

Dendritic Excitability Microzones and Occluded Long-Term Depression After Classical Conditioning of the Rabbit's Nictitating Membrane Response

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Schreurs, Bernard G., Daniel Tomsic, Pavel A. Gusev, and Daniel L. Alkon. Dendritic excitability microzones and occluded long-term depression after classical conditioning of the rabbit's nictitating membrane response. *J. Neurophysiol.* 77: 86–92, 1997. We made intradendritic recordings in Purkinje cells ($n = 164$) from parasagittal slices of cerebellar lobule HVI obtained from rabbits given paired presentations of tone and periorbital electrical stimulation (classical conditioning, $n = 27$) or explicitly unpaired presentations of tone and periorbital stimulation (control, $n = 16$). Purkinje cell dendritic membrane excitability, assessed by the current required to elicit local dendritic calcium spikes, increased significantly in slices from animals that received classical conditioning. In contrast, membrane potential, input resistance, and amplitude of somatic and dendritic spikes were not different in slices from animals given paired or explicitly unpaired stimulus presentations. The location of cells with low thresholds for local dendritic calcium spikes suggested that there are specific sites for learning-related changes within lobule HVI. These areas may correspond to learning "microzones" and are consistent with locations of learning-related *in vivo* changes in Purkinje cell activity. Application of 4-aminopyridine, an antagonist of the rapidly inactivating potassium current I_A , reduced the threshold for dendritic spikes in slices from naive animals to levels found in slices from trained animals. In cells where thresholds for eliciting parallel fiber–stimulated Purkinje cell excitatory postsynaptic potentials (EPSPs) were measured, levels of parallel fiber stimulation required to elicit a 6-mV EPSP as well as a 4-mV EPSP ($n = 30$) and a Purkinje cell spike ($n = 56$) were found to be significantly lower in slices from paired animals than unpaired controls. A classical conditioning procedure was simulated in slices of lobule HVI by pairing a brief, high-frequency train of parallel fiber stimulation (8 pulses, 100 Hz) with a brief, lower frequency train of climbing fiber stimulation (3 pulses, 20 Hz) to the same Purkinje cell. Following paired stimulation of the parallel and climbing fibers, Purkinje cell EPSPs underwent a long-term (>20 min) reduction in peak amplitude (–24%) in cells ($n = 12$) from animals given unpaired stimulus presentations but to a far less extent (–9%) in cells ($n = 20$) from animals given *in vivo* paired training. Whereas 92% of cells from unpaired animals showed pairing-specific depression, 50% of cells from paired animals showed no depression and in several cases showed potentiation. Our data establish that there are localized learning-specific changes in membrane and synaptic excitability of Purkinje cells in rabbit lobule HVI that can be detected in slices 24 h after classical conditioning. Long-term changes within Purkinje cells that effect this enhanced excitability may occlude pairing-specific long-term depression.

INTRODUCTION

The search for neural substrates of learning and memory in model systems has identified a number of sites in mammalian

brain. The use of well-controlled behavioral paradigms, such as classical conditioning, has permitted this search to focus on a relatively small number of areas (e.g., Thompson 1986; for review see Schreurs 1989). Classical conditioning of the rabbit nictitating membrane/eyelid response (NMR) involves the presentation of an innocuous stimulus such as a tone followed by a noxious stimulus such as air puff to or electrical stimulation around the eye (e.g., Gormezano et al. 1962, 1983). Extensive lesion and recording data have implicated the deep nuclei and cortex of the cerebellum in classical conditioning of the rabbit NMR (e.g., Berthier and Moore 1986; Gould and Steinmetz 1996; Gruart and Yeo 1995; McCormick and Thompson 1984; Thompson and Krupa 1994; Yeo et al. 1985b). Lesions of lobule HVI and ansiform of the cerebellar cortex ipsilateral to the stimulated eye disrupt conditioned responses, and, although conditioned responses eventually return, they are of lower frequency and amplitude than before the lesion (e.g., Lavond and Steinmetz 1989; Lavond et al. 1987; Yeo et al. 1985a). Bilateral lesions of lobule HVI abolish or severely impair conditioned responses in trained animals without affecting the unconditioned response, and, even more importantly, bilateral lesions prevent relearning of the conditioned response (Gruart and Yeo 1995). *In vivo* extracellular recordings in and around lobule HVI made during learning have suggested that some Purkinje cells show conditioned response–related increases and others show conditioned response–related decreases in simple spike activity (e.g., Berthier and Moore 1986; Gould and Steinmetz 1996; Thompson 1990).

Information about the tone and air puff normally used during classical conditioning of the rabbit NMR reaches lobule HVI of the cerebellum via pontine nuclei and the inferior olive (e.g., Gould et al. 1993; Steinmetz et al. 1989; Yeo et al. 1985b). The pontine nuclei and inferior olive give rise to mossy fiber and climbing fiber inputs to the cerebellar cortex. Mossy fibers, in turn, give rise to parallel fibers, which together with climbing fibers synapse on the Purkinje cells of cerebellar lobule HVI. Stimulation of the pontine nuclei followed by stimulation of the inferior olive in a classical conditioning procedure using intact rabbits results in conditioned nictitating membrane/eyelid responses (e.g., Steinmetz et al. 1989) and stimulation of parallel fibers followed by stimulation of the climbing fibers in slices supports long-term depression of Purkinje cell excitatory postsynaptic potentials (EPSPs) in rat (Chen and Thompson 1995) and in untrained rabbit (Schreurs et al. 1996).

Studies of plasticity in the cerebellar cortex have focused, in large part, on long-term depression (LTD), a reduction in the size of Purkinje cell EPSPs that results from conjoint stimulation of climbing fiber and parallel fiber inputs to the Purkinje cell (e.g., Crepel and Jaillard 1991; Ekerot and Kano 1985, 1989; Hartell 1996; Ito 1989; Linden and Connor 1992; Sakurai 1987; Schreurs and Alkon 1993; Schreurs et al. 1996). The fact that synaptic plasticity in the form of LTD occurs as a result of stimulation of parallel and climbing fibers (e.g., Chen and Thompson 1995; Ito 1989; Schreurs et al. 1996) and that information about tone and air puff reaches Purkinje cells via parallel and climbing fibers (e.g., Gould et al. 1993; Yeo et al. 1985b) has led to the hypothesis that LTD is a synaptic mechanism underlying classical conditioning of the NMR (e.g., Ito 1989; Thompson 1986).

The purpose of the present experiments was to examine the electrophysiological properties of Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI following classical conditioning of the nictitating membrane response (Schreurs et al. 1991). Specifically, rabbits were given three sessions of paired or explicitly unpaired presentations of a tone and electrical stimulation around the right eye. Twenty-four hours later, we prepared slices of lobule HVI from the right side of the cerebellum and examined a number of Purkinje cell electrophysiological properties including membrane excitability (Schreurs et al. 1991), synaptic excitability (Schreurs and Alkon 1993), and pairing-specific LTD (Schreurs et al. 1996).

METHODS

Behavior

The subjects were 43 adult, male, albino rabbits (*Oryctolagus cuniculus*) weighing ~2.0–2.2 kg. Rabbits were individually housed, given access to food and water, and maintained on a 12:12 light/dark cycle. Animals were allocated randomly to one of two groups in which they received either paired stimulus presentations (Paired, $n = 27$) or unpaired stimulus presentations (Unpaired, $n = 16$). All rabbits received 1 day of preparation and three consecutive days of stimulus presentation. On adaptation day, the rabbits were prepared for periorbital electrical stimulation and recording of nictitating membrane movement and then adapted to the training chambers for the length of time of subsequent training sessions (80 min). Training sessions for Paired subjects consisted of 80 presentations of a 400-ms, 1,000-Hz, 82-dB tone conditioned stimulus (CS) that coterminated with a 100-ms, 60-Hz, 2-mA electrical pulse unconditioned stimulus (US) (Schreurs 1993). Paired stimulus presentations were delivered, on average, every 60 s (range 50–70 s). Sessions for Unpaired subjects consisted of 80 CS-alone and 80 US-alone presentations that occurred in an explicitly unpaired manner delivered, on average, every 30 s (range 20–40 s). Stimulus delivery and data collection were accomplished with the use of a Compaq/ASYST system previously described (Schreurs and Alkon 1990).

Slice preparation

Twenty-four hours after training, the animals were anesthetized deeply with pentobarbital sodium (30 mg/kg) and decapitated. A rapid craniotomy that removed the occipital bone and mastoid processes allowed the cerebellum and brain stem to be detached, removed, and chilled in 95% O₂-5% CO₂ saturated artificial cerebrospinal fluid (ACSF) within ~70–90 s. Next, the area surrounding the right HVI lobule (ipsilateral to the side of training)

was isolated and attached with cyanoacrylate to an agar block in the cutting chamber. The isolated tissue was then immersed in chilled ACSF, and 400- μ m parasagittal slices were cut with a vibrating slicer (Vibratome 1000). After this procedure, slices were incubated in saturated ACSF at room temperature for at least 1 h before being placed in a modified recording chamber in which the ACSF was maintained at 32°C (see Schreurs et al. 1991). The ACSF contained (mM) 124 NaCl, 3 KCl, 1.2 MgSO₄, 2.1 CaCl₂, 1.2 Na₂PO₄, 26 NaHCO₂, and 10 dextrose and was saturated with a mixture of 95% O₂-5% CO₂, which maintained pH at 7.4.

Intradendritic recording

Intradendritic recordings from Purkinje cell dendrites were obtained by advancing a glass microelectrode (Leitz micromanipulator) through the molecular layer of slices of lobule HVI. Only stable recordings from Purkinje cell dendrites with membrane potentials lower than -50 mV and input resistances ≥ 28 M Ω were used (Schreurs et al. 1996). Recordings were made up until 8 h after death. Microelectrodes of thick-walled glass (2 mm OD, 1 mm ID, FHC) were fabricated on a Narishige electrode puller (NE-20), filled with 3 M potassium acetate, and had a dc resistance of 60–120 M Ω . A bridge amplifier (Axoprobe-1A, Axon) was used for all intradendritic recording. The recording electrodes were positioned in the molecular layer with the aid of a binocular dissecting microscope (Wild, magnification up to $\times 50$), which permitted visualization of the different cortical layers. Parallel fibers were stimulated with the use of a bipolar electrode (150 μ m separation, Rhodes) placed in the molecular layer at the cortical edge of the lobule. Climbing fibers were stimulated with the use of a bipolar electrode (100 μ m separation, Rhodes) placed in the white matter of the lobule. Timing of all stimulation parameters including pulse duration, frequency, and train duration was controlled by an Ana-pulse stimulator (302-T, WPI). Constant-current pulses were controlled by stimulus isolation units (0–5 mA, 305-1, WPI).

Synaptic stimulation

The pulse width for both parallel and climbing fiber constant-current stimulation was set at 80 μ s for all protocols. Current values for parallel fiber stimulation and climbing fiber stimulation in slices from both Paired and Unpaired rabbits were adjusted to be as similar as possible while still eliciting comparable (6–8 mV) EPSPs and all-or-none synaptic potentials. This ensured that there was no between-group bias in current strength or response size for the pairing-specific LTD protocol (Schreurs et al. 1996). Threshold measurements for eliciting the standard 6- to 8-mV EPSP as well as for a 4-mV EPSP and for eliciting a Purkinje cell spike were made in a subgroup of cells. Stimulation of parallel fibers and climbing fibers for the pairing-specific LTD protocol occurred at a pulse frequency of 100 Hz for 80 ms (8 pulses) and 20 Hz for 100 ms (3 pulses), respectively. The paired presentation of parallel fiber and climbing fiber trains occurred 20 times at an intertrial interval of 20–40 s with 80 ms of parallel fiber stimulation terminating before the start of 100 ms of climbing fiber stimulation. The pairing-specific LTD protocol was delivered without positive or negative current injection.

Solution application

The potassium inactivating current antagonist, 4-aminopyridine (4-AP, Aldrich), was delivered to the slice via whole-bath perfusion at a concentration of 20–100 μ M.

Data measurement and analysis

Data were recorded on video cassette tape with the use of a PCM-VCR (DX-900, Toshiba) and digitized with the use of

pClamp software (Axon). The majority of Purkinje-cell dendrites revealed autorhythmic spontaneous activity with up to three distinct phases: 1) a hyperpolarized quiescent phase, 2) a more depolarized somatic spiking phase, and 3) a still more depolarized dendritic spiking phase (e.g., Llinas and Sugimori 1980; Schreurs et al. 1991, 1992). Membrane potential was determined as the potential for somatic activity phase (Schreurs et al. 1991, 1996). Input resistance measures were based on a 0.5-nA, 700-ms hyperpolarizing current step. The current necessary to hyperpolarize the dendrite 20 mV below the somatic spike activity level was determined and applied to the membrane in order to determine the dendritic spike threshold. Threshold measurements were based on the specific 700-ms current step required to reach dendritic spike threshold.

Changes in Purkinje cell dendrite EPSPs were expressed as percentage change in EPSP peak amplitude calculated from an average of at least five EPSPs elicited by single parallel fiber pulses at between 0.1 and 0.5 Hz before the stimulation protocol and compared with sets of at least five single parallel fiber pulses first presented at 1 min after the protocol and then every 3 min until at least 21 min after the protocol. The peak amplitude of single EPSPs and all-or-none synaptic potentials was measured during the current step, which hyperpolarized the membrane potential to 20 mV below the level of somatic spiking (Schreurs et al. 1996).

RESULTS

Behavior

Paired subjects ($n = 27$) all showed levels of conditioning in excess of 85% conditioned responses by the end of the 3 days of training, whereas Unpaired subjects ($n = 16$) showed only baseline levels of responding (i.e., <3%) (e.g., Schreurs et al. 1991).

Electrophysiology

A total of 164 of 188 cells that were successfully penetrated met the membrane potential and input resistance criteria and were included in the present experiment. Twenty-four of these cells were recorded with the experimenter blind to the behavioral condition of the animals.

MEMBRANE PROPERTIES. Figure 1 details individual current steps for a Purkinje cell from a Paired and Unpaired rabbit (A) as well as the mean dendritic spike threshold (B) relative frequency distribution of thresholds (C) and threshold current for EPSPs (D) for Paired and Unpaired animals. The individual traces (Fig. 1A) show that at a current step of 0.5 nA neither cell reached dendritic spike threshold, but at a current step of 0.7 nA the cell from the Paired animal did reach threshold. The column graph (Fig. 1B) shows that cells obtained from Paired rabbits ($n = 89$) required a mean current of 1.47 ± 0.05 (SE) nA to elicit dendritic spikes, whereas cells obtained from Unpaired animals ($n = 63$) required a mean current of 1.89 ± 0.07 nA to elicit the same spikes ($P < 0.01$). The relative frequency distribution of threshold values (Fig. 1C) shows clearly that there was a shift to the left (lower thresholds) for cells from Paired animals. In fact, the lowest bin (0.5–0.9 nA) contains only cells from Paired animals (illustrated in the individual traces). The threshold parallel fiber stimulation current for eliciting EPSPs shows that significantly less current was required to elicit a 4- and 6-mV EPSP and an EPSP-induced Purkinje cell spike in slices from Paired animals than in

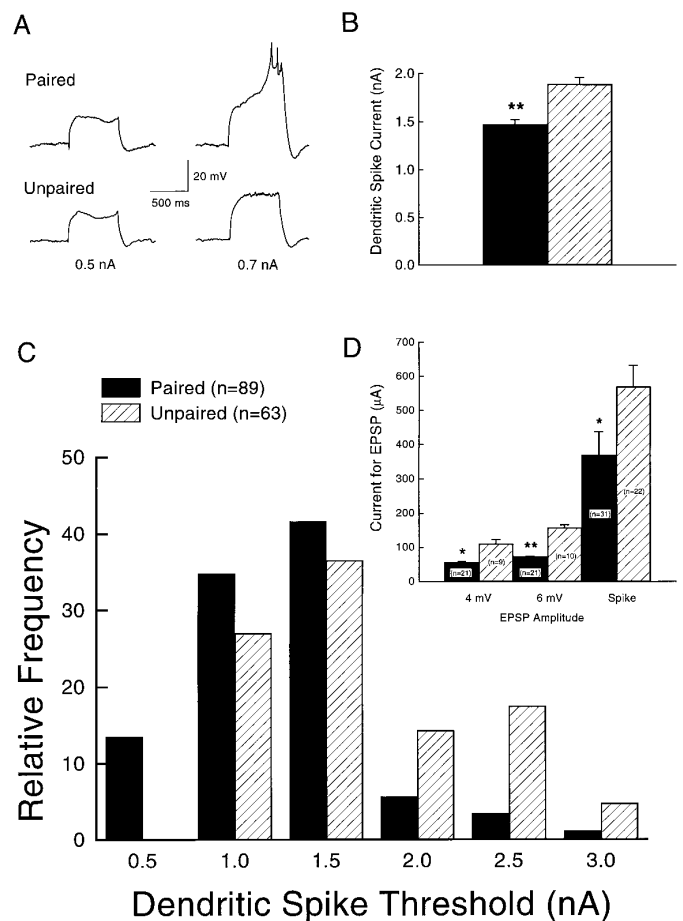


FIG. 1. Effects of classical conditioning on membrane and synaptic excitability. Individual intradendritic recordings (A), group means (B), relative frequency distributions (C) of local dendritic calcium spike thresholds, and parallel fiber stimulation current required to elicit 4-mV and 6-mV excitatory postsynaptic potentials (EPSPs) and an EPSP-induced spike (D) for Purkinje cells from slices of lobule HVI taken from the right cerebellum 24 h after 3 days of Paired training or Unpaired stimulus presentations. Individual recordings show depolarized responses to 700-ms current injections of 0.5 and 0.7 nA for a cell from a Paired animal with dendritic spikes occurring at 0.7 nA and for a cell from an Unpaired animal that remains below threshold at 0.7 nA. The relative frequency distribution shows a clear shift in thresholds to the left for Paired animals with only cells from Paired animals in the lowest threshold bin (0.5–0.9 nA). The threshold current for eliciting EPSPs illustrates that significantly less parallel fiber stimulation is needed to elicit EPSPs in cells from Paired animals than in cells from Unpaired animals.

slices from Unpaired animals ($P_s < 0.05$, 0.01, and 0.05, respectively).

With the exception of the threshold for dendritic spikes, there were no significant differences between cells from Paired subjects ($n = 93$) and cells from Unpaired subjects ($n = 71$) in membrane potential (-57.1 ± 0.45 vs. -58.1 ± 0.5 mV), input resistance (32.5 ± 0.6 vs. 31.3 ± 0.7 M Ω), current required to hyperpolarize the membrane by 20 mV (-0.78 ± 0.02 vs. -0.76 ± 0.03 nA) somatic spike amplitude (6.3 ± 0.6 vs. 6.0 ± 0.7 mV) or dendritic spike amplitude (26.5 ± 1.2 vs. 25.2 ± 1.2 mV).

Figure 2 depicts six sequential parasagittal slices of lobule of HVI (A), an anterior view of the area of the right cerebellar hemisphere from which the slices were obtained (B), and a composite of slices 2, 3, and 4 (C). The sequential slices are depicted so that the rabbit's left folium is on the

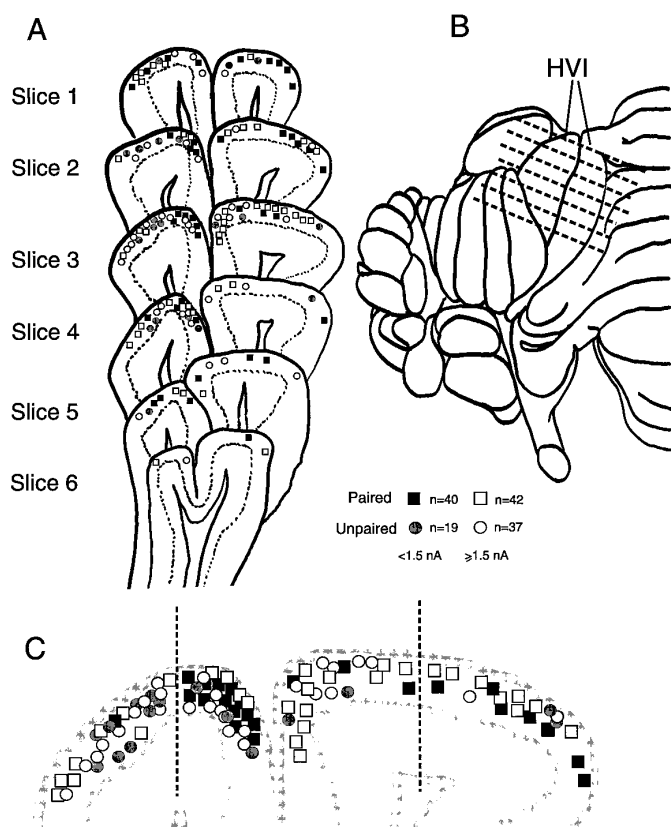


FIG. 2. Membrane excitability is localized to a specific microzone. *A*: recording sites for individual Purkinje cells located on sequential parasagittal slices from Larsell's lobule HVI. *B*: an anterior view of the right cerebellum indicating the area from which the slices were cut (dashed lines). *C*: a composite of slices 2, 3, and 4 showing electrode locations to the left and right of the midline of each folium. The slices are depicted so that the rabbit's left folium of lobule HVI is shown on the left and the rabbit's right folium is shown on the right. The cell locations for Paired (\square and \blacksquare) and Unpaired (\bullet and \circ) animals are based on visual inspection ($\times 12$ magnification) of the electrode position in the slice at the time of recording. Locations are divided between those below (\blacksquare and \bullet) or at and above (\square and \circ) the mean dendritic spike threshold for cells from Paired animals (1.5 nA). The individual slices were obtained by tracing cresyl violet-stained parasagittal sections cut through the right cerebellar hemisphere. Dotted line within each slice indicates the border between the molecular and granular layers.

left, and the rabbit's right folium is on the right. The individual slices show the placement of identified recording sites for cells from Paired and Unpaired rabbits with dendritic spike thresholds above and below the mean dendritic spike threshold for cells from Paired animals (1.5 nA). The composite shows all the electrode locations of slices 2, 3, and 4 combined onto one "slice." Examination of the distribution of cells from Paired animals with thresholds below 1.5 nA (\blacksquare) shows specific aggregations at the medial edge of the rabbit's left folium in slices 2, 3, and 4. In contrast, cells from Paired animals that are above the 1.5-nA threshold and cells from Unpaired animals that are both above and below the threshold appear to be distributed toward the middle and lateral edge of the rabbit's left folium in slices 2, 3, and 4. A statistical comparison of the distribution of electrode placements to the left and right of the midline (dotted line on the composite slice) confirmed that there was a significant aggregation on the medial edge of the rabbit's left folium of cells from Paired animals with thresholds below 1.5 nA

[(χ^2), $P < 0.005$]. There were no significant differences in the distribution of cells from Unpaired rabbits nor in cells from Paired rabbits above the 1.5-nA threshold in either folium.

4-AP. The dendritic spike threshold for five cells from naive animals was recorded before and 15 min after the application of 20–100 μM 4-AP. The mean threshold before the application of 4-AP was 1.8 ± 0.22 nA, and 15 min after application of 4-AP the threshold had dropped to a significantly lower mean value of 1.26 ± 0.21 nA ($P < 0.005$). Because 4-AP is a relatively selective antagonist of I_A at low doses, the present data suggest that the potassium inactivating current I_A may play a role in the lower threshold for dendritic spikes observed in cells from rabbits given paired training.

SYNAPTIC POTENTIALS. The mean amplitude (48.29 ± 0.96 vs. 48.5 ± 1.04 mV) and current (932.5 ± 114 vs. 741 ± 149 μA) for all-or-none synaptic potentials to climbing fiber stimulation were not different for cells from Paired or Unpaired rabbits. As expected, the current (104 ± 7 vs. 108 ± 8.5 μA) required to elicit standard 6- to 8-mV Purkinje cell EPSPs to parallel fiber stimulation was not different for cells from Paired or Unpaired rabbits.

PAIRING-SPECIFIC LONG-TERM DEPRESSION. Figure 3*A* shows an individual record of the long-term depression stimulation protocol with parallel fiber stimulation occurring before climbing fiber stimulation. Figure 3*B* shows six individual EPSPs to parallel fiber test pulses recorded in a cell from a Paired rabbit and in a cell from an Unpaired rabbit before and 21 min after the paired stimulation protocol. Figure 3*C* depicts the mean percent change in EPSP peak amplitude to parallel fiber test pulses over a period of 21 min after the stimulation protocol for Purkinje cells from rabbits previously given either Paired training ($n = 20$ cells) or Unpaired stimulus presentations ($n = 12$ cells). The figure shows that there was a highly significant reduction in EPSP peak amplitude following the paired stimulation protocol for cells from Unpaired rabbits (mean percent change \pm SE, $-24.21 \pm 2.9\%$) compared with cells from Paired animals ($-8.7 \pm 3.35\%$). This significant difference between cells from Paired and Unpaired rabbits lasted across the 21 min after the in vitro paired stimulation protocol [$F(1,30) = 10.52$, $P < 0.005$]. An examination of the data from individual cells revealed that, whereas 11 of 12 cells (92%) from Unpaired rabbits showed depression 21 min after the paired stimulation protocol, 10 of 20 cells (50%) from Paired rabbits showed no depression at the end of the observation interval. In fact, four of the cells from Paired animals showed a mean potentiation of 9.6%.

In three cells that failed to show EPSP depression after our standard pairing-specific long-term depression protocol (i.e., 0% change in EPSP amplitude), we used additional stimulation protocols including parallel fiber stimulation during either constant depolarization sufficient to induce local calcium spikes (Boxall et al. 1996; Crepel and Jaillard 1991; Schreurs and Alkon 1993) or depolarizing steps at 1 Hz for 240 pairings (Chen et al. 1995) to induce EPSP depression. The results suggest that in each case, Purkinje cell EPSPs could be depressed, at least to some extent (range: 8–36% depression), by application of the additional protocols following the pairing-specific long-term depression protocol.

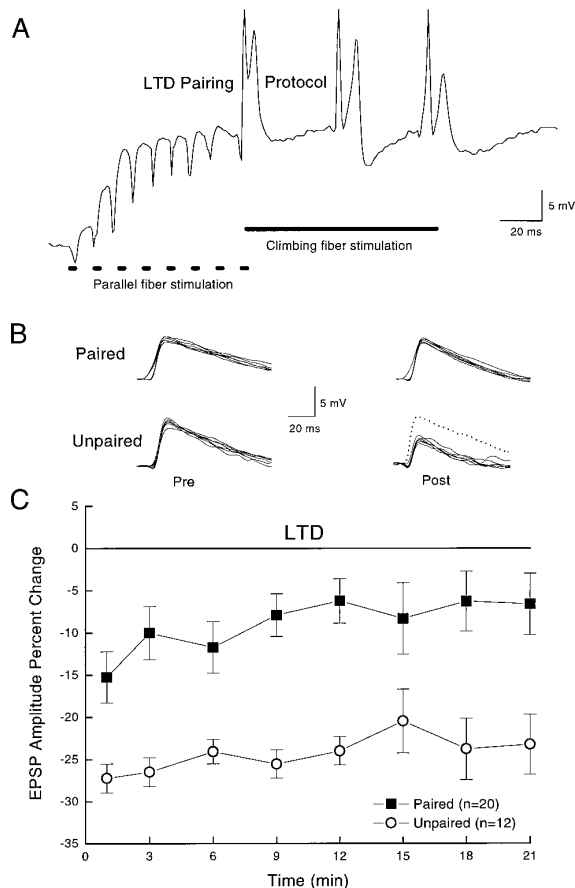


FIG. 3. Effects of classical conditioning on long-term depression (LTD). *A*: recording of 1 pairing of parallel fiber stimulation (80 ms, 100 Hz, 8 pulses) and climbing fiber pairing stimulation (100 ms, 20 Hz, 3 pulses) in the LTD pairing protocol. *B*: individual EPSPs in response to parallel fiber test pulses for a cell from a Paired and an Unpaired rabbit before (pre) and 21 min after (post) the LTD protocol. *C*: mean percent change in EPSP amplitude for cells from trained (Paired) and control (Unpaired) rabbits across the 21-min observation interval following the LTD protocol.

DISCUSSION

The principal findings of the present experiments were as follows. 1) Membrane excitability was higher in Purkinje cell dendrites in lobule HVI of rabbits given paired stimulus presentations than of rabbits given unpaired stimulus presentations. This excitability was indexed, in part, by the lower minimum current required to elicit dendritic calcium spikes in cells from Paired animals than in cells from Unpaired animals. 2) The location of low-threshold cells from rabbits that received paired training suggested that there were specific sites within lobule HVI where learning-related changes took place. These areas may correspond to learning “micro-zones.” 3) The learning-specific increase in excitability could be mimicked in cells from naive animals by blocking the transient outward potassium inactivating current I_A with 4-AP. 4) Threshold current measurements for parallel fiber stimulation showed that the amount of current required to elicit a 6-mV EPSP as well as a smaller 4-mV EPSP and a Purkinje cell spike was lower for cells from Paired animals than cells from Unpaired animals. 5) Purkinje cell EPSPs underwent a significant, long-term reduction in peak amplitude to parallel fiber test pulses following paired stimulation

of parallel and climbing fiber inputs in cells from Unpaired rabbits but not in cells from Paired rabbits.

The results of the present experiments establish that there are learning-specific changes in membrane and synaptic excitability of Purkinje cells in lobule HVI of the rabbit that can be detected in slices 24 h after the end of paired training (Schreurs et al. 1991). The data replicate a previous report by Schreurs et al. (1991), who showed an increase in Purkinje cell membrane excitability in cells from paired animals relative to both unpaired and naive control subjects. Moreover, in the present case, the changes appear to be localizable to specific areas of lobule HVI, and these areas might be termed “learning” microzones (e.g., Chen and Thompson 1995; Hesslow 1994; Ito 1989). Consequently, the data from the present experiments add new evidence to the growing body of literature that has implicated the role of lobule HVI in classical conditioning of the rabbit nictitating membrane/eyelid response (Berthier and Moore 1986; Gould and Steinmetz 1996; Gruart and Yeo 1995; Hesslow 1994; Lavond and Steinmetz 1989; Lavond et al. 1987; Perrett et al. 1993; Schreurs et al. 1991; Thompson 1990; Yeo et al. 1985a) and provide further support for the role of the cerebellum in learning and memory (e.g., Andreasen et al. 1995; Berthier and Moore 1990; Bloedel and Bracha 1995; Fiez 1996; LaLonde 1994; Leiner et al. 1986, 1989; Logan and Grafton 1995; Molchan et al. 1994; Supple and Kapp 1993; Thompson 1986).

The potential role for potassium currents in the learning-specific changes in membrane excitability noted in the present experiments is consistent with observations of the role of potassium channel changes in the invertebrate *Hermisenda* and rabbit hippocampus during classical conditioning (e.g., Alkon 1989; Schreurs and Alkon 1992). In *Hermisenda*, classical conditioning induces an increase in intracellular calcium that causes inactivation of potassium channels. In rabbit hippocampal CA1 pyramidal cells, classical conditioning induces a reduction in calcium-activated potassium current through the cell membrane (e.g., Coulter et al. 1989) in much the same way as in *Hermisenda*. In the cerebellum, local dendritic calcium spikes are correlated with changes in local internal calcium concentration and controlled by transient outward potassium current (I_A) inactivation (Llinas and Sugimori 1980; Midtgaard 1995; Midtgaard et al. 1993). Consequently, it is possible that the increases in dendritic excitability observed in the present experiments are mediated by changes in potassium currents similar to the classical conditioning-induced cellular changes in *Hermisenda* and rabbit hippocampus.

In vivo recordings in and around lobule HVI during classical conditioning of the rabbit nictitating membrane/eyelid have identified cells with activity correlated with the tone, air puff, unconditioned response to air puff, and conditioned responses to the tone (e.g., Berthier and Moore 1986; Gould and Steinmetz 1996; Thompson 1990). In a discrimination experiment, Berthier and Moore (1986) identified 13 of 22 Purkinje cells that had increased simple spike activity correlated with a conditioned response that occurred to either CS^+ or CS^- . Only 5 of 22 Purkinje cells showed decreased simple spikes correlated with a conditioned response to CS^+ or CS^- . In other words, 59% of Purkinje cells recorded in vivo showed increased excitability during conditioned responses, whereas only 23% showed decreased excitability.

These numbers correspond well with a recent study by Gould and Steinmetz (1996), who found that 32 of 142 Purkinje cells (22.5%) in and around HVI identified by their complex spikes had activity that was correlated with conditioning and that 22 of these cells (68.8%) showed increased activity during conditioning and 6 cells (18.8%) showed decreased activity (Gould and Steinmetz 1996, p. 22). In contrast, Thompson (1990) cited preliminary reports in which 31% of Purkinje cells recorded mostly from lobule HVI (p. 166) showed an increase in simple spike activity as a result of classical conditioning, whereas 69% showed a decrease in simple spike activity. Although Thompson (1990) does not provide an anatomic localization of the recording sites, Berthier and Moore (1986) recorded from the right gyrus of lobule HVI and the electrode tracks were located along the medial edge of that gyrus. Consequently, the locus of Purkinje cells in the study by Berthier and Moore (1986) that did show an increase in excitability was located in an area similar to the location of low-threshold cells identified in the present experiments.

It is interesting to note that there appears to be considerable variability between studies in the proportion of cells that show conditioning-specific changes in excitability. One reason for this apparent variability is the criteria used to select these cells. For example, Thompson (1990) found that in trained rabbits, 87% of cells showed changes in simple spike activity to the tone conditioned stimulus. The observed conditioning-specific excitability changes (69% decrease vs. 31% increase) reported by Thompson (1990) are based on these 87% of cells. In contrast, Gould and Steinmetz (1996) found that only 22.5% of cells showed conditioning-specific changes in excitability. However, these cells were selected on the basis of changes in complex spike activity correlated with but occurring before the conditioned response. Berthier and Moore (1986) selected their cells on the basis of changes in simple spike activity that antedated conditioned responses (28.6%), and although 63.6% of these cells had complex spikes, only 13.6% showed conditioning-specific changes in those complex spikes. In other words, the criteria for selecting cells differed rather significantly between Thompson (1990), Gould and Steinmetz (1996), and Berthier and Moore (1986). The Purkinje cells reported in the present experiment were recorded 24 h after training, and the criteria for selection included location (anterior portion of HVI) and intrinsic membrane properties (at least -50 mV and 28 M Ω). Although there was a significant overall increase in excitability for cells from paired rabbits, only 13.5% of these cells had threshold current values that did not overlap with cells from unpaired animals (Fig. 1).

Another possible reason for differences in excitability between in vivo studies and our in vitro experiment concerns the nature of the data obtained from the two different types of studies. Specifically, the in vivo data are based on changes in simple and/or complex spikes resulting from stimulus- or response-elicited activity within an entire integrated network of cells in which there may be presynaptic, postsynaptic, and interneuron changes. In contrast, our experiments remove individual Purkinje cells from the network by hyperpolarization and examine the membrane and synaptic properties of specific dendrites.

The present data show that a form of long-term depression in Purkinje cell EPSPs normally seen in naive animals if

stimulation of parallel fibers precedes stimulation of climbing fibers (Schreurs et al. 1996) is present in unpaired control animals but is absent in 50% of cells from classically conditioned animals. One possible interpretation of our LTD data is that classical conditioning brought about LTD, and once Purkinje cell EPSPs were depressed, no further depression could be induced (e.g., Ito 1989; Shibuki et al. 1996; Thompson 1986). However, there are several pieces of evidence that suggest an alternative interpretation. First, there was no evidence of LTD in our EPSP recordings. In fact, in cells where threshold measurements were made, significantly less parallel fiber stimulation (current) was required to elicit 6-mV EPSPs in cells from Paired animal than in cells from Unpaired animals. In addition, attempts to elicit smaller EPSPs (4 mV) required significantly less current in slices obtained from conditioned animals. Moreover, the level of parallel fiber stimulation required to cause the Purkinje cell to spike was lower in slices from conditioned animals. Consequently, the EPSPs in slices from Paired animals appear to be more excitable rather than less excitable (i.e., potentiated rather than depressed). Second, the lower threshold for dendritic spikes in cells from Paired rabbits also suggests that Purkinje cells in these animals were more excitable rather than more depressed. Third, if cells were not depressed by the pairing-specific LTD protocol, they could be depressed with further stimulation. This finding suggests that learning-induced EPSP potentiation can be depressed with a sufficiently potent protocol (e.g., Hartell 1996). Taken together, these data indicate that classical conditioning 1) enhances dendritic excitability at specific locations, 2) does not induce LTD, and 3) comprises a mechanism that may occlude LTD.

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