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The Role of GABAergic Transmission in Mediation of Striatal Local Field Potentials (LFPs)

by

DREW SEISCIO

Under the Direction of Dr. Andrew Clancy

Abstract

In the present study, electrophysiological and behavioral effects of compromised Gama-Aminobutyric Acid (GABAergic) transmission were investigated in adult Rhesus macaque monkeys (N=2). GABAergic transmission was perturbed in the putamen by administration of a $GABA_A$ receptor antagonist, gabazine (10 and 500 μM), via a microdialysis-local field potential (MD-LFP) probe. Resultant changes in striatal local field potentials (LFPs) were measured as an assay of synchrony.

Gabazine perfusion evoked discrete large amplitude spikes in LFPs in all subjects, and the frequency and shape of individual spikes were concentration-dependent. Pre-treatment with the $GABA_A$ receptor agonist, muscimol (100 μM) blocked the gabazine-induced events, confirming a role for $GABA_A$ receptors in the effects. Behavioral manifestations of gabazine treatment were observed only at the maximum concentration. Unusual facial movements suggested aberrant electrical activity was propagated from striatum to motor cortex, perhaps via reentrant circuits. These results support a role for GABAergic transmission in segregation of striatal circuits.

INDEX WORDS: local field potentials, microdialysis-local field potential probe, gabazine, synchronized oscillatory discharge, Rhesus macaque

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by

Drew Seiscio

A Thesis Submitted in Partial Fulfillment of the Requirement for the Degree of
Master of Science
in the College of Arts and Sciences
Georgia State University

2008

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2008

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List of Abbreviations

AHP =	Afterhyperpolarization
Bg =	Basal Ganglia
DBS =	Deep Brain Stimulation
EEG =	Electroencephalography
Fs =	Fast-Spiking (neurons)
GABA =	Gama-Aminobutyric Acid
GPe =	Globus Pallidus External (segment)
GPi =	Globus Pallidus Internal (segment)
HD =	Huntington's Disease
LFP =	Local Field Potential
MD-LFP =	Microdialysis-Local field Potential (probe)
MSN =	Medium Spiny Neuron
PD =	Parkinson's Disease
SEs =	Synchronized Events
SMA =	Supplementary Motor Area
SOD =	Synchronized Oscillatory Discharge
SNc =	Substantia Nigra Pars Compacta
SNr =	Substantia Nigra Pars Reticulata
STN =	Subthalmic Nucleus
TANS =	Tonically Active Neurons
VLm=	Ventralis Lateralis Pars Medialis
VLo =	Ventralis Lateralis Pars Oralis

Introduction

Circuitry of the Basal Ganglia

The Basal Ganglia (Bg) are subcortical structures known for their contributions to motor activity and movement disorders, including Parkinson's and Huntington's Diseases. Major nuclei of the basal ganglia include input structures such as the striatum (putamen and caudate nucleus) and subthalamic nucleus (STN); output structures such as the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr); and modulatory structures such as the external segment of the globus pallidus (GPe) and the substantia nigra pars compacta (SNc). These nuclei play significant roles not only in motor functions, but also in limbic and cognitive functioning (Alexander *et al*., 1990). Re-entrant circuits of various modalities connect cortex, Bg, and thalamus (Alexander *et al*., 1990). Evidence suggests that these circuits are segregated, i.e. there is little anatomic overlap between functionally different loops, and little correlation between the electrophysiologic activities of neighboring neurons (Gatev *et al*., 2006; Guzman *et al*., 2003; Bergman and Deuschl, 2002; Plenz and Aertsen, 1996.) The specific aim of the present study was to investigate the role of neuronal transmission, primarily via the transmitter Gama-Aminobutyric Acid (GABA), in the segregation of these channels. Local field potentials, (LFPs) were the primary assay used to investigate the integrity of GABAergic transmission, which was manipulated via a GABA-A receptor antagonist and agonist, gabazine and muscimol, respectively.

The motor circuit is representative of the other pathways, in terms of anatomy and physiology (Alexander *et al.*, 1986). The circuit originates in the arcuate premotor area, motor cortex, and somatosensory cortex then projects in succession to the putamen, ventrolateral area of the GPi and caudolateral area of the SNr. Subsequent signals to the ventralis lateralis pars oralis (VLo) and the ventralis lateralis pars medialis (VLm) of the thalamus are relayed back to relevant cortical areas (Alexander *et al*., 1986).

Within each circuit there appear to be a so-called 'direct' and 'indirect' pathways traveling through the nuclei of the basal ganglia (Smith *et al.,* 1998*;* Alexander *et al*., 1990). The direct pathway originates in the striatum and projects to the Bg output nuclei, GPi and SNr. The indirect pathway is more circuitous, involving connections between striatum and GPe, GPe and STN, and between STN and GPi/SNr. The direct and indirect pathways may counterbalance one another (Marsden and Obeso, 1994; Figure #1).

Evidence for Reentrant Circuits

These circuits have been mapped both anatomically and electrophysiologically. Early anatomical experiments used antereograde or retrograde tracing techniques, but more recent studies have employed herpes simplex I virus to label entire circuits via trans-synaptic retrograde transport of labeled viral particles (Middleton and Strick, 2000; Hoover and Strick, 1999; Jorritsma-Byham *et al.,* 1994; Cassel, 1993). For example, trans-synaptic labeling enabled Middleton and Strick (2000) to show the precise cortical origins and termination sites of motor cortico-basal ganglia-thalamo-cortical loops.

Electrophysiological studies with both single-unit and microstimulation approaches have also helped to delineate specific circuits and their function. For example

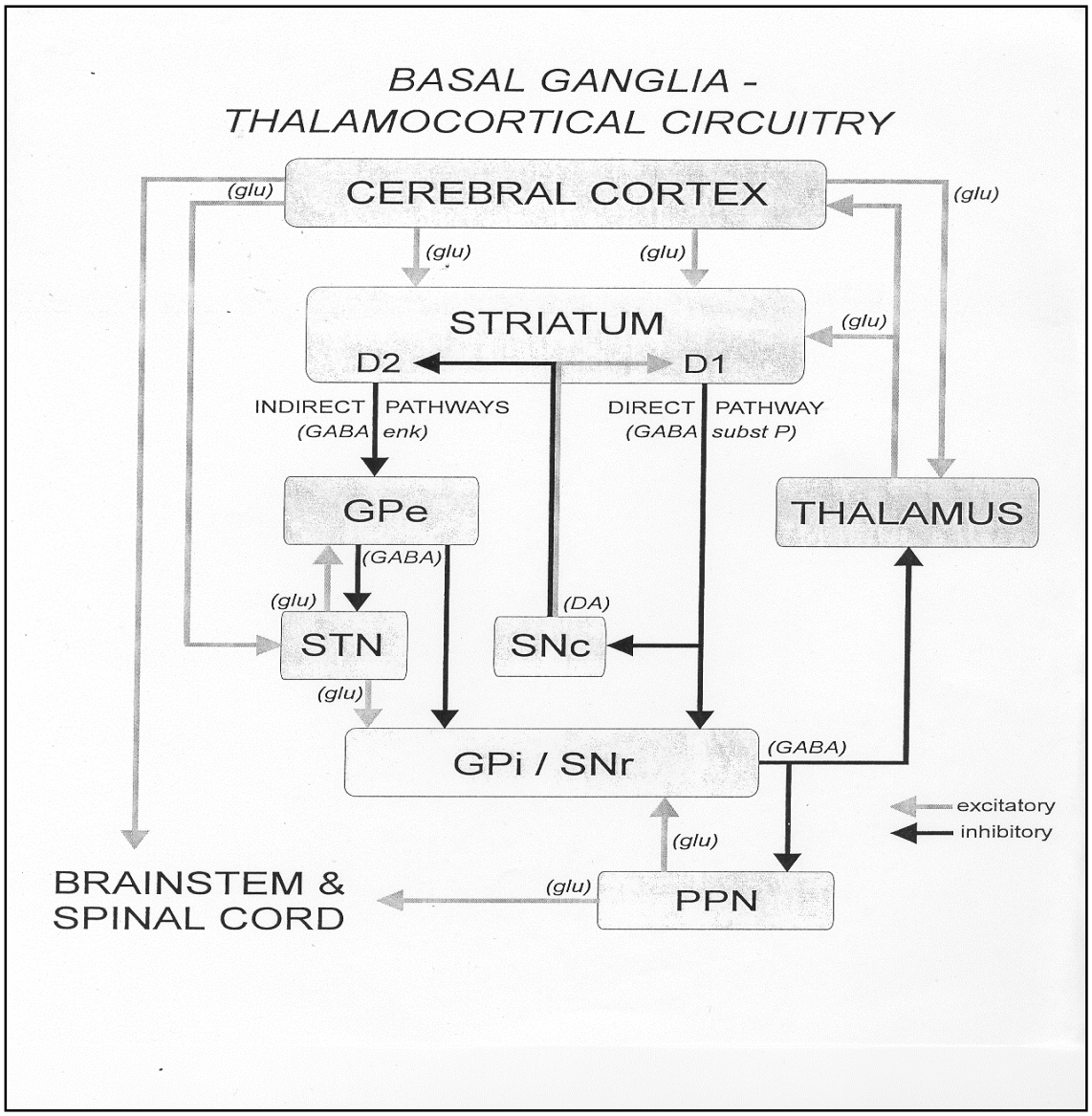


Fig 1:

Direct and Indirect Pathways - The diagram presents an outline of the basic circuitry of the Basal Ganglia, in particular emphasizing the direct and indirect pathways. The direct pathway, primarily mediated by the (dopaminergic) D1 receptor, creates an excitatory effect on movement, and is characterized by the path of striatum to the output nuclei (GPi/SNr) to thalamus to cortex. The more circuitous indirect pathway, primarily mediated by the D2 receptor, creates an inhibitory effect on movement, and is characterized by the path of striatum to the GPe

to the STN to the output nuclei, finally returning to thalamus and cortex. (Source: Alexander *et al*., 1990)

Middleton and Strick (2000) combined single-unit recording with viral tract tracing to explore visual-motor integration in monkey brain (Middleton and Strick, 2000). The majority of neurons recorded during these experiments (working memory tasks) were in the middle third of the pallidum (rostro-caudal dimension). Subsequent tract tracing from that specific region revealed a projection traveling to the SMA, which had been ascribed a role in guiding sequential movements that require working memory (Russo *et al.,* 2002).

Striatal Cellular Makeup

The data reported here result from an investigation of LFPs in the striatum. The striatum is the largest structure in the Bg and is composed essentially of the putamen and caudate nucleus. It is the main input structure, receiving significant afferent projections from virtually all parts of cortex (Parent *et al*., 1995; Gerfen, 1992). Striatal circuitry is comprised of one major type of projection neuron, the medium spiny neuron (MSN), and at least four categories of interneurons (Kawaguchi *et al*., 1995).

The GABAergic MSNs are by far the largest cellular constituents of the striatum, with population estimates ranging from 75 to nearly 98 percent of the total number of striatal cells (Tepper *et al*., 2004; Kawaguchi *et al*., 1995). MSNs project to two different targets (GPe and GPi/SNr) and express various neurotransmitter receptors. MSNs are quiescent under basal conditions (Gatev *et al*., 2006; Guzman *et al*., 2003; Plenz and Aertsen, 1996). Their activity is

strongly driven by cortical and thalamic glutamatergic inputs. These neurons send (inhibitory) axon collaterals to neighboring neurons.

The structure and function of certain populations of striatal interneurons have also been studied extensively. One of the major interests of the present study was the mechanism by which MSNs are electrophysiologically segregated; it is in this area that GABAergic interneurons are thought to play a significant role. Of the three primary classes of striatal inhibitory interneurons, the Fast-Spiking units (Fs) are the best characterized from a variety of parameters (Tepper *et al.*, 2004). Structurally, they possess approximately similar dimensions to MSNs, e.g., cell somata spanning tens of micrometers. Their relevant electrophysiologic profile includes the firing of brief action potentials and possession of large and rapidly peaking AHPs (Tepper *et al*., 2004; Kawaguchi *et al.,* 1995). Another notable characteristic sets these GABAergic neurons apart from other inhibitory influences in the striatum is their spontaneous activity (Mallet, 2005; Bracci, 2003).

Other interneuron populations in the striatum are either lesser known or appear not to be relevant to the present study. Nitric oxide synthetase/somatostatin-containing interneurons and calretinin-containing interneurons are both similar to Fs units in that they use GABA as their primary neurotransmitter, but neither is described as well in the literature. Another group of interneurons, the cholinergic 'Tonically Active Neurons' (or TANs), may play a role in the detection of salient stimuli (Graybiel *et al.,* 1995, Kawaguchi *et al*., 1995).

A Lateral Inhibitory Network

A lateral inhibitory network, potentially producing a striatal center–surround mechanism (similar to that of the retina), has been postulated to play a role in the segregation of neuronal

populations or circuits within the Bg (Alexander *et al.,* 1990; Groves, 1983). This mechanism may operate on a number of levels, including the information paths flowing through the nuclei of the Bg and the level of individual nuclei (Alexander *et al.,* 1990). For example the indirect pathway may provide the inhibitory surround, while the direct pathway supplies the disinhibitory drive.

The extensive local GABAergic axon collaterals of MSNs may be important sources of inhibition in the striatum (Bar-Gad and Bergman, 2001; Parent *et al.,* 1995*;* Gerfen, 1992; Groves, 1983**).** The major targets of these collaterals include other MSNs, and populations of interneurons, namely the Fs subtype (Mallet, 2005; Tepper *et al.,* 2004*;* Bolam *et al.,* 2000). Although far fewer in number, Fs interneurons may also provide inhibition to this network. Terminals from these neurons are anatomically placed to provide highly effective inhibitory post-synaptic potentials (IPSPs), and the strength of these IPSPs is stronger than that of MSN's (Tepper *et al.,* 2004; Jaeger *et al.,* 1995). Regardless of the source of inhibition, be it MSNs, Fs, or a combination, one important function of the inhibitory network is to maintain the segregation of the reentrant circuits described above (Guzman *et al.,* 2003; Plenz and Aertesen, 1996).

GABA Receptors

GABA, the primary neurotransmitter that maintains segregation, acts at either ionotropic $GABA_A$ receptors or, metabotropic $GABA_B$ receptors. $GABA_A$ receptors mediate fast inhibitory transmission, while $GABA_B$ receptors mediate slow inhibitory transmission. The most relevant receptors for our study were $GABA_A$. Thus to manipulate GABAergic transmission we used the competitive and selective $GABA_A$ receptor antagonist gabazine (Jones *et al*., 2001), and the selective $GABA_A$ receptor agonist, muscimol (Jones *et al*., 2001, 1998).

$GABA_A$ receptors, or subunits that comprise the receptors, are located throughout the basal ganglia, including the STN, both pallidal segments, and the striatum (Charara *et al*., 2005; Galvan *et al*., 2004; Smith *et al*., 2000). Subunits of the GABA-A receptor (α1, β2/3 and γ2), have been shown on MSNs and on interneurons (Galvan *et al*., 2004; Fujiyama *et al*., 2000), often in the core of putative GABAergic synapses (Galvan *et al*., 2004; Fujiyama *et al*., 2000).

Consequences of Circuit Desegregation

Segregation of information in specific circuits appears critical to normal behavior (Joel and Weiner, 1994). Damage to this circuitry, either by anatomical lesion or compromise of electrical signaling, may result in pathology. In fact, precise location of an insult or interference may predict the type of resultant pathology (Middleton and Strick, 2000).

The emergence of synchronized oscillatory discharge (SOD) amongst neuronal populations that normally are electrophysiologically segregated may result in a greater correlation of firing patterns amongst these cells, resulting in a reduction of relevant signal quality (Joel and Weiner, 1994). This type of phenomenon may play a role in such syndromes as Huntington's Disease (HD) and Dystonia. Reduced GABAergic transmission within the striatum may also be a factor in these disorders (Fredow and Loscher, 1990). SOD can be studied via recording LFPs.

Local Field Potentials

The type of electrical activities that can be recorded with wires introduced into the brain depend on the physical dimensions of the electrodes. Electrodes with a very small tip (on the order of a few microns) will be sensitive to focally generated activity, but will not be significantly influenced by electrical phenomena generated more than a few hundred

micrometers away from the electrode tip. Such 'micro'-electrodes can therefore be used to record the activities of individual neurons or fibers. The physical dimensions of such electrodes determine their electrical characteristics – they tend to have a high impedance, and act effectively as a high-pass filter with a high cut-off frequency. By contrast, electrodes with larger tip sizes record potentials generated by a much larger volume of tissue (up to several millimeters in size). The physical characteristics of the electrode-tissue interface results in a low electrode impedance, and imparts filter characteristics on these electrodes that relatively favor the recording of lower frequencies. Such electrodes are not sensitive to the activities of individual neurons. Potentials recorded from such 'macro'-electrodes are called 'local field potentials' (LFPs) and reflect the net sum of all electrical activities within the vicinity of the electrode. LFPs are low-frequency potentials, and are influenced by synaptic potentials, synchronized neuronal spiking, and changes in glial membrane potentials (Goldberg *et al.,* 2004, Logothetis, 2003).

In light of our concern with segregation of striatal circuitry, perhaps the most essential attribute of LFPs is that fluctuations in the local field potential may indicate the degree of synchronization between neuronal populations. Boraud and colleagues state that currents must be synchronized in time and space in order to influence the LFP significantly (Boraud *et al*., 2005). LFPs are thought to be 'population windows' of sorts on various aspects of neuronal behavior, and as such are valuable assays. However, the majority of studies conducted to date have been carried out within the cortex, not the Bg. The relative lack of knowledge on Bg LFPs was significant motivation in the development of the present protocol.

In order to investigate the nature of GABAergic control of LFPs in the striatum of the Bg, the present study employed a microdialysis-local field potential (MD-LFP) probe. This probe

enables LFP recording with simultaneous microdialysis sampling. Combinations of LFP recording and pharmacological sampling in the same spatial - temporal frame have not been possible before, mainly due to the fact that traditional dialysis sampling involved the use of a cannula; when both microelectrodes and cannulae were placed in proximity, an untenable signal to noise ratio resulted (Obrenovitch *et al.,* 1994). This circumstance led to the development of microdialysis probes that contained recording electrodes. Originally, their design included only a single, silver chloride wire to record LFPs (Obrenovitch *et al*., 1994). This improvement still left much to be desired in terms of immunity to electrical noise and ability to show neuronal circuit events as truly local phenomena (Darbin *et al*., 2006; Obrenovitch *et al*., 1994). This situation changed significantly with the addition of a second recording electrode to the probe, and a consequent differential grounding setup. Signal to noise ratios were much improved, enabling localization of LFP activity (Darbin *et al*., 2006). The general hypothesis, of the present study, that segregation between striatal channels is actively maintained by GABAergic mechanisms, along with the timely development of the bipolar MD-LFP probe, led to the specific aim of this work: to investigate the contribution of GABAergic mechanisms to the maintenance of segregation of electrical activity in the striatum, as assessed via recording of LFPs.

Materials and Methods

Rationale/Overview

To explore the role of GABAergic inhibition in the maintenance of segregation of activity of neighboring striatal MSNs, we recorded changes in striatal LFPs evoked by local application of GABAergic drugs via reverse microdialysis. Pharmacological manipulations and electrical recordings were made via a combined microdialysis LFP recording, MD-LFP probe.

Two monkeys were used. Surgical procedures were performed to attach a recording chamber to the animal's skull allowing continued access to the brain throughout experimentation. Once the animals recovered from surgery, electrophysiological mapping established anatomical boundaries of the striatum. Resultant maps were used to place the MD-LFP probe in the target area of the motor striatum, i.e., the putamen. After accurate probe placement and an equilibration period, the microdialysis tubing of the MD-LFP probe was used to infuse a GABAergic agonist (muscimol) or an antagonist (gabazine) into the putamen. Before, during, and after the drug application, LFPs were recorded.

Specific Research Design

Animals

Two female Rhesus monkeys (Macaca mulatta) were used in this study, each weighing approximately 5 kg. Animals were housed individually in standard primate cages (Primate Products, Miami, Fl), and had ad libitum access to food and water. Before experimental

procedures began, animals were trained to accept contact and handling by the experimenters. They were also trained to sit in a primate chair for extended periods.

All experimental protocols were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Anonymous, 1996), and the PHS Policy on Humane Care and Use of Laboratory Animals (amended 2002). The Animal Care and Use Committee at Emory University approved all protocols.

Surgical Procedure

In order to place a stainless steel recording chamber (inner diameter 16 mm) on the animal's skull, surgery was conducted under general isoflurane inhalation anesthesia (1-3%) and aseptic conditions. Before setting the chamber, a small circular section of bone was removed with a trephine. The recording chamber allows for chronic access to the brain. In order to access the motor striatum (putamen) the chamber was fixed to the skull stereotactically at an angle of 50° from vertical in the coronal plane guided by the following anatomical coordinates, (A=12 mm, L=10 mm, D = 5 mm). The recording chamber and a head-stabilizing bolt were attached to the skull with dental acrylic.

MD-LFP Set-up

The MD-LFP probe was constructed of microdialysis tubing and fine electrical wires to enable multiple functions (Figure #2). First a specific length of semi-permeable membrane (Hollow Fiber Bundles; molecular weight cutoff: 13,000 Da; Spectra/Por RC; Spectrum Laboratories, Inc., Los Angeles, CA), was cut and closed at one end with an epoxy glue. Then the inlet and outlet tubing of the dialysis probe (length 15 cm, i.d. = 40 µm; o.d. = 103 µm;

Polymicro Technologies, Phoenix, AZ) were added. The inlet tube was placed within 100 μm of the glue cap, extending 2 mm beyond the end of the outlet tube. Following placement of the tubing, two 20 cm segments of ultra- thin wires (Nichrome/Formvar, bare diameter: 0.0020", coated diameter: 0.0026", A-M Systems, Inc.), were placed in the probe. Insulation was carefully removed from one end of the wires, and the ends were soldered to a connector (D-Sub Crimp Contacts – Jameco, USA). The probe end of the wires was de-insulated to a length of 100 μm. The impedances of the wires were matched to lie within a 100 – 200 KΩ range. (Impedance matching improves common mode rejection in the bipolar recording set-up. Common mode rejection refers to the ability of the probe to reject signals common to both wires. This characteristic improves the signal to noise ratio of the activity that the probe records.) The wires were then added to the probe next to the tubing, in such a way as to ensure the wire tips are 2.5 mm from each other.

The entire probe was then inserted into a sleeve of tubing (polyimide) so that a 3 mm segment of both wire tips and inlet/outlet tubing extended out of the sleeve. Ten-millimeter sections of stainless steel tubing (Small Parts, Miami Lakes, FL) were glued to the proximal ends of the inlet/outlet tubing of the probe for connections to a microliter pump. The wires and tubing (stainless steel and polyimide) were then secured in a small plastic holder, which added structural support (CMA, Solna, Sweden).

The system was perfused with aCSF (composition in mM: 147.0 Na^+, 4.0 K^+, 1.0 Mg^{2+}, 1.2 Ca^{2+} and 153.2 Cl^-; CMA, Slona, Sweden), delivered at a rate of 2 μl/min from gas-tight syringes, which were driven by a microliter syringe pump (CMA120; CMA, Solna, Sweden). The pump simultaneously drove two syringes, which were connected to the inlet tubing of the

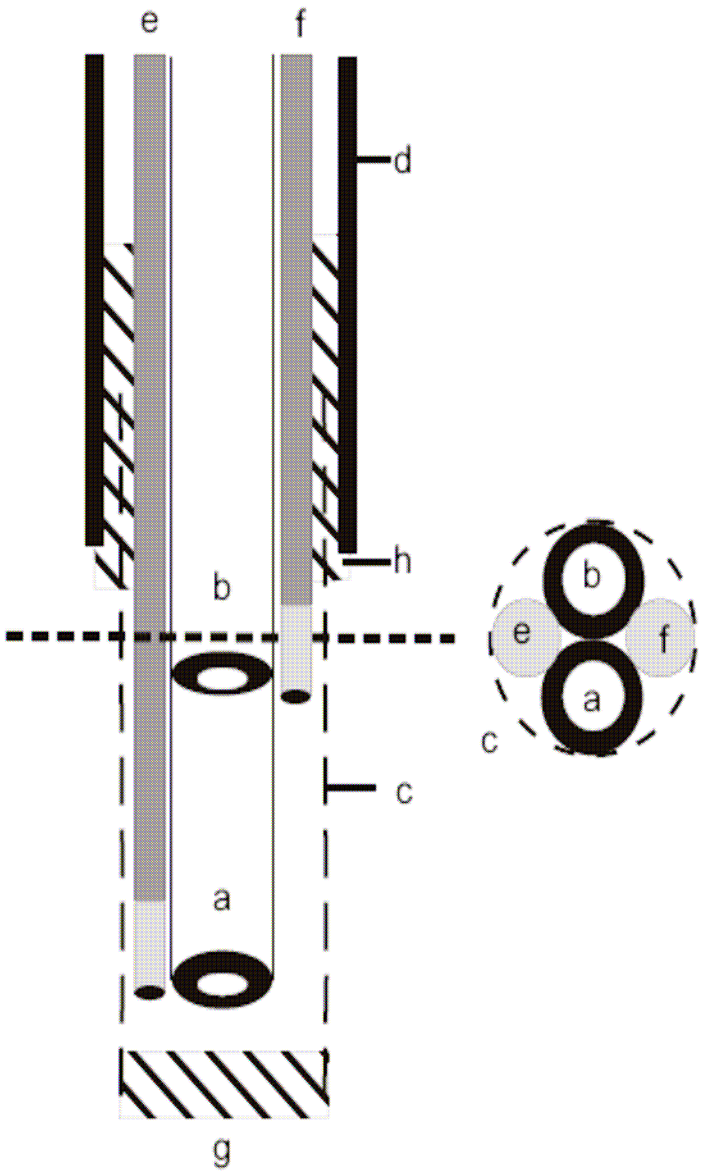


Figure 2:

A schematic representation of the MD-LFP device indicating the following: (a) inlet tube, (b) outlet tube, (c) semi-permeable membrane, (d) polyimide tubing, (e) first wire-electrode, (f) second wire-electrode, (g) glue plug, (h) glue in polyimide tubing 'sleeve'. Source: Darbin, O *et al*., 2006

probe via a liquid switch (CMA110; CMA, Solna, Sweden), which allowed switching between infusion solutions without the introduction of air bubbles.

Sampling Routine

Before the MD-LFP probe was used it was flushed overnight to ensure that the system was properly primed. Thirty minutes before the start of the experiment the system was perfused with a solution of artificial cerebrospinal fluid (aCSF) composed of a composition similar to that described in the previous section. After this perfusion period the probe was placed in the brain via a microdrive and delivered to a relevant target based on mapping data. Once the probe was located correctly, extracellular fluid was allowed to stabilize for a period of sixty minutes (Figure #3). Following stabilization, baseline striatal LFPs were recorded for twenty minutes as a control set of data for all experimental groups. There were five experimental groups or series of injections in this study. They were organized by the following parameters. Group 1 was a control group, in which LFPs were recorded at baseline without drug injection. Three experimental groups (#'s 2, 3 and 4) were recorded in the presence of gabazine (0.1, 10 or 500 μM respectively), introduced to the medium perfusing the microdialysis system starting 40 minutes after the beginning of the recording, i.e., T = 0, and lasting for a period of 20 minutes. The last experimental group (#5), consisted of perfusion with muscimol alone (100 μM) at T = 20 minutes, for a 20 minute period, immediately followed by a 20 minute perfusion with a solution containing both muscimol (100 μM) and gabazine (10 μM). In all groups, except group #1, LFP recording continued for 120 minutes after switching back to regular aCSF.

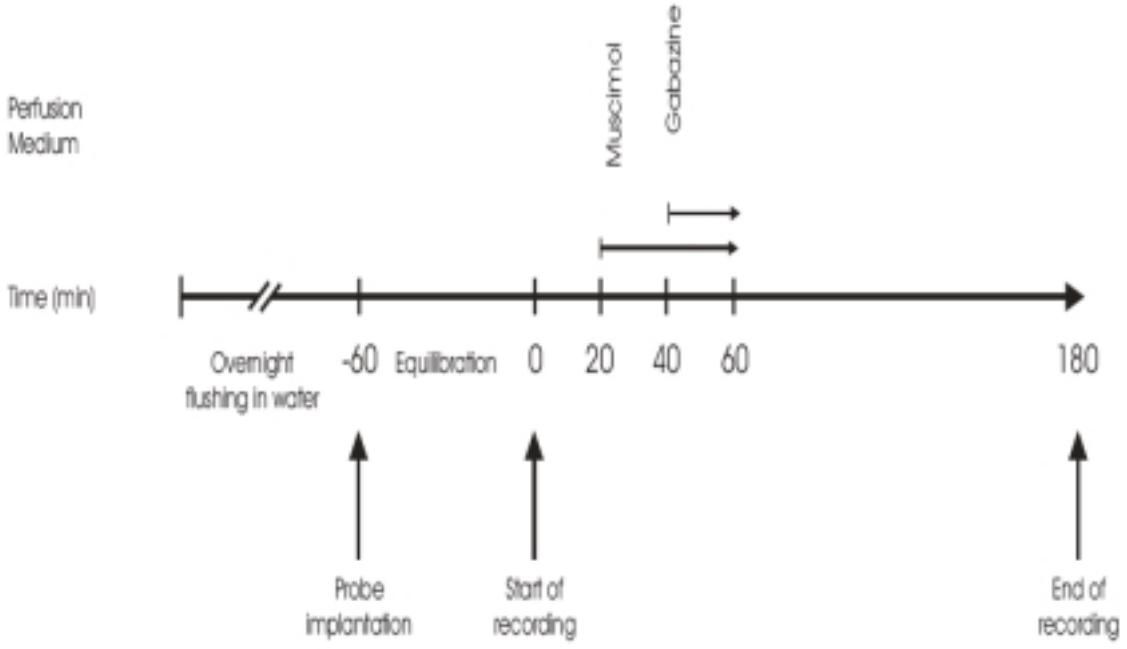


Figure 3:

Schematic representation of the protocol used in this study. First, the MD-LFP probe was perfused overnight using distilled water. Then, the device was inserted into the brain area of interest and a one hour equilibration period was implemented to allow the tissue to recover from the penetration. After this period, the recording session was started and this defined the T_0 of the experiment. Control group (group 1) was recorded for 3 hours without drug injection. Three groups (2,3,4) were treated by infusion of gabazine (0.1, 10 or 500 µM respectively) 40 min after the beginning of the recording for a period of 20 min. The last group (5) was treated at 20 minutes with muscimol (100 µM) alone for 20 min followed by a 20 min period of perfusion with a mixed solution of muscimol (100 µM) and gabazine (10 µM).

Electrophysiological Recording

Two types of electrophysiological signals were recorded: single-unit spiking activity was used to delineate the boundaries of the striatum, and LFPs were used to ascertain the degree of synchronous activity amongst neuronal populations within the striatum. The striatum was mapped using microelectrodes (FHC, Bowdoinham, ME) with an impedance value range of 0.5 – 1 MΩ and standard single-unit recording methods. A microdrive (MO-95B, Narishige, Tokyo, Japan) was used to lower the single microelectrode through the various regions of the brain en-route to the putamen. To avoid damage to the microelectrode tip, we used a guide tube to insert the electrodes past the dura mater. The spiking activity was amplified (DAM-80 amplifier, WPI, Sarasota, Fl), filtered 400 – 10,000 Hz, Krohn-Hite, Brockton, MA), and visualized with a digital oscilloscope (DL 1540, Yokogawa, Tokyo, Japan). The signals were also made audible with an audio amplifier. LFP signals were recorded with an EEG amplifier (Model 8-16 Amplifier, Grass) and low-pass filtered at a range of 0.5 - 75 Hz. LFP recording wires were interfaced with the EEG amplifier via an impedance adaptor (Model HZP High Impedance Probe, Grass, West Warwick, RI).

Histology

At the end of these experiments the animals received an overdose of pentobarbital (100mg/kg) and were then perfused transcardially with cold oxygenated Ringer solution, followed by a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4). The brain was blocked, frozen, and sectioned in the parasagittal plane in 50 μm sections. Sections were stained via cresyl violet. Recording sites were reconstructed based on gliosis patterns and prior electrophysiological mapping data.

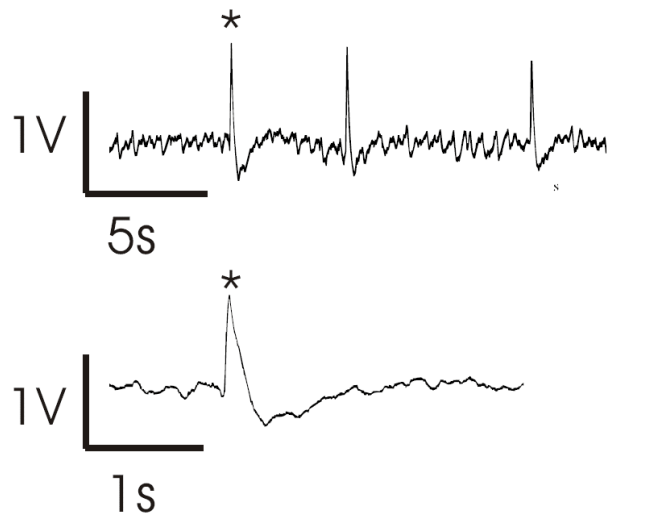
Data Analysis

We observed that gabazine induced rhythmically recurring spike-waves in the striatal LFP record. To generate numerical descriptors of the frequency and duration of these events, we analyzed the data with custom-designed algorithms in Matlab (Mathworks, Natick MA). First the numerical signal was re-sampled to 241 Hz and filtered in the band 0.5-35 Hz. The new signal was then segmented in adjacent 10-second windows. Each individual window was visually analyzed in order to exclude windows with artifacts, to identify the beginning and ending of individual gabazine-induced synchronized events, and to calculate the duration of the gabazine-induced changes (if any). The number of spike-wave events (Figure #8) and their duration (Figure #9) were estimated over 5 minute periods. These data are displayed in the median and 25th – 75th percentile format, via and inter-group comparisons were carried out with non-parametric tests as the data did not follow a normal distribution (Glantz, 1997).

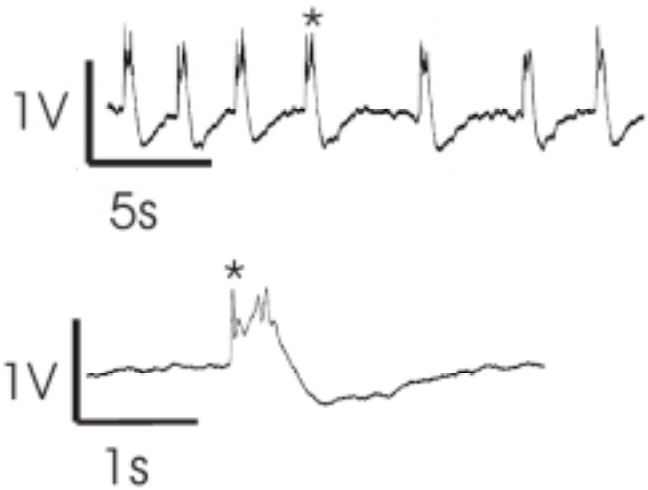
Results

Effects of Gabazine Infusion

GABAergic inhibition was blocked with various concentrations of the $GABA_A$-receptor antagonist gabazine. LFPs recorded from the MD-LFP system were monitored during drug exposure. Gabazine exposure resulted in the emergence of recurring large-amplitude LFP fluctuation. Experiments with various concentrations of gabazine demonstrated a dose-dependent relationship, such that increasing concentrations of gabazine correlated with an increasing frequency and amplitude of the spike-wave events (Figure #4). A dose-dependent relationship was also observed with regard to complexity in shape of the evoked events. From here on these phenomena will be termed synchronized events (SEs). When local areas of the putamen were perfused with concentrations of 10 or 500 μM of antagonist, individual SEs demonstrated a dose-dependent relationship not only regarding amplitude and frequency, but also in terms of complexity of waveform shape (Figures #4 & #5). At both concentration values, SEs increased in amplitude to an extent approaching 3 orders of magnitude. Regarding complexity of shape, at the 10 μM concentration SEs had the shape of a spindle or spike. In contrast, at the 500 μM value, SEs were more complex, (Figure #4b and Figure #5), and were characterized by several spikes during a shift in the baseline potential. One possible reason for increased complexity maybe wider spread of antagonist at higher concentration, and a consequential increase in the number of receptors/cells affected or entrained at small, but slightly varying time frames.



A



B

Figure 4:

Representative samples of synchronized events (SEs) with spike or spike/wave shapes recorded in the striatum of the awake monkey after local application of $GABA_A$ antagonist gabazine using MD-LFP device. (A) Traces recorded after infusion of gabazine at 10 µM. The lower part represents a magnified view of one particular SE (*); (B) Traces recorded after infusion gabazine at 500 µM. The lower part represents a magnified view of one particular SE (*). Increase in gabazine concentration results in an increase in number of spikes and in complexity of their shapes.

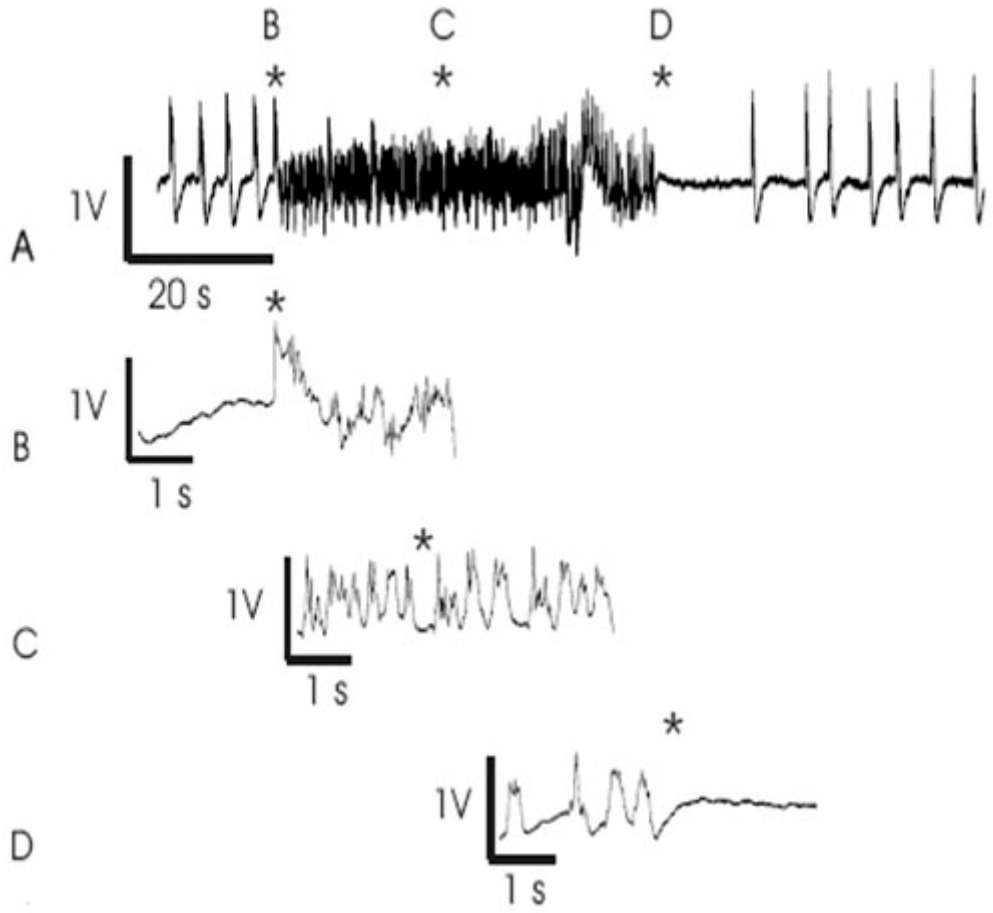


Figure 5**:**

Representative sample of sustained synchronized events (SSEs) recorded in the striatum of an awake monkey after local application of the $GABA_A$ antagonist gabazine at a concentration of 500 μM using reverse microdialysis. Portions of the figure labeled B-D refer to the sections in subplot A marked with an asterisk.

In addition to more complex patterns, we also observed more *sustained* periods of synchronization on both the individual SE level (labeled SSE in Figure #5 and Table I), and in terms of synchronized behavior enduring on a population level (Figures #5, #8, & #9) during infusion of the higher concentration of gabazine (500 μM). On the individual SE level, paroxysmal activity began immediately after an SE began, and consisted of several seconds of high frequency activity at lower amplitude than the preceding SE (Figure #5).

The number of SEs increased overall in a dose-dependent manner (Figure #6). No SEs were seen in the control protocols of the experiments; nor were SEs detected during the injections of 0.1 μM concentration of gabazine. However, at the 10 μM and 500 μM concentrations of antagonist a significant dose-dependent relationship in SE increase was observed. Specifically, the number of SEs observed during the recording sessions increased 193.5% from 10 μM to 500 μM (Figure #6). The timing by which SEs occurred was affected both on a global and individual scale by the concentration of drug perfused. At the 10 μM dosage the number of SEs that occurred within 5-minute increments increased to a peak level of 60 SEs ten minutes after gabazine administration commenced. This maximum was followed by a gradual decline during the rest of the experiment (Figure #7). At a concentration of 500 μM, the maximum value of SEs that occurred per 5-minute increment continued to increase approximately 60 minutes after the start of the antagonist perfusion to a value of 110 SEs. This frequency did not decline over the remainder of the experiment.

SE duration also increased on an individual waveform level, depending on the gabazine concentration in the perfusion medium (Figure #8). With 10 μM gabazine, individual SEs increased in duration less than 10 minutes after commencement of gabazine infusion and culminated in a maximum value of 700 ms at approximately t = 70 minutes. Once attained, this

Condition	Incidence of SEs	Incidence of SSEs	Incidence for partial seizure
Control	**0/4**	**0/4**	**0/4**
Gabazine 0.1 μM	**0/6**	**0/6**	**0/6**
Gabazine 10 μM	**7/7**	**0/7**	**0/7**
Gabazine 500 μM	**5/5**	**4/5**	**2/5**

Table I: Dose-dependence of gabazine effects.

The data are presented in termsof the number of experiments in which SEs, SSEs or behavioral changes were seen.

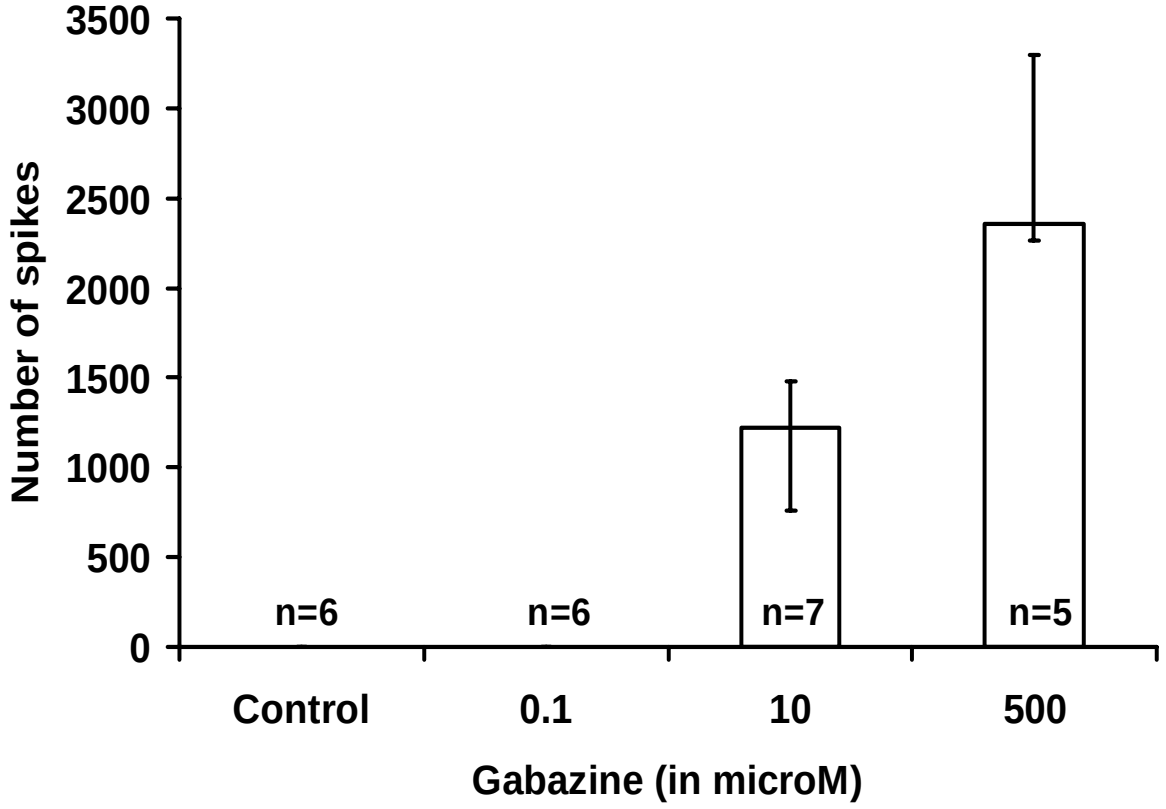


Figure 6:

Dose effect of striatal gabazine administration on number of SEs evoked locally.

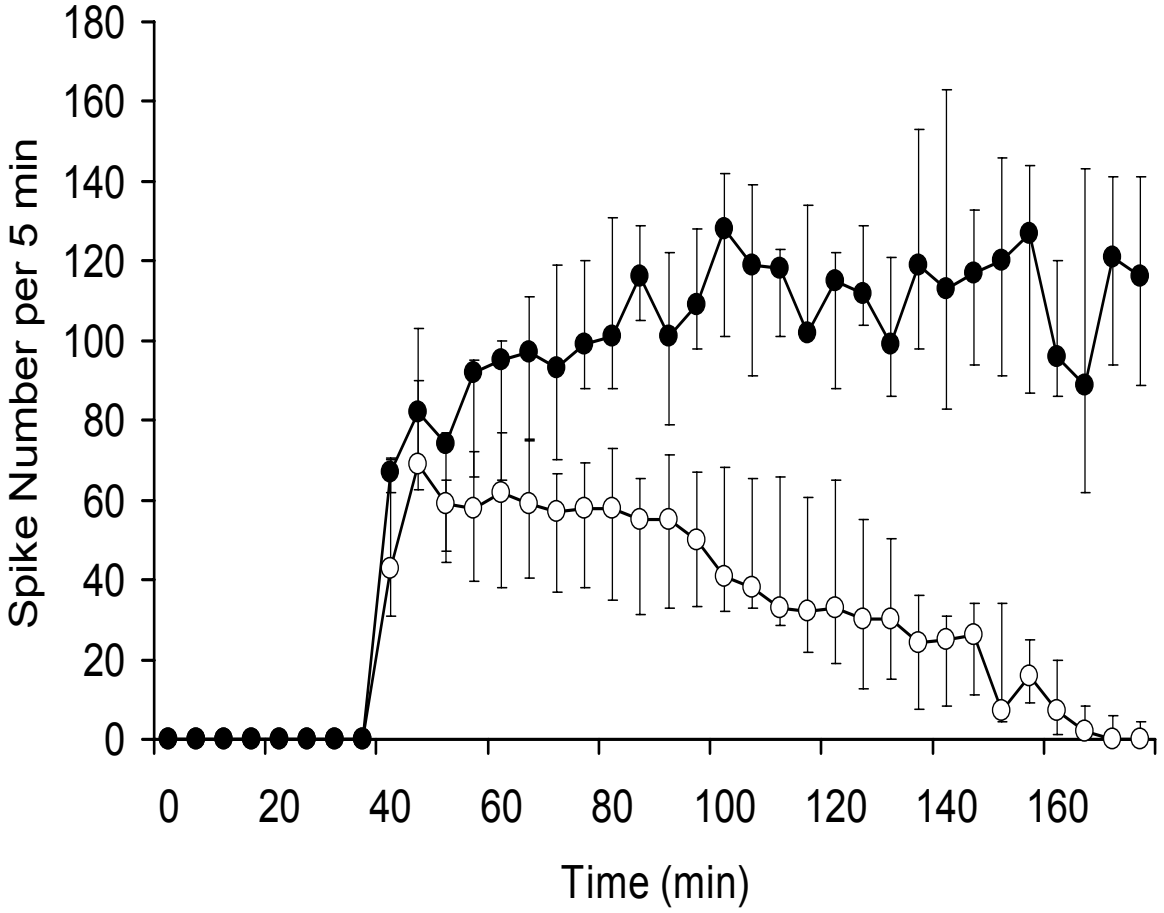


Figure 7:

Time course of SEs occurrence evoked locally by striatal gabazine infusion**.** (○) after infusion of gabazine 10 µM; (●) after infusion of gabazine 500 µM. Data are median and 25th-75th percentiles (*: $p<0.05$).

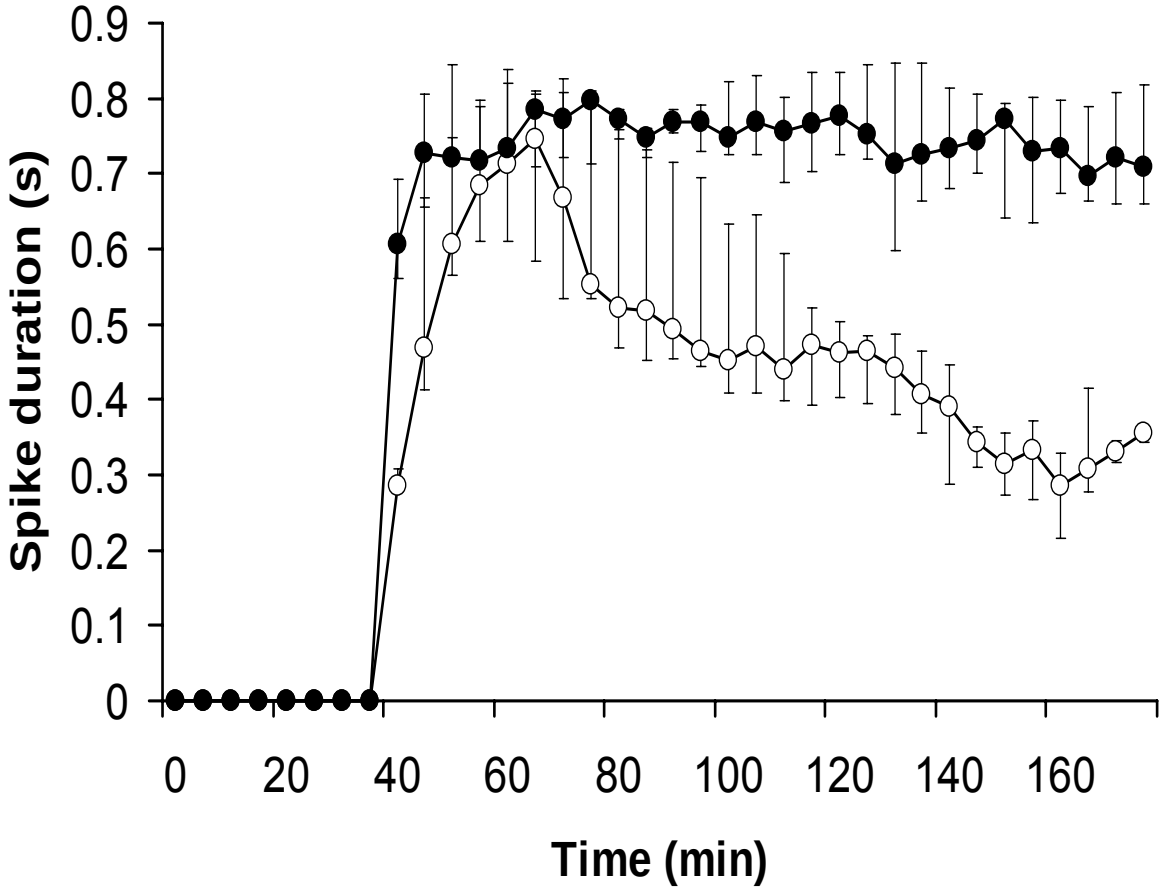


Figure 8**:**

Time course of the duration of SEs evoked locally by striatal gabazine infusion**.** (○) after infusion of gabazine 10 µM; (●) after infusion of gabazine 500 µM. Data are median and 25th-75th percentiles (*: $p<0.05$).

maximal value began to decline soon after and continued for the remainder of the experiment. With 500 μM gabazine, individual SE durations increased at a faster rate, and reached a maximal value between 700 – 800 ms and maintained this plateau for the remainder of the experiment. The greatest duration of individual SEs was not significantly different between lowest and highest concentrations of antagonist, however a plateau was only maintained in the case of the 500 μM value.

Behavioral manifestations were observed only when the highest concentration of antagonist was used. The behavior occurred in two sessions of gabazine infusion, and included subtle facial movements characterized by eye blinking, chewing, and grinding of the teeth. In one experiment, excessive salivation was also observed. These behavioral manifestations were never observed during infusions with the lower concentration values of antagonist, i.e., 0.1 and 10 μM. See Table I for a summary of experiments involving various concentrations of gabazine only.

Experiments with Co-administration of Muscimol and Gabazine:

These experiments examined interactions between muscimol and gabazine in the modulation of LFP events. Muscimol was perfused starting 20 minutes before gabazine 100 μM, and continued for the duration of the gabazine exposure. Under these conditions, effects of gabazine on striatal LFP quality and quantity were blocked
(Figure #9 and Table II).

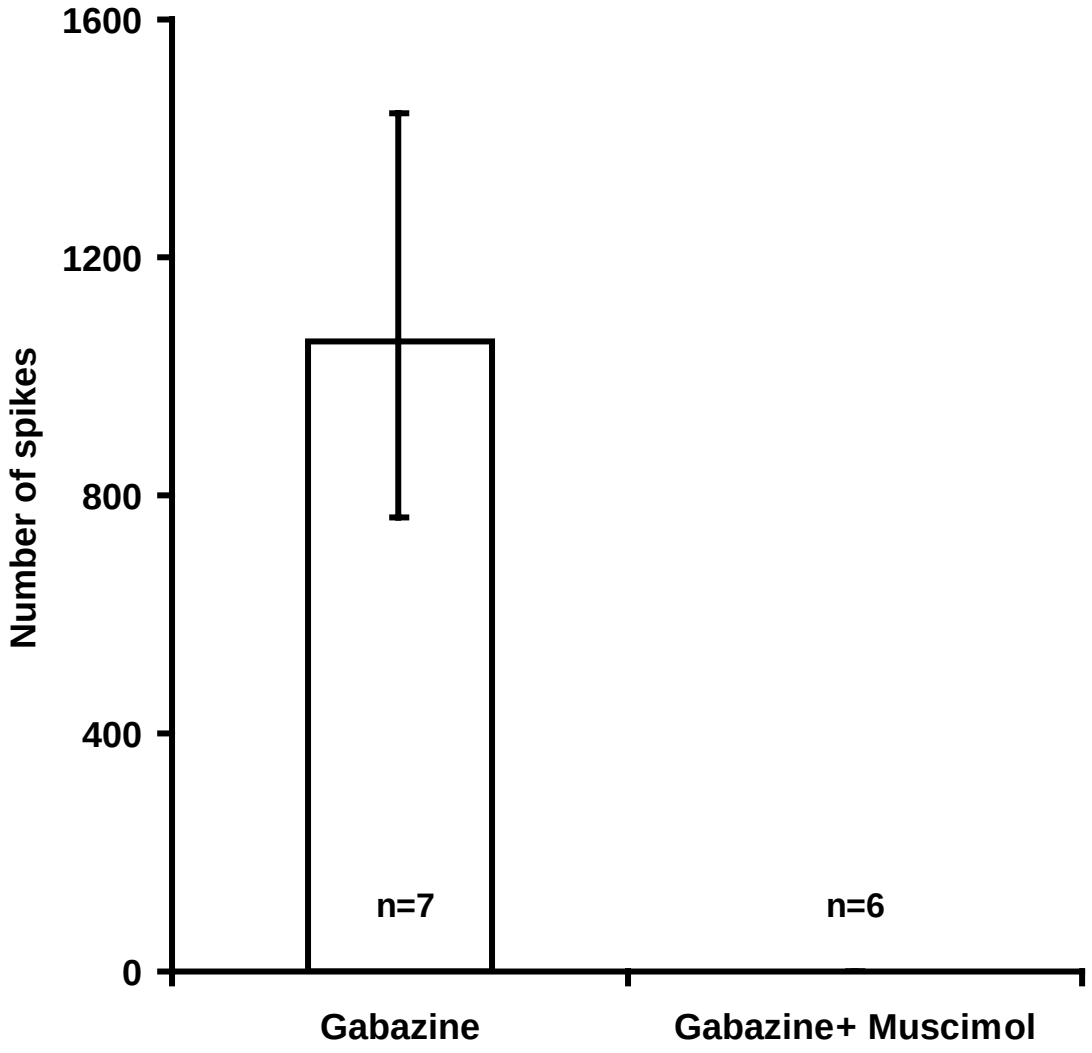


Figure 9:

Effect of pre-infusion of muscimol (100 μM) on SEs number elicited by striatal infusion of $GABA_A$ antagonist gabazine (10 μM).

Pre- and co-treatment	Conditions	Incidence for SE
None	Gabazine 10 μM	7/7
Muscimol 100 μM	Gabazine 10μM	0/6

Table II: Effect of muscimol on gabazine evoked LFP events

Discussion

The present data set reveals a gradient of effects that correlates with levels of inhibition in a striatal/putamen locus. Up to a specific plateau, the higher the concentration of gabazine, the larger in magnitude and more complex in nature the effects observed on the LFP. A role for $GABA_A$ receptors in these gabazine effects is supported by pretreatment with muscimol, which blocked gabazine effects. Overall, these data support a role for GABA in the maintenance of segregation of striatal circuitry, as well as the idea that the integrity of information within striatal channels is ensured in part via lateral inhibitory transmission (Wichmann & DeLong, 2003; Gerfen, 1992).

Specifically, we observed that the frequency of SEs increased in a dose-dependent manner during gabazine infusion, and that SEs were significantly larger in amplitude than the background LFP waveforms. Other parameters that were at least in part concentration-dependent included the time-course of events and the complexity of the shape of evoked SEs. Certain "uneventful" periods were also significant. For example, the lack of SEs in the LFP background during perfusions of the lowest concentration of gabazine is noteworthy. Finally, behavioral manifestations were observed only in the few conditions using the highest concentration of antagonist.

Generally, the data support the hypothesis that strong inhibitory transmission is necessary to prevent the emergence of abnormal oscillations in the LFP. $GABA_A$ receptors are widely distributed in the striatum, located on both MSNs and Fs units (Smith *et al.,* 2001); and therefore, antagonism of the $GABA_A$ receptor should interfere with most GABAergic transmission (in addition to communication mediated by the $GABA_B$ receptor). Not surprisingly then, our results

are consistent with the view that in the alert, resting macaque, GABAergic inhibition is normally present and largely mediated by $GABA_A$ receptors.

Upon diffusion of drug an adequate distance into striatal tissue - consequently binding a sufficient number of $GABA_A$ receptors - a requisite number of striatal cells and synapses terminating on them were disinhibited, allowing for the emergence of the large amplitude LFP events. As alluded to in the introduction, currents must be synchronized in time and space in order to significantly influence the LFP (Boraud *et al*., 2005; Buzsaki, 2004). In theory then, SEs in the LFP reflect a sufficient level of synchronization occurring amongst synapse or neuron populations.

The lack of synchronized events at the lowest concentration of gabazine is noteworthy. This result suggests that there exists a continuum of disinhibition; at low concentrations SEs were not evoked (Faingold *et al.,* 1998; Meldrum & Wilkins, 1984) whereas at higher concentrations SEs became more complex in shape and occurred more frequently. Above this threshold the complexity in shape and the frequency of occurrence of SEs were concentration dependent. The primary limiting factors on the antagonist's effect were most likely its extracellular concentration and its distance perfused from the probe (Hoistad *et al.,* 2002). Essentially then, changes in SE frequency of occurrence and complexity of shape were a function of both of these parameters, although there may have been other more subtle undetected factors**.** The shape of LFP events and their concentration dependent complexity are attributable to the varying quantity of electrically excitable elements contributing to the LFP and to the changing qualitative nature of their contributions.

Co-administration of Gabazine and Muscimol

Gabazine failed to induce LFP events in the presence of muscimol. The rationale behind the co-administration experiments was to increase confidence in gabazine's locus of action and to establish that the perturbations seen in the LFP were primarily due to antagonism of the $GABA_A$ receptor. Given muscimol's less selective nature than gabazine **(**Jones *et al.,* 2001), muscimol may block a diversity of -A- receptor subunit combinations, including those that gabazine exhibits greatest affinity toward. Our results support gabazine's profile in the literature as potent competitive inhibitor of the $GABA_A$ receptor (Lindquist *et al*., 2005).

Although not likely, an 'order-effect' could have influenced the present results. Namely, if a set of experiments had been carried out in which gabazine was applied first, the subsequent administration of muscimol may or may not have inhibited all evoked SEs. Muscimol is described as a potent agonist (Frolund *et al.,* 2002) with consequent intrinsic ability to out-compete the natural agonist. Furthermore, according to Jones and colleagues (Jones *et al.,* 2001 and 1998) muscimol, and agonists like it, may create an 'induced-fit' rearrangement of their receptor's subunits that requires a significant energy outlay for this new configuration to occur. Conversely, antagonists such as gabazine create less involved interactions with their relevant receptors and, consequently, their action is less expensive from an energetic standpoint. This rationale suggests that perfusion of muscimol secondarily to gabazine would have induced limited reversal of the effects evoked herein by the antagonist, primarily because the cost of energetic outlay is favorable toward the mechanism of gabazine. Our findings, while not proving a specific locus of action, do support the assertion that antagonism of the $GABA_A$ receptor generated the emergence of synchronized events.

Results in Light of Physiological Concerns

As mentioned in the introduction, a lateral inhibitory network has been suggested to exist within the striatum (Groves, 1983). The emergence of SEs in our experiments indicates that at least partial, if not significant breakdown of the local inhibitory network occurred. However, cortical motor programs were in all likelihood not disrupted (with certain possible exceptions, see below), as the animals by and large were resting during the protocols. The development of SEs, does indicate that significant synchronization developed in the local vicinity of the probe, primarily caused by the disturbance of GABAergic transmission.

The potential substrate of this network as described in the introduction, is most likely MSNs, Fs interneurons or some combination of these populations. Do these cell populations also contribute to the phenomena observed in the LFP record? MSNs comprise the overwhelming majority of striatal cells (Tepper *et al.*, 2004), thus by sheer number alone they almost certainly contributed to the LFP. Furthermore, since the LFP is a function of a variety of electrical activity, MSN oscillations in membrane potential may have contributed significantly to the events observed in the LFP record. MSN are known to oscillate from a so-called down state (least likely to fire) to an up-state (a membrane potential only a few millivolts away from spiking threshold) (Wilson and Kawaguchi, 1996). Since currents must be synchronized in order to significantly affect the LFP (Boraud *et al.,* 2005), our data may reflect the synchronous oscillations of many MSNs from hyperpolarized states to near threshold. However, MSNs were almost certainly not the only contributors to the LFP. Fs interneurons possess certain characteristics that distinguish them from other striatal populations and make them ideal LFP contributors. These characteristics include an ability to fire spontaneously, significant glutamatergic cortical input, and considerable connections to other Fs cells via gap-junctions

(Mallet *et al.,* 2005; Tepper *et al.,* 2004; Moore & Grace, 2002). Electrotonic coupling via gap-junctions may have played a role in synchronizing the output of Fs cells.

Behavioral Issues

During two experiments with the highest concentration of gabazine, the animals manifested behavioral changes. Although few in number, the fact that oral/facial movements did arise suggests a few interesting possibilities. One, as described above, significant breakdown of an inhibitory network probably occurred allowing for the emergence of synchronized events. This synchrony was almost certainly propagated to relevant areas of cortex and most likely by electrical means, not by diffusion of drug (Hoistad, 2002). The direction of propagation from the striatum could have been either antero- or retrograde. The substrate involved in the retrograde case would be the circuitry that connects cortex with striatum; if anterograde in nature, the direct pathway described in the introduction would be the relevant path (Figure #1).

LFPs Revisited

Regarding more global issues and questions related to the LFP, since this potential is known to propagate over significant distances, on the order of millimeters (Goldberg *et al*., 2004), it seems fair to ask, 'do the SEs in the field potential propagate further in the LFP than the actual spread of the antagonist?' If the LFP is more than just a manifestation of varied electrical activity within the neuropil, if it is actually a signal capable of causing effects (Bullock, 1997), is there any indication of this in the raw data?

In effect, what has been partly carried out in this protocol is to shut down a substantial proportion of the brain's inhibitory mechanism, within a small locus. The resultant lack of

balance as manifested by the large amplitude oscillations in the LFP, should in theory, evoke some sort of homeostatic response from the brain. Since proper brain functioning requires a balance of excitation and inhibition (Moulder *et al*., 2006), one could expect to see evidence of increased GABAergic transmission – metabolic, electrical, and histological – in areas outside of the sphere of influence of the antagonist. For example increases in GABAergic vesicular trafficking and likelihood of release might be detectable (Moulder *et al*., 2006).

The question arises then, is there any evidence in the LFP of a homeostatic response occurring? In other words, are there any subtle manifestations in the LFP reflecting the brain's attempt to compensate for the disturbance in GABAergic transmission? The most fruitful place to look for this answer maybe in sections of the LFP record that take place near the end of the obvious effect of the antagonist. The rationale for looking in this area of the record simply being that the peak effect of the antagonist may mask any other more subtle compensatory activity. Future experiments could also look to sample, via microdialysis, other relevant brain areas outside of the area that is influenced by the MD-LFP probe.

Regarding other subtle aspects of the LFP record, it was evident that at the median concentration of antagonist (10 μM) SE's evoked were simpler in shape than those emerging at the higher concentration. Interestingly, only the SE's of more complex shape were associated with behavioral changes. Obviously, change in shape of the LFP is not the salient cause of the behavior manifestations observed, however, the biological substrate that underlies these changes may be of interest. Are the majority of dose-dependent effects seen in this protocol due solely to greater concentrations of antagonist in the local environment and/or greater distance of diffusion of the antagonist from the probe? Or are there other factors that operate here as well? Although beyond the scope of this work, additional factors possibly include the increased influence of

glutamatergic and dopaminergic transmissions subsequent to the elimination of GABAergic activity.

Further interesting questions regarding the LFP record include: 'why does the sustained and complex paroxysmal behavior illustrated in figure #5 develop?" Is this, too, primarily a function of extracellular drug concentration and perfusion distance from the probe? Unfortunately, the nature and implications of these 'seizure-like' events remain a mystery. Finally, the maximum duration of individual SE's did not change significantly between the median and 500 μM concentrations. Why? Given the significant difference in concentration between the median and highest value, does this result represent some type of plateau that cannot be surpassed without an orders of magnitude increase in concentration of drug and a resultant greater perfusion distance?

Relevance to Human Pathology

Major insights into the behavior of individual neurons have been gained from electrophysiological experiments. Most progress has been achieved by single-unit recording. Single-unit recording offers valuable insights into neuronal behavior, especially regarding the firing rate of units. Firing rates, however, do not give a comprehensive picture of relevant neuronal behavior; emergent oscillation patterns in population activity are also germane. As such, researchers have begun to look at the *pattern*s of the electrical activities of single neurons, or populations of cells, as well as LFP fluctuations (Kuhn *et al.,* 2005). They have found that certain frequency bands of synchronized, oscillatory activity can be deemed either antikinetic or prokinetic (Brown, 2003). For example, Williams and colleagues found 11 – 30 Hz synchronous oscillations in the LFP between STN and GPi, and these nuclei and cortex, in Parkinsonian (PD)

patients undergoing Deep Brain Stimulation (DBS) (Williams *et al*., 2002). Furthermore, Brown broadly defines the frequency bands below 40 Hz as *essentially* antikinetic.

Our results show fluctuations in the LFP that have maximal frequency values of less than 1 Hz at both the median and greatest concentration of the antagonist. These frequency values would probably not be classified as antikinetic (or prokinetic), and so the question remains whether there are any correlations between our results and non–contrived pathological realities, i.e., movement disorders. Some evidence is reported for impaired GABAergic transmission in the progression of various diseases. Two primary examples are Huntington's Disease and some forms of Dystonia. Fredow and Loscher (1990) observed that the symptoms of a dystonic strain of hamsters could be ameliorated or worsened if given drugs that enhanced or impaired GABAergic transmission respectively (Fredow and Loscher, 1990). While Crossman and colleagues found that by impairing GABAergic transmission from the medial putamen to the lateral globus pallidus (via bicuculline) a chorea type symptomology emerged in monkeys. They reasoned that since there is an impairment in GABAergic signaling from the striatum to the globus pallidus in humans suffering from Huntington's Disease (HD) that compromised GABAergic transmission may play at least some part in HD (Crossman *et al.*, 1988).

Variability in Results and Possible Confounds

Two female Rhesus monkeys were tested in this protocol. Due in part to the low number of subjects, significant variability was encountered. Relevant inter-animal differences may include differences in the animal's metabolism, differences in brain architecture and dimensions, and differences in cellular population censuses, e.g., neuronal versus glial ratios. Regarding inter-penetration differences, the primary source of variation was differences in location of the

MD-LFP probe within the putamen from one injection to another. Other sources include experimenter error regarding timing of perfusions and concentrations values of the pharmacological agents. Furthermore, there was some inevitable variation in the construction of one MD-LFP probe and another. One unavoidable limitation of this protocol, namely the fact that only two animals were used, was due to ethical and, in part, economic issues associated with nonhuman primate research. For these reasons multiple observations were carried out in each of these animals, rather than other animal model protocols, e.g., rodent paradigms, in which single observations are carried out in multiple animals.

The number of spikes evoked at various antagonist concentrations showed significant variability especially at the concentration value of 10 μM. In general there appeared to be more variability at the median concentration value throughout all of the various parameters, e.g., total spikes evoked, spike number per 5 minutes and individual spike number. If this interpretation is true, the reason may have to do with a failure to reach a minimum level of disinhibition/excitation (alluded to above) at all, or consistently at the 10 μM value. In the experiments conducted regarding spike number per 5 minutes, there appears to be significant variation in the data at both concentrations values, although the greatest periods of difference manifest at different time periods in the overall experiments, namely, late in the experiment for the maximal concentration value, and early to middle periods for the median value. Reasons why may include variance in the length of activity of the antagonist at the 500 μM concentration and, as above, lack of threshold levels of excitation/disinhibition achieved at the median value. In the data collected regarding individual spike durations, the greatest variability appeared to occur again at the median concentration value and also, in the middle period of the experiment. This occurrence may also be attributed to lack of sufficient antagonism of the $GABA_A$ receptor.

Summary and Conclusions

The data reported here indicate that perfusion of the $GABA_A$ antagonist gabazine at 10 and 500 μM concentrations by reverse microdialysis evokes discrete, large – amplitude spikes in the LFP, which we have termed synchronized events (SEs). These results suggest that the rate of occurrence and the complexity of shape of individual events are dependent on the concentration of gabazine. Behavioral manifestations were seen in only two series of injections using the maximal concentration of antagonist. Pre-administration with the $GABA_A$ receptor muscimol prevented the development of all coarse and subtle manifestations of the gabazine induced LFP events.

The data supported our hypothesis that in order for pathological oscillatory behavior amongst neuronal populations not to emerge, strong inhibitory transmission is necessary. Upon sufficient breakdown of GABAergic transmission, synchrony may develop. Thus the hypothesis that GABAergic mechanisms play a significant role in maintaining the segregation of striatal circuitry is supported by our results. In other words, strong, lateral inhibition within striatum may play a role in prevention of unwanted movements from occurring.

Possible directions to proceed experimentally with the MD-LFP probe include the use of glutamatergic or dopaminergic antagonists and complementary single unit recording in the same locus as the probe. One particularly promising location would be the output nuclei of the Bg. Here the production, and potential propagation, of SEs to cortex could be further investigated. Recording of cortical EEG to more effectively validate the emergence and spread of synchronized oscillatory activity would strengthen the significance of any potential results.

This work may be relevant to the study of human pathologies in part associated with impaired GABAergic transmission. Consequent imbalance in excitation and inhibition resulting

from pathological $GABA_A$ receptor signaling may be conducive to the emergence of certain motor disorders. Huntington's Disease and some forms of Dystonia may be pertinent examples.

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