SYNCHRONIZATION OF PALLIDAL ACTIVITY IN THE MPTP PRIMATE MODEL OF PARKINSONISM IS NOT LIMITED TO OSCILLATORY ACTIVITY

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1. INTRODUCTION

Early studies of the neuronal activity of basal ganglia neurons in the MPTP primate model of the disease (Miller and DeLong, 1987; Filion and Tremblay, 1991) and in human patients undergoing streotaxic surgeries (see reviews in (Lang and Lozano, 1998a; Lang and Lozano, 1998b; Vitek and Giroux, 2000)) focused on changes in the firing rates of these neurons. Firing patterns in the basal ganglia networks are also dramatically altered following MPTP-treatment. There is an increase in the percentage of neurons that discharge in bursts. These bursts are either irregular or oscillatory (periodic) and have been found in the striatum (Raz et al., 1996), STN (Bergman et al., 1994), GPe and GPi (Miller and DeLong, 1987; Filion and Tremblay, 1991; Boraud et al., 1998; Raz et al., 2000; Bergman et al., 1994; Nini et al., 1995; Wichmann et al., 1999) and recently also in primary motor cortex (Goldberg et. al, this volume). Physiological studies in human PD patients have found cells whose discharge is modulated in the tremor frequency range in the thalamus, in GPi and in the STN (Hutchison et al., 1997; Lenz et al., 1988; Magnin et al., 2000)

Multiple electrode studies revealed that in normal behaving animals most of the crosscorrelograms between pallidal neurons are flat, indicating that the neuronal pairs are functionally uncorrelated. However, physiological studies in the globus pallidus of MPTP-treated monkeys demonstrate that the crosscorrelograms become peaked and mainly oscillatory (Nini et al., 1995; Raz et al., 2000). Similar findings of increased oscillatory synchronization within primate brains following MPTP-treatment have been found among striatal TANs and between the TANs and pallidal neurons (Raz et al., 1996; Raz et al., 2001). However, it seems that while the discharge of BG cells has a strong spectral component in the tremor frequency range, the synchronization between cells tends to be within a higher frequency range (Raz et al., 2000; Raz et al., 2001). Many neuronal pairs in the STN of human PD patients (Levy et al., 2000) were also synchronized in a higher frequency range (15-30 Hz).

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It was therefore proposed (Bergman et al., 1998) that an increase in synchronization between neurons in the basal ganglia contribute to all clinical features of Parkinson's disease, and not only to tremor. This conclusion was challenged by recent study of Ron Levy et al (Levy, 2001) who use double electrode set-up to test the correlations of 163 pairs of neurons in the output structures of the basal ganglia of human parkinsonan patients. In five patients without limb tremor during the surgical procedure none of the 88 pallidal pairs displayed synchronous activity. Synchronized activity was observed in 25% of pallidal pairs recorded from three patients with limb tremor; however, this was limited to the tremor related oscillatory activity. We therefore decided to directly test the question whether pallidal activity in the MPTP treated monkeys is limited to oscillatory activity only.

2. METHODS

A vervet monkey (Monkey Q, *Cercopithecus aethiops aethiops*, female, weight 3.8 kg) was trained to perform a self-initiated button-pressing task. After training, a Cilux recording chamber (18mm internal diameter) was attached to the skull over a trephine hole to allow access to the GP. During recording sessions the monkey's head was immobilized, and eight glass-coated tungsten microelectrodes (impedance 0.3-1.2MΩ at 1000 Hz) confined within a cylindrical guide (1.65 mm inner diameter) were advanced into the GP. Electrodes were advanced separately (EPS, Alpha Omega, Nazareth, Israel) and optimally placed in the vicinity of GP cells. Cells were selected for inclusion in the study only according to their isolation quality and optimal signal to noise ratio of the recorded spike. The output of each electrode was amplified with a gain of 5-20K and band-pass filtered with a 300-6000 Hz 4-pole Butterworth filter (MCP+, Alpha-Omega, Nazareth, Israel). The electrical activity recorded from each electrode was sorted and classified on-line using a template-matching algorithm implemented by a PC-based spike sorter (MSD, Alpha-Omega Engineering, Nazareth, Israel). Spike trains detected by this system, behavioral events and other measurements of the monkey's behavior, were recorded at 12KHz resolution.

Parkinsonism was induced with systemic injections of the MPTP neurotoxin (Aldrich, Milwaukee, WI, USA). The treatment comprised of consecutive 5 intra-muscular injections of 0.4 mg/kg/day. The monkey developed severe Parkinsonism five days after initiation of MPTP treatment. Recordings were then resumed 4 days after the last injection. After 14 days of recordings in the parkinsonian state, we initiated daily dopamine replacement therapy. Here, we report only on the results of the recording before the morning dose of the treatment.

Correlated activity was estimated using the crosscorrelograms of pairs of recorded cells. Only crosscorrelograms of pairs recorded by different electrodes were included (Bar-Gad et al., 2001). The correlograms were calculated for ±5000 ms offset, using 1 ms bins including recording edge normalization. We tested the null hypothesis of independent activity (i.e., flat crosscorrelogram) using two methods: a. Searching for

significant peaks or troughs b. Searching for periodic oscillations in the crosscorrelograms. A crosscorrelogram was considered non-flat if it contained either a significant peak or trough or a significant periodic oscillatory process.

The monkey's health was monitored by a veterinarian, and it's fluid consumption, diet and weight were monitored daily. All procedures concerning animals were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996), and with the Hebrew University guidelines for the use and care of laboratory animals in research, supervised by the institutional animal care and use committee.

3. RESULTS

Bradykinesia, flexed posture and lower limb dystonia appeared on the third day of the MPTP treatment. Akinesia, freezing and paradoxical kinesia developed on the forth day with tremor and rigidity appearing only on the fifth day. By this day the monkey has lost all ability to perform the behavioral paradigm, to self feed and reached the state of severe Parkinsonism. The clinical situation of the monkey was stable for all the recording period. The clinical "off" state, e.g., before the morning dose of the dopaminergic replacement therapy was not significantly different from the drug naive MPTP state.

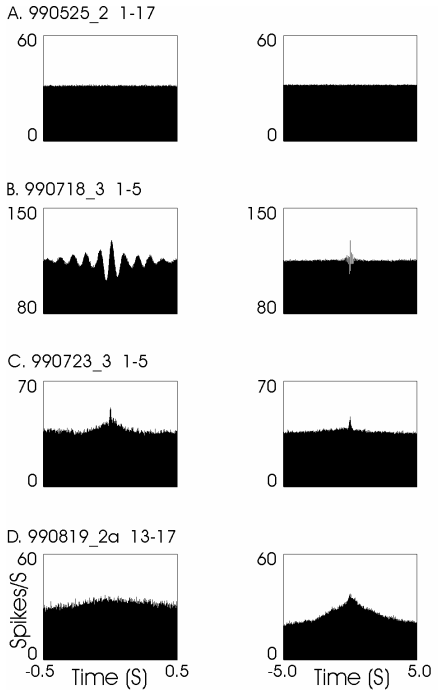

Figure 1: Examples of cross-correlation functions of GP cells in the normal (A) and the MPTP-treated monkey (B,C,D).

Before the MPTP treatment less than 20% of the cross correlograms were non-flat, and only few of these were oscillatory (see example of flat correlogram at figure 1a). In the parkinsonian state almost 50% of the cross correlograms were non-flat. Two third of the non-flat correlograms were oscillatory (figure 1b), mainly at 10-12 Hz. In the 'off' periods of the treated monkey the percentage of total correlated pairs was higher than in

the parkinsonian state, however it seems that the fraction of oscillatory correlations was lower (~40%) and long range non oscillatory correlations were very common (figure 1c,d). The time range of the correlated activity was between fraction of second (figure 1c to few seconds (figure 1d and 2).

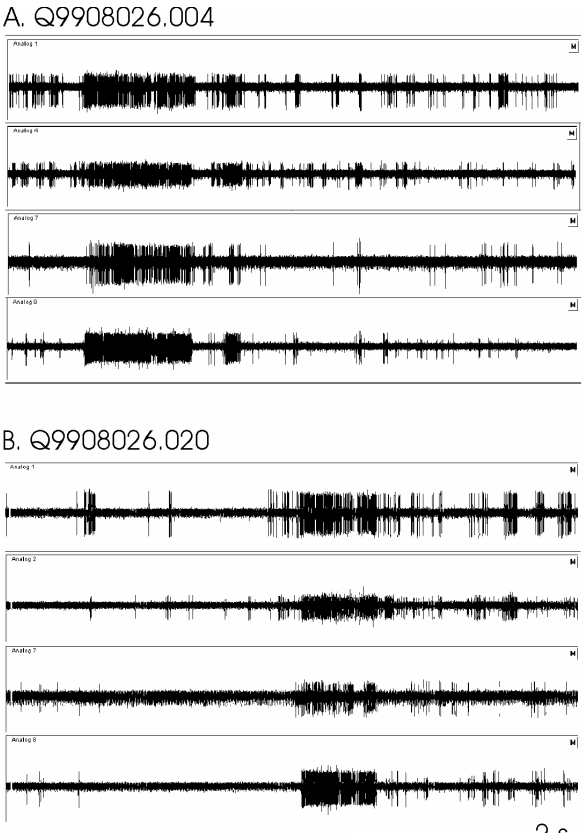

Figure 2: Examples of simultaneous recording of the activity of four cells in the globus pallidus of MPTP treated monkey**.** The two examples were sampled at an interval of ~20 minutes. Synchronous bursts were observed in a-periodic fashion every 1-2 minute along the continuous recording.

4. DISCUSSION

Previous MPTP primate studies (Nini et al., 1995; Raz et al., 2000) and human data (Hurtado et al., 1999; Levy et al., 2000) have shown increase in neuronal synchronization in the basal ganglia of MPTP monkeys and human Parkinson's disease patients. We extended these studies to larger timescale, regarding an offset of ±5.0 s in contrast with the ±0.5-1.0 s offset in the previous studies. In addition we investigated for the first time the neuronal synchronization under chronic dopamine replacement therapy, a condition that better represent the vast majority of Human patients with Parkinson's disease.

Our results confirm the increase in neuronal synchronization after induction of Parkinsonism and show that this increased level of synchronization is maintained in the 'off' states of the chronically L-DOPA treated monkey. A preliminary analysis of the cross-correlation functions indicate that although clinically identical, the neuronal synchronization in the L-DOPA naïve parkinsonian state may be of a different nature than the neuronal synchronization in the 'off' state of the chronically treated monkey. Whereas in the L-DOPA naïve monkey the dominant proportion of the correlations are oscillatory, after chronic exposure to dopamine replacement therapy approximately half of correlations are non oscillatory and are of long timescale nature. These findings might suggest that a gradual modification of the intrinsic neuronal properties and circuitry of the basal ganglia is taking place under chronic dopamine replacement treatment. In this point, we cannot give a simple answer to the differences between our data and the human data (Levy, 2001). The fact that the number of non-oscillatory synchronized activity increased in the levodopa treated monkey is surprising, since all human patients were on dopamine replacement therapy before the surgery. There are some major differences between the two set of experiments, first and most clearly in the species (human and monkeys), the natural history of the disease (idiopathic vs. MPTP induced) and finally in the duration of the recording. For ethical reasons the duration of recording of the neural activity in Human is made as short as possible. Thus the average recording time of Levy et. al. is 70 s, whereas it is more than 20 minutes in the monkey. It could be that pallidal synchronization is an intermittent phenomenon as the neuronal oscillations in the basal ganglia, and therefore need more prolonged recording time to be detected. We therefore suggest that abnormal neuronal synchronization in output nuclei of the basal ganglia is a major contributor to the clinical signs of Parkinsonism in the primate MPTP model and that the desynchronization of pallidal output should be a key factor in future therapeutics of Parkinson's disease.

5. ACKNOWLEDGEMENTS

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