Pharmacology Biochemistry & Behavior, Vol. 11, pp. 499–503. Printed in the U.S.A.

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

FRANCISCA VÁZQUEZ, CAROLINA TÉLLEZ, PABLO DE LA MORA AND HÉCTOR BRUST-CARMONA

Departamento de Fisiología, Div. de Investigación, Facultad de Medicina, U.N.A.M., México 20, D.F.

Received 13 July 1979

VÁZQUEZ, F., C. TÉLLEZ, P. DE LA MORA AND H. BRUST-CARMONA. *Modification of motor activity, passive avoidance conditioning and evoked potentials by microinjections of picrotoxin in both caudate nuclei in cats.* PHARMAC. BIOCHEM. BEHAV. 11(5) 499–503, 1979.—Inhibition of movements is the result of the caudate nucleus (CN) activity. This action seems to be necessary for passive avoidance conditioning (PAC). Much data indicates that GABA has inhibitory effects in the CN. The present report provides further evidence for GABA inhibition in the CN. In cats, the acquisition session of a PAC response was carried out following the bilateral administration of 6 μg of picrotoxin or saline in the CN. Twenty four hours later (1st test session) the animals were placed in the security compartment and remained there during 600 sec. In the second test, the cats injected with picrotoxin crossed to the other compartment while the subjects injected with saline did not move. Another series of acute experiments were also carried out. Evoked potentials (EP) were recorded in the CN produced by nucleus centralis medialis electrical stimulation. After picrotoxin application the first and second peaks, which could represent EPSPs, increased while the third peak, which could represent IPSPs, decreased. In conclusion, it seems that GABAergic intracaudate transmission maintains a lower level of neuronal discharges and probably participates in the maintenance of a PAC but not in its acquisition.

Basal ganglia Caudate nucleus Passive avoidance conditioning Evoked potential in CN
Blockage of inhibition by picrotoxin GABA in passive avoidance conditioning

THE PARTICIPATION of the caudate nucleus (CN) in the neuronal circuitry responsible for the acquisition and retention of a motor conditioned response (MCR) has been demonstrated by use of electrolytic lesions [8], topically applied KCl 3M [25], and locally injected anesthetics [3]. It has also been shown that CN is essential for the maintenance of inhibitory conditioning [2]. Therefore, it has been assumed that CN holds the necessary elements to activate the circuitry responsible for the performance of a MCR as well as those required to inhibit this circuitry [4]. The inhibitory influences observed in electrograms and motor activity by the CN, [10, 11, 17] could be responsible for the behavioral modification which occurs when an animal learns not to pass from one compartment to the other, passive avoidance conditioning (PAC). Different experiments have shown modifications of PAC by striatum lesions [26,34] and stimulation [35].

Furthermore, the existence in the CN of substances which may have inhibitory actions such as gamma aminobutyric acid (GABA) [14,23] has been demonstrated. Thus the inhibition of motor activity might be exerted through the actions of GABA in the CN. This inhibition could be eliminated by blocking GABA actions through local application of picrotoxin. A loss of such inhibition could then be revealed by: (a) an increment in spontaneous motor activity; (b) a blockage of a learning process which requires inhibition of the motor activity and (c) modifications in evoked potentials produced in the CN by electrical stimulation of the n. centralis medialis of the thalamus. The present results obtained support these postulations.

METHOD

Sixteen cats of either sex (2.5 to 3.5 Kg body weight) were used. Stainless-steel cannulae were implanted stereotaxically into the anterior rostral-dorsal part of the head of the caudate nucleus (A.P. 19, L 3.5, H 4.5) following the Jasper and Ajmone-Marsan atlas [20] under Pentobarbital anesthesia and aseptic surgical conditions and fixed permanently to the skull with acrylic dental cement. In 7 cats bilateral microinjections of 6 μg of picrotoxin dissolved in 5 μl of 0.9% NaCl were performed after 5–8 days. In the other 9 cats only 0.9% NaCl (5 μl) was also bilaterally applied. Each injection was made in 100–120 sec. Immediately after, the gross spontaneous motor activity was observed and some reflex responses were clinically tested. After 10 min, the acquisition of a one

trial passive avoidance conditioning (PAC) was tested. 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 W bulb and was provided with an electrifiable grid made of 0.5 cm diameter stainless steel bars separated by 2 cm intervals and connected to a high impedance stimulator (Nucleo-electronica). Three min after placing a cat in the first compartment, 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 (14–15 mA and 0.1 sec of 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. Twenty four hours later, without any treatment the latency to cross from the one compartment to the other (test trial) was again measured over a maximum waiting time of 600 sec (learning criterion). Twenty four hours later a second test of PAC was performed 10 min after bilateral CN injections of either picrotoxin (N -6) or 0.9% NaCl (N–4). Latencies of the animals injected with saline or picrotoxin were compared by the U test and latencies before and after the nociceptive stimulation were compared with the Wilconson test [31].

In the same animals acute experiments were performed one or two 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 (20 mg IP). 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. Two bipolar stainless steel electrodes were lowered through drilled holes in the acrylic and the bone, one aimed at n. centralis medialis (A 10, L 0.5, H 0.5) and the other 3 mm behind the cannula into the head of CN (A 16, L 4, H 4.5). The first electrode was used for electrical stimulation: a square pulse, (0.1 msec duration and 10 V, Grass S 88 with SIU 6) was repeated 14 times once every two seconds. The second electrode was used to detect the electrical changes which were amplified by a Grass AC P511 differential input preamplifier with a bandwidth of 0.1 Hz to 30 Hz. The preamplifier output was fed into an Ampex Model PR-500 tape recorder. The output from the tape recorder was continuously monitored during the experiment. Sets of 14 responses were recorded for subsequent analysis. 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. Six sets of the average of 10 responses obtained one every 2 min were taken as controls and then bilateral microinjections of either 6 μg or 18 μg of picrotoxin were performed. The evoked potentials in CN were recorded first every two min during the first 20 min, thereafter every 5 min for the following 100 min and finally every 10 min for the next one or two hours. The effect of picrotoxin upon the evoked potentials was 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 [31].

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 10% Formalin with potassium ferrocyanide [15]. 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 negatives in an enlarger [16].

RESULTS

During the first few minutes (4–8) after the picrotoxin microinjections the animals stayed quiet cleaning themselves many times, and then started walking and suddenly bursts of violent motor activity appeared. Sometimes, they moved very quickly in a straight line tumbling over obstacles. They ran with the extremeties flexioned, very near to the floor which could be termed flying response on the floor. Sometimes, animals bumped against the wall of the cage but continued moving in one direction despite such collisions. In some animals salivation, midryasis and increased respiratory frequency were observed. It is interesting to mention that no cat had convulsive activity or presented rage with the dose of 6 μg. They were easy to handle except during the flying response. The postural reflexes tested: tonus, tendinous, righting and supporting, were not different from control cats injected with NaCl. In the acquisition session of PAC the cats crossed from one side to the other with an average latency of mean=75 sec (range 7–180) which was very similar to the control (mean=61.7, range 45–80) animals. The difference was not statistically significant ($U=8$, n_1 4, n_2 7). In the first session, 24 hr later, without any treatment, both groups of cats (injected the day before with NaCl or picrotoxin) did not cross from one compartment to the other in 600 sec (learning criterion). The Wilconson test showed that the difference between the latencies of each animal during the first test and the acquisition trial was statistically significant ($n=6$, $t=0.00$, $p<0.05$). Twenty four hr later in the second test session, the 6 animals injected again with 6 μg of picrotoxin 10 min before the session, moved to the other compartment with an average latency of 401.5 sec, which compared with the 600 sec of the 4 cats injected with saline solution revealed a statistically significant difference at the level of $p<0.05$ ($U=4$, n_1 4, n_2 6). Figure 1 illustrates these results.

The evoked potentials, bipolarly recorded in the CN by n. centralis medialis stimulation were very similar to the previously described by Diez-Martínez *et al.*[12,13]. In general, they consisted of a first positive peak with a latency of 9 ± 2.5 msec followed by a second peak more frequently negative with 24 ± 5 msec latency and then a third peak with a latency of 44 msec which was more frequently positive but was negative in few experiments. In some cases, another late peak of 100 or more msec latency was recorded. In 5 cats changes in the evoked potentials were observed after injecting 6 μg of picrotoxin however, these changes were small and undefined, and the dose of 18 μg was utilized instead. Figure 2 illustrates the small increment of peak one. After the application of 18 μg (usually 6 min) the first peak increased very clearly as is shown in Fig. 2. The difference in the magnitude before and after was statistically significant at the level of $p<0.05$ ($N=95$, $X=21$, $Z=5.34$). A more constant and more intensive increment was observed in the second peak. The increment of the magnitude produced by the application of picrotoxin is statistically significant at the level

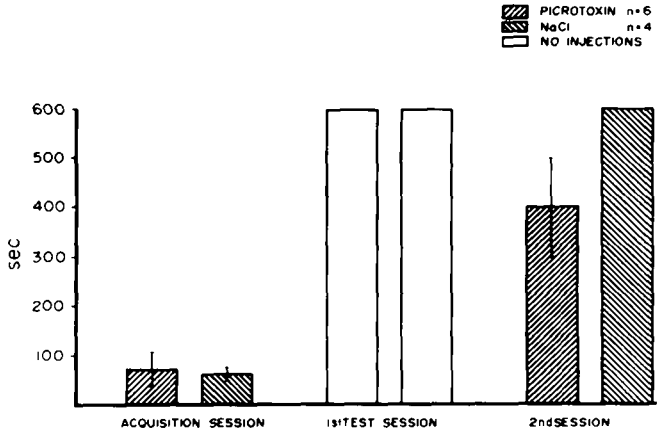

FIG. 1. The columns represent the average latency (vertical line, standard deviation) to cross from the security to the punishment compartment of cats injected with either saline or picrotoxin, 10 min before the acquisition session. Note that there is no difference. The latency is over 600 sec during the first test in both groups of cats. In the second test session, the cats injected with saline, maintain similar latencies, whereas the ones injected with picrotoxin showed decreased latencies.This difference is statistically significant at the level of $p<0.05$.

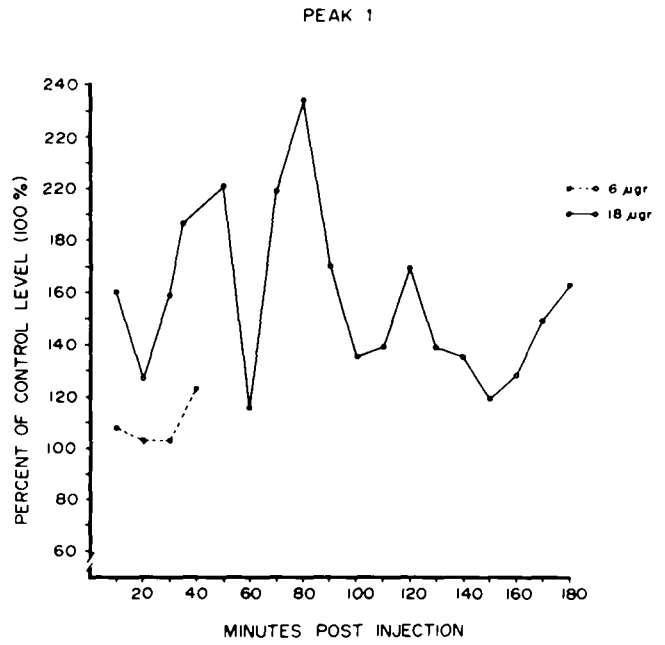

FIG. 2. Illustrates the enhancement (expressed in percentage), in the magnitude of peak one, after picrotoxin microinjection (6 or 18 μg) in both caudate nuclei. The average magnitude of 6 samples of 10 evoked potentials each, during the control period was taken as 100%.

of $p<0.05$ (N=152, X=38, Z=6.08). Figure 3 illustrates the increment of peak two after picrotoxin application in contrast with the small change after a saline solution microinjection.

In contrast the third peak decreased significantly $p<0.05$, N=82, X=24, Z=3.6) during the first 40 min. Figure 4 illustrates this decrement in magnitude of peak 3. Dur-

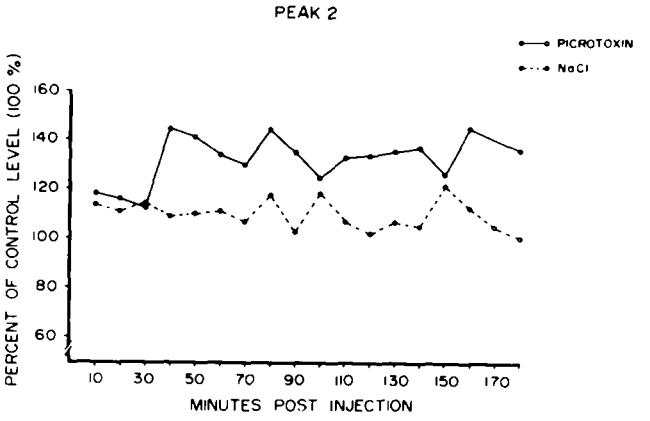

FIG. 3. Illustrates the enhancement (expressed in percentage), in the magnitude of peak two, after picrotoxin or saline solution microinjection in both caudate nuclei. The average magnitude of 6 samples of 10 evoked potentials each during the control period for saline or picrotoxin was taken as 100%.

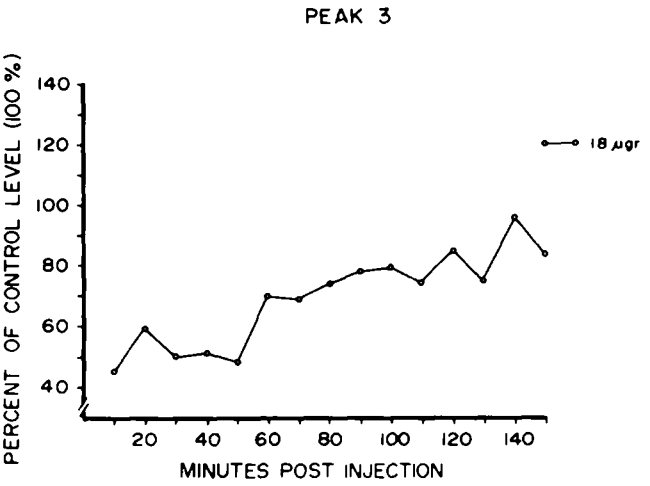

FIG. 4. Illustrates the diminution (expressed in percentage), in the magnitude of peak three, after picrotoxin microinjection in both caudate nuclei. The average magnitude of 6 samples of 10 evoked potentials each during the control period was taken as 100%.

ing the following 40 min in some animals the peak stayed small, increased in some, and was not significantly different from the control level.

In some experiments the effects of increasing the frequency of CMN stimulation were tested. It was observed that the first peak could follow frequencies up to 100–150 Hz. The second peak usually followed up to 50 Hz. Finally, in only one cat, at the end of the experiment, Pentobarbital at a dose of 10 mg/Kg of body weight was injected (IP). It was observed that the second and third peaks increased. With a second dose these modifications persisted but were less evident. With a third dose the evoked potential decreased and almost vanished, disappearing completely with a fourth dose, which in total amounted only to the usual anesthetic dosage.

The coronal brain sections showed that the tip of the cannulae as well as the recording electrode were in the head of

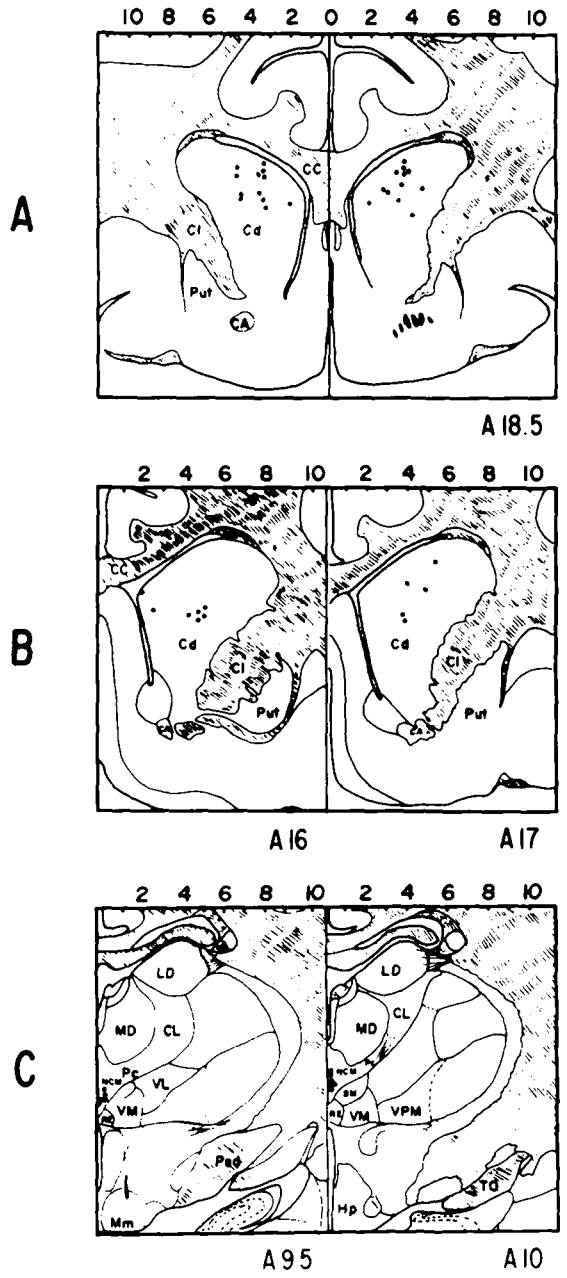

FIG. 5. Illustrates the tip placements of the cannulae in the head of the caudate nucleus anterior 18.5 (A); the recording electrode in Anterior 16 and 17 (B); and the stimulating electrode in nucleus centralis medialis in A 9.5 and A 10 (C), according to the atlas of Jasper and Ajmone-Marsan [20]

the CN as is illustrated in Fig. 5. The location of the stimulating electrode was in the nucleus centralis medialis (Fig. 5C).

DISCUSSION

From previous descriptions on the effects of CN stimulation [10,17] the CN has been assigned an inhibitory electrophysiological and behavioral function. Furthermore, lesions of CN produced an increment of locomotor activity modifying maze learning or PAC in rats [24,29] and in monkeys [9]. Different authors have also indicated that the striatum exerts descending inhibitory influences upon some spinal motor functions [30], the substantia nigra [27], as well as modulatory actions upon cortical and spinal cord activity [1]. Roemer 1978 [28] described that the tonically sustained inhibition of the CN upon cerebral areas is lost after picrotoxin applications. In contrast Wachtel *et al.* [33] illustrated that GABA injections into the rostral neostriatum in rats inhibited motor activity, whereas injections of picrotoxin increased it. They described this enhanced motility as continuous, coordinated, and forward directed movements, very similar to the effects described in this paper. We observed an increment in well coordinated movements consecutively to picrotoxin microinjection in both CNs. Sometimes, associated with collateral effects such as salivation, piloerection, mydriasis, crouching and plaintive crying. Similar effects were obtained by Cools [6] after stimulation of CN with frequencies of 30/sec.

The increment in motor activity was so important that even once the cats had learned not to cross from one compartment to the other, they did it under picrotoxin effect, being unable to inhibit the motor activity and avoid the nociceptive stimulation. It is noteworthy that the animals acquired the passive avoidance response under the picrotoxin effects.

The lack of inhibitory actions in the CN is also revealed by the increment in some of the peaks of the CN's evoked potentials. These evoked potentials show similar morphology and latencies as those described by Diez-Martínez *et al.* [12,13]. Similar evoked potentials in the CN were described by Hull *et al.* [18,19] and Buchwald *et al.* [5] and suggest that the 25–50 msec latency peak might represent EPSP and the following one IPSP. The first component of the EP described here could represent presynaptic activity. It appeared early (9 ± 2.5 msec), and followed frequencies up to 100 Hz, which are features ascribed to this type of response [7]. The second and third component might represent the EPSP-IPSP sequence. The picrotoxin microinjection in both CNs produces an increment of the first and second peaks with a decrement of the third peak. Both effects are statistically significant ($p<0.05$). Similar modifications of the EP recorded in the CN following stimulation of the anterior thalamic ventral nucleus have been observed by Spehlmann *et al.* [32]. Therefore, the increment of the first two components with a decrement of the third one, seems to fit well with the hypothesis that the blockage of GABAergic interneurons inside the CN increases the EPSPs and decreases the IPSPs produced by their inputs. Precht and Yoshida [27] described that the evoked potential in the substantia nigra produced by CN electrical stimulation decreased after IP picrotoxin application. Picrotoxin also blocks the CN's inhibitory effect upon the entopeduncular nucleus [22]. Furthermore, Ladinsky *et al.* [21] suggested an intracaudate neuronal circuitry which involves dopamine and acetylcholine as well as GABA.

In conclusion, the experiments reported here give further support to the hypothesis that the CN is part of a circuit responsible for learned motor responses which depend on inhibitory actions and suggest that this effect depends on GABAergic intracaudate transmission.

ACKNOWLEDGEMENT

We thank Ms Martha García and Ms Ingrid Mascher for their technical assistance.

REFERENCES

1. Amato, G., V. La Grutta, L. Melitello and F. Enia. The function on the caudate nucleus in the control of some paroxystic activities in the neuroaxis. *Arch. Int. Physiol. Biochem.* 77(3): 465–484, 1969.
2. Brust-Carmona, H. and I. Zarco-Coronado. Instrumental and inhibitory conditioning in cats. II. Effects of paleocortical and caudate nucleus lesion. *Boln. Inst. Estud. méd. biol. Univ. nac. Méx.* **27:** 61–70, 1971.
3. Brust-Carmona, H., R. Prado-Alcalá, J. Grinberg-Zylberbaum. Bloqueo reversible de respuestas condicionadas motoras por la aplicación de anestésicos locales en el núcleo caudado. *Boln. Inst. Estud. méd. biol. Univ. nac. Méx.* **27:** 109–117, 1971.
4. Brust-Carmona, H., R. Prado-Alcalá, J. Grinberg-Zylberbaum, J. Alvarez Leefmans and I. Zarco-Coronado. Modulatory effects of acetylcholine and catecholamines in the caudate nucleus during motor conditioning. In: Neurohumoral Coding of Brain Function. *Adv. in Behav. Biol.* **10:** 171–187, 1974.
5. Buchwald, N. A., D. D. Price, L. Vernon and C. D. Hull. Caudate intracellular response to thalamic and cortical inputs. *Expl Neurol.* **38:** 311–323, 1973.
6. Cools, A. R. Chemical and electrical stimulation of the caudate nucleus on freely moving cats: the role of dopamine. *Brain Res.* **58:** 437–451, 1973.
7. Chang, H. T. The evoked potentials. In: *Handbook of Physiology. Section I: Neurophysiology*, edited by American Physiological Society. 1959, Vol. 1: 299–313.
8. Chorover, S. L. and C. G. Gross. Caudate nucleus lesions. Behavioral effects in the rat. *Science* **141:** 826–827, 1963.
9. Davis, G. D. Caudate lesions and spontaneous locomotion in the monkey. *Neurology* **8:** 135–139, 1958.
10. Delgado, J. M. R. *Physical Control of the Mind: toward a Psychocivilized Society*. 1st. edition. New York: Harper and Row Publ., 168, 1971.
11. Denny-Brown, D. *The Basal Ganglia and their Relation to Disorders of Movement*. London: Oxford University Press, 1962.
12. Diez-Martínez, O., M. García Muñoz, G. Prieto, J. A. Roig and H. Brust-Carmona. Sensory afferents to the caudate nucleus. *Physiol. Behav.* **16:** 727–733, 1976.
13. Diez-Martínez, O., J. A. Roig, J. E. Vargas, J. Sepúlveda and H. Brust-Carmona. Neural pathway mediating somatic evoked responses in the caudate nucleus of cats. *Physiol. Behav.* **19:** 269–276, 1977.
14. Fahn, S. and L. J. Coté. Regional distribution of gamma-aminobutyric acid (GABA) in brain of the rhesus monkey. *J. Neurochem.* **15:** 209–213, 1968.
15. Green, J. D. A simple microelectrode for recording from the central nervous system. *Nature, Lond.* **182:** 902, 1958.
16. Guzmán, C. F., V. M. Alcaraz and A. Fernández. Rapid procedure to localize electrodes in experimental neurophysiology. *Boln. Inst. Estud. méd. biol. Univ. nac. Méx.* **16:** 29–31, 1958.
17. Heuser, G., N. A. Buchwald and E. J. Wyers. The 'caudate spindle' II. Facilitatory and inhibitory caudate-cortical pathways. *Electroenceph. clin. Neurophysiol.* **13:** 519–524, 1961.
18. Hull, C. D., G. A. Bernardi and N. A. Buchwald. Intracellular responses of caudate neurons to brain stem stimulation. *Brain Res.* **22:** 163–179, 1970.
19. Hull, C. D., N. A. Buchwald, D. D. Price and G. A. Bernardi. Intracellular responses of caudate neurons to temporally and spatially combined inputs. *Expl Neurol.* **38:** 324–336, 1973.
20. Jasper, H. H. and C. Ajmone-Marsan. *A Stereotaxic Atlas of the Cat*. Nat. Res. Council of Canada, Ottawa, 1954.
21. Ladinsky, H., S. Consolo, S. Bianchi and A. Jori. Increase in striatal acetylcholine by picrotoxin in the rat: evidence for a Gabaergic Dopaminergic-cholinergic Link. *Brain Res.* **108:** 351–361, 1976.
22. Obata, K. and M. Yoshida. Caudate evoked inhibition and actions of GABA and other substances on cat pallidal neurons. *Brain Res.* **64:** 455–459, 1973.
23. Okada, Y., C. Nitsch-Hassler, J. S. Kim, I. J. Bak and R. Hassler. Role of gamma-aminobutyric acid (GABA) in the extrapiramidal motor system. I Regional distribution of GABA in rabbit, rat, guinea pig and baboon CNS. *Expl Brain Res.* **13:** 514–518, 1971.
24. Peeke, H. U. S. and M. J. Herz. Caudate nucleus stimulation retroactively impairs complex maze learning in the rat. *Science* **173:** 80–82, 1971.
25. Prado-Alcalá, R. A., J. Grinberg-Zylberbaum, J. Alvarez-Leefmans and H. Brust-Carmona. Suppression of motor conditioning by injection of 3M KCl in the caudate nuclei of cats. *Physiol. Behav.* **10:** 59–64, 1971.
26. Prado-Alcalá, R. A., J. Grinberg, L. Arditti, M. M. García, H. G. Prieto and H. Brust-Carmona. Learning deficits produced by chronic and reversible lesions of the corpus striatum in rats. *Physiol. Behav.* **15:** 283–287, 1975.
27. Precht, W. and M. Yoshida. Blockage of caudate-evoked inhibition of neurons in the substantia nigra by picrotoxin. *Brain Res.* **32:** 229–233, 1971.
28. Roemer, R. A., W. W. Baker and D. Zeianovic. Differential effects of intracaudate injection of carbachol and picrotoxin on somatosensory evoked potentials. *Brain Res.* **140:** 182–187, 1978.
29. Rothman, A. H. and S. D. Glick. Differential effects of unilateral and bilateral caudate lesions on side preference and passive avoidance behavior in rats. *Brain Res.* **118:** 361–369, 1976.
30. Segundo, J. P., E. E. Migliano and J. A. Roig. Effect of striatal and claustral stimulation upon spinal reflex and strychnine activity. *J. Neurophysiol.* **21:** 391–399, 1958.
31. Siegel, S. *Estadística no Paramétrica*. Ed. Trillas, 1976.
32. Spehlmann, R., K. Norcross and E. J. Grimmer. GABA in the caudate nucleus: a possible synaptic transmitter of interneurons. *Experientia* **33:** 623–625, 1977.
33. Wachtel, H. and N. E. Andén. Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. *Naunyn-Schmiedeberg's Arch. Pharmac.* **302:** 133–139, 1978.
34. Winocur, G. Functional dissociation within the caudate nucleus of rats. *J. comp. physiol. Psychol.* **86:** 432–439, 1974.
35. Wyers, E. J., H. V. Peeke, J. S. Williston and M. J. Herz. Retroactive impairment of passive avoidance learning by stimulation of the caudate nucleus. *Expl Neurol.* **22:** 350–366, 1968.