-UCS during 40 days (■) or when it was never associated with the UCS during a conditioning period of 11 days (□). B: evolution of the amplitudes of the motor responses (deg of angular deviation). Curves with filled and open dots have the same meaning as curves in A. C: evolution of the UCS averaged motor responses recorded 25 times during the conditioning sessions.
the distal forelimb. It must be noted that stimulation applied at this cortical point induced an elbow flexion. The electrode placed in the deep cortical layers (III and V) recorded a negative wave after IN stimulation corresponding to excitation of the neurons of these layers with a latency of 2 and 3 ms (Sasaki and Prelevic 1972; Sasaki et al. 1970). In the primary somatosensory cortex (SI), no responses were detected after the stimulation applied in the IN (CS) (Fig. 4C, SI).

The UCS evoked a response in the RNm with a mean latency of 8.2 ms (Fig. 4D). The response was similar to that recorded in cats with the cerebellum and the motor cortex removed, indicating that it could be transmitted by the direct spinorubral pathway (Padel and Relova 1988).

Additional support for this interpretation is provided by the fact that no somaesthetic responses appeared in the cerebellar region of the VI. (Fig. 4D), which received the same IN input as the RNm through axon collaterals (Toyama et al. 1967). In the motor cortex, the UCS applied to the dorsal forearm evoked a negative wave in the deep layers with a latency of ~6 ms. This response was maximal in the region where the stimulation induced a forelimb flexion confirming the existence of a sensorimotor loop according to topographic relationships. In the SI, the response to the UCS appeared with a latency similar to that obtained in the MI.
Identification of the various components of the VL and RNm responses to IN stimulation

We attempted to accurately identify the components of the responses evoked in the RNm and the VL by the IN stimulation. First, a stimulation with pulses at high frequency was applied in the IN. A stimulation with two pulses separated by an interval of 2 ms (500 Hz) produced pre- and postsynaptic field potentials in the VL (Fig. 5A, 3rd trace). The amplitude of the presynaptic response to the second shock remained unchanged, whereas the postsynaptic excitatory response was considerably diminished. The decrease
of the postsynaptic wave is also seen (Fig. 5A, 4th trace) when the interval between the two shocks is as long as 8 ms (125 Hz). Considering that in the thalamus the excitatory postsynaptic component is generally followed by an inhibitory one (Uno et al. 1970), it is likely that the second response is diminished because of the inhibitory effect that follows the negative excitatory wave evoked by the first shock. In the RNm, where the postsynaptic component is only excitatory (Toyama et al. 1967), a high frequency of stimulation (500 Hz) did not enable a distinction to be made between the postsynaptic wave and a possible late component of the presynaptic volley, because the respective amplitudes of the two components remained constant, even with a high frequency of stimulation (Fig. 5B). A second test was used in which the motor cortex was stimulated before the IN so that the cerebellar response appeared during the inhibitory phase of the corticorubral response (Fig. 5, Ca and Cb) (Jeneskog and Padel 1983; Tsukahara and Kosaka 1968). Under these conditions, no modification was observed in the presynaptic response (Fig. 5, Cb and Cc), whereas the postsynaptic RNm wave was decreased, as can be seen from the superimposition of two expanded traces (Fig. 5Cc) of the interpositoral responses illustrated in Fig. 5Cb (St.1 and St.2 + St.1). Statistical analysis was carried out on the RNm responses evoked in the three different conditions. The first pair of bar graphs represent the mean amplitudes of the pre- and postsynaptic waves of the RNm response evoked in 10 successive trials, in which the IN stimulation was applied alone (St.1). The second pair of bar graphs corresponds to the mean amplitudes of the same RNm responses when the cortical motor area was stimulated before the IN. The third pair of bar graphs illustrates the pre- and postsynaptic NRm waves evoked by IN stimulation given alone after the two tests described above. The drop in amplitude of the postsynaptic waves evoked in the St.2 + St.1 situation, with respect to those found in the situation in which St.1 was given alone before the 10 St.2 + St.1 trials, was found to be highly significant (t = 4.15, df = 18, P < 0.01).

Evolution of the postsynaptic field potentials in the relays of the interpositospinal system

It was of prime importance in the present study to accurately analyze the characteristics of the motor responses and to identify the cerebral regions specifically involved in the elbow conditioned movement. Electrophysiological and stereotaxic methods were used to locate the neurons activated by stimulation of the IN within each of the thalamic, rubral, and cortical relays that caused flexion of the forearm. However, because each body segment is controlled by several distinct sites in the IN (Cicirata et al. 1989; Rispal-Padel et al. 1982), the placement of the electrode delivering the CS might slightly vary from one animal to another. As a consequence, although the neuronal circuits studied in each of the animals did produce elbow flexion, they could have been situated in slightly different regions of the IN and its relays. Before being pooled together, the data collected in the four animals were examined separately and compared to evaluate their degree of similarity. Figures 4, 5, 7A, 8, A–D, 9, 10, and 11, which illustrate the responses gathered from a single animal and were used for the assessment of the results, are representative of the data collected from all the animals.

The mean electrophysiological plots (n = 20) presented in Fig. 6 correspond to the field potentials and to the motor responses evoked by IN stimulation simultaneously in all the cerebral sites of cat ICI either before conditioning (Fig. 6, Before) or after conditioning (Fig. 6, After). The superimposition of the plots obtained before or after (Fig. 6, Sup.) indicates that the relay structures of the cerebellar efferent pathways are not all affected in the same way by conditioning. The changes in amplitude of the motor responses are accompanied by variations that affect the cortical (Fig. 6, Mlc) and rubral (Fig. 6, RNm) responses but that do not involve the thalamic responses (Fig. 6, VL). The presynaptic response recorded in the VL and the RNm did not undergo any variations (Fig. 6, RNm and VL). This similarity between the data obtained in the two nuclei is not surprising, because the presynaptic volleys originate from the same interpositus neurons with collateralized axons (Toyama et al. 1967; Tsukahara et al. 1967). Moreover after the acquisition period, the similarity in the amplitude of these presynaptic responses evoked by identical stimulating currents stresses the stability of IN neurons excitability throughout the entire duration of the experiment (3 mo). In the VL, the postsynaptic interpositothalamic response also remained constant, indicating that neither the interpositothalamic transmission nor the excitability of the thalamic neurons changes after conditioning.

On the other hand, the amplitude of the postsynaptic component of the interpositoral response was strongly affected by the conditioning. On the mean plot (n = 20) of the cerebellorubral potentials recorded before conditioning (Fig. 6, RNm, Before), the amplitude of the postsynaptic potential field represents 12.73% of that of the presynaptic potential. At the end of conditioning, (Fig. 6, RNm, After), the postsynaptic wave was increased and the ratio reached 34.27%. The superimposition of the plots obtained before and after conditioning (Fig. 6, RNm, Sup.) confirmed that the change in ratio between the amplitudes of the pre- and postsynaptic potentials was not due to a presynaptic alteration. The curve illustrating the evolution of the surface areas of the postsynaptic waves versus the duration of conditioning (Fig. 8E) shows that the areas double with respect to their initial values indicating either a facilitation of the cerebello-rubral transmission or an increase in the excitability of the rubral neurons.

The amplitude of the the MI responses following IN stimulation also increased with conditioning, but the increases were not so great as those seen for the postsynaptic rubral responses (Fig. 6, Mlc). In the example taken from cat ICI and presented in Fig. 6 (Mlc), the MI responses increased by 37% of their initial value, whereas the increase was 200% for the postsynaptic component of the rubral response. The increases produced in the CNS were associated with a large increase of the flexion motor response of the forelimb, rising in angular value from 0.96 to 2.96° (Fig. 6, Mvt.). Despite a difference in their magnitude, changes in the RNm as well as those observed in the motor cortex (Meftah and Rispal-Padel 1994) paralleled the evolution of the motor responses (Fig. 8).
Examination of the changes produced in the rubral response by conditioning

The pre- and postsynaptic components of the rubral responses were clearly separated after each of the shocks applied at 500 Hz, in the pulse train that composed the CS (Fig. 7A). During conditioning, the presynaptic responses (incoming volley) did not differ at any time, as shown from the mean daily plots ($n = 25$) sampled every 5 days. The postsynaptic component remained stable up to the 5th day and then steadily increased. Superimposition of the plots recorded on the 1st and 20th days illustrates the increase of the amplitude that affects each of the postsynaptic responses evoked by each pulse of the train. Another example of the averaged analog traces recorded in a different cat (LIS) and showing the evolution of the postsynaptic rubral responses.
during the test sessions carried out before and after each of the critical conditioning periods (see METHODS) is given Fig. 7B. Like the presynaptic wave recorded from cat KAS (Fig. 7A), the one recorded from cat LIS (Fig. 7B) did not vary during the conditioning. However, the dynamic period of acquisition (Fig. 7Ba) was marked by an increase of the amplitude of the postsynaptic component, which rose 57% above the initial value measured before conditioning. The difference can be best assessed (Fig. 7Ba, Sup.) from the superimposed plots of the recordings made before (Fig. 7Ba, Before) and at the end of (Fig. 7Ba, After) the acquisition period of conditioning. This increase persisted during the consolidation period (Fig. 7Bb). During extinction, the effects reversed after a few days, giving way to a decrease in the postsynaptic wave (Fig. 7Bc). Superimposition of the two plots, recorded before the acquisition and the last day of the extinction (15th day of an extinction produced by the reversal of the conditioning procedures, i.e., UCS-CS with an interval of 900 ms) (Fig. 7Bd) enabled an evaluation of “long-term traces” left in the interpositorubral circuit. The postsynaptic rubral response evoked after extinction was still 43% greater than that obtained before conditioning.

**Time course of the effects of conditioning on the motor and cerebellorubral responses**

During the acquisition period, comparison of the time course of these two types of responses (Fig. 8, B and D) shows that the changes involving the motor responses (which also depend on thalamocortical synaptic modifications) precede the modifications of the postsynaptic component of the interpositorubral response (Fig. 8, Cb and D). The latter only started to increase after 5 days of conditioning, whereas the motor response parameters steadily increased from the 2nd or 3rd days (Fig. 8, R and D). Thereafter, the amplitudes of the motor and rubral responses followed a similar time course. Finally, at the end of the acquisition and during the consolidation, the rubral postsynaptic responses as the motor responses became stable. The motor responses reached a maximum amplitude of ~3° between the 10th and 13th days and 100% of occurrence on the 16th day. At the end of conditioning, after 38 days of spontaneous extinction (in the present example no reversal of the conditioning procedure was used), a slow decrease of the amplitude of the postsynaptic rubral responses was observed to parallel the decrease of the motor response parameters. The final values of the motor response amplitude and of the postsynaptic rubral responses remained much higher (40%) than those of the initial responses. When extinction was produced with the backward paradigm (UCS given 900 ms before CS), the decrease of the conditioning effects was also progressive but was accelerated with regard to the decrease observed during spontaneous extinction (Fig. 8E). The postsynaptic rubral responses presented their lowest amplitude between 9 and 14 days after the beginning of the extinction instead of ~30 days for the two procedures, respectively. Their amplitude values were always 41 ± 7% (n = 3) greater than those found at the beginning of conditioning. As in the case of the spontaneous extinction, in the UCS-CS condition the motor response decreased in parallel with the postsynaptic rubral responses. At the end of the extinction period, the parameters of these motor responses also remained greater than their initial values; the differences between the 1st and the 15th day were 45 ± 15% for the occurrence and 32 ± 6% for the amplitude (Fig. 3, A and B) (see also Rispal-Padel and Meftah 1992). Although the motor parameters and the amplitude of the postsynaptic responses in the RNm were strongly increased during conditioning, the presynaptic cerebellorubral response remained unchanged (Fig. 8, Ca and Cd), suggesting that the excitability of the IN neurons did not vary during the conditioning.

Similar data were found for all the animals (Fig. 8, E and F). The mean amplitude of the postsynaptic waves recorded in the RNm reached 165 ± 17% (n = 4) after 11 days during the CS-UCS conditioning and its greatest value reached 193 ± 22% on the 13th day (Fig. 8E). For the statistical analysis (Fig. 8F), only the responses collected from the two cats subjected to the CS alone before the CS-UCS paradigm were evaluated (n = 2). Ten mean amplitude values of the responses recorded on the 1st and 11th days of the CS alone paradigm were considered. For the CS-UCS paradigm, as acquisition was achieved between the 15th and the 17th day, the values were taken on the 15th day. But the statistical difference between the data collected on the 11th day with the two paradigms was already highly significant. The statistical difference between the data collected on the 11th day of the CS alone paradigm and those collected the 15th day of the CS-UCS paradigm (t = 4.28, df = 18, P < 0.001) was also significant. The data mentioned above indicate that the CS-UCS association was essential for the induction of plasticity both in the behavioral responses (percentage and amplitude of the motor responses) and in the postsynaptic field potentials.

**Statistical analysis of the motor and rubral response parameters during conditioning**

In an earlier study (Meftah and Rispal-Padel 1994) we showed, through statistical analysis, that the variations seen in the cerebellorubral field potentials were strongly correlated with those of the motor responses. This result led us to suggest that the synaptic modifications affecting the cerebellothalamocortical pathway could represent one of the neurobiological correlates of associative conditioning. To determine whether the changes in the interpositorubrospinal circuit efficacy play a similar role, we further analyzed the correlation between motor performance and the interposito rubral potential magnitude (Fig. 9). The high correlation coefficients found in the present study suggested that, like the motor cortex, the RNm contains plastic elements modified by the effects of conditioning. In three of the four animals studied, the correlation coefficients between the areas of the postsynaptic field potentials and those of the percentages of motor responses were calculated. They were r = 0.92, n = 20, P < 0.001; r = 0.77, n = 21, P < 0.001; and r = 0.93, n = 20, P < 0.001. The same areas of the rubral field potentials were also well correlated to the amplitude of the movements: r = 0.76, n = 20, P < 0.001; r = 0.89, n = 21, P < 0.001; and r = 0.88, n = 20, P < 0.001.

The correlations calculated on the data collected from cat KAS showed that there was no causal relationship between
the variation of the presynaptic cerebellorubral responses and the amplitude (r = 0.22, n = 20, P = 0.34) or the percentage of the movements (r = 0.03, n = 20, P = 0.9) (Fig. 9, A and C). However, strong correlations were found between the variations in the postsynaptic responses and the amplitude (r = 0.76, n = 20, P < 0.001) as well as the percentage (r = 0.92, n = 20, P < 0.001) of the motor responses (Fig. 9, B and D). This could indicate a common origin for the changes affecting the movements and the interpositoral postsynaptic field potentials, although there is no relationship between the motor changes and the IN neuron excitability.

Identification of the neuronal elements in the interpositoral pathway modified by conditioning

Tests carried out before and after conditioning revealed that the effects produced were restricted to a clearly specific group of interpositoral fibers. They also indicated that the increase in the rubral postsynaptic field potentials was
produced by a change in the synaptic transmission between the interpositus fiber terminals and the neurons of the RNm.

Figures 6, 7, and 8, D and E, show that the amplitude of the presynaptic interpositorubral responses recorded in all animals remained constant throughout conditioning. The presynaptic response amplitude (Fig. 10Ba) plotted against the IN stimulating current intensity obtained before and after conditioning (Fig. 10C) are practically superimposable. The ratios of the values with which these curves were plotted remained close to 1, whatever the intensity of the stimulation, indicating that the state of excitability of the IN neurons did not change during the conditioning procedures. In the RNm, the increase of the postsynaptic responses (Fig. 7B) observed during acquisition (Fig. 7Ba, Sup.) is due to changes that only affected the connections between the interpositus neurons to which the CS was applied and the rubral neurons. Examination of the curves shown in Fig. 10D shows that this effect is not caused by a phenomenon generalized to all interpositorubral synapses. Identical stimulation intensities caused a greater amplitude in the postsynaptic responses of the RNm after conditioning. However, this process did not affect the transmission of all the interpositorubral connections. The ratios of the amplitudes before and after conditioning express the enhancement factor. Figure 10D shows that the enhancement factor did not increase evenly for all the stimulation intensities: for low stimulating currents it was equal to 1, then it increased sharply to a value of 3 between 40 and 60 μA, i.e., values close to the stimulation intensity used for the CS (55 μA); this could indicate that the only synaptic connections modified were those coming from the interpositus neurons situated in the immediate vicinity of the stimulation electrode and activated by application of the CS. For the present animal, no effects appeared after conditioning for stimulating currents <25 μA. This could be explained by the fact that the neurons located at the tip of the RNm electrode received their cerebellar projections from a population of neurons situated close to the tip of the IN electrode but not exactly at its contact. In the other animals, increase of the enhancement factor began with current intensities of 13, 17, and 35 μA, respectively. The decrease of the enhancement factor that appeared with stimulating currents >60 μA and its further stability at a value of 2, up to the beginning of the plateau of the curve (~120 μA), suggests that the connections of the interpositus neurons located at greater distances from the stimulating electrode and solicited with stimulation currents stronger than the current used during conditioning were not modified. It must be noted that a second increase of the enhancement factor was never observed even with higher stimulating current intensities (up to 250 μA) that were able to excite the whole population of IN neurons.

Examination of the rubral neuron excitability before and after conditioning

Two complementary electrophysiological tests were used to check the excitability of rubral neurons. The first was a study of the invasion of the rubral neurons by antidromic activation. This is a classic test commonly used to evaluate the neuronal excitability with intracellular and extracellular electrophysiological methods (see review by Eccles 1957; Eccles et al. 1970, 1971; Jankowska et al. 1968). The stimulation electrode was implanted contralaterally in the rubrospinal pathway, after its decussation, at levels P5.5, L4.5, and
H6 (Fig. 11Aa). The electrode used to record the antidromic response in the RNm was that used, during conditioning, to record the pre- and postsynaptic field potentials evoked by the CS. The antidromic response, which was formed of a negative wave with a latency of 0.4 ms and a peak at 0.6 ms, followed each shock of a high-frequency stimulation train (500 Hz) (Fig. 11Ab). Deducting the time necessary for fiber activation (~0.3 ms) (Blair and Erlanger 1936) from the onset and peak latencies of the negative wave, and estimating the distance separating the rubral neurons from the stimulation electrode at 12 mm, the conduction velocities calculated for the rubrospinal axons were between 40 and 120 m/s. Although the conduction time was too short to allow a precise measurement, these values are consistent with previous measurement of the conduction velocity (Eccles et al. 1975; Jeneskog and Padel 1983; Padel et al. 1972; Tsukahara et al. 1967). It can be seen (Fig. 11C) that there was no modification of the antidromic response during conditioning, indicating the absence of any change in the rubral neuron excitability.

In the second test we investigated the efficacy of rubral stimulation in the production of a forearm flexion before and after conditioning (Fig. 11Ba). The electrode previously used to record the field potentials evoked by the stimulation of the IN was used in this examination to stimulate the RNm neurons. It is presumed from previous experiments that the metallic semimicroelectrodes we have used directly activate the cell somas (Rispal-Padel and Grangetto 1977; Rispal-Padel et al. 1982). Several stimulation intensities were applied so as to test both the neurons near the electrode and those further away. No significant modifications appeared in the amplitudes or in the time courses of forearm flexions of cat KAS after conditioning (Fig. 11Bb). The mean values, calculated for different stimulation intensities, for all the conditioned animals are represented by bars in Fig. 11Bc. This second test, therefore confirms the stability of rubral neuron excitability during the dynamic phase of conditioning.

Finally, the last indication that the excitability of the rubral neurons was unaltered was given by the observation of the similarity of the basal activity recorded with the same electrode and in the same place in the RNm before (Fig. 11Ca) and after (Fig. 11Ch) conditioning. During both recordings the behavior of the animal was stable and its state of wakefulness did not present any detectable changes.

**DISCUSSION**

This study shows that in the adult animal, in a simplified and well-defined sensorimotor circuit, the efficacy of the cerebellar transmission to the neurons of the RNm can be modified by classical conditioning. The associative modifications doubled the field potential amplitude evoked by low-current stimulation of the IN in a small population of RNm neurons. Overall, the tests carried out show that these increases in the central responses do not result from a change in the biophysical characteristics (membrane properties) of the rubral and interpositus neurons. The constancy of rubral and interpositus neuronal excitability was demonstrated by the stable amplitude of the presynaptic interpositorubral re
response and by the unchanged amplitude of the forelimb flexion in response to RNm stimulation. The latter test shows, in addition, that neither the rubrospinal transmission nor the excitability of the spinal neurons were affected by the conditioning. It can be hypothetized that the increases in motor responses evoked by the IN stimulation are due to
the plasticity of the cerebellorubral connections. Thus the cerebellorubral synapses present properties of plasticity that can be demonstrated by associative conditioning procedures; these properties are similar to those observed in the corticorubral connections by others (Murakami et al. 1988; Oda et al. 1988; Tsukahara et al. 1981). Some plasticity of the cerebellorubral pathway had already been observed in the immature animal after a period of recovery following the destruction of a portion of the RNm afferents (Lim and Leong 1975; Tsukahara and Fujito 1981). Our study shows that it can also be produced in the adult animal with an intact nervous system.

**Conditions for induction of plasticity in the cerebellorubral connections**

In the present experiment, synaptic changes involving the cerebellorubral connections only appeared under particular conditions. During conditioning, the expression of plasticity in the red nucleus seems to be closely dependent on the degree of activation of the afferent pathway in which it occurs. This suggestion is supported by a comparison of our results with those of Tsukahara (Tsukahara et al. 1981) and Oda (Oda et al. 1988). One of the essential conditions for the induction of synaptic plasticity in the RNm appears to be the repetitive activation of the CS afferent pathway. This plasticity is therefore likely to be "activity dependent."

Another important aspect of red nucleus plasticity involves the temporal aspect of the CS and UCS association between the events produced by the two paired stimulations. When the CS was delivered alone, repeatedly, 125 times daily over 11 consecutive days, no modifications occurred, either for the motor responses (Fig. 3, A and B) or for the postsynaptic field potentials in the RNm (Fig. 8E). Moreover, at the end of the acquisition period, when extinction procedures were applied, i.e., dissociation of CS-UCS pairing, the increase of the two types of responses stopped and their amplitudes returned to values close to, but still greater than, those they initially had. The result was identical whether it was a simple dissociation (CS alone) (Fig. 8B) or a temporal dissociation when the order CS-UCS was reversed and the CS no longer announced the imminent application of the UCS (Rispal-Padel and Meftah 1992). It also appears that the activity-dependent changes of the interposito-rubral connections can only occur when the activation of the RNm neurons by the CS coincides with excitation from another afferent pathways. These data clearly show the associative character of the motor and synaptic changes observed in this experiment.

At the cellular level, changes in the synaptic efficacy of IN effector pathways seem to be the result of mechanisms complying with Hebb's rules (Hebb 1949). The conjunction of a presynaptic activation of the IN fiber terminals and a postsynaptic activation of the RNm neurons could be produced either by the only activation of the CS pathway or by the convergence of facilitations generated by the CS and UCS pathways on the RNm neurons, which could contribute to produce associative synaptic modifications (Brown et al. 1990) in the cerebellorubral synapses.

In this experiment, the conjunction of pre- and postsynaptic activations could be first produced in the cerebellorubral connections during the CS application. The CS train of shocks applied to the IN could build up a progressive depolarization of the rubral neurons that could be concomitant with the activation in presynaptic cerebellar axon terminals due to the last shocks in the train. This mechanism should be able to bring about the conjunction of pre- and postsynaptic activations in a single afferent pathway to RNm neurons.

A second mechanism of pre- and postsynaptic activations in the cerebellorubral connections could be achieved by the convergence of distinct afferent pathways activated through internal and external loops. The contribution of the transcortical loop in which the RNm is included (Allen et al. 1974, 1977; Padel 1993) could occur during conditioning via the interpositothalamocorticorubral circuit, which is activated by the CS at the same time as the interposito-rubral pathway. The excitatory synaptic connections in the various relays of the circuit can allow the cortical excitation of the RNm neurons with latencies on the order of 4-7 ms. This time is sufficiently short for the depolarization or activation of the rubral neurons through this loop to precede the end of the activation of the cerebellorubral synapses by the CS. It must be noted that pre- and postsynaptic coactivation should also occur in the VL, which also receives cortical inputs. The fact that no modifications occur in the interpositothalamic synapses can be explained by the presence of intrathalamic inhibition.

There are still two other central loops involving coactivation of the interpositus terminals and the rubral neurons: the cerebellocorticopontointerposito-rubral and the cerebellopon-tointerposito-rubral loops. From the motor cortex, which is excited by the CS via the VL, inputs transmitted through the precerebellar and the cerebellar nuclei can cause the rapid reactivation of the RNm neurons before the end of the CS direct interposito-rubral excitation. Indeed, the cortico-cerebellar pathway is characterized by its rapidity (7-8 ms) (Allen et al. 1976) and the time of transmission in this pathway is slightly shorter than the duration of excitation produced in the RNm by the CS. The closed loop between the IN, the pontine precerebellar nuclei and the RNm, i.e., the "reverberating circuit" disclosed by the use of picrotoxin (Tsukahara et al. 1983), presents the same temporal characteristics as the cerebellocorticocerebellar loop. However, it must be noted that the pre- and postsynaptic coactivation in the RNm that can be brought about by these pathways must be of short duration because it is interrupted by Purkinje cell inhibition of cerebellar nuclear neurons.

Taking only the delays into consideration, all these central circuits appear to be possible candidates for inducing plasticity of the interposito-rubral synapses by this Hebbian mechanism. We have, however, observed that application of the CS alone, even though it was able to activate these circuits, was not sufficient to generate the synaptic changes. The pairing of the CS with the UCS was indispensable for the appearance of changes in the efficacy of the interposito-rubral transmission. However, because the plasticity of the interposito-rubral synapses seems to be activity dependent, the conjunction of the pre- and postsynaptic coactivation in RNm by the repetitive activation of these pathways by the CS must be of extreme importance even when CS and UCS are paired.

The convergence in the RNm, of the CS, and UCS activa
tions could also be achieved by two peripheral feedback loops, which must be taken into account. They could prolong the activations evoked by the CS until the time when the UCS excitation is triggered in the red nucleus. They are both involved in the transmission of effects evoked from the cutaneous and muscular receptors. One of them is composed of the transcerebellar peripheral feedback loop (Allen and Tsukahara 1974; Padel 1993), in which the rubral neurons are included. This loop can be stimulated by the slight contraction, triggered in the muscles of the forelimb by the CS, that causes the excitation of the muscle receptors. The reactivation of the rubral neurons by the influx coming from these receptors can appear at the moment the UCS is applied and can converge with its facilitatory effects because the shortest time lapeses between the periphery and the cerebellum are of 5–7 ms and the peak of their unimodal distribution is 14–16 ms (Eccles et al. 1975). Pre- and postsynaptic coactivation can also occur at that moment in the interpositorubral connections and reinforce the efficacy of their transmission. As in the case of the transcerebellar central loops, the duration of excitation in this peripheral feedback loop would be rapidly truncated by the inhibitory effects of the Purkinje cells. This imposes a temporal constraint on the duration of the convergent excitation.

The second circuit passing through the periphery and the spinal centers includes the ascending spinorubral pathway, which is the most likely candidate for long-lasting facilitations induced by CS and UCS. Excitation of the receptors corresponding to high-threshold muscle and of the cutaneous afferents (Padel et al. 1986) can produce, in the RNm neurons, powerful long-lasting discharges reaching from 25 to 150 ms (Padel and Relova 1988). The pathway is direct between the spinal cord and the rubral neurons, bypassing the cerebellum, and therefore is not affected the Purkinje cell inhibition. Moreover, its participation in the induction of synaptic plasticity in the efferent cerebellar circuits could explain the absence of similar changes in the interpositorubral synapses. Thalamic neurons that relay cerebellar outputs do not receive direct spinal projections (Berkeley 1983; Jones 1985).

It should also be noted that, in addition to the involvement of the loops described above in maintaining the effects of the CS, the temporal association of the CS and the UCS effects can be attained via long-duration intracellular events. Triggered by the CS, these events seem to be able to explain the associative character of the synaptic changes evoked in classical conditioning in invertebrates (Abrams and Kandel 1988). Similar events could happen in the RNm because the rubral spikes are followed by afterhyperpolarizing potentials present a rising time that is slightly longer than 10.220.32.247 on October 26, 2016 http://jn.physiology.org/ Downloaded from
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those of the interpositorubral pathway (Fujito et al. 1983), but there could be an overlap in the locations of cerebellar and spinal synaptic contacts on the membrane of RNm neurons.

Function of the cerebellorubral synaptic plasticity

The demonstration of synaptic plasticity at the cerebellorubral (present paper) and thalamocortical levels (Meftah and Rispal-Padel 1994; Rispal-Padel and Meftah 1992) supports the possibility that, in addition to the commonly accepted critical cerebellar site involved in motor learning (Albus 1971; Clark et al. 1992; Glickstein 1992; Ito 1972; Lisberger 1988; Marr 1969; McCormick et al. 1981; Thompson 1988; Thompson et al. 1983; Yeo 1991; Yeo and Hardiman 1992), there are varied memory support sites in the pathways through which the new motor programs are expressed. This synaptic plasticity appears to be “activity dependent,” because it needs, for its induction, the frequent activation of the conditioned sensorimotor pathway, and it could be associated with the refinement of the pathways involved in the newly learned movements. The question arises as to whether the cerebellorubral plasticity acts from the earliest phases of the acquisition period of the new conditioned responses or whether it becomes involved later and participates in the retention and the adjustment of performance.

Our results show that during associative conditioning, this plasticity, revealed by the change in amplitude of the post synaptic field potentials, appears during a period that can be considered as the phase of acquisition (period of increase of the percentage of responses evoked by the CS). This appears to be in agreement with several other data that support the suggestion that the RNm might be involved in the acquisition of the memory traces (Berthier and Moore 1986; Bromily 1948; Desmond and Moore 1982; Mauk and Thompson 1987; Poltrew and Zelioni 1930; Rosenfield and Moore 1985).

In other respects, the RNm could also be involved in the storage of a component of the memory trace because, when the conditioning was resumed after a hiatus of >5 days, the plastic changes were shown to persist in the interpositorubral synapses. This suggestion has already been proposed on the basis of results showing that previously learned responses are not lost after bilateral cerebral decortication (Desmond and Moore 1991; Oakley and Russell 1977; Rosenfield and Moore 1983).

In agreement with the idea that the RNm is essential in movement execution and in updating of the motor command (Allen and Tsukahara 1974; Padel 1993; Padel and Steinberg 1978) the cerebellorubral synaptic changes could be involved in the adjustment of the movement parameters. Indeed, these changes present topographic relationships and their emergence and time course parallels changes of the conditioned motor responses. The plasticity of the interpositorubral synapses would consequently have a preponderant role in the execution of the motor command of new learned responses. This could also explain some previous results (Chapman et al. 1990; Krupa et al. 1993; Smith 1970).

Although the synaptic plasticity of the cerebellorubral connections, like that of the corticorubral synapses, justifies the possible involvement of the RNm in several aspects of motor learning, it must be noticed that conditioning of the nictitating membrane response was still possible in animals after complete decortication and decerebellation (Bracha et al. 1993, Kelly et al. 1990, see also reviews of Bloedel and Bracha 1995 and Bloedel et al. 1991), indicating that perhaps other pathways could still be able to form sensorimotor loops in the RNm for the conditioning of some specific reflexes.

In conclusion, the RNm, which is known for its powerful influence on somatic motricity, appears to have a particular position with regards to systems related to motor learning and consequently to the generation of the mnemonic trace: three principal afferent pathways, coming from the cerebral cortex, the cerebellum, and the spinal cord or periphery, make synaptic contacts onto the rubrospinal neurons. Repetitive interactions on clusters of rubral neurons, between the cerebellar and the sensory inputs, produce a synaptic plasticity that could be of an activity dependent type. The synaptic plasticity of the interpositorubral pathway evidenced in the present experiment could be one of the neuronal correlates of the adaptation processes of the motor command. It could also take part in the refinement of the pathways involved in newly learned movements; this would reflect the RNm participation in some aspects of the motor learning.

The authors thank M. Coulmance for dealing with the computer programming of the conditioning stimulation sequences and the data processing, and Dr. Yves Padel for invaluable scientific advice during the course of this study. They are most grateful to Dr. Allan Smith for criticisms of the manuscript.

E. M. Meftah was supported by the Fondation pour la Recherche Medicale. The work was also funded by the Conseil Regional Midi-Pyrenees. Address for reprint requests: L. Rispal-Padel, Centre de Recherche Cereveaux et Cognition, CNRS, UMR 9940, Faculte de Medicine de Toulouse Rangueil, 133, route de Narbonne, 31602 Toulouse, France.

Received 7 July 1995; accepted in final form 28 December 1995.

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