THE MECHANISM OF INHIBITION IN RAT NEOSTRIATUM IN BRAIN SLICE PREPARATION

by

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A DISSERTATION

Submitted to
Michigan State University
in partial fullfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Anatomy

1981

ABSTRACT

THE MECHANISM OF INHIBITION IN RAT NEOSTRIATUM

IN BRAIN SLICE PREPARATION

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The purpose of the present investigation was to determine if inhibition is present in the neostriatum in vitro, and if present, to analyze and describe the mechanisms underlying the response.

Extracellular and intracellular recording was performed in 350-450 um thick parasaggital slices of rat neostriatum (caudate-putamen) maintained in vitro.

Local stimulation of the neostriatum in vitro, elicits a field potential usually consisting of two negative components: N-1, latency 0.5-2.0 msec and N-2, latency 1.5-5.0 msec, both ranging in amplitude from 0.5 to 1.0 mV. In paired shock experiments, when a maximal N-2 test response is preceded by a conditioning stimulus (of equal duration and amplitude), total reduction of the N-2 test response occurs at interstimulus intervals (ISIs) less than 4 msec.

The initial response recorded intracellularly following local stimulation of neostriatum is a depolarization. This response was determined to be a monosynaptic excitatory postsynaptic potential (EPSP). In paired shock experiments reduction of test EPSP amplitude occured over ISIs of 3 to 38 msec. Action potentials were also blocked in double shock experiments over the same time course.

In 17% of the 63 neurons in the test group, hyperpolarizing potentials were observed following EPSPs evoked by local stimulation of neostriatum. The hyperpolarizing potentials were determined to be inhibitory postsynaptic potentials (IPSPs) based on their response to intracellular current injection. The time course of the IPSPs ranged between 25 and 50 msec.

In 23 neurons, a pulsed injection of depolarizing current was used to trigger an action potential (AP). The AP thus triggered, was used to condition a test orthodromic EPSP. Test EPSPs conditioned in this manner were shunted over ISIs less than 45 msec.

In order to examine the nature of a possible transmitter involved in this inhibition, double shock tests were performed while the slices were perfused with medium containing GABA antagonists bicuculline methiodide, picrotoxin, or penicillin-G. It was found that double shock performed under these conditions resulted invariably in potentiation rather than the inhibition of test EPSPs. For all concentrations of GABA antagonists used, EPSP 1/2 width values increased when compared to control.

Inhibition in the neostriatum in vitro is demonstrated as reduction in test EPSP amplitude in double shock tests, by the presence of IPSPs, and by the shunting of EPSPs conditioned by an AP triggered by direct depolarization. Neurons exhibiting these three forms of inhibition were intracellularly labelled with HRP, and identified as medium spiny neurons.

ACKNOWLEDGMENTS

I would like to thank my wife and parents for their perpetual support and encouragement throughout the course of this study.

I would like to express my appreciation and highest regards to my mentor Dr. S.T. Kitai. It was he who enabled me to to build the slice laboratory in which these experiments were performed. I thank him for sharing with me his vast knowledge and expertise in the field of neuroscience, without his guidance none of the work presented here would have been possible.

I thank my friends and colleagues who assisted me during this set of experiments. I thank Dr. M.R. Park for his assistance in designing the beginning stages of the experiments and for his clear insight and interpretation of the problems that confronted us. I thank Dr. C.J. Wilson for his timely advice, continual support and his aid in the statistical analysis of the pharmacological data presented here. I thank my friend and classmate Dr. H.T. Chang for his skillful assistance in the histology laboratory.

FORWARD

Portions of the experimental findings reported here have been previously reported in one of the following communications:

Lighthall, J.W., M.R. Park, and S.T. Kitai. Evidence for inhibition in Rat Neostriatal Brain Slices. Soc. Neurosci. Abstr., Vol. 6, 187. 1980

Lighthall, J.W., M.R. Park, and S.T. Kitai. Inhibition in slices of rat neostriatum. Brain Research, 212:182-187. 1981

Lighthall, J.W. and S.T. Kitai. Recurrent GABAergic Inhibition in Rat Neostriatal Brain Slice. Soc. Neurosci. Abstr., Vol. 7, 247. 1981

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Chapter 1. INTRODUCTION

Electrophysiological investigations of neostriatum, performed in vivo, have observed a pattern of excitation and inhibition following either intrinsic stimulation or stimulation of extrinsic afferents (7,35,40,43,48,49,63,76,77).

Excitatory postsynaptic potentials (EPSP) evoked from stimulation of afferents to the neostriatum are observed to be followed by inhibitory postsynaptic potentials (IPSPs) having a duration of 80-300 msec (7,35,43,63,77). Also interpreted as an indication of inhibition in the neostriatum is the reduction in amplitude of a test EPSP when it is preceded by a single afferent conditioning stimulus. The time course of this inhibition is 75 to 250 msec (43,76,77). Local stimulation of the neostriatum likewise results in suppression of evoked field potentials lasting up to 280 msec (49). Intracellular studies indicate that local stimulation of the neostriatum elicits EPSPs followed by IPSPs 175-300 msec in duration (48). In general, the time course of inhibition made evident in any of the aforementioned ways in the neostriatum in vivo following either stimulation of afferents or local stimulation is greater than 50 msec and less than 300 msec. The events underlying this long period of inhibition in vivo are now beginning to be more clearly understood. Current electrophysiological data acquired from identified neostriatal neurons in vivo, indicate that the long

duration inhibition elicited by stimulation of substantia nigra may represent a temporary inhibition of a tonic cortico-striatal excitatory discharge rather than some inhibitory process intrinsic to the neostriatum (78).

It is becoming increasingly apparent, based on the avalible in vivo data, that the long duration inhibition (more than 50 msec) is not likely to be intrinsic to the neostriatum. Only recently recurrent inhibition of a much shorter duration (less than 35 msec) has been observed in neostriatal medium spiny neurons using intracellular recording techniques (58). In this study recurrent inhibition was demonstrated in the following way. The intracellularly recorded neuron is depolarizied by injection of a square wave of depolarizing current. The resulting action potential thus triggered at the soma would propagate in the anterograde direction along the axon and throughout the plexus of axon collaterals and causing the release of transmitter at axonal junctional sites of the neuron. The resulting inhibition was found to effectively shunt EPSPs evoked by stimulation of substantia nigra over a 35 msec time course. The recurrent inhibition is blocked by antagonists of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), the tramsmitter substance apparently utilized by the neostriatal medium spiny neuron (67).

Inhibition elicited by activation of a single neuron implies this response may be mediated by axon collaterals of the same neuron which make synaptic contact with the dendrites of that same neuron. Anatomical evidence of this form of synaptic contact, between the axon and dendrites of the same neuron, has been identified on rat

neocortical pyramidal neurons and has been termed an autaptic synapse (75). The inhibition resulting from activation of autapic synapses would represent a unique form of recurrent inhibition, intrinsic to the neostriatum (58). In addition to autaptic recurrent inhibition, a more general form of mutual inhibition among neighboring medium spiny neurons has also been proposed to occur in the neostriatum in vivo (58). This form of inhibition is proposed to be mediated by inhibitory axon collaterals of medium spiny neurons making synaptic contact with neighboring medium spiny neurons. Direct anatomical evidence of axo-dendritic synaptic profiles between identified medium spiny neurons indicates that mutual inhibition among members of this cell type can occur (79). The duration of the inhibition elicited in the neighboring post-synaptic neuron should follow a similar time course as that demonstrated for autaptic recurrent inhibition of medium spiny neurons (58). However, no short duration (less than 35 msec) inhibitory conductance change has been observed in neostriatum in vivo (48,49), nor in vitro (53,54), following local stimulation. In addition, the ionic mechanism underlying the short duration autaptic inhibition elicited by direct depolarization of a medium spiny neuron remains unexplained.

The brain slice preparation is one method of studying circuitry intrinsic to a specific nucleus. The neostriatum in vitro is a nucleus in which extrinsic loop circuits are open. The responses recorded intracellularly following local stimulation of neostriatum in vitro reflect the interaction between elements intrinsic to the neostriatum, and fibers terminating in, or passing through that nucleus. The portions of efferent fibers and fibers of passage

remaining in the slice preparation are physically isolated from their nuclei of origin. This arrangement precludes the possibility that the stimulus delivered to the slice will be integrated by circuits extrinsic to the neostriatum. The local responses obtained in the neostriatum in vitro should therefore represent a less complex intranuclear interaction between those elements remaining intact in the slice, rather than a complex internuclear response. Moreover, the nature of the slice preparation allows the investigator to accurately manipulate the extracellular enviornment of the impaled neuron. Alterations in the electrolyte balance and introduction of neuropharmacological substances to the medium bathing the slices is easily performed in this preparation. Therefore, in addition to intrinsic circuitry, the brain slice may by the ideal preparation for the study of neurotransmitters present in a specific nucleus and the postsynaptic conductance changes resulting from their action. The specfic mechanisms involved in the responses recorded in the neostriatum maintained in vitro, once established, may aid in the interpretation of those obtained in vivo.

To date investigations performed, in vitro, in neostriatum detected only excitation following local stimulation and denied the existence of inhibition as noted in vivo (53,54). The absence of inhibition was interpreted from a facilitation of test EPSPs observed in double shock experiments (53,54). This contrasts to the reduction in amplitude of the test response as noted in vivo (43,76,77). More recently, in experiments performed by Misgled and associates (55), a disfacilitation of test EPSPs in double shock was observed in vitro. However, this observation was not intrepreted as an a indication of

inhibition, due to the lack of a measurable inhibitory conductance change superimposed on the test response (55). In addition, the responses evoked through local neostriatal stimulation were proposed by the authors to be generated solely by cholinergic local circuit neurons (53,54).

The purpose of the present study is to investigate the responses in the neostriatum, maintained in vitro, following local stimulation in order to determine if inhibition is present, at both the intracellular and extracellular level, as shown in vivo.

Objectives:

When beginning the electrophysiological investigation of a system in a new preparation it is important to fulfill the following objectives:

1. Extracellular recording techniques are utilized to examine the characteristics of the field potentials elicited by local stimulation of neostriatum. During extracellular recording changes in the field potential in response to variations in divalent cation concentration (i.e. [Ca+2] and [Mg+2]), stimulus intensity and frequency should be analyzed in order to determine if the response is generated by synaptic or non-synaptic potentials. In addition, in order to determine if inhibition is detectable extracellularly, double shock experiments are performed. A reduction in amplitude of the test response in double shock may indicate the presence of inhibition extracellularly.
2. Intracellular recording is performed in order to examine the postsynaptic responses in neurons following local stimulation of the neostriatum. The intracellularly recorded potentials are examined

during intracellular injection of constant depolarizing and hyperpolarizing current in order to determine the conductance changes generating response. To establish if the intracellular response is monosynaptic, it is important to examine any variations in the latency of the response during changes in stimulus intensity.

3. During intracellular recording double shock is performed in order to determine if inhibition is dectectable at this level. Reduction in the amplitude of the test response in double shock may indicate the presence of inhibition. The time course over which the test response amplitude is reduced may reflect the duration of the inhibition.

4. To establish if autaptic recurrent inhibition is functioning in this preparation, experiments are performed in which a neuron is activated by intracellular current injection. The effect of direct depolarization neuron on the amplitude of orthodromic potentials is measured before and after current is injected. A reduction in the amplitude of a test response following the direct activation of the neuron may indicate the presence of autaptic recurrent inhibition.

5. If inhibition is detected by one of the aforementioned methods, it is important to verify the neurotransmitter mediating the response. During intracellular recording, the slices are exposed to possible neuropharmacological antagonists of the inhibition, in an attempt to block the response. If the inhibitory response is blocked by one or more specific antagonists of a neurotransmitter, that neurotransmitter may be responsible for the inhibition observed.

6. The neurons which exhibit inhibition during intracellular recording are identified by intracellular labelling. Experiments are performed in which the impaled is intracellular injected with the

enzyme horseradish peroxidase. Neurons recovered following intracellular labelling may then be identified on the basis of their morphological characteristics.

Chapter 2. METHODS

Adult male rats (Long-Evans) were killed by decapitation. The brain was removed and a core measuring 2.5 mm high (vertcal) by 5.5 mm wide (AP), and containing neostriatum (caudate-putamen) from both sides was obtained by pressing a coring device (a brass tube with rectangular cross section) through the brain from right to left. The tissue was then extruded from the coring device with a brass plunger. The core was placed on a tissue chopper where 4 to 6 parasagittal slices, 350-450 um thick were cut from each caudate-putamen. The brain slices were mostly neostriatum with portions of subcortical white matter and cerebral peduncle located dorsal and ventral. The slices were then transferred to a nylon support mesh in a chamber (see Fig. 1,2) containing 3.5 ml of medium. The perfusion medium used in the experiments consisted of the following (in mM): NaCl 124.0, KCl 5.1, MgSO4-6H2O 1.3, CaCl2 2.5, KH2PO4 1.25, NaHCO3 26.0, and d-glucose 10.0. Total time between decapitation and placement in the chamber was 3-5 min. The media was adjusted to just contact the bottom surface of the slices and perfused through the chamber at a flow rate of 0.5 ml/min. The pH was adjusted to 7.3-7.4 by bubbling a gas mixture of 95%CO2/5%O2 through the perfusate. The osmolarity of the medium was measured prior to each experiment with an automatic osmometer (Osmette A, Precision Systems Inc.) and adjusted to 305+—5 mOsm. The time required for complete exchange of medium in the

Figure 1. Photographs of chamber used in neostriatal slice preparation.

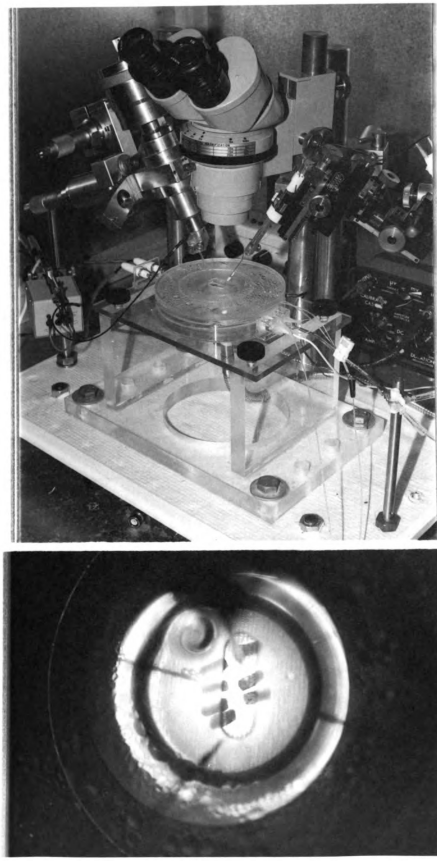


FIGURE 1

Figure 2: Diagram of chamber used in in vitro preparation. A: water bath chamber; B: recording chamber; C: lid cover; D: water chamber lid. Calibration bar equals 2 inchs.

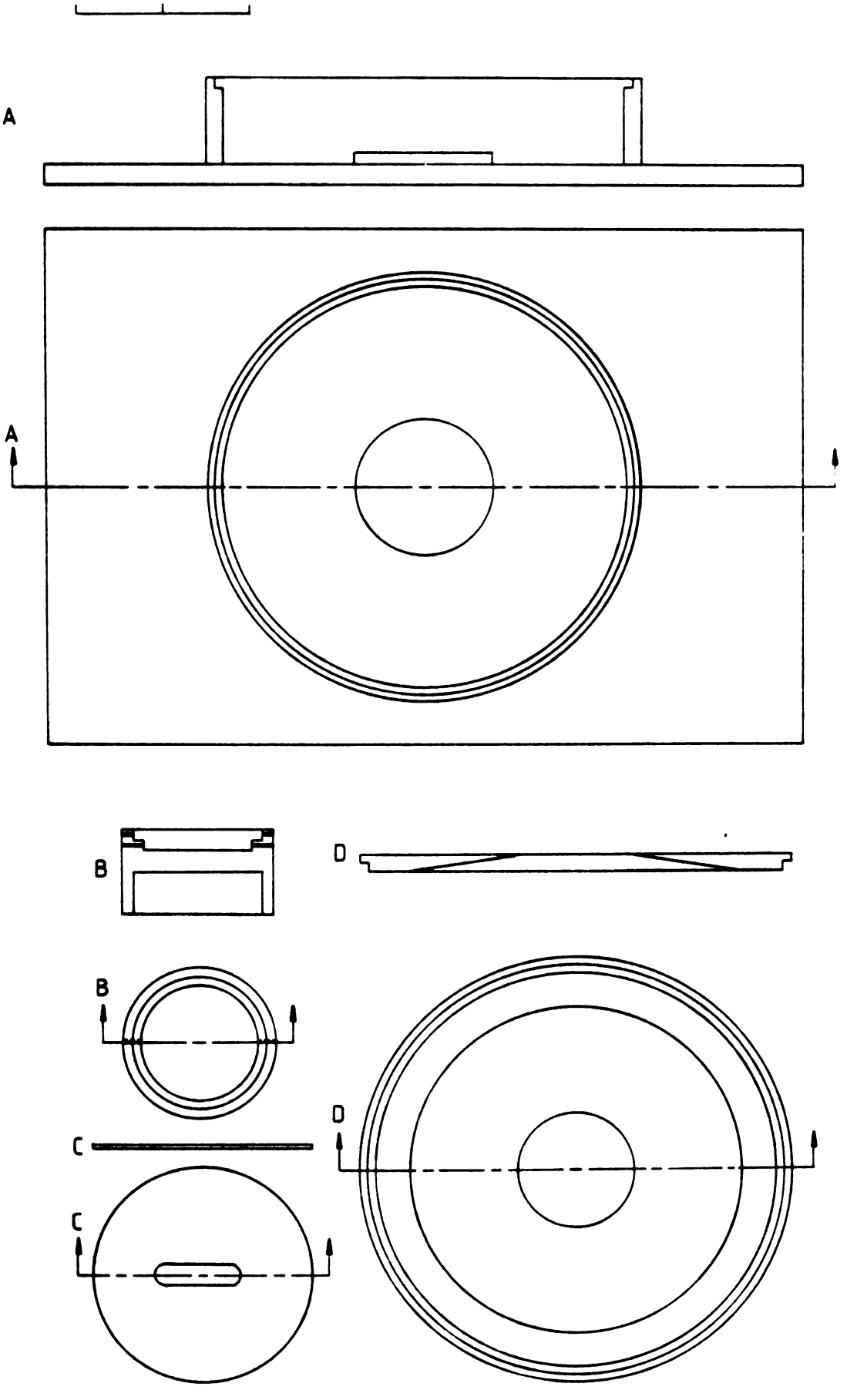


FIGURE 2

recording chamber was 10-12 min. Surrounding the chamber a water bath, whose temperature could be controlled to within 0.25 °C, warmed the medium and a mixture of 95%02/5%C02 gas which was bubbled through the bath continuously. Experiments were performed at temperatures between 35 and 37 °C.

For direct bipolar stimulation of neostriatum, twisted pairs of 80 um diameter nichrome wire insulated to within 100 um of the tip and with a center to center distance of 120 um, were lowered to just beneath the upper surface of the slice. An analog stimulator (WPI) and stimulus isolation unit was used to deliver pulses 0.05-0.30 msec in duration and 5-90 V in amplitude to the stimulating electrodes.

Glass microelectrodes filled with 2 M NaCl (DC resistance 5-10 um) were used for recording extracellular field potentials. Intracellular recording was performed using glass microelectrodes (WPI capillary tubing with inner filament) with a tip diameter §0.1 um, filled with 2M potassium methylsulfate and selected on the basis of a DC resistance of 50 to 120 Mohm. Beveled glass microelectrodes, filled with horseradish peroxidase (HRP; Sigma type VI) 2M in Tris buffer (pH 7.6) and 0.5 M potassium methylsulfate (DC resistance 30 to 90 Mohm) were used for intracellular labelling of neurons with HRP. HRP injection was performed using 1-5 nA rectanglar depolarizing pulse with a 50% duty cycle. The recording glass microelectrodes were visually positioned 0.5 to 2.0 mm from the stimulating electrodes using a micromanipulator (Narishge) and with the aid of a dissection microscope (Nikon). The microelectrodes were connected to a high input resistance biological amplifer with an active bridge circuit (WPI; M701) which allows measurment of membrane potentials and

simultaneous intracellular injection of constant current. Membrane potentials were fed to DC and AC coupled amplifiers and displayed on an oscillscope (Tektronix 565) from which photographic records were made.

For those experiments in which HRP injection was to be performed, the slices were placed on a filter paper disc resting on the nylon support mesh in the recording chamber, rather than resting directly on the mesh. This permitted the slices to be removed from the recording chamber without mechanical disrupation. The filter paper allows the slices to be fixed without curling (in addition to the absence of any impressions normally left by the nylon support mesh on the bottom surface of the slice). Slices containing HRP injected neurons were allowed to fix overnight in 2.5% gluteraldehyde at 4°C. The slices were than porcessed the next morning using the following protocol:

1. Wash in 0.15 M phosphate buffer 3X10 min. each.
2. Wash in 0.1 M Tris made isoosmotic (~320 mOsm) by addition of glucose 3X5 min. each.
3. Incubate in 0.1 M Tris-glucose with 0.5% cobalt chloride 2X10 min. each.
4. Wash in 0.1 M Tris-glucose w/o coblat chloride 2X5 min. each.
5. Wash in 0.1 M phosphate buffer 2X10 min. each.
6. React for peroxidase activity in 0.1 M phosphate buffer with 0.05% diaminobenzidene (DAB; Sigma), and H2O2 0.06% 2X20 min. each.
7. Wash in 0.1 M phosphate buffer 2X5 min. each.
8. Dehydrate in EtOH, clear in xylene, mount whole in

Histoclad. Developed neurons were photographed with the aid of a microscope and drawing tube reconstructions were also made (Fig. 11).

Slices were exposed to control medium or medium containing one of the following GABA antagonists: picrotoxin (Sigma; No. P-5753) 0.10-0.05 mM, bicuculline methiodide (ICN Pharmaceuticals, Inc.; No. 20684) 0.10-0.05 mM, or penicillin-G (Sigma; PEN-NA) 2000 I.U./ml.. When used in these concentration, there was no effect on either the pH or the osmolarity of the perfusate.

Chapter 3. RESULTS

Field Potential Analysis:

Low intensity stimulation of neostriatum elicits a field potential usually consisiting of two negative components: N-1, latency 0.5-2.0 msec and N-2, latency 1.5-5.0 msec, both ranging in amplitude from 0.5 to 1.0 mV. Stimulus intensity and/or duration can be increased to elicit a maximum N-2 response ranging in amplitude from 1.0 to 3.6 mV (mean 1.72 mV, S.D. 0.82 mV, n=10) with a latency of 1.7-5.0 msec (mean 2.57 mV, S.D. 0.78 mV) (Fig. 3, upper trace). Increasing stimulus frequencies from 1 Hz to 20 Hz has the effect of reducing the amplitude of N-2. In contrast N-1 is unattenuated by high frequency stimulation up to 100 Hz. Reduction of the N-2 response also occurs in high magnesium and low calcium medium (8.0 mM [Mg2+] and 0.3 mM [Ca2+]; normal values were 1.3 mM [Mg2+] and 2.5 mM [Ca2+]). The susceptibility of N-2 to the above manipulations of stimulation and electrolyte concentration indicates that this component of the field is synaptically driven.

In paired shock experiments, when a maximal N-2 test response is preceded by a conditioning stimulus (of equal duration and amplitude), total reduction of the N-2 test response occured at interstimulus intervals (ISIs) less than 4 msec. Fig. 3, top trace, shows an example of a control N-2 test response. When preceded by a conditioning stimulus (first arrow in the bottom trace), this response

Figure 3. Neostriatal evoked field potential: Electrical stimulation of neostriatum elicits a field potential shown in the upper trace, downward arrows indicate the two components of the field potential N-1 and N-2. Bottom trace shows a reduction of the test N-2 response in paired shock. Upward arrows indicate onset of stimulus. The time course of recovery of the N-2 response in double shock is depicted in the graph. Data from 10 recording sites were pooled. Each point represents mean recovery of N-2 test amplitude to control levels at that ISI +/- 1 msec. Vertical bars indicate standard errors of the means. Negativity is downward, and positivity is upward in this and all subsequent figures.

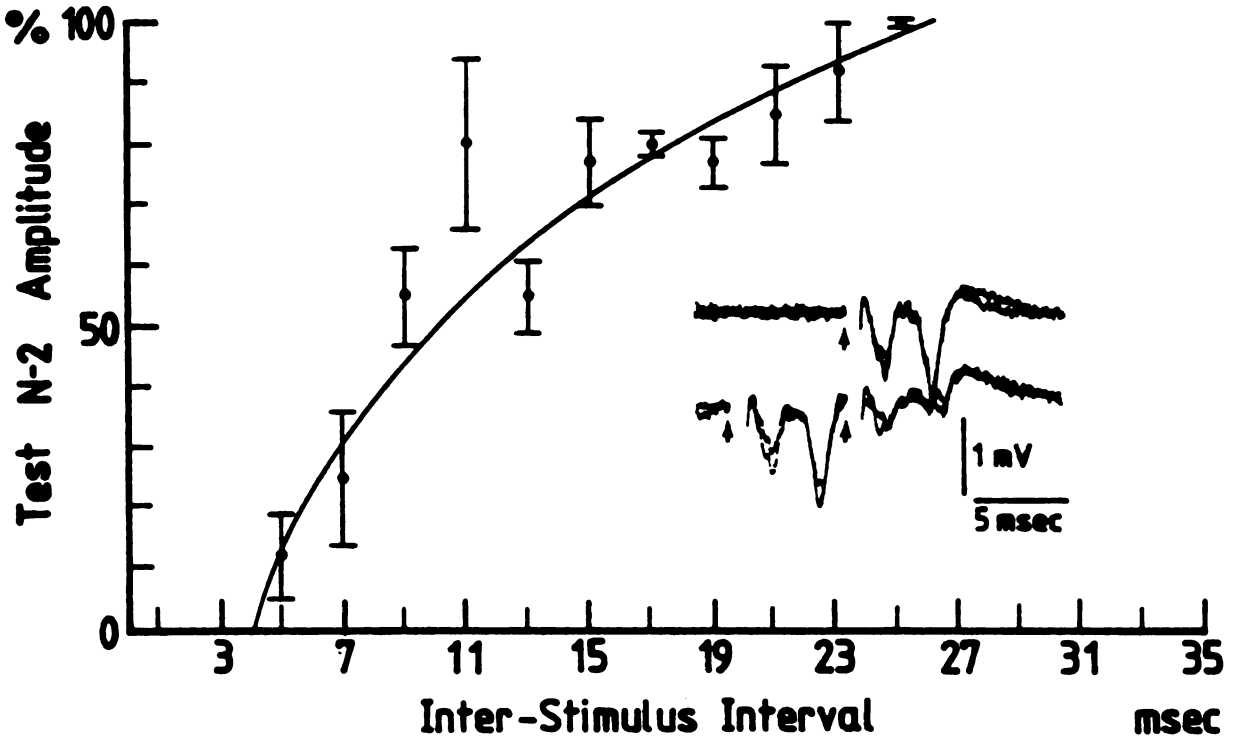

% 100
50
0
Test N-2 Amplitude
3
7
11
15
19
23
27
31
35
Inter-Stimulus Interval
msec
1 mV
5 msec

FIGURE 3

was reduced to 10% of the control amplitude at an ISI of 23-27 msec. The time course of recovery of the N-2 response in double shock is depicted in the graph in Fig. 3. The amplitude of test N-2 responses returned to control level at ISIs of 25-30 msec. Inhibition of test responses was observed in all the cases tested. When stimulus parameters are set to evoke a submaximal N-2 response, double shock resulted in slight potentiation of, or has no effect on the N-2 test response.

Intracellular Analysis:

Neostriatal neuron impalement was signaled by a transmembrane potential as large as 67 mV. Of the 157 neurons impaled, 40% or 63 neurons were deemed satisfactory on the basis of a stable membrane potential of at least 40 mV, and the ability to generate action potentials 40 mV in amplitude, with a duration of less 2.5 msec. Intracellular analysis shows the initial response recorded following local stimulation of neostriatum to be a depolarizing potential. The response increased in amplitude during injection of constant hyperpolarizing current and decreased in amplitude with constant depolarizing current, which may indicate the response is an excitatory postsynaptic potential (EPSP)(Fig. 4E-F, broken line A). The current-voltage relationship for this EPSP is plotted in the graph in Fig. 4C, curve a. The reversal of this EPSP occured during intracellular injection of 0.25 nA of depolarizing current. The latency of the EPSPs was invariant to incremental steps of stimulus intensity (Fig. 4A, B; Fig. 5), an indication of monosynaptic activation. For the 63 neurons recorded in control medium EPSP

Figure 4. A monosynaptic EPSP-IPSP sequence is elicited by stimulation of neostriatum: A and B show the response of a neuron to incremental steps of stimulus intensity. Low intensity stimulation elicits only an EPSP (middle and top trace), increasing neostriatal stimulus intensity evokes a EPSP-IPSP sequence (lower three traces in A and B). Both the EPSP and IPSP increase in amplitude with constant latency in response to incremental steps of stimulus intensity indicating monosynaptic activation. The effect of constant current injection on the amplitude of the EPSP-IPSP is shown in traces E-H and plotted in C. D: Increasing hyperpolarizing current (0.5 nA, constant current) eventually elicits an action potential. All records shown are recorded from a single neuron.

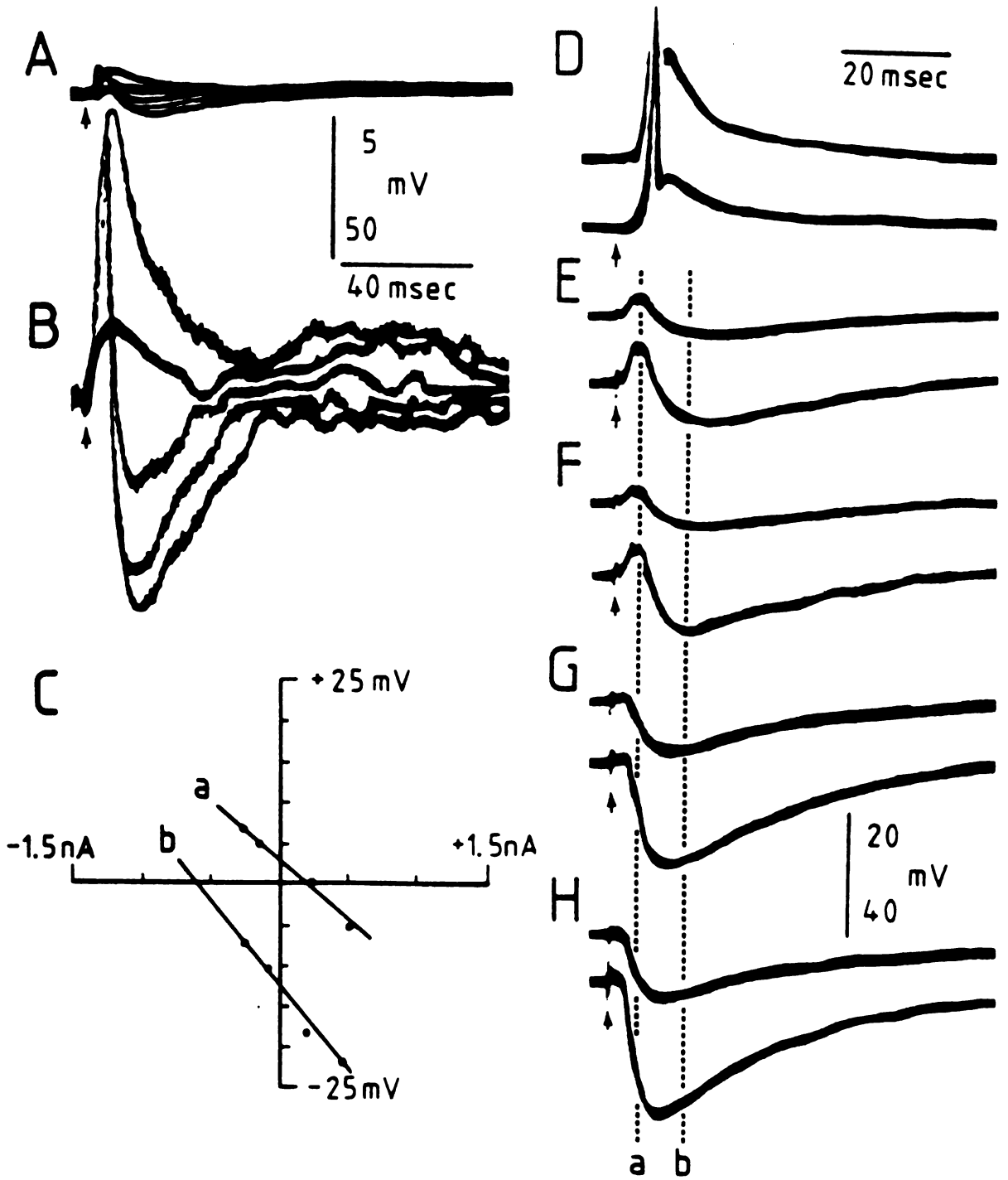

A
B
5
mV
50
40 msec
C
+25 mV
a
-1.5nA
b
+1.5nA
-25mV
D
20 msec
E
F
G
H
20
mV
40
a
b

FIGURE 4

Figure 5: Neostriatal evoked EPSP responding to incremental steps of stimulus intensity with increasing amplitude and constant latency is shown in A and B (high gain AC and DC records respectively).

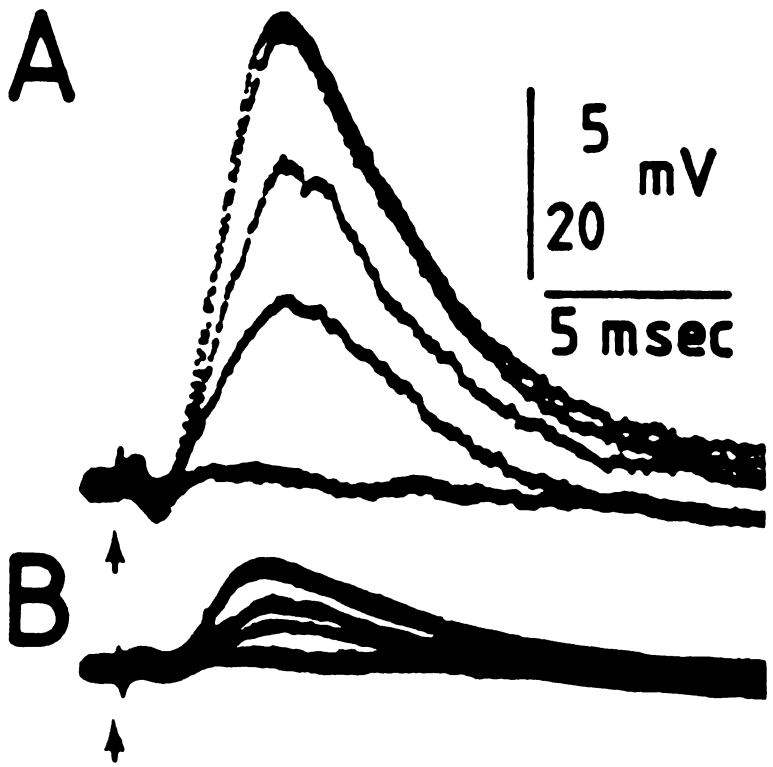


FIGURE 5

configuration data was pooled to yield the following: latency (as measured from stimulus artifact)=2.18 msec (SD 0.32); amplitude=6.57 mV (SD 1.67); time to peak (measured from the onset of the response)=3.27 msec (SD 0.85); time from peak to 1/2 decay =4.82 msec (SD 1.15); and half-width=6.82 msec (SD 1.25).

In paired shock experiments, when a test EPSP is preceeded by a conditioning stimulus of equal amplitude and duration (indicated by the first arrow in Fig. 6A,B), reduction of test EPSP amplitude occured over interstimulus intervals (ISIs) of 3 to 38 msec (Fig. 6C). Fig. 6A demonstrates this phenomenon. Test EPSP amplitude is reduced 35% at a 10 msec ISI (indicated by the second arrow in Fig. 6A) as measured from the base line trajectory of the conditioning response. In this neuron the test response returned to the amplitude of the conditioning response (control) at an ISI of 36 msec (indicated by the sixth arrow). Failure of summation of test EPSPs with conditioning responses occured in all 63 neurons maintained in control medium. The lack of summation of a conditioning EPSP with a test EPSP was interpreted as an indication of inhibition.

Action potentials (APs) were also shunted in double shock experiments (Fig. 6B). The time interval in which orthodromic test APs are shunted closely matches the time course of inhibition of test EPSPs in double shock experiments.

In 17% of the 63 neurons in the test group, hyperpolarizing potentials were observed following EPSPs evoked by local stimulation of neostriatum (Fig. 4 and 7). The hyperpolarizing potentials were always preceeded by an initial period of excitation and could occur in the absence of an AP. The effect of constant current injection on

Figure 6. Time course of inhibition of neostriatal evoked EPSPs and action potentials in paired shock: A: Neostriatal evoked conditioning EPSP (first arrow indicates stimulus artifact of conditioning response) shunts test EPSPs at ISIs of 9.5 to 36.5 msec, both high gain AC and DC records are shown. APs triggered by test EPSPs are blocked by a conditioning orthodromic AP in B. Arrows indicate onset of stimulus. Time course of test EPSPs inhibition is depicted in C. Data were pooled from 63 neurons. Each point represents mean per cent recovery to control amplitude at that ISI +/- 1 msec. Vertical bars represent standard errors of the means.

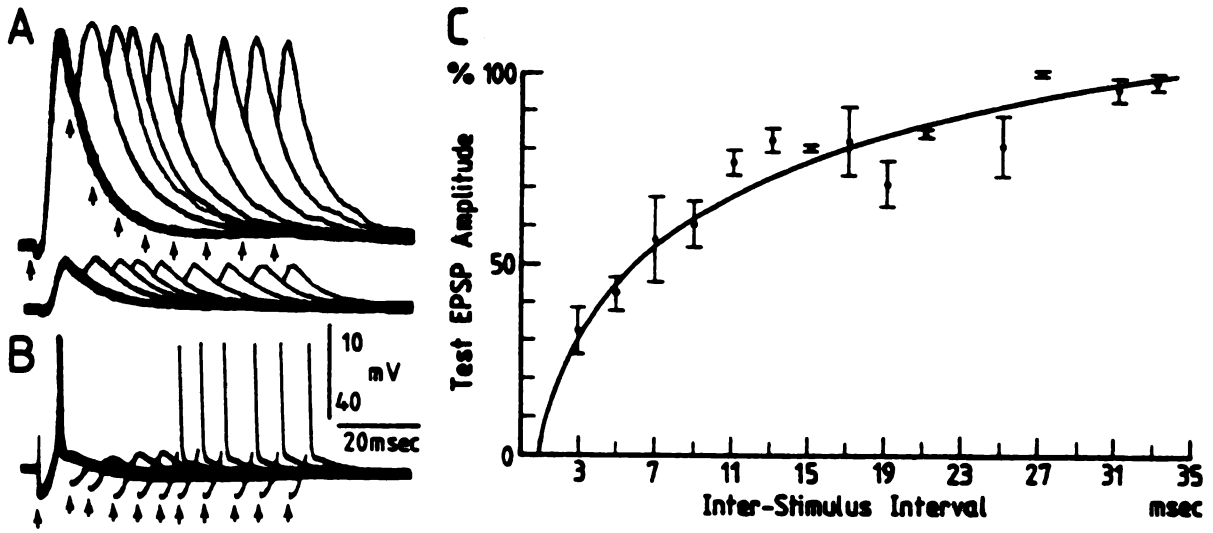

A
B
10
mV
40
20msec
C
% 100
50
0
Test EPSP Amplitude
3
7
11
15
19
23
27
31
35
Inter-Stimulus Interval
msec

FIGURE 6

Figure 7: High frequency (250 Hz) stimulation in F-I elicits an IPSP. K and M show the effect of 1.7 nA of constant hyperpolarizing and depolarizing current on the amplitude of the control IPSP in L. Extracellular control shown in J.

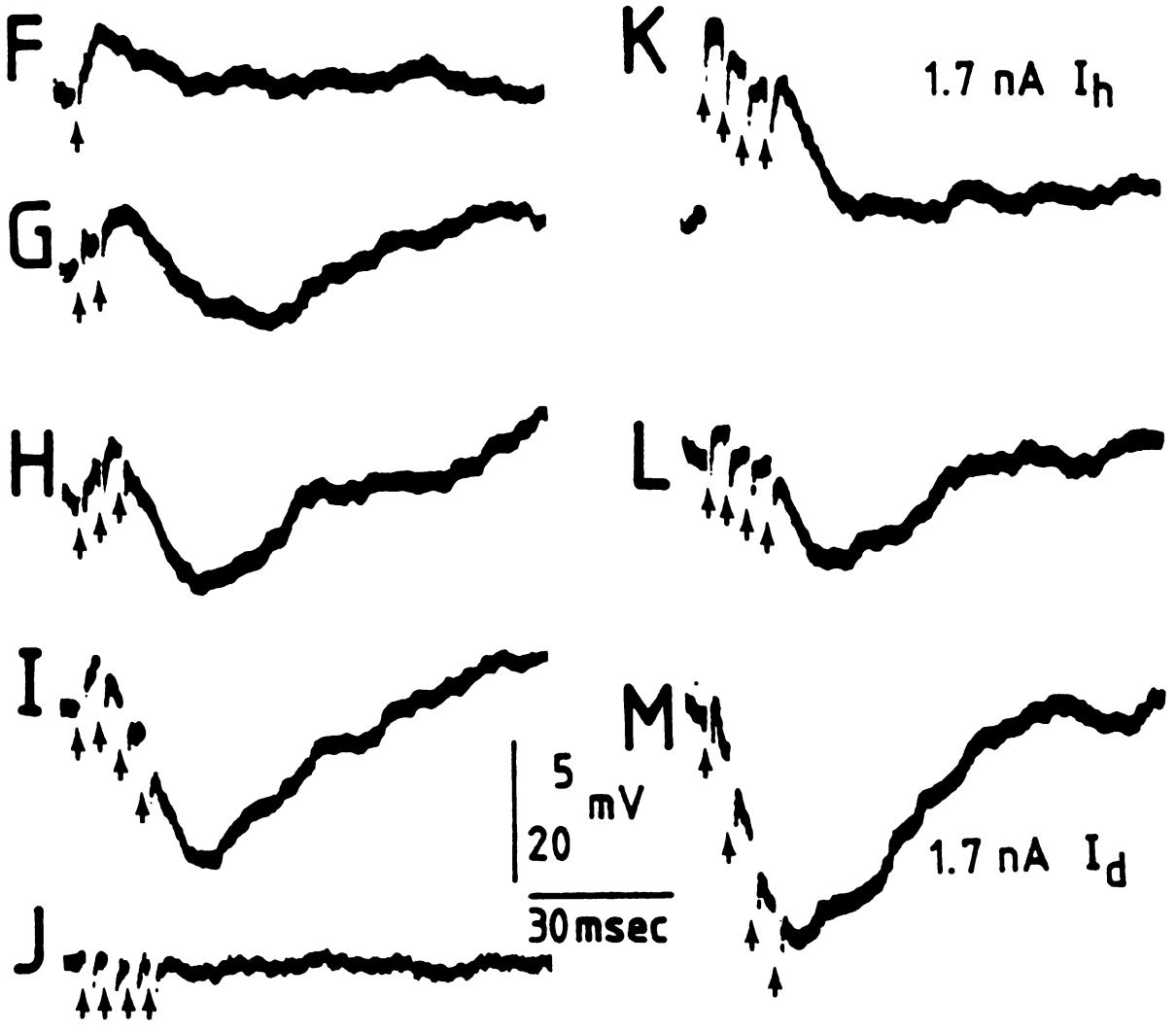


FIGURE 7

both the EPSP and hyperpolarizing potential are shown in the series of traces in Fig. 4E-H. The amplitude of the hyperpolarizing potential increased during intracellular injection of constant depolarizing current (Fig. 4G,H) and decreased in amplitude following hyperpolarizing current injection (Fig. 4E,F). The level of current required to reverse the polarity of the hyperpolarization was extrapolated from Fig. 4C, line b as 0.6 nA hyperpolarizing. The presence of the hyperpolarization in the absence of an AP, and the manner in which the response is affected by intracellular current injection indicates it may be an inhibitory postsynaptic potential (IPSP). The onset of the IPSP is masked by the EPSP preceeding it in the traces in Fig. 4A,B,E-H. The temporal relationship of these two responses makes the latency determination of the IPSP diffucult to resolve. The amplitude of the IPSP was incremental to increasing stimulus intentsity. The point where the trajectory of the response crossed resting potential appeared to exhibit a constant latency; however, it is impossible to determine, based on the present data, if the the IPSP is monosynaptic. The current-voltage plot for both the EPSP and IPSP is shown in Fig. 4C, the data points were obtained from the traces shown Fig. 4E-H. Sufficient hyperpolarizing current was passed into this neuron to trigger an AP from the EPSP. No after hyperpolarizing potential was apparent following the action potential when the membrane potential is artifically displaced to a more negative value.

In some neurons high frequency stimulation elicited IPSPs. A single local stimulus evoked an EPSP 2.6 mV in amplitude, with no apparent after-hyperpolarization (Fig. 7F). Increasing stimulus

frequency (from 1 to 250 Hz) elicited a hyperpolarization approximately 30 msec in duration (Fig. 7G-I). The amplitude of the hyperpolarizing potential increased with constant depolarizing current (1.7 nA, Fig. 7M) and reversed with constant hyperpolarizing current (1.7 nA, Fig. 7K) when compared to control (Fig. 7L), confirming the response is an IPSP.

Evidence of recurrent inhibition:

A pulsed injection of depolarizing current (2-10 msec duration, less than 2nA amplitude) was used trigger an AP in 23 neurons. The AP triggered from the depolarizing pulse was used to condition a test EPSP. Test EPSPs conditioned in this manner were shunted over ISIs less than 45 msec. Fig. 8A,B show an example of this phenomenon. The traces in Fig. 8A represent an unconditioned (control) EPSP evoked by local neostriatal stimulation, exhibiting an amplitude of 15.5 mV. When this response was conditioned by a AP triggered by a depolarizing pulse in Fig. 8B, the conditioned EPSP was reduced by 35% when compared to the control in Fig. 8A. The interval between onset of conditioning AP and test EPSP was 13.5 msec (Fig. 8B). Orthodromic APs were also shunted by a conditioning AP triggered by intracellular current injection as shown in Fig. 8C-F. Increasing neostriatal stimulation intensity in Fig. 8C triggered an AP 78 mV in amplitude and 1.8 msec in duration. This AP was shunted when it was conditioned by a AP triggered by intercellular current injection in Fig. 8D. The test EPSP was delivered 10 msec after the onset of the conditioning AP. The resulting EPSP in Fig. 8D measures 10 mV in amplitude. Test EPSP amplitude increased to 14.5 mV when the ISI is increased to 20

Figure 8. Recurrent inhibition follows AP triggered by depolarizing current pulse: DC and high gain AC records of a control test EPSP evoked by stimulation of neostriatum are shown in A. In B, reduction of test EPSP amplitude occurs when conditioned by a AP triggered from a intracellular depolarizing current pulse (1 nA, 8 msec depolarizing pulse). The time between AP onset and stimulus onset in B is 13.5 msec. Dotted lines in A and B represent control base line. Control AP triggered from neostriatal evoked EPSP in C is shunted in D when conditioned by an AP triggered by a depolarizing current pulse (1 nA, 8 msec depolarizing pulse). Time between conditioning AP onset and stimulus in D is 10 msec. E: At a 20 msec ISI the test EPSP amplitude increases but the action potential is blocked. F: At a ISI of 60 msec the inhibitory effect no persists. To verify that the shunting of neostriatal evoked EPSPs is not due to a long lasting conductance resulting from action potential currents two tests were performed and are demonstrated in G and H. Twin threshold depolarizing pulses trigger action potentials in G. No effect on the second action potential is observed at a 13.5 msec ISI, this may indicate AP currents are not responsible for shunting of test EPSPs and APs (i.e. B, E-F). Hyperpolarizing current pulses (1 nA, 5 msec) provide a measure of input resistance (H, lower trace). Input resistance is not reduced following a single AP triggered by a depolarizing pulse (1 nA, 10 msec; H, upper trace).

msec in Fig. 8E and finally triggered an AP at a 60msec ISI in Fig. 8F (the records in Fig. 8A-F were recorded from a single neuron).

In order to verify that inhibition of test neostriatal EPSPs is a result of synaptic potentials rather than voltage and/or current dependent conductance changes triggered by depolarization of the soma, two tests were performed. Twin threshold depolarizing current pulses were delivered through the recording electrode within the range of ISIs in which shunting of test EPSPs and APs normally occur. The trace in Fig. 8G shows no change in amplitude or duration of a test AP triggered by intracellular current injection when preceeded by an identical depolarizing pulse (4msec, 1 nA) which also triggered an AP. The interval between the onset of the first and second AP was 13.5 msec (Fig. 8G). In the second test, no change in conductance was observed following an AP triggered by direct activation of the soma as measured by passing a train of hyperpolarizing current pulses (1nA, 2 msec given at 2 msec intervals) as shown in Figure 8H.

Effect of GABA antagonists on the inhibition observed:

In order to examine the nature of a possible neurotransmitter involved in this inhibition, double shock tests were performed while the slices were perfused with medium containing the GABA antagonists bicuculline methiodide, picrotoxin, or penicillin-G. It was found that double shock, performed in this condition, resulted invariably in potentiation of test EPSP amplitude. The response in Fig. 9A shows a control double shock test performed in normal perfusion medium. In this neuron shunting of the test EPSP was demonstrated at an 11 msec ISI. In the same slice, following 20 min exposure to 0.05 mM

Figure 9. Inhibition of test EPSPs is blocked by GABA antagonist bicuculline methiodide: Control double shock in A demonstrates inhibition of the test EPSP (indicated by the second arrow). B: Exposing the same slice to perfusate containing bicuculline methiodide (0.05 mM) results in a 20% potentiation of the test EPSP amplitude when compared to the conditioning EPSP amplitude . The time course of the potentiation of the test EPSP is shown in the records in C. Test EPSP amplitude returns to the conditioning EPSP amplitude at a 28 msec ISI (indicated by the sixth arrow in C). D: Graphic representation of EPSP potentiation at various ISIs. Three concentrations of bicuculline methiodide were tested and are represented by the three lines. The three concentrations used were 0.01 mM (lower line, open diamonds), 0.05 mM (middle line, solid circles) and 0.10 mM (upper line, open circles). Each line represents data pooled from 10 neurons exposed to each concentration. Inhibition of test EPSPs in control perfusate is shown for comparison in the lower portion of the graph in D. Each point represents either mean per cent potentiation or inhibition at that ISI +/- 1 msec, vertical bars indicate standard errors of the means. Potentiation of the test response in E, during exposure to perfusate containing 0.05 mM bicuculline methiodide, is abolished in F following 1 hr wash with control perfusate. The records in E and F were recorded from the same neuron. High gain AC and DC records (upper and lower traces) are shown in each case. Calibration in B applies to all traces.

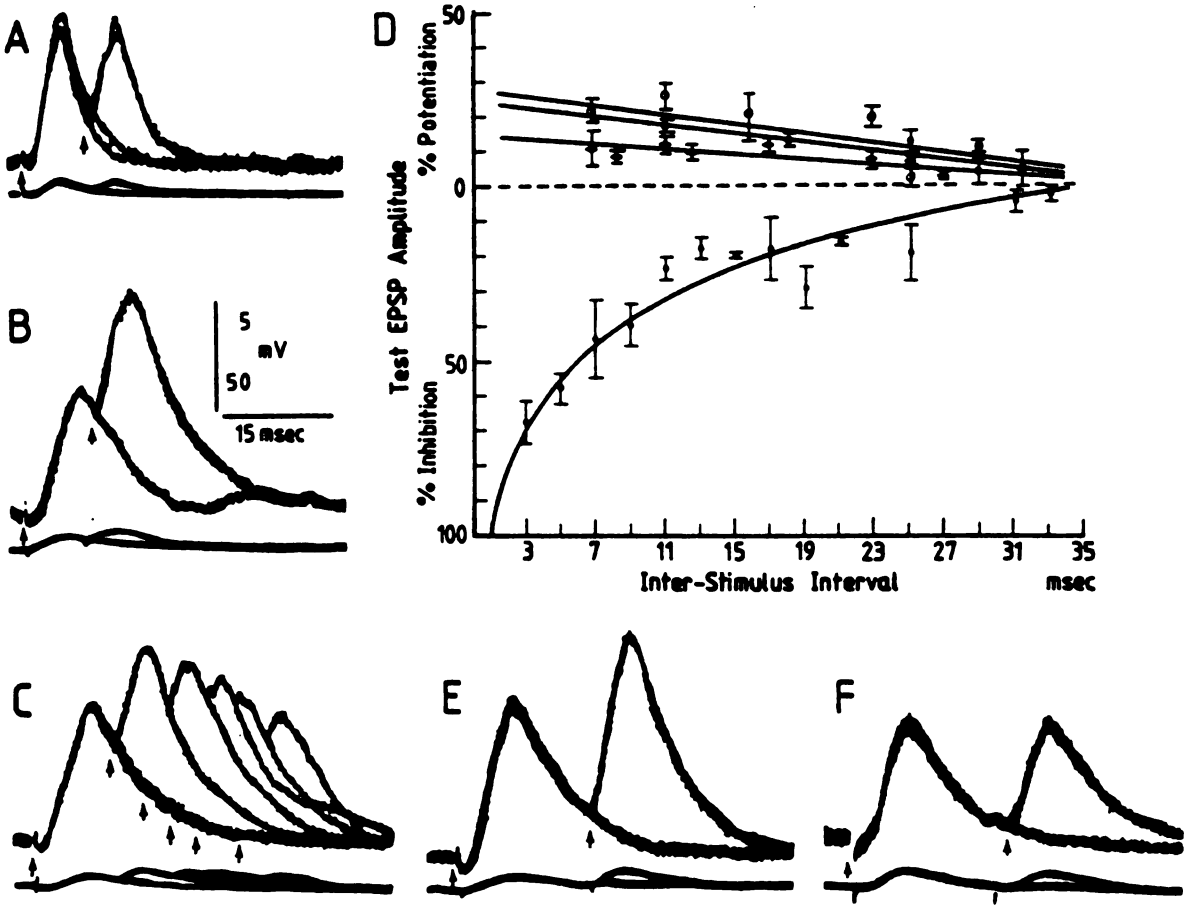

A
B
5
mV
50
15 msec
C
D
% Potentiation
0
% Inhibition
50
100
Test EPSP Amplitude
3
7
11
15
19
23
27
31
35
Inter-Stimulus Interval
msec
E
F

FIGURE 9

bicuculline methiodide, test EPSP amplitude was potentiated by 22% at the same ISI when compared to the conditioning response in Fig. 9B. The time course of the potentiation is shown in the traces in Fig.9C. The test EPSP amplitude in Fig. 9C returned to control level at an ISI of 37 msec. The potentiation of test EPSPs was also observed in medium containing 0.01mM and 0.10 mM bicuculline methiodide (Fig. 9D). The dose dependence of the three concentrations of bicuclline methiodide on the potentiation of test EPSP amplitude is statistically significant ($F=22.7$, $df=4,27$, $p \leq 0.01$)(Table 1B) when measured at an 11msec ISI. The potentiation of the amplitude of the test EPSP illustrated in the traces in Fig. 9E which occured in medium containing bicuculline methiodide (0.10 mM), was abolished in the same neuron following 1hr. wash in control medium (Fig. 9F). For all concentrations of bicuculline methiodide tested, EPSP 1/2 width values were increased when compared to control values (Table 1A). This effect can be seen by comparing the traces in Fig.9A to those in Fig. 9B. The increase in 1/2 width values in neurons exposed to GABA antagonists may reflect the absence of a conductance change that is normally present in control EPSPs. The effect of IPSPs on the 1/2 width of EPSPs is similar, and may be observed in Fig. 9A,B.

The GABA antagonist picrotoxin was also tested, and was observed to have a similar effect on test EPSP amplitude and 1/2 width. Prior to addition of picrotoxin to the perfusate, double shock resulted in inhibition of test EPSPs (Fig. 10A). In the same neuron, following addition of 0.01 mM picrotoxin to perfusate, double shock resulted in potentiation rather than inhibition of test EPSPs (Fig. 10B). At a 9 msec ISI (second arrow in Fig. 10B) test EPSP amplitude was 13

Figure 10. Inhibition is also blocked by the gaba antagonists pictrotoxin ans penicillin-G. Control double shock in A resusts in inhibition of test EPSPs. Potentiation of the test response in B occurs following 30 min. exposure to perfusate containing 0.01mM picrotoxin. Test response amplitude returns to control EPSP amplitude at a 35 msec ISI. C: In another neuron, double shock results in potentiation of the test EPSP to trigger an action potential. Test response amplitude returns to control levels at a 55 msec ISI, and action potentials are no longer triggered (in C). The records in D show potentiation of test EPSPs also occurs in perfusate containing penicillin-G (200 I.U./ml). The potentiation exhibits a time course of 35 msec (D). E: In the same neuron, when stimulus intensity is increased potentiation of the test response is pbserved at a 40 msec ISI. At a 25 msec ISI, potentiation causes an action potential to be triggered off the test response (in F). High gain AC and DC (upper and lower traces) are shown. Calibration in C applies to all traces.

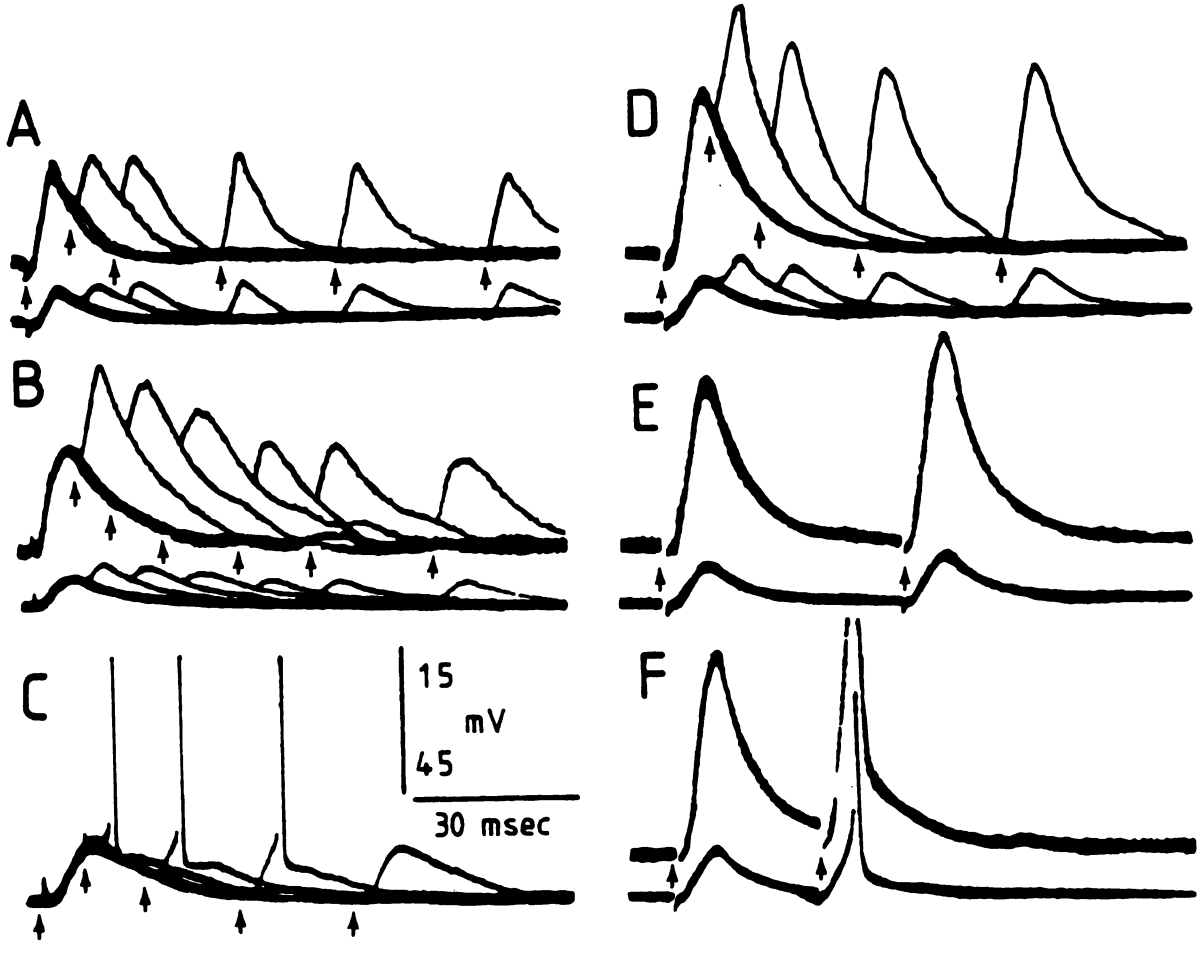


FIGURE 10

TABLE 1

A. mean 1/2 Width in msec

Control EPSPs	X= 6.82 (n=63, SD 1.25)
Bicuculline	
0.10 mM	X=11.14 (n=21, SD 2.56)
0.05 mM	X= 9.23 (n= 9, SD 1.44)
0.01 mM	X= 9.82 (n=17, SD 2.86)
Picrotoxin	
0.10 mM	X=10.67 (n= 9, SD 0.58)
0.05 mM	X=15.20 (n= 7, SD 3.42)
0.01 mM	X=12.40 (n=10, SD 2.07)

B. mean % of control EPSP amplitude at an 11 msec ISI

Control EPSP	X= 0.76 (n=14, SD 0.20)
Bicuculline	
0.10 mM	X= 1.11 (n=15, SD 0.09)
0.05 mM	X= 1.09 (n= 9, SD 0.10)
0.01 mM	X= 1.07 (n=11, SD 0.07)
Picrotoxin	
0.10 mM	X= 1.22 (n= 9, SD 0.16)
0.05 mM	X= 1.20 (n= 7, SD 0.11)
0.01 mM	X= 1.13 (n=10, SD 0.07)

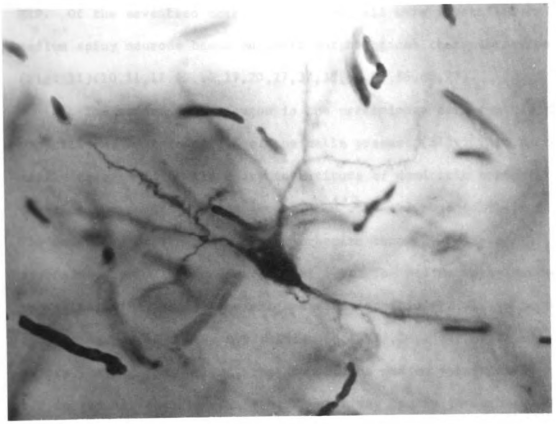
mV, a 23% potentiation as compared to the conditioning EPSP. Test EPSP amplitude returned to control amplitude at ISIs greater than 25 msec (Fig. 10B). In another neuron, test EPSPs reached spike threshold to trigger APs over the same time course (Fig. 10C). The potentiation of test EPSPs varied with the concentration of picrotoxin used (0.01-0.10 mM), and was shown to be statistically significant at an 11 msec ISI ($F=31.66, df=3,20, p \leq 0.01$)(Table 1B). The 1/2 width of EPSPs in medium containg picrotoxin increased by 36-45% dependent on the dose (Table 1A). The potency of picrotoxin was observed to be greater than bicuculline methiodide over the doses tested (0.01-0.10 mM)(Table 1A).

The effect of penicillin-G on the inhibition was observed to mimic those of bicuculline methiodide and picrotoxin. Fig. 10D-F show the response of a single neuron to double shock in a slice exposed to perfusate containing 2000 units/ml penicillin-G. A test neostriatal EPSP is potentiated 10% (second arrow Fig. 10D) when it was preceeded 11.5 msec by a conditioning EPSP (first arrow Fig. 10D). Test EPSP amplitude returns to conditioning EPSP amplitude at a 37 msec ISI (fifth arrow in Fig. 10D). In the same neuron, increasing neostriatal stimulus intensity elicited a test EPSP potentiated 30% at a 40 msec ISI. At the same stimulus intensity, test EPSP amplitude reached threshold to trigger an AP when the ISI was shortened to 30 msec.

Identification of neurons which exhibit inhibition:

Neurons which exhibited the ability to shunt test EPSPs following local stimulation in double shock tests, or as a result of direct intracellular depolarizition, were intracellularly labelled with

Figure 11. Photomicrograph and drawing tube reconstruction of HRP injected neuron which exhibited the ability to shunt neostriatal test EPSPs. Neuron displays morphological characteristics of neostriatal common medium spiny cell type.



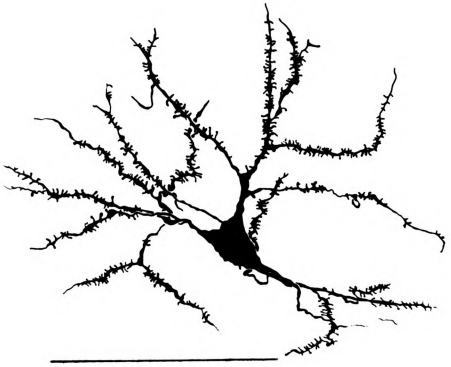


FIGURE 11

HRP. Of the seventeen neurons recovered, all were identified as medium spiny neurons based on their morphological characteristics (Fig. 11)(10,11,12,13,14,19,20,27,37,38,47,52,56,60,79).

The medium spiny neuron is the predominate cell type in the neostriatum, comprising 96% of the cells present (37). This neuron is easily recognized by its heavy investiture of dendritic spines, covering the dendrites from about 20 um from the soma to their tips. The dendrites of the medium spiny arborize through a sphere approximately 500 um in diameter (41,64). The medium spiny neuron is known, based on both anatomical and electrophysiological, to be the projection neuron of the neostriatum (8,10,23,24,26,41,64,72,73). Before leaving the nucleus, the axon of the medium spiny neuron gives off an extensive collateral plexus which may ramify through the same space occuppied by its dendrites, in some cases to contact the dendrites of neighboring medium spiny neurons (79).

Chapter 4. DISCUSSION

Evidence for two forms of recurrent inhibition have been presented. The first is a special form of recurrent inhibition envolving axon collaterals and dendrites of a single neostriatal neuron. This form of inhibition is elicited by direct intracellular depolarization of a single neuron. In the neostriatum in vivo, inhibition evoked through, and as a result of activation of a single neuron is proposed to be mediated by autaptic synapses (58). The second type of recurrent inhibition is a more general form involving the axon collaterals and dendrites of neighboring neostriatal neurons.

The mutual inhibition of neighboring neostriatal neurons is evoked by local stimulation of the neostriatum, demonstrated as a reduction in amplitude of test EPSPs in double shock experiments. Both forms of recurrent inhibition are illustrated in Fig. 12.

Neurons which demonstrated both forms of inhibition were intracellularly labelled with HRP. All neurons recovered following HRP injection, exhibited morphological charactertics of the medium spiny neuron (10,11,12,13,14,19,20,27,37,38,47,52,56,60,79). Because of the preponderance of this cell type in the rat neostriatum (4,11,12,47,52,56,60) and the striking physiological similarity in all the recorded neurons, the physiological data presented here was most likely acquired from medium spiny neurons.

In addition to the medium sized spiny neuron there are at

least 5 other identified cell types present in the neostriatum: The large aspiny neuron comprising less than 1% of the total neuron population in the neostriatum; three other medium sized cells together making up 3%; and the small neuron comprising the remaining 1% (13,37).

The cellular distrubution in the neostriatum of man, monkey, cat and rat, unlike hippocampal or cerebral cortex, exhibit a homogenous orginization (1,2,11,12,14,19,20,27,36,37,38,47,56,60,61). The lack of a strict geometric or laminar distribution of the neurons and their associated afferents and efferents preclude selective stimulation of a specific group of fibers or neurons in the slice preparation. However, the portion of those efferent structures remaining in the slice are physically isolated from their nucleus of orgin. This arrangement allows the investigator to record from a neostriatum in which extrinsic loop circuits are open. Therefore, the responses recorded extracellularly or intracellularly following local stimulation of neostriatum reflect the interaction between intrinsic neostriatal elements and their related afferents and respective efferents without the involvement of circuits extrinsic to neostriatum.

Extracellular Analysis:

The local stimulation of neostriatum elicited a field potential consisting of two components N-1, and N-2. The susceptibility of the N-2 component to increasing stimulus frequency and alterations in divalent cation concentration (i.e. [Mg+2], [Ca+2]) partially confirms previous reports that a portion of this component

represents underlying synaptic activity in the slice. In contrast, the N-1 component is unaffected by these manipulations indicating the response is non-synaptic and may refelect AP currents of presynaptic fibers in the vicinity of the recording electrode. In double shock experiments, performed during extracellular recording it was observed that shunting of the N-2 portion occured over a 20-30 msec time course (Fig. 3). Shunting of the N-2 component only occured when high stimulus intensity was delivered to the slice. The lack of shunting during sub-maximal stimulation may indicate that the medium spiny neurons are receviving insufficient excitatory input to generate APs. If the inhibition is mediated by axon collaterals of GABAergic projection neurons, as proposed in the present investigation, sufficient activation of these inhibitory fibers would be required to effectively block cellular activity. Because of the preponderance of the medium spiny neuron in the neostriatum of the rat (4,11,12,47,52,56,60), the synaptic portion of the field may be an indirect indication of the activity of this cell type. Increasing stimulus intensity would result in the activation of a greater number of afferent fibers in the slice. If the afferent fibers are excitatory, this could result in depolarization of the medium spiny neurons and subsequent activation of their axon collaterals resulting in the inhibition of neurons receiving their input. Also, increasing stimulus intensity should lead to direct activation of the axons and associated collaterals of medium spiny neurons. Direct activation of neostriatal efferent axons would also elicit inhibition in the neostriatum, following local stimulation.

In order to identify the sorce of N-2 component of the field

potential, experiments have been performed in which attempts to isolate the neostriatum, prior to the preparation of the slice, from its extrinsic inputs by either surgical or by pharmacological methods (53,54). No changes in the extracellular field potential were observed in these experiments (53,54), however, at no time were all the extrinsic inputs to the neostriatum abolished simultaneously in the same slice. From this observation it was concluded by Misgled and associates that the synaptic events underlying the N-2 portion of the field potential are generated by neurons intrinsic to the neostriatum. The neurotransmitter mediating this portion of the field potential was also investigated by Misgeld (53,54,55). Based on extracellular data alone, it was proposed that the N-2 component reflects excitatory conductance changes, mediated by acetylcholine (53,54,55). In the same group of experiments, no effect of GABA antagonists on the N-2 portion of the field potential was detected extracellularly. This observation lead the investigator to conclude that GABA, the apparent neurotransmitter utilized by the medium spiny neuron (67), is not excerting its influence in the neostriatum (54,55). It was found by the present investigator that changes in intracellularly recorded responses following exposure to GABA antagonists were not reflected in the extracellular field potential. That is, neurons in which inhibition was blocked, resulting in potentiation rather than the inhibition of test EPSPs in double shock experiments, no consistent effect was observed on test N-2 field potentials. This may indicate that the mass potentials recorded extracellularly do not give an accurate indication of conductance changes occuring in an individual neuron. Activation of the terminal

portions of known extrinsic excitatory afferents that remain intact in the slice are the most probable sorce of excitation in this preparation. Although a portion of the response contributing to the N-2 component of the field potential may be attributed to cholinergic inter-neurons, it not plausible based solely on extracellular data, that they alone generate this response.

Previous investigators (53,54,55) propose that the non-synaptic portion, or N-1 component of field potential is generated by antidromic activation of neurons. Local stimulation of neostriatum should also activate neostriatal efferents, thus leading to the antidromic activation of the impaled neuron. Surprisingly few APs were judged, in the present investigation, to be antidromic, based on collision experiments performed intracellularly. A low incidence of antidromicity among medium spiny neurons has been demonstrated, in vivo, following stimulation of substantia nigra (22,43). The lack of antidromic activation in vivo is proposed to be due to a low safety factor encountered by the AP at collateral branch points, or the initial segment of the axon of the medium spiny neuron (41). The lack of antidromic activation of medium spiny neurons, in vitro, may be a combination of these factors, and may also involve the relative location of the axon of the impaled neuron with the stimulating electrodes. If the axon of the impaled neuron was located in an area receiving subthreshold levels of stimulating current, no spike would be triggered in that fiber. In the present study, during extracellular recording, when stimulating electrodes were visually positioned in a fiber bundle of the internal capsule, the amplitude of the N-1 potential increased as the recording electrode was moved

toward that fiber bundle. Although indirect, this observation may indicate the N-1 component of the field potential reflects the activity of fibers remaining in the slice, rather than the antidromic activation of neurons, as proposed by Misgeld et al (53,54). It was concluded from the present study that the field potential, recorded following local stimulation of the neostriatum in vitro, does not give a reliable indication of the activity of the neurons in the slice This would indicate, until better guidelines for interpreting the field potential are determined, that further investigation of the neostriatum in vitro must be performed using intracellular recording techniques.

Intracellular Analysis

EXCITATION:

The initial response recorded intracellularly following local stimulation of neostriatum is a depolarization. This depolarizing potential was determined to be EPSP based on intracellular current injection (Fig. 4E-H). The amplitude of the depolarization, shown in Fig. 4, increased during intracellular injection of constant hyperpolarizing current, and decreased during injection of constant depolarizing current, which may indicate this response is an EPSP. However, the amplitude of the depolarization was reduced to its reversal potential by intracellular injection of 0.25 nA of depolarizing current. This value was extrapolated form Fig. 4C, curve a. During the present investigation the input resistance of

neostriatal neurons maintained in vitro varied between 35 and 57 Mohm. The maximum depolarization that could be recorded by injection of 0.25 nA of current would be between 9 and 14 mV above resting potential. These levels of depolarization are well below the equilbrium potential of an EPSP generated by a combined conductance increase to potassium and sodium ions (34). The low level of current required to reverse this response, may indicate the depolarization is produced by a non-specific increase in conductance to a variety of ions (i.e. K+, Na+, Cl-); or , the response may indeed be an EPSP generated by increased gNa+ and gK+, immediately followed by an IPSP, generated by increased gCl-. The hyperpolarizaion that follows the initial depolarization in Fig. 4A,B,E-H increased in amplitude during intracellular injection of constant depolarizing current and decreased in amplitude during injection of constant hyperpolarizing current (Fig. 4E-H). The amount of hyperpolarizing current required to reverse this component of the response was extrapolated from Fig. 4C, curve B, as 0.6 nA. The level of hyperpolarization resulting from the injection of 0.6 nA of current (i.e. 21 to 34 mV below resting potential) is within the range of the reversal potential of an IPSP generated by increased gCl- and gK+ (34). Due to the temporal relationship of the EPSP and the IPSP, it is impossible to accurately determine the onset of the IPSP from the data presented here. However, the ease with which the initial depolarization was reversed, and the presence of a hyperpolarization following this response, may indicate the depolarization is an EPSP, the conductance change of which overlaps with the IPSP following it.

EPSPs responded to incremental steps of stimulus intensity

with increasing amplitude and invarinant latency indicating monosynaptic activation (Fig. 5). Convergent monosynaptic EPSPs are elicited in medium spiny neurons in vivo, following stimulation of cerebral cortex, substantia nigra, dorsal raphe, and intralaminar thalamic nuclei (40,43,44,59,65,76,77). The neurotransmitters envolved in this excitation in vivo remain to be clearly defined. The transmitter substance released from cortical fibers terminating in neostriatum are not completely understood. Apparently, glutamic acid may function as the transmitter in the pathway, and be responsible for the excitation observed (16,51,74). A portion of the fibers comprising the nigrostriatal pathway have been shown to utilize dopamine as their neurostransmitter (17,18). Although there is controversy surrounding the effect of dopamine on neostriatal neurons, a number of investigations show an inital excitation recorded intracellulsrly in neostriatum following stimulation of substantia nigra. This observation is supported by a limited series of studies which detect a depolarization in neostriatal neurons during iontophoretic application of dopamine (6,30,41). Increasing evidence indicates the neurotransmitter serotonin as the substance liberated by fibers from the dorsal raphe terminating in the neostriatum (27). Monosynaptic EPSPs are recorded in medium spiny neurons following stimulation of dorsal raphe and recently this response was shown to be serotonergic (59,76,77). At this time the neurotransmitter involved in the excitation elicited by thalamic stimulation awaits identification.

In vitro, the initial excitation recorded following local stimulation is most likely a result of activation of these excitatory

fibers remaining in the slice. Previous attempts to isolate the sorce of excitation propose the response is intrinsic to neostriatum and is mediated by cholinergic interneurons (53,54,55). Acetylcholinestrase (AChE) as used as a marker for acetylcholine, has been demonstrated histochemically in neostriatum. In the neostriatum of DFP (diisopropylflurophosphate) treated animals, the large aspiny neuron is unique in its intense staining for AChE (9). The small neuron of neostriatum stained lightly for AChE, and the medium sized cells ,in general, did not stain (9). This finding is supported by more recent immunohistochemical studies studies obtained with antibodies directed against purified choline acetyltranferase (CAT), a more specific marker of cholinergic neurons than AChE (39). The CAT containing neurons were idenitified as large aspiny cells which possess few dendrites and few dendritic spines (39). There are discrepincies between recent CAT studies and eariler investigations utilizing the same technique that suggested that some medium sized neurons in neostriatum also contain AChE (28,50). However, it is generally agreed that the large aspiny neurons, rather than the medium sized neurons, are the cholinergic interneurons of the neostriatum (45). In view of the small number of large aspiny cell types in the neostriatum in situ it may follow that the number of aspiny cells in the slice and their associated dendrites and axons remaining in the slice would be incomplete. If it is assumed that the large aspiny neuron is the major sorce of cholinergic excitation in the neostriatum, and the sole sorce of excitation in the slice preparation as proposed by Misgeld and associates, it would be required of the aspiny neuron axon collateral plexus to have an extensive ramification throughout the

nucleus to make synaptic contact with all medium spiny neurons of the neostriatum.

INHIBITION:

Evidence of inhibition is shown intracellularly to occur in the neostriatal slice preparation in three forms.

The first form is demonstrated as a reduction in test EPSP amplitude when it is preceeded by a conditioning EPSP (Fig. 6). The lack of summation of EPSPs in double shock may be interperuted as a indication of inhibition. The shunting of test EPSPs occured in all neurons in the test group. Of these neurons 83% exhibited shunting in the absence of a detectable inhibitory conductance change. The ability of neostriatal neurons to shunt orthodromic test responses in the absence of a detectable inhibitory conductance change confirms both previous in vivo and in vitro data (7,43,44,55). Test EPSP amplitude returned to control amplitude at ISIs of 30-45 msec. Test APs were also blocked by a conditioning orthodromic stimulation over the same time course (Fig. 6B). In vitro, shunting of test EPSPs following local stimulation of neostriatum may be indirect evidence of a general form of mutual inhibition, involving the convergence of inhibitory input from one or more neurons onto a single neuron.

The second form of inhibition is demonstrated, in vitro, as a reduction in amplitude of a test neostriatal evoked EPSP when it is preceded from 10 to 50 msec by a AP triggered at the recording site by intracellular current injection (Fig.8A, B). The AP thus triggered would propagate throughout the axon collateral plexus of the neuron in turn activating inhibitory terminals contacting the dendrites of the

depolarized neuron. The subsequent inhibition effectively shunts neostriatal evoked EPSPs and APs. The relatively short (less than 50 msec) duration of the inhibition elicited by depolarization of the soma, matches the time course of recurrent inhibition demonstrated in neostriatal medium spiny neurons in vivo (58). Inhibition of test orthodromic responses in neostriatal neurons following and as a direct result of the their own activation, is a special type of recurrent inhibition, proposed by Park et al (58) to be mediated in neostriatum in vivo by autaptic synapses, a proposal experimental findings presented here support.

IPSPs, the third form of inhibition in neostriatum in vitro, were present in a small percentage of the neurons tested. IPSPs were always preceeded by EPSPs and are present in the absence of an AP (Fig. 4). Due to the temporal relation of the EPSP with the IPSP, it is impossible to measure the onset of the inhibitory response from the present data. Because of this, it is diffucult to determine if the IPSP is mediated by a monosynaptic or a polysynaptic pathway. The point at which the trajectory of the inhibitory response crosses the resting potential (Fig. 4A, B) may exhibit a constant latency, however it is difficult to resolve at this time base. If the IPSP is indeed monosynaptic, high frequency potentiation of this response (Fig. 7) may refelct the temporal summation of unitary IPSPs rather than the temporal facilitation of a polysynaptic pathway.

The duration of the IPSPs was observed to be similar to the time course of shunting of test EPSPs in double shock and inhibition elicited by direct activation of soma (i.e. less than 50 msec). The presence of a post-synaptic conductance change, the amplitude of which

increased during application of constant depolarizing current and decreased in amplitude in response to hyperpolarizing current, indicate the inhibition is post-synaptic rather than a pre-synaptic inhibition or disfacilitation of a tonic excitatory input (34).

The duration of the IPSPs and the time course of the shunting of test EPSPs in the absence of a measurable conductance are quite similar (i.e. less than 50 msec) suggesting that the shunting is mediated by a similar inhibitory mechanism as that underlying the IPSP. No prolonged period of increased conductance was observed to follow APs triggered by either orthodromic stimulation or by intracellular current injection, which may indicate a non-synaptic mechanism is responsible for shunting of test EPSPs. This is consistent with our finding that a test AP triggered from a threshold depolarizing current pulse is not shunted over ISIs which normally shunt test orthodromic EPSPs or APs following direct activation of the soma. The inability of the electrode to detect a conductance change following depolarization of the soma does not preclude the existance of conductance changes occuring at a site electrotonically isolated from the recording site (66,71). Depolarization induced voltage and/or conductance changes are known to regulate firing patterns of neurons (3,5,15,29,31,45,71), and to mimic the effect of an IPSP (33), however the inability of the recording electrode to detect such a change makes judgement regarding its involement difficult at best. The shunting in medium spiny neurons following an AP triggered by direct depolarization of the soma is most likely the result of activation of inhibitory axon collaterals rather than a non-synaptic mechanism. The absence of an IPSP following the triggering of an AP

at the soma may be due to the paucity of autaptic synapses recuring on the medium spiny neuron, and due to the electronic location of those present. The presence of IPSPs following local stimulation of neostriatum lends indirect support to the hypothesis that the number of inhibitory inputs received from neighboring medium spiny neurons is greater than the number of autaptic synapses normally present. Although indirect this experimental finding may indicate that mutual inhibition among medium spiny neurons is the predominate form of inhibition occuring in the neostriatm, and the mechanism mediating the short term inhibition in vitro.

GABA antagonists:

Immunohistochemical and electron-microscopic investigation of glutamate decarboxylase (GAD) content of the neostriatum, an enzyme involved in GABA synthesis, show terminals and cell bodies of spiny neurons contain this enzyme (67). Ultrastructurally, the GAD containg neurons resemble those of the common medium spiny neuron (78). The output of the neostriatum is carried by fibers of medium sized neurons (10,23,24,26,41,64,72,73), these fibers are inhibitory at their termination sites globus pallidus and substantia nigra, and this inhibition is blocked by GABA antagonists (63,80,81). In the present investigation, neurons which exhibited inhibition following either local stimulation or direct intracellular activation were labelled with HRP and identified as medium spiny neurons based on their morphological characteristics (Fig. 11)(10,11,12,13,14,19,20,27,37, 38,47,52,56,60,79). The GABA antagonists bicuculline and picrotoxin have been shown to also block the recurrent inhibition of medium spiny

neurons in vivo (58).

The effect of GABA antagonists on shunting of test EPSPs in double shock indicates GABA, identified as the possible neurotransmitter of the medium spiny neuron (67), as the neurotransmitter responsible for the short term inhibition in vitro. Although these antagonists utilize different postsynaptic mechanisms to block the effect of GABA (61,70), there effect on the short duration inhibition was identical (Fig. 9,10). Bicuculline, picrotoxin, and penicillin-G produced potentiation rather than inhibition of the test response in double shock. In some cases APs were triggered from potentiated EPSPs, however no bursting or rythmic firing was detected. In addition to potentiation, broadening of EPSPs was also observed, indicating the blockade, or absence of a inhibitory conductance change normally present in control cases. The potentiation of EPSPs may reflect the normal subthreshold postsynaptic response of a neuron that is not receiving a synchronous excitatory afferent input. The method of stimulation generally used in physiology experiments results in synchronous activation of a specific group of afferents, or the synchronous stimulation of different groups of afferents. The response elicited following either form of stimulation may not be a accurate representation of normally occuring postsynaptic responses. The potentiation of temporally related EPSPs may exemplify a normal postsynaptic activity of a medium spiny neuron which is receiving no inhibitory input from itself or from neighboring neurons, and may indicate a form of postsynaptic integration normally performed by this neuron type.

The sequence of excitation and inhibition recorded

Figure 12. Summary Diagram: Schematic representation of a simple neuronal circuit consistent with our findings is shown. Orthodromic excitation is inhibited by autaptic or mutual recurrent inhibition. This inhibition is mediated by axon collaterals of medium spiny neurons. Closed triangles represent inhibitory synapes, while open triangles represent excitatory synapes.

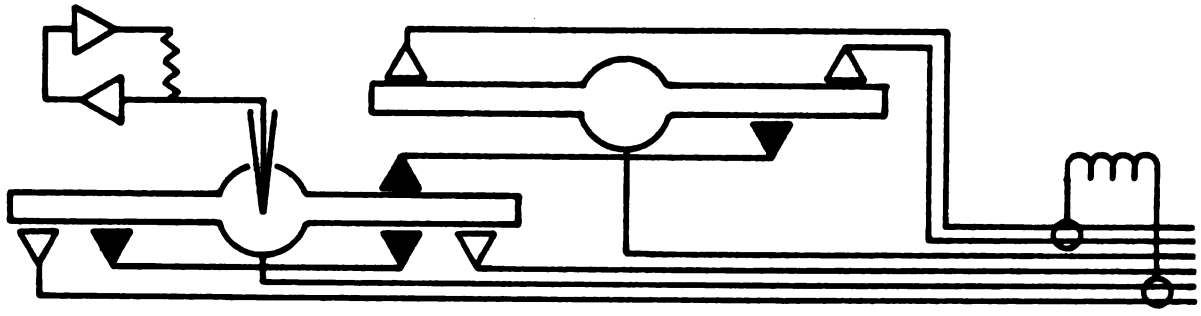


FIGURE 12

intracellularly following local stimulation of neostriatum may be explained if the circuit in Fig. 12 is considered. Local bipolar stimulation of the neostriatum is indiscrete, activating both afferent and efferent structures simultaneously (to control against direct stimulation of an impaled neuron, the distance between recording and stimulating sites was always greater than 0.50 mm). Local activation of excitatory afferents in turn elicits a monosynaptic EPSP in the impaled neuron. Although the EPSP is subthreshold, this does not rule out the the possibility that neighboring medium spiny neurons are deopolarzied to threshold levels. The axon collaterals of medium spiny cells have been shown to make synaptic contact with neighboring medium spiny neurons (79). If a sufficent number of neighboring medium spiny neurons making contact with the impaled neuron were activated, the resulting IPSP could effectively shunt the subsequent test EPSP. The shunting of the test EPSP would occur in the absence of an AP, a proposal supported by our data. In addition, this arrangement could result in the EPSP-IPSP sequence observed. This response would also be elicited by the activation of a inhibitory local circuit neuron, or inhibitory neurons other than the medium spiny, however the techniques used do not allow delineation to be made. The presence of an IPSP following a subthreshold EPSP may indicate subthreshold electrotonic potentials in neighboring medium spiny neurons underly the inhibition in the postsynaptic neuron. The presence of dendrodendritic synapses have been reported in the neostriatum, however their occurence is rare (61). Examples of presynaptic dendrites have been reported in monkey, cat and rat (37,60,61). This structure apparently is associated with processes of

aspiny III cells (61). However, an extensive electron-microscopic study of the synaptic terminals contacting medium spiny neurons in rat neostriatum intracellularly labeled with HRP show only axodendritic and axosomatic synapses present (79).

Mutual inhibition of neighboring medium spiny neurons may be responsible for the general type of short term inhibition elicited by local stimulation of neostriatum in vitro. Where the inhibition a result of autaptic (75) synapses in general, activation of the soma preceeding the inhibition would be required.

Initial investigation of neostriatum in vitro performed by Misgeld et al. (53,54) detect only excitation following local stimulation. Lack of a inhibitory component in the responses observed in these early investigations may be attributed to the [Ca++] of the perfusate (53,54). Facilitation rather than inhibition of test EPSPs in double shock was observed in slices exposed to medium containg 1.2 mM Ca++ (53,54). This is in constrast to more recent data acquired from slices perfused with medium containg 2.4 mM Ca++ which demonstrate a lack of facilitation of test EPSPs in double shock (55). However, the lack of facilitation or linear summation of test EPSPs was not interpreted as a indication of inhibition (55). Presumablily the interpretation was based on the absence of a measurable inhibitory conductance change superimposed on the EPSP (55). Although there is contraversy surrounding the presence or absence of a Ca++ activated, K+ dependent GABA release mechanism operating in the neostriatium (57), it has been proposed to function in other preparations (33). Preliminary work investigating the effect of [Ca++] on the shunting phenomenon indicate the absence of inhibition in neostriatal neurons

in double shock paridigm in medium containing less than 1.7 mM Ca++ (personal observation). However, at this time we have no conclusive data to explain the relationship between [Ca++] and inhibition in the neostriatal slice preparation.

Concluding Remarks:

In conclusion, the present in vitro data demonstrate a pattern of excitation and inhibition is elicited following local stimulation of neostriatum or following and as a result of direct activation of a single neostriatal neuron. The inhibition observed exhibits a relatively short time course (i.e. less than 50 msec), similar to that reported for recurrent inhibition in vivo. The experimental findings presented here support previous reports that the inhibition is mediated by axon collaterals of neostriatal neurons, and is therefore intrinsic to that nucleus (58).

The evidence presented here establishes recurrent GABAergic inhibition as the mechanism underlying the short term inhibition in the neostriatum in vitro, and therefore delineates one and perhaps the major function of the extensive axon collateral network possessed by the medium spiny neuron. The short duration inhibition is intrinsic to the neostriatum, the existance of longer duration inhibition observed in vivo following stimulation of either local structures or extrinsic afferents (7,21,35,40,43,44,48,49,65,76,77), suggests that the long duration is due to circuits extrinsic to the neostriatum.

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