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Modification of Motor Activity, Passive Avoidance Conditioning and Evoked Potentials by Microinjections of Strychnine in Both Caudate Nuclei in Cats

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TELLEZ-VILLAGRA, C., F. VAZQUEZ, P. DE LA MORA AND H. BRUST-CARMONA. *Modification of motor activity, passive avoidance conditioning and evoked potentials by microinjections of strychnine in both caudate nuclei in cats.* PHARMAC. BIOCHEM. BEHAV. 14(2) 193–199, 1981.—Passive avoidance conditioning (PAC) seems to depend on inhibitory actions within the caudate nucleus (CN). Thus topical application of strychnine could block those influences. In cats, with permanently implanted cannulae in the head of the CN, bilateral microinjections of 100 μg of strychnine produced a stereotyped behavior of grooming and running with the extremities flexed to dark places. When placed into a two compartment chamber for a PAC acquisition they would not move from the dark compartment to the illuminated one. After decreasing the illumination some cats crossed and the acquisition test was performed. Twenty four hours later they showed the learned response. A second retention test was not modified by another strychnine application. These results contrast with those obtained by picrotoxin microinjections which disrupt the retention of a PAC. However, strychnine affects the evoked potentials (EP) recorded in CN by n. centralis medialis (NCM) stimulation by decreasing a positive peak, which probably represents post-synaptic inhibition and increasing the late positive component. In the lateral geniculate body strychnine affected a late positive wave and in the occipital cortex it increased the initial negative peaks and decreased the late positive ones of the potentials evoked by flash stimulation. These findings suggest a more subtle role of the CN in the regulation of visual information, which is probably related with the attention processes.

Evoked potentials in CN, VC and LGB Caudate nucleus Passive avoidance conditioning
Glycine in passive avoidance conditioning Visual responses enhancement by strychnine

THE ROLE of the caudate nucleus (CN) in the regulation of motor activity has been accepted [4,9], as well as the fact that learning requiring this type of motor regulation can be affected by lesions of the CN [2,3]. Several authors [20, 21, 24] have shown that lesions of the CN produced learning deficits in the retention of passive avoidance conditioning, i.e., a higher rate of errors by entering the punishment compartment. On the other hand, bilateral microinjections of picrotoxin into caudate nuclei in cats produced a marked enhancement of spontaneous motor activity [23] and hence retention deficits in a passive avoidance conditioning. Similar injections in cats immobilized with gallamine triethiodide (Flaxedyl) produced an enhancement of the primary components of the potentials evoked in CN by electrical stimulation of n. centralis medialis (NCM). These results were interpreted as the result of presynaptic inhibition blockage in the CN. However, almost no presynaptic terminals have been described in the CN, as compared with the amount of possible post-synaptic inhibitory terminals described [15]. On the other hand, it has been suggested that strychnine blocks the inhibitory neurotransmitter in the post-synaptic structures; for example, at the spinal cord level [1], the reticular formation [13], and other cerebral regions [7, 16, 17]. Therefore, we decided to investigate whether or not the topical application of strychnine in both caudate nuclei would disrupt the functional organization of the intracaudate circuits. The effects of strychnine microinjections into the CN by selectively blocking the post-synaptic inhibition could be disclosed by: (a) enhancement of stereotyped motor responses; (b) modifications of a learning process requiring inhibition of motor activity and (c) enhancement of the later components of the potentials evoked in CN by stimulation of nonspecific thalamic pathways. The results are quite interesting; some stereotyped motor responses change and the animals become very sensitive to light. This sensitivity is shown in their behavior as well as by the evoked potentials recorded in the

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lateral geniculate body (LGB) and the occipital cortex (OC). However, the animals retain the necessary motor inhibition to show passive avoidance conditioning.

METHOD

Twenty-eight cats of either sex (2.5 to 3.5 kg body weight) were used. Stainless steel cannulae were implanted stereotaxically into the head of both caudate nuclei (A 16, L 4.5, H 4.5) following the Jasper and Ajmone-Marsan Atlas [14] under pentobarbital anesthesia (35 mg/kg) and fixed permanently to the skull with acrylic dental cement. In 19 cats bilateral microinjections of 100 μg of strychnine dissolved in 5 μl of 0.9% NaCl were performed after 4 days. In five cats (controls) only 0.9% NaCl (5 μl) was applied bilaterally. Each injection was made in 100 sec. During the following 10 min the gross spontaneous motor activity was observed and some reflex responses (placing, jumping, tendinous, pupilar) were clinically tested. Immediately after, the cats were submitted to passive avoidance conditioning (PAC) training. A two compartment chamber, with the compartments separated by a sliding door, was used for the training and testing session. One of the compartments (50×40×40 cm) had an even floor. The other compartment, of the same size, was continuously illuminated by a 40, 10 or 2 W bulb, depending on the experimental design, and was provided with an electrifiable grid made of 0.5 cm diameter stainless steel bars separated at 2 cm intervals and connected to a high impedance stimulator (Núcleo-electrónica). Three minutes after placing a cat in the first compartment (without light), the sliding door was opened and the latency to cross into the second compartment was measured. Once the animal had crossed, the door was closed and a nociceptive stimulus (4–5 mA, 0.1 sec duration) was applied to the paws. Two additional shocks were delivered at 60 sec intervals. After the third shock, the cat was allowed to escape into the first compartment and was then removed after 3 min. Of the 19 cats trained in this way, 8 were trained with a 40 W, 6 with a 10 W and 5 with a 2 W bulb. Twenty-four hours later, without any treatment, the latency to cross from one compartment to the other was again measured over a maximum waiting time of 600 sec (learning criterion).

Twenty-four hours later the five cats trained with a 2 W bulb, as well as 4 of the 5 control cats, were submitted to a second retention test of PAC 10 min after bilateral CN microinjections of strychnine and 0.9% NaCl, respectively.

In each group latencies before and after the nociceptive stimulation were compared with the Wilcoxon test [22] and the latencies of the animals injected with saline were compared with those of the animals injected with strychnine using the U test.

In 7 animals (3 of the same population and 4 naive ones) acute experiments were performed three days later. Tracheostomy was performed under ether anesthesia and artificial respiration was begun. Ether was then discontinued and animals were placed in a stereotaxic apparatus. Animals were then immobilized with gallamine triethiodide (Flaxedyl). Exposed skin, and tissue surrounding stereotaxic pressure points were infused every 60–90 min with xylocain solution (1%), as well as the area around the tracheostomy incision. Body temperature was maintained at 37–38°C with an electric heating pad. In all the experiments heart frequency was continuously monitored and no important changes were observed which could have indicated pain or suffering in the animals. Two hours prior to the acute experiments, atropine sulphate 1% was applied to each conjunctival sac to keep the pupils dilated.

Visual stimulation consisted of one flash every 2 sec produced by a flash lamp situated at 40 cm in front of the animal. Stimulation intensity was set at "8" on the Grass Photostimulator PS22.

N centralis medialis (A 9, L 0.5, H 0.5) was electrically stimulated. A square pulse of 0.1 msec duration and 1–1.5 mA intensity (Grass S88, SIU) was repeated 14 times, once every two seconds. This stimulation pattern was repeated several times.

The evoked potentials were recorded through stainless steel bipolar electrodes aimed at CN (A 18.5, L 4.5, H 4.5) LGB (A 7.5, L 9.5, H 2.5) and OC (A −1, L 4). In the 4 naive cats a cannula-electrode was also implanted in the CN (A 16, L 4.5, H 4.5). All the electrical changes were amplified by a Grass AC P511 differential input preamplifier, with a bandwidth of 1 Hz to 10 kHz. The preamplifier output was fed into an Ampex Model PR-500 tape recorder.

Control recording consisted of 6 groups of 14 potentials each evoked by NCM stimulation. Light and electrical stimulation were applied alternately at 2 min intervals. Bilateral microinjections of 100 μg of strychnine dissolved in 5 μl of 0.9% NaCl were performed immediately. After the microinjections the recordings were continued every 2 min for the first 20 min and then every 5 min for the following 2 hr. Later on, sets of 10 responses were averaged and the latency and magnitude of the different peaks of each potential were measured using a PDP 11/40 computer, and the average magnitudes and standard deviations were plotted. The effects of strychnine upon the evoked potentials were assessed comparing the magnitude of each peak of each sample after the injection, with the average magnitude of the control period (6 samples of 10 potentials each). The difference was evaluated with the sign test [22].

At the end of the experiment a lethal dose of pentobarbital was given to the animals and the brains perfused first with saline solution (0.9%), and then with Formalin with potassium ferrocyanide [11]. The brains were kept in Formalin 10% for at least one week and then sectioned coronally (50 μ) on a freezing microtome. To localize cannulae and electrode placements, photographic prints were made of the sections by using them as a negative in an enlarger [12].

RESULTS

Behavioral Observations

During the 10 minutes following the strychnine microinjection the cats showed constant grooming, and walked with the extremities flexed keeping their bodies near the floor. They also showed a tendency of running in this way towards the dark places of the laboratory. Their gross behavior and clinically tested reflexes were normal. A slight midryasis was observed but the pupilar reflex was normal. None of the animals presented convulsive activity.

Passive Avoidance Conditioning

The five control cats (100%) treated with 0.9% NaCl crossed to the punishment compartment (illuminated by a 40 W bulb) during the acquisition session with an average latency of 64 sec and received the nociceptive stimulation (Fig. 1A).

During the acquisition session, the cats (N=8) pretreated

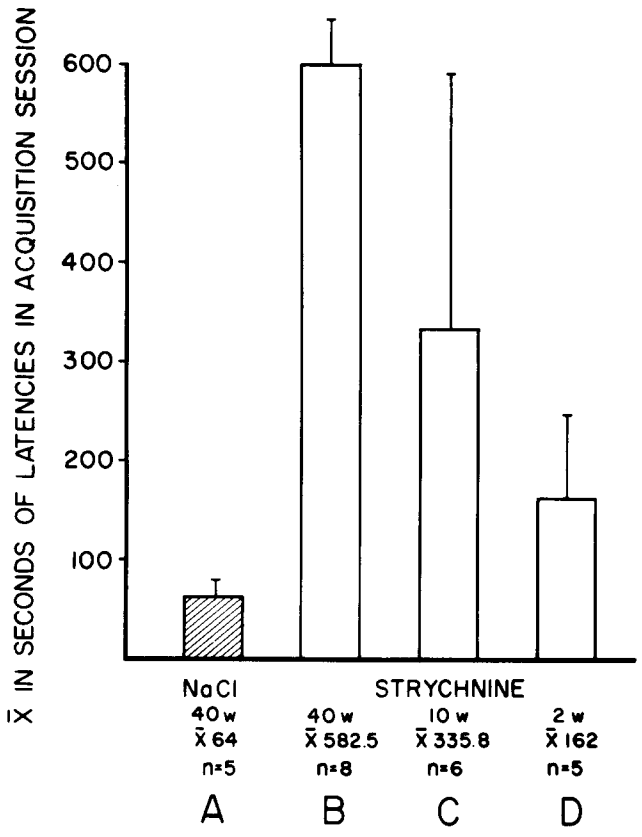

FIG. 1. Mean latencies to cross from the security compartment to the punishment one in the different groups of cats. (A) 10 min after the NaCl microinjection; (B) 10 min after the microinjection of 100 μg of strychnine with the punishment compartment illuminated by a 40 W bulb; (C) 10 min after the strychnine microinjection and a 10 W illumination and (D) 10 min after the strychnine microinjection and a 2 W illumination. Note that the cats of the second group (B) do not cross to the illuminated compartment.

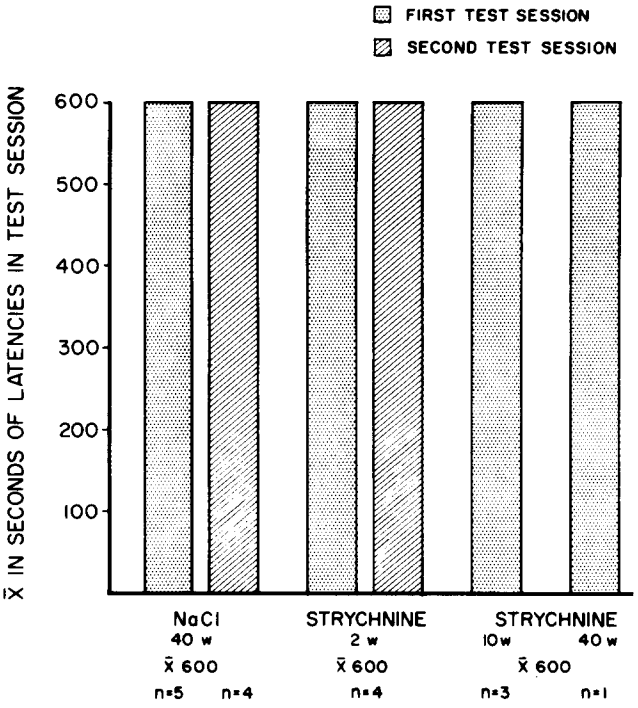

FIG. 2. Latencies in seconds for the first and second retention tests of the animals treated with strychnine or NaCl for a second time. Note that the animals do not cross to the punishment compartment indicating that they acquired the PAC, retaining it even after a second strychnine microinjection.

with strychnine and which had to pass into the punishment compartment illuminated with a 40 W bulb did not cross, except for one (13%); therefore their mean latency was of 582.5 sec (Fig. 1B). When the punishment compartment was illuminated with a 10 W bulb, of the 6 cats pretreated with strychnine, 3 (50%) crossed, giving a mean latency of 335.8 sec (Fig. 1C). And finally, when the punishment compartment was illuminated by a 2 W bulb, 4 of the animals (80%) crossed giving a mean latency of 162 sec (Fig. 1D).

The difference between the latencies of this latter group, exposed to a 2 W bulb, and the group exposed to a 40 W bulb, is statistically significant at the level of $p=0.015$ ($n_1=5$, $n_2=8$, U=4.5).

All the animals that crossed into the punishment compartment received nociceptive stimulation (unconditioned stimulus).

When the punishment compartment was illuminated by a 10 W bulb, the strychnine injected group of cats crossed with a mean latency of 335.8 sec during the acquisition session, and during the retention test, 24 hr later, the latency was over 600 sec (learning criterion). If we compare these latencies, the difference is statistically significant at the level of $p<0.05$ (T=0, N=3, Z=3.5), indicating that they acquired the PAC.

The other experimental group, exposed to a 2 W bulb had a shorter latency during the acquisition session (mean=162 sec) and a much greater one during the first retention test (mean=600), which is statistically significant at the level of $p<0.05$ (T=0, N=4, Z=1.82) indicating that this group also acquired the PAC. During the second retention test, after a second strychnine microinjection, this group showed again a latency over 600 sec, which is statistically significant at the level of $p<0.05$ (T=0, N=4, Z=1.82).

The group of cats trained under the effects of NaCl had a shorter latency (mean=64) during the acquisition session than during the retention test (mean=600). The difference is statistically significant at the level of $p<0.05$ (T=0, N=5). Only four of these cats received a second NaCl microinjection and were submitted to a second retention test showing a latency greater than 600 sec. These results are the same as those obtained with the second strychnine microinjection. The histogram of Fig. 2 illustrates these results.

Electrophysiological Observations

The morphology of the potentials recorded in CN evoked

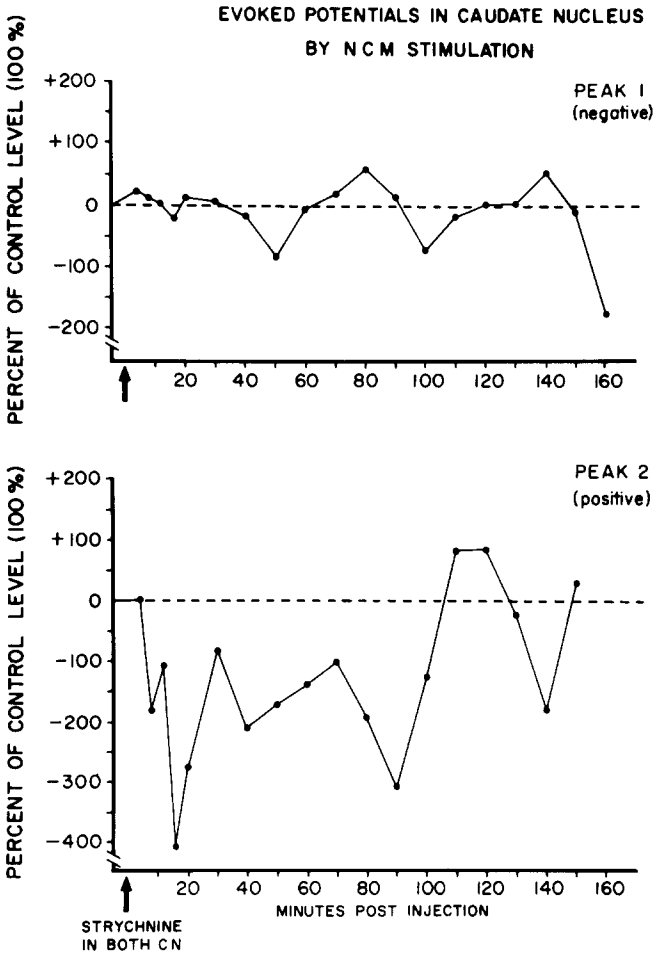

FIG. 3. Permanence of peak 1 (upper part) and the decrement of the positive peak of the evoked potentials recorded in CN after the microinjection of strychnine in both caudate nuclei (lower part). In this and following figures the control amplitude was taken as 100%.

by stimulation of the NCM remained constant throughout the experiments. They consisted of three peaks and a plateau. No differences in the evoked responses recorded either through the bipolar electrodes farther away from the cannula or from the cannula-electrode were observed. Hence it can be stated that no "short circuit" is produced in the recording electrodes by the injected liquid. The first peak was negative with a mean latency of 9.8 msec. The second peak was positive with a mean latency of 26 msec. And the third peak was negative with a mean latency of 71.8 msec. The plateau was positive with a mean latency of 153 msec, measured when it reached maximum amplitude.

Comparing the magnitudes of the peaks before and after the strychnine application, by means of the sign test, only the second peak and the plateau showed a significant change.

The second peak diminished significantly at the level of $p=0.01$ (N=18, X=4), and Fig. 3 illustrates the results obtained in five cats. The plateau showed a significant increase at the level of $p=0.001$ (N=18, X=1).

The potentials evoked by light stimulation and recorded in the occipital cortex were variable, although those shaped by four peaks were predominant. The first two peaks were negative with a mean latency of 22.6 and 40.6 msec, respectively. These were followed by two positive peaks of 90 and 158.2 msec, respectively. After the strychnine microinjection into the CN the magnitude of all of the peaks varied. The first two negative peaks increased significantly at the level of $p=0.004$ (N=18, X=3) and $p=0.001$ (N=18, X=2), respectively (Fig. 4), while the two positive peaks diminished at a statistically significant level of $p=0.004$ (N=18, X=3).

The potentials evoked by light stimulation and recorded in the lateral geniculate body were morphologically constant. They started with a negative wave with a mean latency of 22.5 msec. At a higher sampling velocity of the computer, this first peak appeared on its ascending phase to be shaped by a set of 2 to 3 biphasic waves; however the analysis was made taking all of them as the first negative peak. A second positive peak of more duration was also analyzed which had a mean latency of 60 msec. This positive peak was also shaped by several biphasic waves but the analysis was made of the peak as a whole. Finally, a third negative peak with a mean latency of 124.8 msec was observed. After the application of strychnine into the CN only the positive peak changed. Its magnitude increased significantly at the level of $p=0.004$ (N=18, X=3). Figure 5 illustrates the results obtained in 6 cats.

Figure 6 illustrates the locus of microinjection in CN (A); the recording in CN (B) and in lateral geniculate body (C) as well as the stimulation sites in NCM (D) obtained by projecting the histological sections upon the Jasper and Ajmone Marsan Atlas.

DISCUSSION

The small effect of strychnine, at the used dosage, upon the motor activity which at no time produced convulsive activity is noteworthy. All the reflex responses tested were normal, except for the stereotyped behavior of grooming and the running with the extremities flexed. Parts of this stereotyped behavior have been described as resulting from the electrical stimulation of the CN by Cools *et al.* in 1971 [6]. A very interesting observation was that the cats sought the dark places of the laboratory after the microinjection of strychnine. This tendency was even clearer during the acquisition session of the PAC in which the animal had to pass from a dark place to an illuminated one. Most of the cats did not cross. The direct relation with the light intensity is shown by the fact that when the luminosity was diminished then the cats did cross to the other compartment. These results suggest that the CN plays a role in the regulation of "luminosity perception" or in the organization of the responses related with luminosity. It has indeed been described that electrical stimulation of the CN might facilitate or inhibit responses to light, depicted either by evoked potentials or unit activity recorded at the occipital cortex [10]. The potentials evoked by flash stimulation and recorded in the visual cortex showed that the initial negative components, which could represent neuronal depolarization (excitation), increase consecutively in response to strychnine microinjection into both caudate nuclei. In the lateral geniculate body the first components of the EP do not seem to be affected by strychnine applied to the CN, but the late components increased, suggesting that the effect is upon the responding neurons of the LGB and not upon the afferent signals coming from the retina. All of this favours the postu-

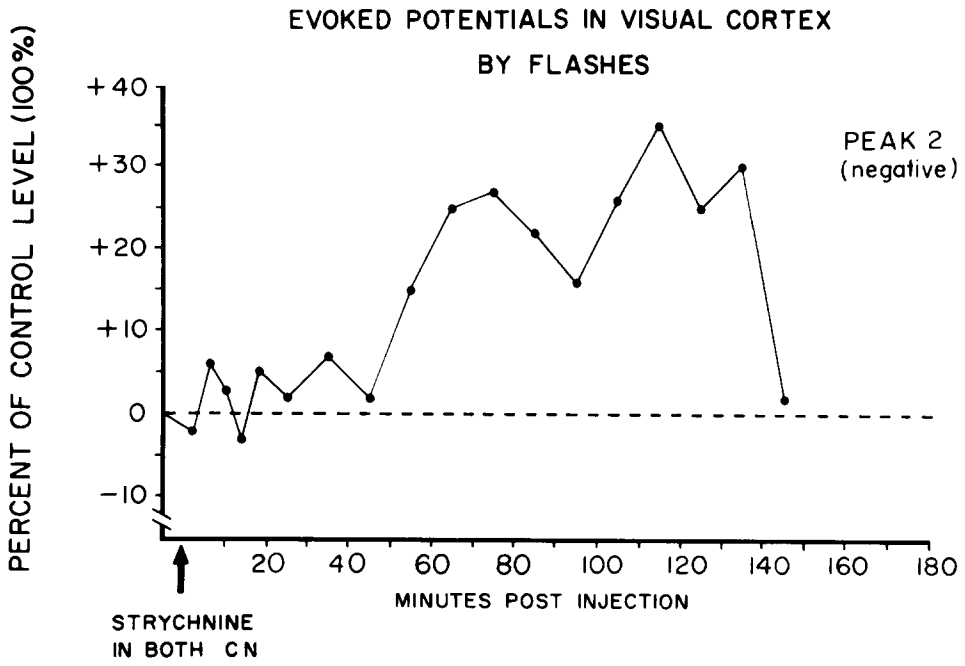

FIG. 4. Increment of the negative peak after the microinjection of strychnine in both CN. Each point represents the average amplitude, expressed in percent, of 6 cats.

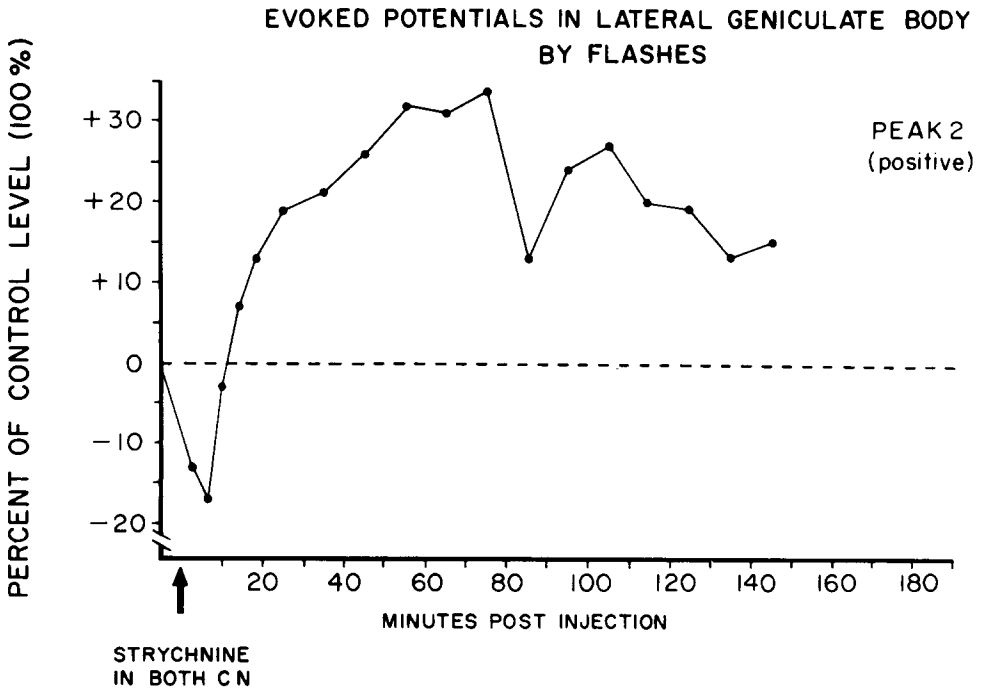

FIG. 5. Enhancement of the positive peak after the microinjection of strychnine in both CN. Each point represents the average amplitude, expressed in percent, of 6 cats.

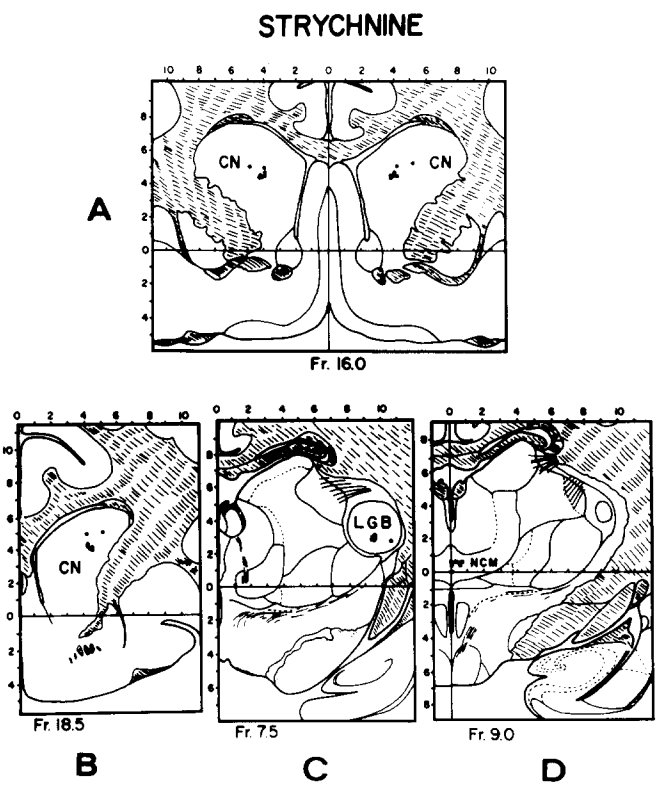

FIG. 6. Locus of microinjection in CN (A); the recording in CN (B) and LGB (C) as well as the stimulation in NCM (D) sites, obtained by projecting the histological sections upon the Jasper and Ajmone Marsan atlas [14].

lation that the CN exerts a regulatory influence, mainly inhibitory, upon the light responses at the geniculate body and visual cortex level, although it is unknown how and when this regulatory mechanism functions normally.

Despite these possible disrupting effects of strychnine upon the neuronal circuitry of the CN, the animals crossed to the punishment compartment and received the US but did not cross during the retention test which means that they were able to acquire the PAC, and they continued to show the PAC even after a second strychnine microinjection. This result contrasts with the effects obtained with picrotoxin, which rendered the animals incapable of inhibiting their motor activity and therefore they crossed to the punishment compartment where they had previously received the nociceptive stimulation [23].

From the aforementioned, it is suggested that the GABAergic action of the CN is important for the persistence of the inhibitory actions related with motor learning responses. In contrast, glycine, the inhibitory mediator which competes with strychnine, would be less important for these functions. On the other hand, the fact that some substances are effective while others are not, indicates that their actions are specific and not just the result of any intracaudate treatment.

It is interesting to discuss the effect of strychnine upon the potentials evoked by NCM stimulation, in which the second peak diminishes significantly. This peak is comparable to those described by Buchwald *et al.* [5], who described them as possible inhibitory post-synaptic components. The magnitude of enhancement of the late positive peak could indicate an increased intracaudate excitation. This seems to coincide with the observation that at the occipital cortex and lateral geniculate body level the magnitude of some components of the evoked potentials increased significantly, suggesting the release of an inhibitory effect. In conclusion, it seems likely that the neuronal intracaudate organization is highly related with GABAergic transmission for motor inhibition. Numerous authors have described the presence of intracaudate GABA [8,19]. Ladinsky *et al.* [18] suggested that GABA is involved in the intracaudate neuronal circuitry, which depends on the acetylcholine and dopamine contents, as has been previously proposed [3]. In contrast, the inhibitory transmission through glycine seems to be more related to regulation of perception and attention processes.

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