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A Nitric Oxide Synthase Inhibitor Delays the Formation of Learning-Related Neural Activity in the Cerebellar Interpositus Nucleus During Rabbit Eyelid Conditioning

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ALLEN, M. T. AND J. E. STEINMETZ. *A nitric oxide synthase inhibitor delays the formation of learning-related neural activity in the cerebellar interpositus nucleus during rabbit eyelid conditioning.* PHARMACOL BIOCHEM BEHAV 53(1) 147–153, 1996. – Inhibiting nitric oxide synthesis with L-nitro arginine methyl ester (L-NAME) has been found to retard the acquisition of the classically conditioned rabbit eyelid response. In the present study, rabbits received an SC injection of either L-NAME (an inhibitor of nitric oxide synthase) or its inactive stereoisomer, D-NAME, an hour before each daily training session. L-NAME retarded both the acquisition of conditioned responses and associated learning-related neural activity in the interpositus nucleus. There was no effect of L-NAME on spontaneous interpositus activity recorded during a period of time before trial onset. These findings indicate that nitric oxide may play a role in neuronal processes associated with the acquisition of the classically conditioned rabbit eyelid responses.

Classical conditioning Cerebellum Nitric oxide Plasticity Retrograde messenger

NITRIC oxide has recently been discovered to be a messenger molecule in the mammalian central nervous system (CNS) (2,5–7,11,12,15). It is now thought to be the first in a new family of transmitters that are light lipophilic gases that can pass through the plasma membranes of neurons, glia, and blood vessels and affect cellular functions in nearby cells (15). Nitric oxide is synthesized from L-arginine by the enzyme nitric oxide synthase. Nitric oxide synthase has been found in high concentrations in the CNS (2,5). The highest concentration of nitric oxide synthase within the CNS has been found in the cerebellum with the next highest concentrations in the olfactory glomeruli and the hippocampus (2).

Nitric oxide has been found to play a role in a form of synaptic plasticity known as long-term depression (14). Cerebellar long-term depression (LTD) is a decrease in synaptic transmission from parallel fibers to Purkinje cell in cerebellar cortex, which comes about when there is conjunctive activation of parallel fiber inputs and climbing fiber inputs to Purkinje cells (9). These two pathways were previously found to produce nitric oxide (14,16). Because NO has been found to be necessary for LTD in the cerebellum, and because exogenous NO or even cGMP can be substituted for climbing fiber input to bring about LTD, it seems likely that NO plays a role in LTD (14).

Based on data showing that NO plays a role in LTD in the cerebellum and the hypothesis that LTD may be involved in cerebellar motor learning, in an earlier experiment we looked at the effects of blocking the synthesis of NO on classical conditioning of the rabbit eyelid response, a model motor learning paradigm that involves the cerebellum (3). A number of previous experiments have shown that the cerebellum, especially the interpositus nucleus (INP), is important for the learning of the conditioned eyeblink (4,10,17). We found that daily SC injections of an NO synthase inhibitor, L-nitro argine methyl ester (L-NAME) (10, 25, and 75 mg/kg of body weight), an hour before conditioning, retarded the acquisition of classically conditioned eyelid responses (3). This retardation resulted from an L-NAME-induced reduction of the percentage of CRs early in training, thus increasing the number of trials necessary to reach conditioning criterion. L-NAME had no

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effect on the percentage of conditioned responses seen in control rabbits previously conditioned to criterion with D-NAME, the inactive stereoisomer of L-NAME. This finding indicated that the inhibition of nitric oxide synthesis affected only the acquisition and not the expression of conditioned responses.

The effect of L-NAME was likely not a simple performance deficit that affected generation of the reflexive eyeblink. The unconditioned response (UR) amplitudes did not differ between sessions with D-NAME or with L-NAME and did not differ across the D-NAME or the L-NAME groups (1,3). Also, the lowest concentration of NO in the CNS has been found in the medulla (5). Because URs are elicited by way of the reflex pathway in the medullary brainstem, specifically from the caudal regions of the sensory trigeminal to the abducens and accessory abducens and facial motor nuclei (see Ref. 17 for review), it is not likely that these reflexes would be directly affected by inhibiting NO synthase activity.

Because our earlier study showed that L-NAME retarded the acquisition of the classically conditioned rabbit eyelid response, in the present experiment we tested the effects of NO synthesis inhibition on the classical conditioning-related activity known to appear in the cerebellar interpositus nucleus during the acquisition of the conditioned eyelid response. In short, by monitoring interpositus activity, we hoped to determine whether L-NAME's effects were apparent afferent or at the level of the cerebellar nuclei, or conversely whether its effects were exerted at a point efferent to the nuclei.

METHOD

Subjects

Sixteen male New Zealand albino rabbits were used in this study. They weighed on average 2.2 kg (range, 1.77 to 2.55 kg). They were housed in individual cages, had food and water available ad lib, and were maintained on 12 h light/dark cycles.

Surgery

Using aseptic techniques, each rabbit had a pair of multiple unit recording electrodes (1–2 MΩ impedance) surgically implanted into either the right or left interpositus nucleus of the cerebellum. Rabbits were first given an SC injection of xylazine (6 mg/kg), then given an intramuscular injection of ketamine (60 mg/kg) 15 min later. The rabbits were then placed in a standard stereotaxic head holder on the operating table that positioned the bregma skull landmark 1.5 mm dorsal to the lambda skull landmark. Additional IM doses of the ketamine/xylazine mixture were administered at 45 min intervals. During the surgery, a hole was drilled into the rabbit's skull 5 mm lateral and 1 mm anterior to lambda. Two small holes were drilled anterior for the implantation of stainless steel ground screws, and stainless steel surgical wire was wrapped around the screws to serve as the grounding leads in a plug assembly used during recording. A pair of electrodes were then lowered into the interpositus nucleus. Neural activity was monitored as the electrodes were lowered to stereotaxic position and correct placement was verified by noting activity characteristic of the deep cerebellar nuclei. Stereotaxic position was 0.7 mm anterior, 5.5 mm lateral, and 14.5 mm ventral to lambda. After the final positions of the electrodes were determined, they were cemented into place with dental acrylic. The leads from the electrodes and the leads from the grounding screws were then attached to the gold pins of a plug assembly used to connect to amplifiers during subsequent recording sessions. A 1 cm screw was also cemented to the head stage and used to secure an air hose holder during subsequent conditioning sessions.

Each rabbit was given a week to recover from surgery before adaptation was begun. To adapt the rabbits to restraint and to the conditioning chambers, each rabbit was placed in a standard plexiglas restraint box for 30 min on one day and for 60 min the next day. After the second adaptation session, the rabbits were given a 0.33 cc SC injection of xylazine and stainless steel wires were placed in the upper eyelid musculature. The ends of the wire were twisted together and gold pins were attached. These pins served as electromyographic (EMG) recording electrodes for monitoring the eyeblink responses of the rabbits during subsequent training.

Behavioral Training and Neural Recording

Classical conditioning of the rabbit eyelid response was accomplished by daily conditioning sessions consisting of 120 total trials. Ninety percent of the trials (i.e., 108 trials) were paired trials during which the onset of a 348 ms, 1 kHz, 85 dB tone preceded the onset of a 99 ms, 3 psi, corneal air puff. The remaining 12 trials were tone-alone test trials. The parameters for the paired trials included a 249 ms pre-CS period before the tone onset. The tone CS onset then occurred and was followed 249 ms later by the onset of the air puff US. The tone and air puff were on simultaneously for 99 ms and were terminated together. For trials on which only the tone was presented, the 249 ms pre-CS period was followed by a 348 ms tone. The time interval between trials randomly ranged from 20 to 30 s with an average of 25 s.

EMG activity was recorded from the musculature controlling the upper eyelid, amplified, then integrated before being sent to a computer. The integrated EMG activity was calibrated with a potentiometer so that outer eyelid movement could be expressed as millimeters of movement. A conditioned response (CR) was scored if movement of greater than 0.5 mm was seen during the CS period.

The plug assembly implanted during surgery contained the leads from the two recording electrodes and the ground connections. This assembly was connected to a dc amplifier within the conditioning chamber that filtered the signal between 300 and 3000 Hz, amplified the signal 10×, then routed the signal to an external ac amplifier for an additional 100× gain. Output from the secondary amplifier was sent to a window discriminator that discriminated 3–5 action potentials (at least 2 : 1 signal : noise ratio) on the basis of amplitude only. The discriminated pulses were then routed to a computer that controlled stimulus delivery and also the online acquisition of behavioral and neural data. For each trial, the computer displayed a tracing of the integrated EMG activity along with a histogram of the multiple unit activity for each electrode site time-locked with the behavioral response. On each trial, the computer collected data over a 747 ms period divided into 3 ms bins (i.e., the analog behavioral data was polled once every 3 ms while the discriminated multiple-unit data were summed in 3 ms epochs).

Drug Administration

L-NAME and its inactive stereoisomer D-NAME were obtained from Sigma Chemicals (St. Louis, MO). Rabbits were given SC injections of either L-NAME for the experimental group or D-NAME for the control group (25 mg/kg of body weight) 1 h before each daily conditioning session began. This dose was selected because we previously found it to be effec-

tive in retarding acquisition of the conditioned response while having less variable effects than lower or higher doses tested (e.g., 10 or 75 mg/kg) (3). For the L-NAME group, each of the rabbits were run under the influence of L-NAME for eight consecutive sessions. Each rabbit then received D-NAME for two consecutive sessions. The D-NAME group received eight consecutive sessions of training with D-NAME, followed by two sessions with L-NAME.

A subgroup of the L-NAME group ($n = 4$) were used to assess the effects of L-NAME on spontaneous interpositus nucleus activity. Before the first conditioning session, these rabbits were placed in the chamber, and spontaneous INP activity was recorded during the pre-CS period using 120 "blank" trials during which no CSs or USs were presented. The rabbits were then injected with L-NAME and 1 h later the first session of training began. In this fashion two sessions of spontaneous pre-CS period INP activity were recorded; one session without drug injection and one session with drug injection.

After completing the training, the positions of the recording electrodes were marked by passing 100 μA of dc for 10 s. The rabbits were overdosed with an IV injection of pentobarbital (4 cc) and perfused via the ascending aorta with 0.9% saline solution followed by 10% formalin. The brain was then removed and preserved in a 30% sucrose/10% formalin solution for a week. Frontal portions of the brain were removed, the brain was embedded in albumin/gelatin, and serial coronal 80 micron sections through the cerebellum were taken, mounted on slides, and stained with cresyl violet and potassium ferrocyanide.

Data Analysis

A number of behavioral measures were taken and analyzed including percentage CRs, CR amplitude, CR area, CR onset, and UR amplitude. The CR and UR amplitudes for each trial were defined as the maximum departures of the calibrated, integrated EMG signal (expressed in mm) from a baseline level calculated during the 249 ms pre-CS period that occurred during the 249 ms CS and 249 ms US periods, respectively. The CR area on each trial was calculated as the summed differences from baseline of the integrated EMG measured every 3 ms during the 249 ms CS period. The CR onset was defined as the first point in time after CS onset when the behavioral response exceeded 0.5 mm.

Discriminated neural activity from the interpositus nucleus on each trial was collected in 3 ms bins during the 249 ms pre-CS period, 249 ms CS period, and 249 ms US period, creating 249 bin peristimulus histograms of unit activity for each trial. The relationship between the behavioral responses and the increase in neural activity in the interpositus nucleus was analyzed by computing the t-scores for the neural activity and cross-correlations between the behavioral responses and unit records. The integrated EMG activity was averaged for each block of 10 trials for each session, and the record of discriminated neural activity was summed across each block of 10 trials. The pre-CS behavioral average was reported for each 10 trial block. The behavioral onset was determined by finding the first point where EMG activity indicated a eyeblink of at least 0.5 mm. The unit records were computed as difference t-scores by dividing the 83 three ms bins for the pre-CS period into eight bins consisting of a single 13 ms bin followed by seven 10 ms bins. Each of these eight bins were compared to the corresponding bins in the CS period by subtracting the pre-CS value from the CS value. T-scores were calculated for the US period in the same fashion. If a significant t-score was calculated within a 10 trial block, the behavioral and unit records were cross-correlated by shifting the alignment between the behavior and unit records in an ordered fashion to determine when the highest correlation occurs between the behavior and unit records. The behavioral record was aligned with a unit record so the unit pre-CS period was matched to the behavioral CS period and the unit CS period was matched to the behavioral US period. This was the starting point for the cross-correlation and was designated as a time value of -249. A Pearson product moment correlation was computed between the unit and behavioral records. The unit record was then shifted 6 ms toward the start of the data sets and the Pearson r was correlated again. The behavior data are shifted with a Pearson r being calculated at each point in the shift until the unit CS period was aligned with the behavioral pre-CS period. After all 83 shifts, the 10 highest correlations were reported with the time value at which they occurred.

The behavioral and neural data were statistically analyzed using mixed ANOVAs with group assignment (L-NAME vs. D-NAME) serving as a between factor and training sessions serving as a within factor. Significant effects were further analyzed with Tukey HSD tests (all $ps < 0.05$).

RESULTS

Behavioral Data

The data presented here were obtained from seven INP sites in the L-NAME group and five INP sites in the D-NAME group. Consistent with a previous study (3), the percentages of CRs across the 10 acquisition sessions differed significantly between the L-NAME and D-NAME groups, $F(1,10) = 28.08$, $p < 0.01$. As shown in Fig. 1, the D-NAME group learned the conditioned response faster than the L-NAME group and had a higher asymptotic level of responding. There were significantly higher percentages of CRs later in training for both drug groups as compared to early in training, $F(9,90) = 24.92$, $p < 0.01$. There was also a significant Group $\times$ Sessions interaction between group membership and session, $F(9,90) = 4.91$, $p < 0.01$, demonstrating the slower acquisition rate in the L-NAME injected rabbits.

Because the previous study of eyelid conditioning indicated that the CRs for the L-NAME groups were of low amplitude (3), characteristics of the CRs were compared for the D-NAME control group and the L-NAME experimental group. The average amplitudes, areas, and onset latencies for trials when CRs were seen in the D-NAME and L-NAME groups are shown in Fig. 2.

Analysis of onset latencies revealed a significant effect for Group; the L-NAME group had a much longer latency to CR onset than the D-NAME group, $F(1,10) = 19.61$, $p < 0.01$. Analysis of CR areas and CR amplitudes also revealed significant main effects for Group. The L-NAME group had CRs with smaller areas, $F(1,10) = 6.34$, $p < 0.05$, and smaller amplitudes, $F(1,10) = 13.62$, $p < 0.01$, than the D-NAME control group. A significant Session effect observed when CR amplitudes were analyzed revealed that CR amplitudes were significantly higher later in training, $F(9,90) = 2.55$, $p < 0.05$. There were no significant differences in UR amplitudes between the two drug groups, $F(1,10) = .009$, $p > 0.05$.

Rabbits given L-NAME during the first eight sessions were given D-NAME during the last two sessions, while rabbits given D-NAME during the first eight sessions were given L-NAME during the last two sessions. No significant changes in performance were seen in the two groups over the last two

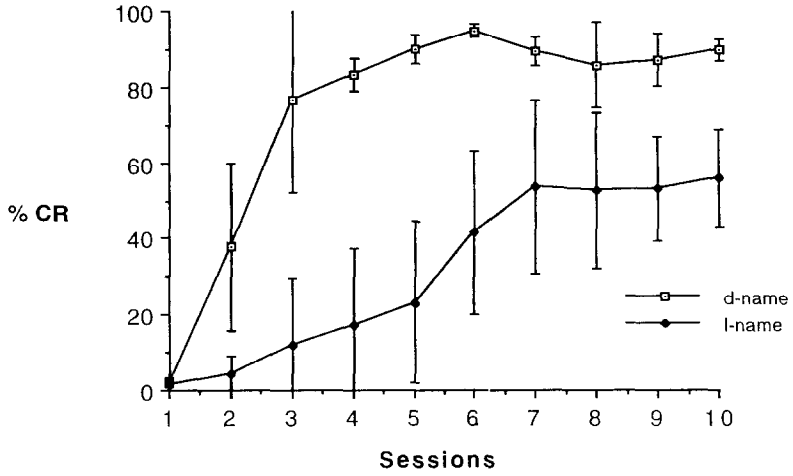

FIG. 1. Percent CRs recorded from rabbits given 8 days of training under D-NAME followed by 2 days of training under L-NAME (open squares) and from rabbits given 8 days of training under L-NAME followed by 2 days of training under D-NAME (filled diamonds). Error bars depict SEM.

sessions and no changes in performance were noted when Session 8 was compared to Session 9 for each group. These data provide two important findings. First, because conditioned responding did not improve when rabbits were switched from L-NAME to D-NAME, it is likely that the effects of L-NAME continued for a period of days after the injections were discontinued. Second, because conditioned responding did not decrease when rabbits were switched from D-NAME to L-NAME, it is clear that L-NAME did not affect retention of the learned response.

Neural Data

There were no significant differences in spontaneous INP activity measured one session before and one session after L-NAME injection. Only the INP activities recorded during the pre-CS period were compared because the tone- and air-puff-evoked activity recorded during conditioning trials contributed to CS and US period activity.

The standard scores of neural activity were significantly retarded in the L-NAME group as compared to the D-NAME, $F(1,10) = 2.33$, $p < 0.05$. The L-NAME rabbits did not develop significant learning-related activity until about the sixth session (see Table 1). In fact, the sixth session is when the behavioral CRs were present. The D-NAME rabbits on average exhibited conditioned responses and learning-related unit activity on the second day. The neural recording data are in close agreement with the behavioral data depicted in Fig. 1. It was on the second day for the D-NAME group and the sixth day for the L-NAME group that both groups showed about 40% conditioned responses. These were the two days when significant amounts of learning-related increases in neural activity were seen. These data indicate that a rather close correspondance between the appearance of behavioral CRs and learning-related activity in the INP existed in both the D-NAME and L-NAME groups. Examples of the retardation in the acquisition of both the conditioned response and the associated increase in INP activity are shown in Fig. 3. Neither the conditioned response or the increased INP activity were exhibited until the sixth training session in this rabbit.

Figure 4 depicts the highest correlations obtained between behavioral responses and cerebellar activity on Sessions 1, 3, and 6 for L-NAME and D-NAME rabbits plotted against the onset latencies recorded for the behavioral responses. Data points found before the vertical line indicate the occurrence of CRs, while data points found above the horizontal line indicate positive correlations that were found between the behavioral and neural response topographies. Note the rather tight clustering of data points in the upper left quadrant of the scatterplot of the D-NAME rabbits by Session 3. This indicates that by Session 3, the D-NAME rabbits formed amplitude-time course neuronal models that were positively correlated with the behavioral response. By contrast, the L-NAME rabbits did not show this pattern of unit activity until Session 6, and, in addition, showed a distribution of correlations that were shifted closer to the US onset. These data indicate that the appearance of the neuronal model characteristic of CR-related activity in the interpositus nucleus was delayed in L-NAME rabbits.

DISCUSSION

In a previous experiment, we reported that CR acquisition during classical eyelid conditioning was impaired by injections of L-NAME although the UR amplitudes were not affected by drug injection (3). The present study exactly replicated this earlier study and extended these behavioral results by showing disruptions in CR area and CR onset. Furthermore, the present experiment showed that retardation of conditioned responding was accompanied by a retardation in the formation of CR-related activity in the INP. These data are consistent with the idea that L-NAME may be disrupting neuronal activity in the cerebellum that is associated with acquisition of the classically conditioned eyelid response.

The learning curves exhibited by the rabbits under the influence of L-NAME were very similar to the learning curves

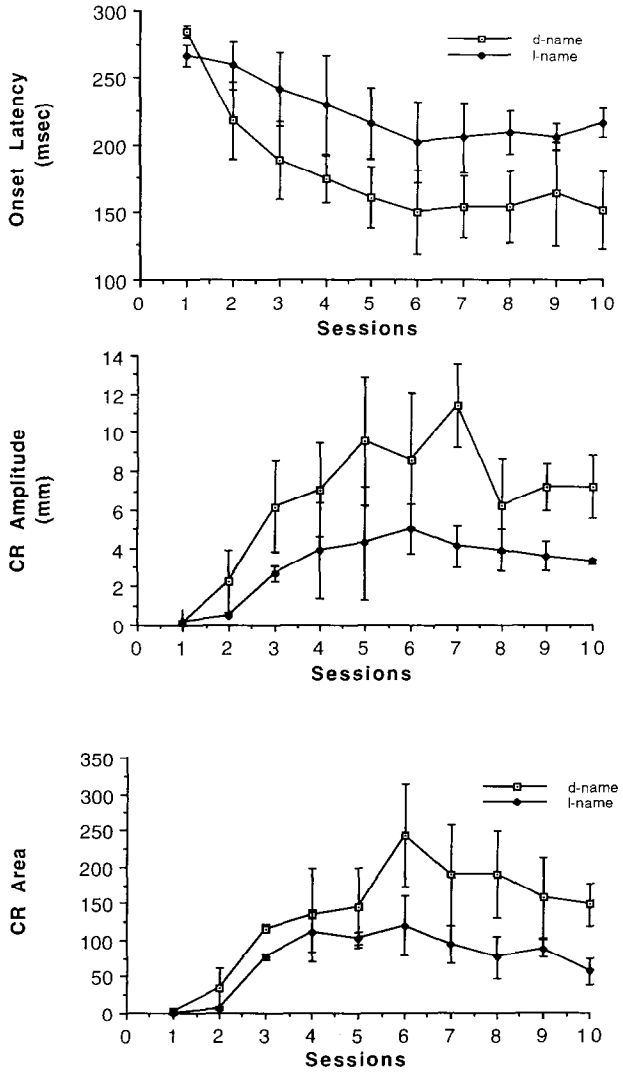

FIG. 2. CR amplitudes (Top), CR areas (Middle), and CR onset latencies (Bottom) recorded from D-NAME (open squares) and L-NAME (filled diamonds) rabbits. Error bars depict SEM.

of rabbits given selective lesions of the cerebellar cortex (10). This suggests that L-NAME might act by impairing synaptic processes in the cerebellar cortex (14). However, this impairment seems to only affect acquisition because rabbits given L-NAME after training showed no decreases in the percentage of CRs. Apparently, inhibiting the synthesis of nitric oxide after the CR has been acquired has no detrimental effect on the production of the CR. However, the L-NAME group did appear to reach an asymptotic level responding which is significantly lower than the D-NAME control group. This deficit may have occurred because we allowed only 10 sessions of training. If we had continued to train the L-NAME group for additional sessions, they may have eventually reached the same level of responding as the D-NAME control group. Finally, the L-NAME injections did not significantly alter the UR, the reflexive response to the air puff, indicating that the

TABLE 1

INTERPOSITUS MULTIPLE UNIT ACTIVITY COMPUTED AS STANDARD SCORES FOR THE THIRD AND FOURTH QUARTERS OF THE CS PERIOD (CS3 AND CS4, RESPECTIVELY) FOR THE D-NAME AND L-NAME GROUPS FOR ALL 10 SESSIONS OF TRAINING

	CS 3		CS4	
Session	D-NAME	L-NAME	D-NAME	L-NAME
1	1.28	0.83	1.25	0.69
2	2.40	1.47	2.10	1.57
3	2.10	0.89	2.07	1.19
4	2.27	1.05	2.02	1.11
5	1.76	1.48	1.65	1.49
6	1.54	2.10	1.55	2.33
7	1.50	1.73	1.72	2.18
8	2.05	1.70	2.16	2.14
9	1.54	2.09	1.60	2.69
10	1.70	1.80	1.86	1.96

effect of inhibiting NO synthesis was on retarding the acquisition and expression of the CR and not on the reflexive response.

The differences seen in CR onset, area, and amplitude be-

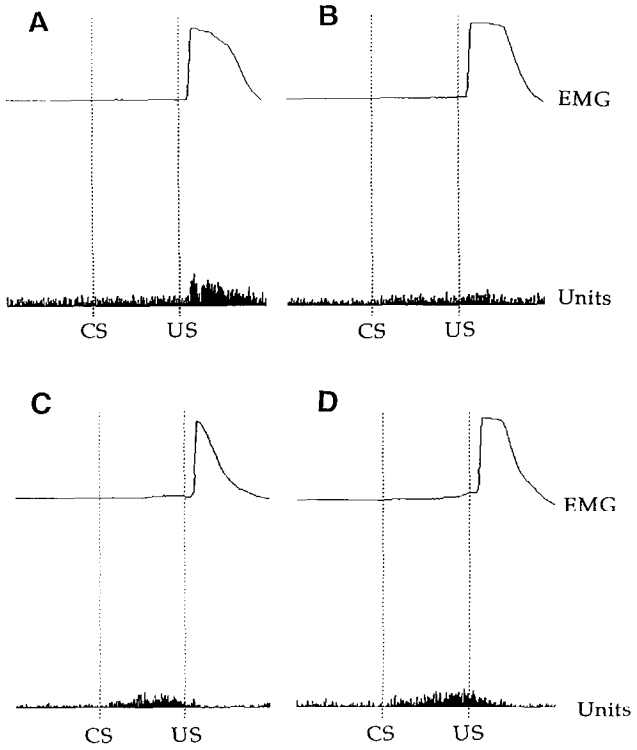

FIG. 3. Examples of behavioral (top traces) and neural (bottom traces) responses for a rabbit given L-NAME before each training session. Averaged integrated EMG signals and summed peristimulus histograms of INP activity are shown for this rabbit on (A) Session 1, (B) Session 3, (C) Session 5, and (D) Session 6.

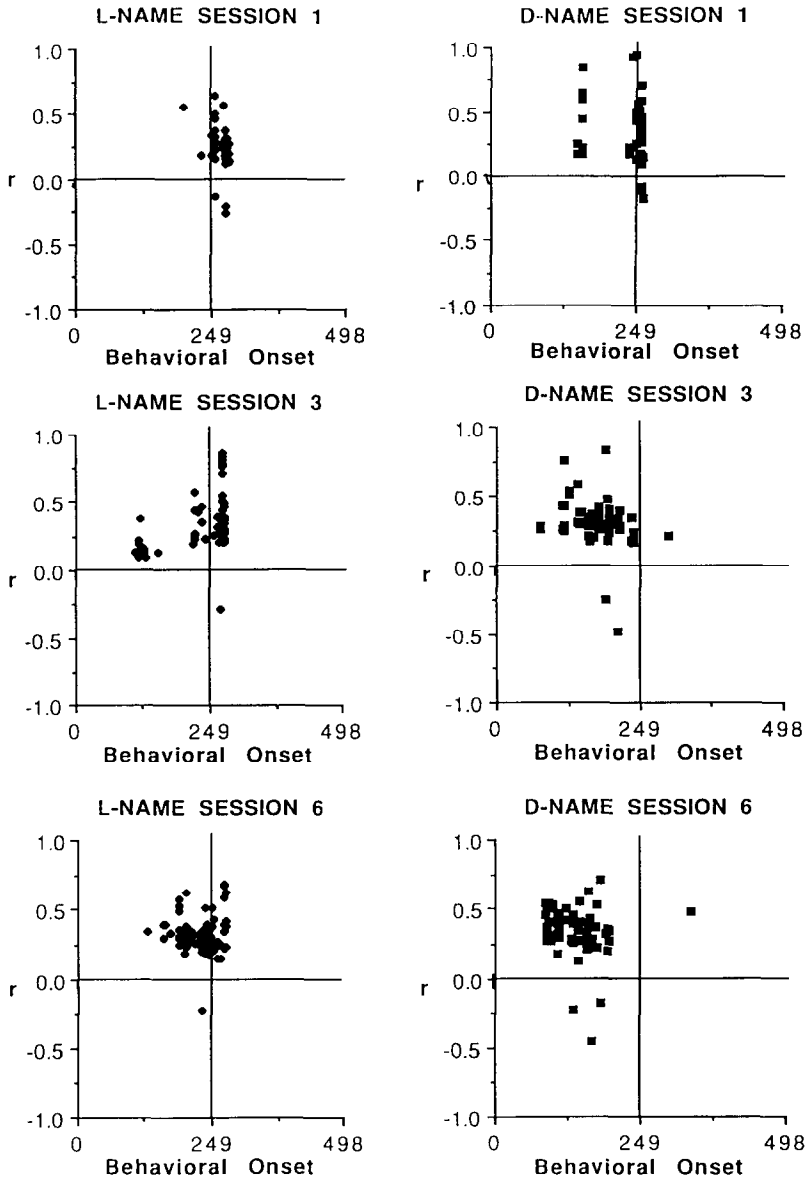

FIG. 4. Scatterplots depicting the relationship between onset of the behavioral CR and the maximum correlation calculated for the topographies of the behavioral and neural responses (see text for details). Scatterplots for Sessions 1, 3, and 6 are shown for L-NAME (left column) and D-NAME (right column) rabbits.

tween the D-NAME and L-NAME groups showed that inhibiting NO synthase not only retarded acquisition of the conditioning response but also resulted in a deficit in the performance of the CR. L-NAME rabbits on average made CRs later than the D-NAME controls and also showed a delay in the appearance of learning-related activity in the INP as compared to the D-NAME controls. In fact, there was a very close correspondance between when CRs were observed and when learning-related INP activity was first seen. These data suggest that L-NAME exerted its actions by disrupting cerebellar activity. The low amplitude and small area CRs demonstrated by the L-NAME group were similar to the CRs found in rabbits with cerebellar cortical lesions (10). This similarity further suggests that the effects of L-NAME may be on the cerebellar cortex or in structures afferent to the cortex.

The present recording results indicate that after L-NAME injections the acquisition of learning-related activity in the INP was impaired as well as the appearance of the CRs. There were no changes in spontaneous activity in the INP when the activity was compared before and after the injection of L-NAME, indicating that the action of L-NAME was not to change spontaneous INP activity but rather to alter activity related to the acquisition of the CR. The finding that L-NAME retarded not only the formation of increased INP ac-

tivity associated with the conditioned eyeblink but also lengthened the latency between the onset of INP activity and the onset of the behavioral response, indicates that the site of action of the nitric oxide inhibitor, L-NAME, is at or afferent to the deep cerebellar interpositus nucleus. If the site of action of L-NAME was efferent to the interpositus nucleus (i.e., in the red nucleus or brainstem motor nuclei), one would expect to see the normal increases in INP activity.

A possible confounding variable that needs to be explored is the effect of inhibiting nitric oxide on the vasculature associated with the CNS. Nitric oxide's biological role was first described as an endothelium-derived relaxant factor or EDRF (6). For example, there is normally an increase in blood flow into the somatosensory cortex when peripheral nerves are stimulated. Northington et al. (11) found that inhibiting nitric oxide synthesis uncoupled this increased blood flow and increased neural activity. It may be possible that the effects seen in the cerebellum following systemic administration of L-NAME were due to a decreased blood flow to an area that normally had an increase in both activity and blood flow during conditioning. We plan to address this question in the future by administering L-NAME directly into the cerebellar cortex by iontophoresis and by using NO synthase inhibitors that do not affect blood flow. Also, there are several isoforms of NO synthase, including two calcium-regulated isoforms of the enzyme, neuronal NO synthase and endothelial NO synthase, which may be involved in learning-related processes. For example, using mice for which the gene encoding the neuronal isoform of NO synthase was disrupted by gene targeting, O'Dell et al. (13) demonstrated that somewhat normal hippocampal LTP could be observed after weak tetanic stimulation, LTP that could be blocked by NO synthase inhibitors. Immunocytochemical studies revealed that the endothelial form of NO synthase was expressed in hippocampal CA1 neurons, thus suggesting that the endothelial isoform was involved in NO generation within the mouse hippocampus.

In summary, the novel form of neurotransmitter, nitric oxide, has been implicated as playing a role in a variety of learning paradigms. These paradigms include the Morris Water maze (3), aversive taste conditioning in chicks (8), and the classically conditioned eyelid response (1,3). The inhibition of nitric oxide had been found to retard the acquisition of the classically conditioned eyelid response (3) but had no effect on the unconditioned response (1). We have now found that the appearance of the learning-related increase in neural activity recorded in the deep interpositus nucleus is retarded along with the development of CRs.

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REFERENCES

1. Allen, M. T.; Steinmetz, J. E. A nitric oxide synthase inhibitor retards acquisition of the classically conditioned rabbit eyelid response. Soc. Neurosci. Abstr. 18:1220; 1992.
2. Bredt, D.; Hwang, P.; Snyder, S. Localization of nitric oxide synthase indicating a neural role for nitric oxide. Nature 347:768–770; 1990.
3. Chapman, P.; Atkins, C. M.; Allen, M. T.; Haley, J. E.; Steinmetz, J. E. Inhibition of nitric oxide synthesis impairs two different forms of learning. Neuroreport 3:567–570; 1992.
4. Clark, G. A.; McCormick, D. A.; Lavond, D. G; Thompson, R. F. Effects of lesions of cerebellar nuclei on conditioned behavioral and hippocampal neuronal responses. Brain Res. 442:97–104; 1984.
5. Forstermann, U.; Gorsky, L. D.; Pollock, J. S.; Schmidt, H. H.; Heller, M.; Murad, F. Regional distribution of EDRF/NO-synthesizing enzyme(s) in rat brain. Biochem. Biophys. Res. Commun. 168:727–732; 1990.
6. Garthwaite, J.; Charles, S. L.; Chess-Williams, R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intracellular messenger in the brain. Nature 336: 385–388; 1988.
7. Garthwaite, J.; Garthwaite, G.; Palmer, R.; Moncada, S. NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. Eur. J. Pharm. 172:413–416; 1989.
8. Holscher, C.; Rose, S. P. R. An inhibitor of nitric oxide synthesis prevents memory formation in the chick. Neurosci. Let. 145:165–167; 1992.
9. Ito, M.; Sakurai, M.; Tongroach, P. Climbing fibre induced depression of both mossy fibre responsivity and glutamate sensitivity of cerebellar purkinje cells. J. Physiol. (London) 324:113–134; 1982.
10. Lavond, D. G.; Steinmetz, J. E. Acquisition of classical conditioning without cerebellar cortex. Behav. Brain Res. 33:113–164; 1989.
11. Northington, F. J.; Matherene, G. P.; Berne, R. M.. Competitive inhibition of nitric oxide synthase prevents the cortical hyperemia associated with peripheral nerve stimulation. Proc. Natl. Acad. Sci. USA 89:6649–6652; 1990.
12. O'Dell, T.; Hawkins, R.; Kandel, E.; Arancio, O. Test of the roles of two diffusable substances in long term potentiation. Proc. Natl. Acad. Sci. USA 88:1285–1289; 1991.
13. O'Dell, T. J.; Huang, P. L.; Dawson, T. M.; Dinerman, J. L.; Snyder, S. H.; Kandel, E. R.; Fishman, M. C. Endothelial NOS and the blockade of LTP by NOS inhibitors in mice lacking neuronal NOS. Science 265:542–546; 1994.
14. Shibuki, K.; Okada, D. Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature 349: 326–328; 1991.
15. Snyder, S. H. Nitric oxide: First in a new class of neurotransmitters. Science 257:1898–1902; 1992.
16. Southam, E.; Garthwaite, J. Climbing fibres as a source of nitric oxide in the cerebellum. Eur. J. Neurosci. 3:379–382; 1991.
17. Steinmetz, J. E.; Lavond, D. G.; Ivkovich, D.; Logan, C. G.; Thompson, R. F. Disruption of classical eyelid conditioning after cerebellar lesions: Damage to a memory trace or a simple performance deficit? J. Neurosci. 12:4403–4426; 1992.