The role of the subthalamic nucleus in the response of globus pallidus neurons to stimulation of the prelimbic and agranular frontal cortices in rats

L.J. Ryan and K.B. Clark

Department of Psychology, Oregon State University, Corvallis, OR 97331-5303, USA

Received January 24, 1991 / Accepted May 10, 1991

Summary. We investigated how the cerebral cortex can influence the globus pallidus by two routes: the larger, net inhibitory route through the neostriatum and the separate, smaller, net excitatory route through the subthalamic nucleus. Stimulation (0.3 and 0.7 mA) of two regions of frontal agranular (motor) cortex and of the medial orbitofrontal cortex centered in the prelimbic cortex typically elicited one or more of the following extracellularly recorded responses in over 50% of tested cells: an initial excitation (approximately 6 ms latency), a short inhibition (15 ms latency) and a late excitation (29 ms latency). Some other cells responded with an excitatory response only (18 ms latency). The excitatory responses largely arise from the subthalamic route. Kainic acid or electrolytic lesion of the subthalamic nucleus eliminated most excitatory responses and greatly prolonged the duration (16 vs 50 ms) of the inhibition. Subthalamic neurons typically showed one or more of the following responses to cortical stimulation: an early excitatory response (4 ms latency), an inhibitory period (9 ms) and a late excitatory response (16 ms). The early response was seen after motor cortex but not prelimbic stimulation. The timing of the globus pallidus and subthalamic responses suggest the operation of a reciprocal inhibitory/excitatory pathway. Two reciprocal interactions were indicated. First, pallidal inhibition may disinhibit the subthalamus and, via a feedback pathway onto the same pallidal cells, act to terminate the neostriatal-induced inhibition. Second, there may be a feedforward pathway from pallidal cells to subthalamic neurons to a different group of pallidal cells. This pathway could act to suppress competing responses. Thus the subthalamicus may have three actions: 1) an early direct cortical and 2,3) later reciprocal feedforward and feedback excitatory antagonism of the neostriatal mediated inhibition of globus pallidus.

Key words: Subthalamus – Globus pallidus – Prelimbic cortex – Motor cortex – Basal ganglia

Introduction

Information arising from the cerebral cortex can influence basal ganglia output by two distinct routes. The larger route comprises excitatory projections to the neostriatum, which are thought to use glutamate or aspartate as neurotransmitter (Divac et al. 1977; Dube et al. 1988; Kitai et al. 1976; McGeer et al. 1977). The neostriatum, in turn, projects to the globus pallidus, the entopeduncular nucleus and the substantia nigra. The majority of these projections are thought to be inhibitory (Deniau et al. 1976), using GABA, enkephalin, dynorphin and substance P as neurotransmitters (Gerfen and Young 1988; Kita and Kitai 1988; Penny et al. 1986). Thus the net effect of cortical activation on these output cells is largely inhibitory.

Some information can also reach these output stations by way of the subthalamic nucleus. Projections from widespread areas of motor and somatosensory cortex reach the subthalamic nucleus (Canteras et al. 1990; Rinvik et al. 1979), whereas projections from association, limbic and other sensory areas do not. The motor and somatomotor projections are branches of corticobulbar and corticospinal projections, thus much of the cortical information reaching the subthalamic nucleus arises from a largely separate pool of neurons than reach the neostriatum (Donoghue and Kitai 1981; Giufrida et al. 1985; Jinnai and Matsuda 1979; Wilson 1987). These projections, too, are excitatory and use glutamate or aspartate (Kitai and Deniau 1981; Nauta and Cuenod 1982; Rouzaire-Dubois and Scarnati 1985). The majority of subthalamic neurons, at least in the rat, send collaterals to all three of the neostriatal targets (Deniau et al. 1978; Van Der Kooy and Hattori 1980). Indeed the presence of bifurcating projections to the substantia nigra and the entopeduncular nucleus rather than non-overlapping topographic projections indicates that these are distinct anatomical structures and not part of a common structure divided by a white matter tract as some have suggested (e.g., Albin et al. 1989).

Until recently it was thought that the subthalamic projections were inhibitory, but recent evidence has sug-
gested that its projections are excitatory and use an excitatory amino acid (Robledo and Feger 1990; Kitai and Kita 1987). This has lead to suggestions that the subthalamic nucleus provides an important excitatory drive to the output of the basal ganglia. Recent lesion studies based on this idea demonstrate that destruction of the subthalamic nucleus can relieve symptoms of experimental Parkinson’s disease induced in monkeys by the neurotoxin, MPTP (Bergman et al. 1990).

The subthalamic nucleus and the globus pallidus are reciprocally related. Thus information from the cortico-neostriatal-pallidal pathway may influence the subthalamic nucleus via projections from the globus pallidus to the subthalamic nucleus. This pathway is thought to be inhibitory and to use GABA as a transmitter (Kita et al. 1983; Rouzaire-Dubois et al. 1980). Fine structural details of these reciprocal projections are lacking. Though cortically receptive regions of the subthalamic nucleus and the globus pallidus appear to be in register (Groenewegen and Berendse 1990), it is not known, for instance, if subthalamic neurons only project to globus pallidal neurons that return projections to that same cell (feedback) or whether projections from cells in one structure influence neurons other than those that return direct projections to the first set of cells (feedforward). The feedforward route clearly exists for globus pallidus to subthalamic nucleus to substantia nigra and entopeduncular nucleus projections.

In this study we examine how information flows from two regions of agranular frontal cortex and from medial orbitofrontal cortex centered on the prelimbic cortex to the globus pallidus by examining the timing of neuronal excitation and inhibition in the globus pallidus and the subthalamic nucleus. These regions were chosen for two reasons. First, the motor cortex, but not the prelimbic cortex, projects to the subthalamic nucleus. Second, the prelimbic cortex projects extensively to the neostriatal striosomes, though also, but more weakly, to the matrix (Donoghue and Herkenham 1986; Gerfen and Young 1988; Gerfen 1989) whereas the motor cortex projects primarily to the neostriatal matrix. Thus, these inputs may be part of separate channels in the basal ganglia (Alexander et al. 1986). In addition, the effects of both excitotoxic and electrolytic lesion of the subthalamic nucleus on globus pallidus responsiveness were examined.

**Methods**

Adult male black-hooded Long-Evans rats (n = 38) were anesthetized with 1.25 gm/kg, ip, urethane. Bipolar stainless steel stimulating electrodes (200 μ polymer insulated) were stereotaxically implanted into two regions of frontal agranular cortex (FRI and FRII of Zilles, 1985) at coordinates A: 3.5, L: 2.0 and 3.0, D: 1.5 below cortical surface (according to the atlas of Paxinos and Watson 1986). And additional electrode was implanted in the medial orbitofrontal cortex centered in the Prelimbic cortex (area Cg3 of Zilles, 1985) at A: 3.5, L: 0.6, D: −2.3. In most animals electrodes were implanted in all three sites, in the earliest animals, two electrodes only were implanted, one always in prelimbic cortex and the other in one of the motor cortex sites. In animals from which neurons in the subthalamic nucleus were to be recorded, an additional stimulating electrode was implanted into the globus pallidus near the typical pallidal recording site (A: −0.3, L: 2.9, D: −5.0).

Extracellular single unit recordings were obtained from either the globus pallidus or subthalamic nucleus using glass micropipettes (4–8 MΩ). Every neuron that was encountered and that could be held for the duration of the testing was studied. The unit activity was recorded onto audio tape and analyzed either on or off line. Subthalamic nucleus neurons were identified by antidromic activation from the globus pallidus using standard criteria (latency, frequency following and especially collision intervals (Fuller and Schlag 1976)). Some neurons that could not be antidromically activated but were obtained deeper on a track with a confirmed subthalamic neuron were also judged to be in the subthalamic nucleus. In a typical experiment, spontaneous activity of the neuron was recorded for 3 min and the firing rate was determined. Each cortical site was stimulated at two currents (0.3 and 0.7 mA, 0.2 ms duration monopolar pulses at 1 Hz) for 2 min in a counterbalanced sequence. The single unit activity was A/D converted at 16.67 kHz. Peristimulus time histograms of unit firing were constructed using 0.96 ms bins for 50 ms prior to the stimulus and continuing for approximately 180 ms following the stimulus. Typically 100 stimuli were used for each histogram. Latency estimates of event onsets and offsets were made manually. In most cases events began and ended abruptly. In other cases a criterion of 3 consecutive bins with a response at least 50% greater or less than the comparable pre-stimulus frequency signalled an event change. The first bin of such a change was used for the latency estimate. Return to the baseline rate defined the event offset.

Excitotoxic lesions of the subthalamic nucleus and vicinity were made by positioning a 30 g stainless steel cannula at coordinates A: 4.4 from lambda, L: 2.5, D: −7.5, and infusing 0.3-0.4 μL of 1.25 μg/μL kainic acid in 0.9% saline over 2 min. The animals were anesthetized with 60 mg/kg, ip, sodium pentobarbital with 1.0 mg/kg atropine methyl nitrate adjunct and, following surgery, received 20000 units, im, of Penicillin G suspension (Crysticillin brand) and nitrofurazone ointment on the wound margins. The animals were tested as above 2–4 days later. Electrolytic lesions (n = 2) were made by positioning a stimulating electrode to this same site and verifying the location by antidromically activating globus pallidus neurons. A current of 2.0 mA was passed for 10 s to make the lesion. In these animals only neurons responsive to FRII stimulation were analyzed.

Lesion location, stimulation sites and recording sites were confirmed histologically. After recording, all animals were deeply anesthetized and perfused intracardially with 10% formalin. The brains were sectioned and stained with neutral red.

**Results**

**Effects of cortical stimulation on globus pallidus neurons**

Extracellular recordings were made from 133 cells in 14 animals. Approximately 10% (12 of 133) of the cells were antidromically activated from one or more cortical site. These cells were excluded from the following analysis.

During the first 50 ms following cortical stimulation four different responses were observed (Fig. 1). All of these response types were seen from each stimulation site (and at each current). The earliest event (6–8 ms onset latency) was an increase in the probability of firing. This was typically followed by an intense period of neuronal inhibition (14–17 ms latency). This response was typically followed by a period of neural excitation (26–32 ms latency). These three events occurred in various combinations (see Fig. 1).