Articles in PresS. J Neurophysiol (August 13, 2008). doi:10.1152/jn.90574.2008

Neuronal activity of the human subthalamic nucleus in the Parkinsonian and non-Parkinsonian state

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Abbreviated Title: Neuronal activity of the human STN

Keywords: Parkinson's disease, subthalamic nucleus, microelectrode recordings, Essential Tremor, Basal Ganglia, neuronal oscillations

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Number of figures: 6
Number of tables: 3
Number of pages: 15
Word count: 4815

Abstract

We recorded resting state neuronal activity from the human subthalamic nucleus during functional stereotactic surgeries. By inserting up to five parallel microelectrodes for single- or multiunit recordings and applying statistical spike sorting methods, we were able to isolate a total of 351 single units in 65 patients with Parkinson's disease (PD) and 33 single units in 9 patients suffering from essential tremor (ET). Among these were 93 pairs of simultaneously recorded neurons in PD and 17 in ET, which were either detected by the same (n=30) or neighboring microelectrodes (n=80).

Essential tremor is a movement disorder without any known basal ganglia pathology and with normal dopaminergic brain function. By comparing the neuronal activity of the STN in patients suffering from PD and ET we intended to characterize for the first time changes of basal ganglia activity in the human disease state, that had previously been described in animal models of Parkinson's disease We found a significant increase in the mean firing rate of STN neurons in PD and a relatively larger fraction of neurons exhibiting burst-like activity compared to ET. The overall proportion of neurons exhibiting intrinsic oscillations or interneuronal synchronization as defined by significant spectral peaks in the auto- or crosscorrelations functions did not differ between PD and ET when considering the entire frequency range of 1-100 Hz. The distribution of significant oscillations across the theta (1-8 Hz), alpha (8-12 Hz), beta (12-35 Hz) and gamma band (>35 Hz), however, was uneven in ET and PD as indicated by a trend in Fisher's exact test (p=0.05). Oscillations and pairwise synchronizations within the 12-35Hz band were a unique feature of PD.

Our results confirm the predictions of the rate model of Parkinson's disease. In addition, they emphasize abnormalities in the patterning and dynamics of neuronal discharges in the Parkinsonian STN, which support current concepts of abnormal motor loop oscillations in Parkinson's disease.

Introduction

Several lines of evidence indicate, that abnormal neuronal activity of the subthalamic nucleus (STN) plays a pivotal role in the pathophysiology of Parkinsonian motor symptoms: Lesioning or deep brain stimulation (DBS) of the STN dramatically reduce akinesia, tremor and rigidity in 1,2,3,6-tetra-hydropyridine (MPTP)-treated monkeys (Benazzouz et al. 1993; Bergman et al. 1990) and patients with Parkinson's disease (Volkmann 2007). Recordings from the subthalamic nucleus in MPTP monkeys, on the other hand, exhibit characteristic changes in the firing behaviour of STN neurons with the appearance of Parkinsonian symptoms after striatal dopamine depletion. The mean firing rate of STN neurons increases from approximately 20 Hz to 30-40 Hz after MPTP intoxication (Bergman et al. 1994; Wichmann et al. 1994b; Wichmann and Soares 2006) in keeping with the popular rate model of the basal ganglia (Albin et al. 1995; DeLong 1990).

However, several clinical observations, in particular the effects of functional stereotactic procedures (Marsden and Obeso 1994), are difficult to reconcile with an exclusive role of firing rate in the pathophysiology of movement disorders. Other features such as the firing pattern (Boraud et al. 2002) or interneuronal synchronization (Bergman and Deuschl 2002; Bergman et al. 1998) may be more important for a normal flow of information through the basal ganglia and determine pathological states (Hammond et al. 2007). The most prominent abnormalities in the discharge pattern of MPTP monkeys are the development of periodic bursting or oscillatory activity in STN and internal globus pallidus (GPi) (Bergman et al. 1994; Wichmann et al. 1994a) and the increase of synchronous firing in simultaneously recorded neurons (Nini et al. 1995; Raz et al. 2000).

Microelectrode recordings are routinely performed during functional neurosurgery and provide a unique opportunity to confirm the observations from the primate MPTP-model in patients with Parkinson's disease (PD). The reported mean firing rates of subthalamic neurons between 33 and 64 Hz in awake and unmedicated PD patients (Hutchison et al. 1998; Magarinos-Ascone et al. 2000; Magnin et al. 2000; Rodriguez-Oroz et al. 2001) lie roughly within the range observed in MPTP-treated monkeys.

Most neurons exhibit an irregular discharge pattern. Oscillatory single cell activity was described as tremor-locked 3-7 Hz bursting of STN or GPi neurons (Hutchison et al. 1998; Hutchison et al. 1997; Rodriguez-Oroz et al. 2001), but also as beta-band oscillations (15-30 Hz), which were modified by voluntary movements or dopaminergic medication (Levy et al. 2002a). Microelectrode and local field potential recordings in unmedicated PD patients have not only demonstrated the synchronization of this oscillatory activity within but also between basal ganglia nuclei (Brown et al. 2001; Levy et al. 2002b).

In summary, these data suggest, that the electrophysiological activity of the STN in Parkinson's disease is similar to that described in MPTP monkeys. The pathophysiological interpretation of microelectrode recordings in Parkinsonian patients, however, has so far been limited by the lack of a control group. For obvious reasons invasive recordings from normal humans without any disease will never be available for comparison.

In our center microrecordings are routinely performed to explore the ventrolateral thalamus and subthalamic area of patients with essential tremor, before implanting therapeutic DBS electrodes into the thalamic ventrointermediate (VIM) nucleus and underlying subthalamic fiber tracts (Herzog et al. 2007). During these explorations we were able to record from a limited number of subthalamic neurons in ET patients and to compare their activity to recordings from patients with Parkinson's disease undergoing implantation of DBS electrodes into the STN. Classical essential tremor is one of the most prevalent neurological disorders and is clinically defined by a slowly progressive action tremor in the absence of other neurological symptoms, especially Parkinsonian features (Deuschl et al. 1998). In almost half of the patients intention tremor evolves together with other subtle signs of cerebellar dysfunction like ataxia and movement overshoot over many years. The motor impairment imposed by intention tremor is typically the reason for patients to seek surgical treatment, once medical alternatives have failed. ET is most likely caused by abnormal functioning of the olivocerebellar motor pathways (Deuschl and Bergman 2002; Deuschl and Volkmann 2002). Although this classical view of ET as a pure functional-metabolic disorder without any structural abnormality has recently been challenged by more rigorous neuropathological studies reporting signs of neurodegeneration in the cerebellum and brain stem (Louis and Vonsattel

2008), there has never been any evidence for an involvement of dopaminergic pathways or basal ganglia nuclei in the disease. Hence, we considered ET an appropriate non-Parkinsonian control condition for characterizing resting state neuronal activity of the human subthalamic nucleus in comparison to Parkinson's disease.

Patients and Methods

Intraoperative microelectrode recordings were obtained in 65 patients suffering from advanced PD (mean age 60 ± 8.6 years; disease duration 16 ± 6.7 years; 41 male/24 female) and 9 patients (mean age 70 ± 4 years; disease duration 34 ± 16.2 years, 7 male/2 female) with medically intractable essential tremor. All PD patients suffered from severe akinetic-rigid motor symptoms in the medication-off state (Unified Parkinson's disease rating scale (UPDRS) motor score: 46 ± 15 points in the off-state), which were highly levodopa sensitive (UPDRS motor score: 20 ± 11 points in the best"-on-state). They had been selected for surgery to treat drug-refractory hypokinetic fluctuations or dyskinesias according to the established in- and exclusion criteria for DBS (Lang et al. 2006). 31 patients suffered from a moderate to severe (UPDRS item 20 ≥ 2) off-period resting tremor in at least one extremity. ET patients had been selected for VIM-DBS to treat a severe, drug-refractory kinetic tremor in at least one upper extremity (Raethjen et al. 2004) as defined by a score of at least 2/4 on the postural and intention tremor items of the Fahn–Tolosa–Marin tremor rating scale (TRS). None of them suffered from a resting tremor or had clinical signs of Parkinsonism. The average severity of tremor as assessed by the TRS (part A) was 18 ± 7 points. Clinical scores were obtained during a standardized and videotaped neurological examination within one week before surgery.

The decision to perform surgery was not influenced by the participation within this study. The protocol had been approved by the local Ethical Committee and all patients gave informed consent.

Surgical and microrecording procedure

The details of the stereotactic procedure and the intraoperative microrecordings have been reported previously (Herzog et al. 2007; Steigerwald et al. 2005). In all patients antiparkinsonian or antitremor medication were withheld for at least 12 hours before surgery. The entire neurophysiological mapping procedure was performed under local anesthesia without sedatives. We used stereotactic magnetic resonance imaging (MRI) and a combination of a landmark based and direct imaging for target and trajectory planning. After a burr hole anterior to the coronal suture was prepared according to the planned entry point, up to five parallel rigid cannulas were inserted into a Ben-Gun microelectrode holder and microdrive. The central trajectory of the Ben-Gun-Holder was directed at the anatomical target, while the other four were equally spaced around with a center to center distance of 2.0 mm. Up to five stainless microelectrodes (FHC, Bowdoinham, ME) were simultaneously advanced in increments of 0.5 mm or less. Amplification, visual display and audiomonitoring of the signal were handled by the Leadpoint microrecording system (Medtronic Inc, Minneapolis, USA). The analog output of this system was fed into a second stage biosignal amplifier (TPM, Lüneburg, Germany) for band-pass filtering (0.3-10 kHz) with a gain of 100. Recordings were digitized using the CED 1401 system (sampling rate of 25 kHz; Cambridge Electronic Design, Cambridge, UK) and stored for off-line analysis on a personal computer.

The theoretical coordinates of the subthalamic nucleus (STN) were 11-13 mm lateral to the intercommissural line, 4-6 mm below and 2-3 mm behind the midcommissural point. In patients with ET we targeted the subthalamic area below the ventrointermediate thalamic nucleus (VIM: 14-15 mm lateral to intercommissural line, 0 mm below and 6 mm behind the midcommissural point). From these coordinates it is evident that depending on the individual angulation of the trajectory the anterior or medial electrode within the “Ben-Gun” arrangement could possibly enter into the subthalamic nucleus. The STN forms the ventral border of the aubthalamic area and identifying typical STN discharges when slowly advancing the microelectrodes beyond the anatomical target helps to delineate the boundary of the subthalamic white matter tracts, which in our experience represent the optimal target for treating intention tremor in ET (Herzog et al. 2007). Only in those ET patients, for whom the stereotactic planning

suggested that at least one of the trajectories would also pass through the more ventro-medial STN without a risk of injuring vessels, the recordings were extended to identify the depth of the STN neurophysiologically. After the microelectrode mapping of the STN region the electrodes were pulled back to the subthalamic area or the thalamus in ET patients to perform test stimulations. We did not implant therapeutic electrodes into the subthalamic nucleus, nor did we perform test stimulations within this region in ET patients, because the intended target, which most likely corresponds to the zona incerta and prelemniscal radiation, lies dorsal to the nucleus (Herzog et al. 2007).

A MATLAB (Mathworks, Germany) routine was programmed to reconstruct the AC-PC-based coordinates of all neuronal recording sites based on the depth of the microelectrode tip (readings from the microdrive), the stereotactic MRI coordinates of the AC and PC and the anteroposterior and mediolateral angulation of the trajectory. Only those neurons in ET, whose stereotactic position fell within the 95% confidence interval of the recordings sites in PD-patients, were included in the comparative analysis. The stereotactic coordinates of each recording site were transferred into the corresponding plates of the Schaltenbrand-Wahren-atlas (Schaltenbrand and Wahren 1977). The fusion of atlas and recording sites was strictly based on standard stereotactic techniques and no corrections were made for the individual boundaries of nuclei detemined by neurophysiological methods or interindividual differences in scaling. Thus, possible deviations between the intended and the true stereotactic position of the microelectrode tip, brain shift after openinng the dura and the variability between the individual brain and the atlas morphology, cause uncertainties with respect to the true localization of the recording sites. The goal of this analysis, however, was not a precise anatomical superimposition onto a standard atlas brain but rather a comparison of the recording sites of ET and PD patients. Such a comparative analysis should be able to detect possible anatomical biases despite the localizational errors, because they should equally affect both groups.

Intraoperatively, the dorsal border of the STN was identified by an increase of background noise and typical large amplitude irregular spike activity after passing the subthalamic white matter and zona incerta. Neuronal activity was recorded for at least 30 seconds at each depth, while the patients were lying

relaxed with their eyes open and without performing voluntary movements. Resting tremor was infrequently seen under this condition in PD–patients, because we selected patients with predominantly akinetic symptoms and severe off-period rigidity counteracting tremor.

Analysis of neuronal activity and statistics

The recordings were analysed offline using the Spike2 software version 5 (CED, Cambridge, UK). Given the high cellular density of the STN, we restricted the spike sorting procedure to those recordings with stable single- or multi-unit activity that clearly exceeded the amplitude of the background noise. Single unit activity (SUA) was discriminated by threshold spike detection and template matching, controlled by cluster analysis with principal component analysis and final visual inspection. Only SUA, which could be recorded for at least 20sec or with at least 350 spikes, were further analyzed.

The statistical characterization of SUA included instantaneous and mean firing rate (1 sec bins), interspike interval (ISI) histogram, autocorrelation and crosscorrelation of simultaneously recorded units (1 msec bin).

The firing pattern of each neuron was predetermined based on the visual inspection of the spike train and the ISI histogram (ISIH). The final classification was observer independant and relied on several statistical parameters of the ISI distribution (Gernert et al. 1999; Hassani et al. 1996): The asymmetry index (AI), which is the ratio of the mode to the mean ISI and provides information on the shape of the ISIH, the variance and the coefficiant of the variance (CV) of ISIs. According to these variables the firing patterns were classified into the following three types: (1) A bursting or burst-like firing pattern consisting of intermittent grouped firing separated by periods of pauses or low-frequency tonic activity. The ISIHs of these neurons were characterized by a positively skewed distribution (AI < 0.75), i.e., by a large fraction of short interspike intervals (ISI) representing „intraburst“ firing overlapping with variably longer „interburst“ intervals. With more regular bursting actvity the shape of these ISIH was shifted to a double-peaked (bimodal) distribution representing the short regular intraspike intervals and longer regular interburst intervals. (2) An irregular firing pattern characterized by a flat and broad based, random

distribution of the ISI (CV > 85), which sometimes showed mild positive skewness. (3) Regular tonic firing characterized by ISIHs with a typical bell-shaped distribution and a small variance as indicated by an asymmetry index close to unity and a CV of the interspike interval < 90. SUA classified as bursting were further analyzed for their burst features by the burst surprise method (Legendy and Salcman 1985) with a burst surprise value ≥ 5.

Auto- and crosscorrelation functions (1000ms window, bin size 1ms, offset 500ms) were reconstructed for each SUA and neuronal pairs and analyzed by the method of Raz et al. (Raz et al. 2000) to detect significant oscillations and synchrony. In brief, correlations (in case of autocorrelation the trough of the refractory period ±10ms around time zero was removed first to reduce high-frequency noise) were low-pass filtered (100Hz) and the DC offset was removed. Only peaks between 1 and 100Hz of the power spectra (Fast Fourier transformation, 1.953Hz resolution, hamming window) exceeding 5-times the standard deviation around the mean power were considered significant (Fig. The oscillatory activity detected by this method was classified according to the peak frequency of the power spectrum into theta (1-8 Hz), alpha (8-12 Hz), beta (12-35) or gamma (>35 Hz) band activity, similar to the terminology used for EEG or local field potential recordings.

All statistical comparisons were conducted with JMP 6.0 (SAS Institute Cooperation, Cary, USA). Continuous variables of the PD and ET group were compared using ANOVA. For categorical data we used the Chi-square test or Fischer's exact test, where applicable. The level of significance for all statistical tests was defined as less than 0·05 (two-tailed). Unless otherwise noted, all group values of continuous variables are reported as mean ± standard deviation.

Results

We isolated a total of 351 SUA in patients with PD and 33 SUA in ET with an average recording duration of 50±28 seconds. Owing to the different stereotactic approaches SUA in ET patients were on average slightly more posterior (-3.9±1.8 mm sagittal distance from MCP) and dorsal (-2.1±1.2 mm vertical distance from AC-PC plane) compared to PD (-2.8±1.6 mm sagittal distance from MCP; -3.1±1.6

mm vertical distance from AC-PC plane), but because of the large variability there was still extensive topographical overlap of the recording sites within STN (Fig. 2).

Discharge pattern and frequency

Spikes recorded from the STN had a characteristic biphasic shape with an initial negative phase. Based on the ISI distribution three main patterns of neuronal discharges were differentiated (Fig. 3): The majority of neurons exhibited a burst-like firing pattern with a positively-skewed distribution of the ISI, resulting in a small asymmetrie index (AI) and intermediate coefficient of variance (n=258, 67.2%; AI = 0.58 ± 0,11; CV = 133.9 ± 2.44). 126 neurons (32.8%) had non-bursting activity. Among these, 60 SUA (15.6%; AI = 0.42 ± 0.17; CV = 185.9 ± 5.07) were characterized by a high CV and broad, random ISI distribution reflecting irregular activity and 66 neurons (17.2%, AI = 0.83 ± 0.05; CV = 73.3 ± 4.83) by the bell-shaped ISI distribution of tonic activity with a very low CV.

The relative proportion of these discharge patterns differed significantly between the PD- and the ET-group (chi-square-test, $p<0.001$, see Fig. 4). Burst-like activity dominated in PD (70%), whereas the proportion of burst-like versus non-bursting SUA was reversed in ET (36 vs. 64%). Because we did not find a particular topographical distribution of the different discharge patterns within the subthalamic nucleus of PD-patients (see Fig 6C & D), the group differences were unlikely the result of a recording bias within STN. The burst characteristics of neurons with burst-like discharge in PD did not differ significantly from burst-like neurons in ET (Table 1). Only a trend was seen towards longer interburst intervals in ET.

An overall ANOVA with the factors DISEASE (PD vs. ET) and PATTERN (bursting vs. non-bursting) was conducted on the mean discharge rate of all SUA. It indicated significant independent effects of both factors on the mean firing rate, with SUA in ET firing at a significantly lower rate than in PD and bursting cells having in general higher discharge rates than non-bursting cells (DISEASE $p<0.0005$; PATTERN: $p<0.001$; PATTERNxDISEASE, $p=0.0107$). Whereas SUA in PD-patients had a mean frequency of 40.5 ± 23.3 Hz, the average discharge rate in ET was only 19.3 ± 20.1 Hz (Table 2,

Figure 5). Again, topographical analysis ruled out, that the group differences resulted from a sampling bias due to an uneven spatial distribution of firing rates within the STN (see Fig, 6A & B).

Oscillation and synchronization

SUA with significant peaks in the power spectrum of their autocorrelation (see methods and Fig. 1 B & C), were referred to as oscillatory. When considering the entire analysed bandwidth from 1 to 100 Hz the proportion of oscillatory SUA was almost equal in PD and ET (25.1% vs. 18.2%). Table 3 summarizes the distribution of significant oscillations across the theta, alpha, beta and gamma band. This distribution differed between ET and PD as indicated by a strong trend in Fisher's exact test (p=0.05). Oscillations in the theta-band were common in either group (PD 79.5%; ET 50.0%). However, oscillations within the alpha and beta-band were only found in PD and the relative proportion of gamma-band oscillations was markedly lower in PD (9.1%) than in ET (50.0%). In both groups significant oscillations within the gamma-band were restricted to frequencies above 60Hz, no significant peaks were found in the lower frequency range. When stratifying by discharge pattern, it became apparent that in neurons with theta and beta oscillations bursting characteristics predominated (n=71/81; 87.6%), whereas gamma oscillations were commonly found in non-bursting cells (n=8/11; 72.7%). Oscillatory activity in general was equally distributed throughout the STN, but beta oscillatory SUA were exclusively found within the dorsal portion of the STN (average coordinates: -1.9±1.0 mm sagittal distance from MCP; -1.6±1.2 mm vertical distance from AC-PC plane; 12.2±1.2 mm lateral to MCP).

To evaluate whether these oscillations were a mere phenomenon of single cell activity or rather represented synchronized activity within the STN neuronal network, we looked for the presence of synchronization within pairs of simultaneously recorded neurons using cross-spectral analysis. Overall, we were able to simultaneously record from 93 pairs of SUA in PD patients (see Fig. 1 D; 25 from the same, 68 from different electrodes) and 17 pairs in ET (5 same, 12 different electrodes). 17/93 (18%) of these pairs in PD and 2/17 (12%) in ET exhibited significant synchronization of their activity within the entire bandwidth from 1 to 100 Hz. The small number of synchronized pairs did not allow any

meaningful statistical analysis on their distribution across frequency bands, but similar to the autocorrelation analysis we only found synchronized beta-activity in SUA recorded from PD patients (Table 3).

Relation between neuronal discharge properties and clinical symptoms

To relate the severity of parkinsonian symptoms to neuronal discharge properties we calculated the average frequency of all SUA and an oscillation index (number of SUA with oscillation / total number of SUA) for each individual patient. Only those PD patients with more than five isolated SUA were included in this analysis (n=26). We did not find any significant correlation between the total UPDRS-motor score (part III) or symptom subscores for tremor, rigidity or akinesia with the frequency of neuronal discharges or the oscillation index. Oscillatory SUA were not more frequent in PD patients with rest-tremor than in those without. Neither did we find a difference between between PD patients with or without resting tremor in the frequency distribution of oscillatory SUA or in the relative proportion of the discharge patterns.

Discussion

The present study describes the characteristics of resting state neuronal activity in the subthalamic nucleus of patients with Parkinson's disease and Essential tremor. It is unique in using for the first time recordings from the STN of patients not affected by PD as control data, which allows verifying the concepts of basal ganglia dysfunction derived from primate models of Parkinsonism in the human disease state.

In agreement with the rate model of basal ganglia function, which predicts a disinhibition of the STN in the Parkinsonian state (Alexander et al. 1990; Bergman et al. 1994), we found significantly higher firing rates of subthalamic neurons in PD compared to ET. Our mean discharge frequency of 40.5 Hz in PD patients lies within the range of previous reports (Hutchison et al. 1998; Magarinos-Ascone et al. 2000; Magnin et al. 2000; Rodriguez-Oroz et al. 2001), and roughly doubles the rate found in ET (19.3

Hz). The possibility, that the lower mean frequency in ET is an artifact by recording from a circumscribed part of the STN with a lower discharge rate, is very unlikely. A previous report described a lower discharge rate in the ventral portion of the STN (Rodriguez-Oroz et al. 2001). We were not able to reproduce this result in our topographical analysis. But even if the discharge rate had a rostrocaudal gradient, we should have recorded predominantly from the faster discharging, dorsal regions of the STN in ET, because the STN marks the lower border of the subthalamic area, which we explored electrophysiologically to delineate the target for optimal tremor control (Herzog et al. 2007).

Another hallmark of the Parkinsonian state was a relative increase of burst-like activity in the STN compared to ET. Based on the ISI distribution we classified 70 % of single unit activities in PD as bursting, Rodriguez and colleagues (Rodriguez-Oroz et al. 2001) using a different analysis approach and terminology described “irregular discharges with silent periods or pauses” in 60.5% and tonic discharges in 24% of STN neurons recorded from PD patients. The properties of the first type match our group of “burst-like” activity. This close coincidence in the relative proportion of different discharge types supports the validity of our findings in PD. The shift from non-bursting to bursting activity in PD most likely reflects a change in the preferred functional state of a single neuronal population, because histological differences in the STN of PD and ET patients have not been described and the neuronal morphology and structure of the STN is relatively homogenous in monkeys (Parent and Hazrati 1995) rendering an anatomical sampling bias implausible.

Interestingly, we found specific features of intrinsic oscillatory activity associated with the bursting and tonic discharge mode: Gamma band oscillations dominated in non-bursting SUA, whereas beta-band and lower frequency oscillations were linked to the bursting pattern. This could explain the higher proportion of SUA with gamma-band oscillations in ET and the exclusive finding of alpha and beta-band oscillations in neuronal activity recorded from PD patients. Previous studies using local field potential (LFP) recordings from the human basal ganglia have emphasized two distinct operating modes characterized by the dominance of either synchronized beta (<30 Hz) or gamma band (>60 Hz) oscillations (Brown and Williams 2005). The two frequency bands are inversely affected by the execution

or inhibition of movement, and differentially expressed according to the prevailing level of dopaminergic activity. Levodopa shifts the peak frequency of LFP in Parkinson's disease from below 30Hz to above 70Hz (Brown et al. 2001). During voluntary movements gamma-band activity is enhanced and 8-30Hz activity is suppressed (Cassidy et al. 2002; Levy et al. 2002a) suggesting a pro- (movement fascilitating) and antikinetic (movement inhibiting) function of the two bands. The differential distribution of neuronal oscillations across frequency bands in our study of ET and PD patients does in principle support the concept of abnormal oscillatory binding in PD. However, the relative proportion of cells exhibiting intrinsic oscillations or interneuronal synchronization within the beta-band, was much smaller in this study compared to previous reports (Levy et al. 2002a; Levy et al. 2000; 2002b). Levy and colleagues observed synchronized 15-30 Hz oscillations in 28 out of 82 pairs (34%) of simultaneously recorded STN units in PD. The authors emphasized the importance of tremor for the emergence of beta-band oscillations, because the majority of synchronous cells were recorded from five patients with resting tremor in the operating room, whereas no synchronous pairs were found in non-tremulous patients. In our group of patients moderate to severe resting tremor was relatively infrequent with only 31 patients (47%) exhibiting a score≥2 on item 20 of the UPDRS for any body segment and 25 patients having a total score≤2 on item 20 indicating a pure akinetic-rigid type of Parkinsonism. The 139 units recorded from these 25 patients without tremor did not differ from the remaining group in the prevalence of bursting activity, theta or beta oscillations. This is in accordance to a recent study of the same group which found beta oscillations also in PD patients without tremor (Weinberger et al. 2006).

A limitation of our study, however, is the lack of tremor recordings during the surgery. We were therefore not able to determine the amount of tremor locked activity within the 3-10 Hz or transient changes of the neuronal discharge pattern in relation to intermittent tremor. In addition, methodological issues in detecting oscillatory single unit activity (Rivlin-Etzion et al. 2006) and in particular the very conservative statistical threshold for significant spectral peaks in the auto- and crosscorrelations, which however was also used in studies by other groups (Raz et al. 2000), may have caused an underestimation of oscillatory and synchronous discharge behaviour in this study. Although this could compromise the

comparability of the results with previous studies, it should not affect our internal comparison of the physiological characteristics in PD and ET as the same criteria were applied to both groups.

Another important methodological issue, which needs to be considered in the comparison of human and animal recordings, is the behavioural context of the experiments. We recorded neuronal activity intraoperatively in the resting state, after instructing the patient to withhold any voluntary movements. Moreover, we did not specifically monitor the level of attention during the recordings such that drowsiness may have occurred. In contrast, most studies in the monkey report recordings in unrestrained animals or even during the performance of trained motor tasks (Raz et al. 2000). Other limitations of our study include the relatively small sample of neurons recorded in ET, which has to be attributed to the small number of patients, in whom the stereotactic trajectories led into the STN region without risk of injuring blood vessels, the possible impact of an anatomical recording bias and the fact that we were comparing two disease states, although ET has never been associated with abnormal basal ganglia function.

Despite these limitations, our study confirms several predictions of the MPTP model of Parkinsonism: Firing rates of STN neurons were increased in PD compared to ET patients closely matching the frequency ranges observed in monkeys before and after MPTP intoxication (Bergman et al. 1994; Wichmann and Soares 2006). Likewise, we found a shift towards bursting-type activity in PD, which was also described in recordings from GPi or STN of the primate model (Bergman et al. 1994; Wichmann et al. 1994a; Wichmann and Soares 2006). Our ANOVA analysis with independent effects of disease and discharge type on firing rate, corroborates recent identical findings in several extrastriatal basal ganglia nuclei of monkeys by Wichmann and colleagues (Wichmann and Soares 2006), who also emphasized the complexity of changes in the timing of cellular activity after MPTP intoxication. In contrast to the animal model, however, we did not find global differences in the proportion of neurons exhibiting intrinsic oscillatory activity or interneuronal synchronization (Nini et al. 1995; Raz et al. 2000) in PD or ET. Both disease states rather differed in the relative fraction of neurons with theta, alpha, beta or gamma-band activity. The large proportion of neurons exhibiting theta band activity around 4 Hz in

PD patients in our study has not been described in the MPTP monkey, where 10 Hz activity dominates (Nini et al. 1995; Raz et al. 2000; Wichmann and Soares 2006).

In summary, we were able to confirm several predicted key features of abnormal subthalamic neuronal activity in the Parkinsonian state compared to patients without any symptoms of Parkinsonism, which consist in an overall increase in discharge rate and complex changes in the patterning and dynamics of neuronal firing.

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Acknowledgement

This study was funded by Deutsche Forschungsgemeinschaft, SFB 654 (project A7) and by the German Ministry of Research and Technology (Kompetenznetz Parkinson, FK: 01GI0201).

Tables

Table1: Burst Characteristics in PD and ET

	Asymmetrie index	**Burst frequency [/min]**	**Burst duration [msec]**	**Spikes in burst [n]**	**Frequency in burst [Hz]**	**Interburst interval [sec]**
	Mean ±SD	*Mean ±SD*	*Mean ±SD*	*Mean ±SD*	*Mean ±SD*	*Mean ±SD*
PD	0,58 ±0,11	6,16 ±7,1	164 ±167	20 ±19	110,8 ±83,3	3,87 ±5,91
ET	0,55 ±0,15	6,04 ±6,0	225 ±172	31 ±34	113,5 ±88,5	1,64 ±2,35
	n.s	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2: Temporal characteristics of neuronal discharges in the subthalamic nucleus

	Discharge pattern				**Mean firing rate [Hz]**		**Interspike-interval Variation coefficient**	
			n	*(%)*	*Mean±SD*	*range*	*Mean±SD*	*range*
PD	Bursting	Burst-like	246	(70)	42,9 ±20,0	(11-123)	1,34 ±0,39	(0,8-3,3)
	Non-bursting	Irregular	43	(12)	13,9 ±5,6	(1-25)	2,04 ±0,52	(0,9-2,9)
		Tonic	62	(18)	49,2 ±29,7	(5-135)	0,74 ±0,16	(0,3-1,4)
	Total		***351***		***40,5 ±23,3***	***(1-135)***	***1,327 ±0,52***	***(0,3-3,3)***
ET	Bursting	Burst-like	12	(36)	38,4 ±23,1	.(11-88)	1,32 ±0,39	(0,9-2,0)
	Non-bursting	Irregular	17	(52)	7,3 ±2,4	(4-11)	1,41 ±0,39	(0,9-2,5)
		Tonic	4	(12)	12,9 ±2,8	(10-16)	0,71 ±0,1	(0,6-0,8)
	Total		***33***		***19,3 ±20,1***	***(4-88)***	***1,29 ±0,43***	***(0,6-2,5)***

Table 3: Distribution and mean frequency of significant peaks in the power spectral analysis of autocorrelations and crosscorrelations of single unit activity.

			bandwidth (Hz)			
			1 – 8	**8 – 12**	**12 – 35**	**> 35**
Oscillatory activity in Autocorrelation			**theta**	**alpha**	**beta**	**gamma**
	PD	n	70	2	8	8
		(%)	(79.5)	(2.3)	(9.1)	(9.1)
		peak frequency (Hz)	4.2±1.4	9.7±0.1	17.9±6.0	84.9±6.5
	ET	n	3	0	0	3
		(%)	(50.0)	(0)	(0)	(50.0)
		peak frequency (Hz)	4.0±0.4	-	-	82.7±12.8
Oscillatory activity in Crosscorrelation						
	PD	n	8	0	2	7
		(%)	(47.1)	(0)	(11.8)	(41.2)
		peak frequency (Hz)	5.6±1.4	-	18.4±4.4	75.9±15.2
	ET	n	1	0	0	1
		(%)	(50)	(0)	(0)	(50)
		peak frequency (Hz)	4.5	-	-	66,5

Figure legends

Fig. 1: Example of spike sorting and postprocessing of single unit activity in a recording sweep from a PD patient. (A) Template matching and principle component analysis (PCA) identified the activity of two units with different spike shape and separate clusters in the PCA (upper panels) from the raw recording (lower trace). The sorted spikes are displayed above the raw trace. (B, C) For each unit the interspike interval histogram (upper graph) and the autocorrelation (middle) graph is calculated. The power spectrum of the autocorrelation (lower graph) indicates significant oscillatory activity in the theta-band for both units. (D) For pairs of simultaneously recorded units the crosscorrelation is computed (left), which also exhibits a significant peak within the theta band of the power spectrum (right).

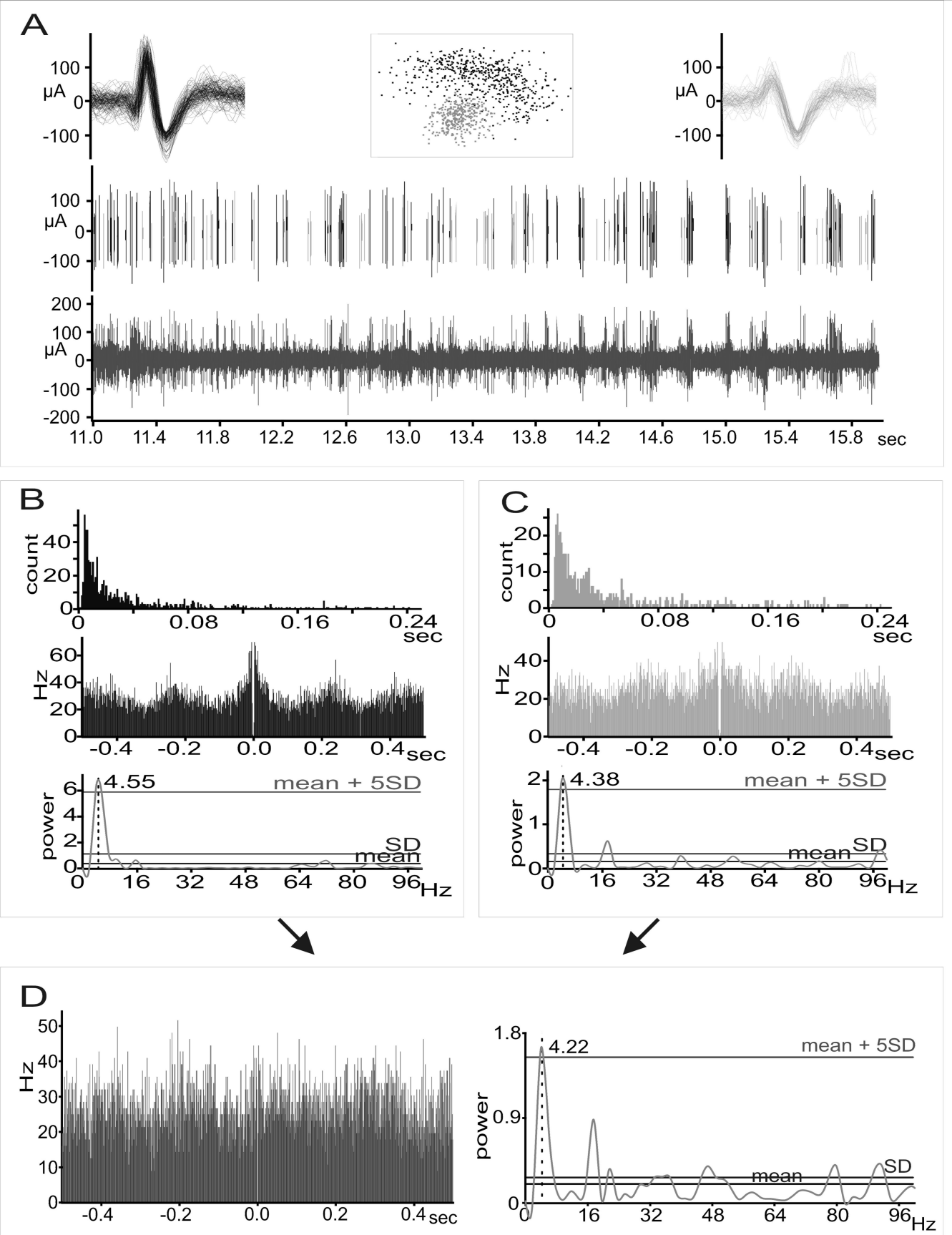
Fig. 2: The stereotactic positions of the recording sites of single unit activity in patients with Parkinson's disease (crosses) and Essential tremor (boxes) are superimposed onto the corresponding coronal (left) and sagittal (right) section of the Schaltenbrand Wahren atlas.

Fig. 3: Discharge patterns of subthalamic neurons of PD and ET patients. Trains of discriminated spikes drawn as raster plots (50 spikes for each, resulting in different time bars shown under the raster plot) and, below, interspike interval histograms (ISIHs) illustrate the three different types of discharge patterns.

Fig. 4: The relative fraction of the three discharge patterns among single unit activity recorded from PD and ET patients is displayed in a bar graph. The percentage of burst-like activity (white) was significantly increased at the expanse of less irregular activity (grey background) in PD compared to ET (chi-square test, $p<0.001$).

Fig. 5: Distribution of the mean frequencies of all STN neurons in patients with Parkinson's disease (PD) and essential tremor (ET). Each dot represents the mean frequency of an individual SUA. The box plots summarize the distribution with the ends of the box indicating the 25th and 75th quantiles and the line across the middle of the box identifying the median sample value. The whiskers extend to the 95% confidence limits. The mean discharge rate of single units recorded in PD was significantly higher than in ET (Mann-Whitney-U-test, $p < 0.001$).

Fig. 6: A bivariat analysis showed no correlation between the recording position and the mean frequency (A, B) or the discharge pattern (C, D) of single units recorded in Parkinson's disease.


A
100
μA 0
-100
100
μA 0
-100
100
μA 0
-100
200
100
μA 0
-100
-200
11.0 11.4 11.8 12.2 12.6 13.0 13.4 13.8 14.2 14.6 15.0 15.4 15.8 sec
B
count 40 20 0
0 0.08 0.16 0.24 sec
Hz 60 40 20 0
-0.4 -0.2 0.0 0.2 0.4 sec
power 6 4 2 0
4.55
mean + 5SD
SD
mean
0 16 32 48 64 80 96 Hz
C
count 20 10 0
0 0.08 0.16 0.24 sec
Hz 40 20 0
-0.4 -0.2 0.0 0.2 0.4 sec
power 2 1 0
4.38
mean + 5SD
mean
SD
0 16 32 48 64 80 96 Hz
D
Hz 50 40 30 20 10 0
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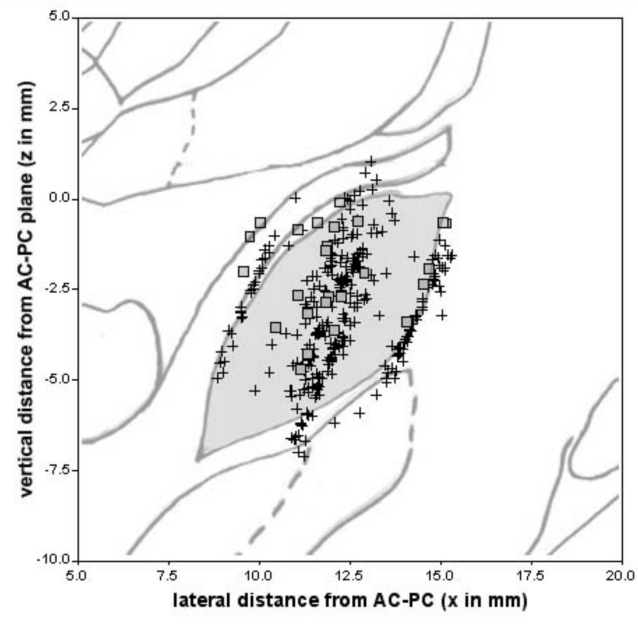
mean
SD
0 16 32 48 64 80 96 Hz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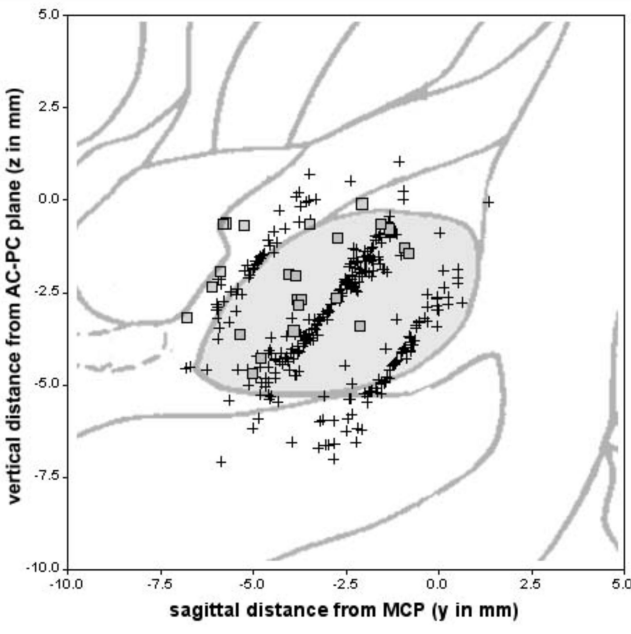
0.0
-2.5
-5.0
-7.5
-10.0
5.0
7.5
10.0
12.5
15.0
17.5
20.0
lateral distance from AC-PC (x in mm)


vertical distance from AC-PC plane (z in mm)
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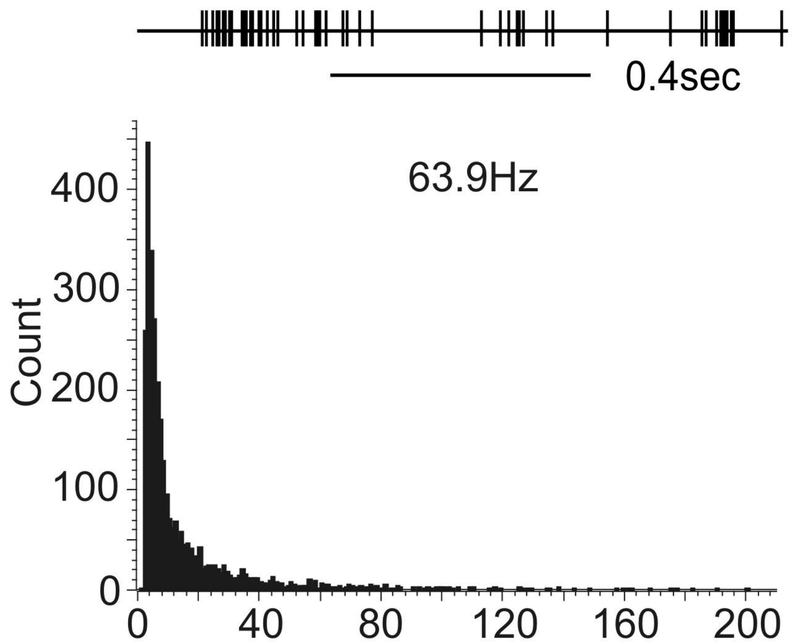
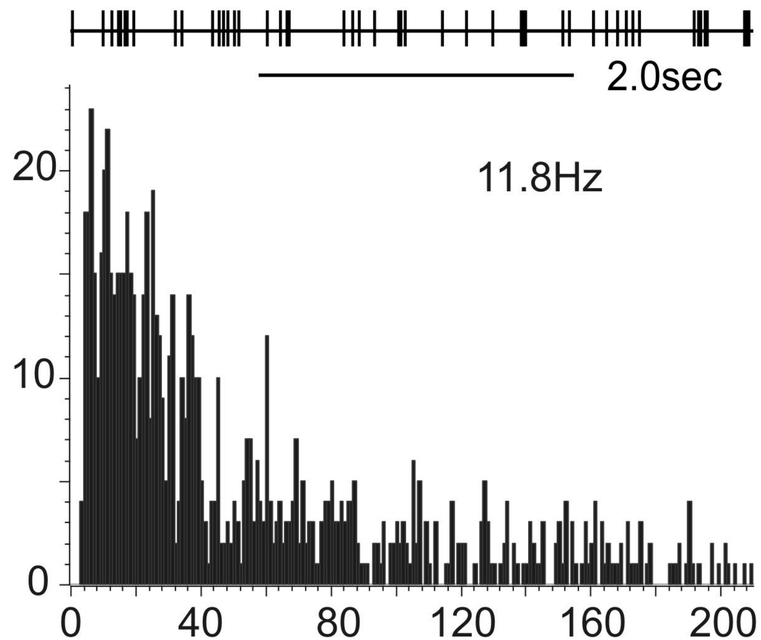
-2.5
-5.0
-7.5
-10.0
-10.0
-7.5
-5.0
-2.5
0.0
2.5
5.0
sagittal distance from MCP (y in mm)

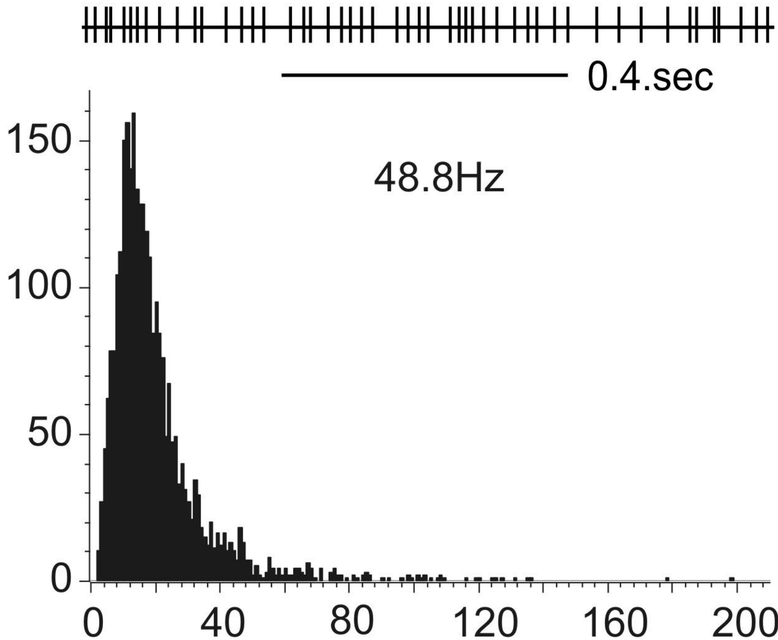
burst-like | **non - bursting**

irregular | *tonic*

PD







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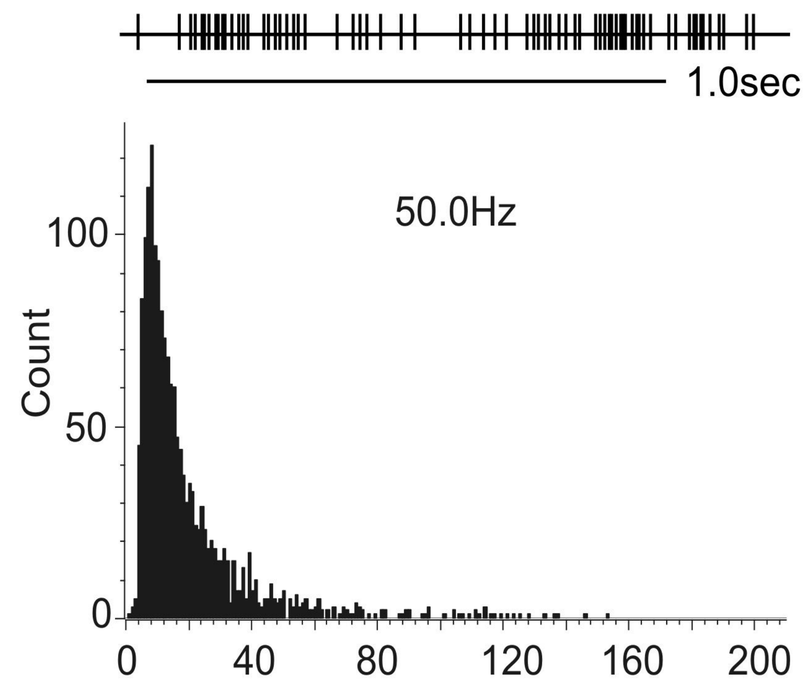
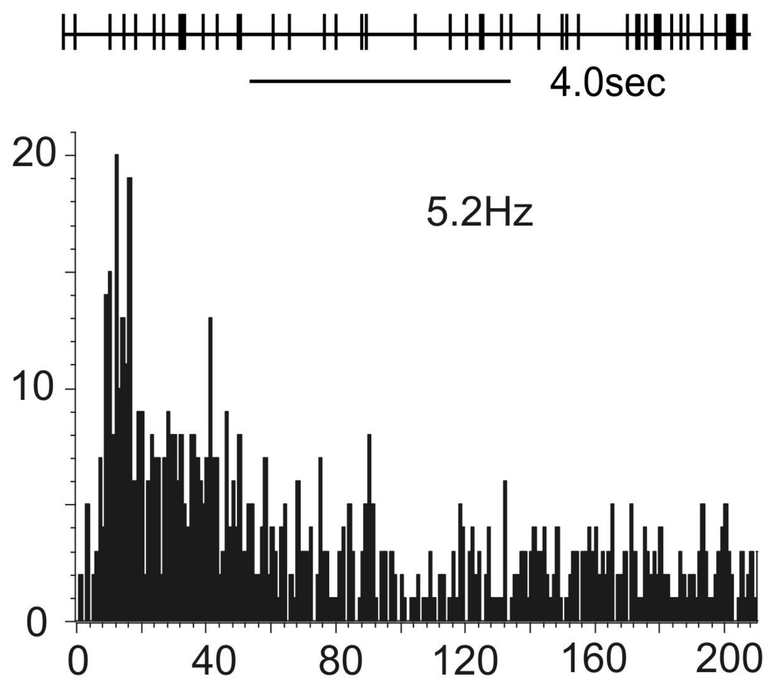
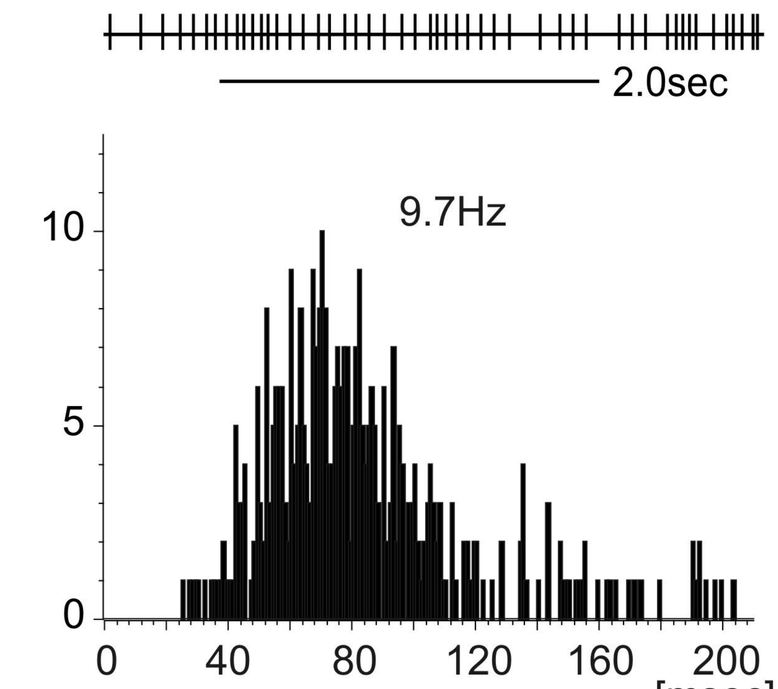
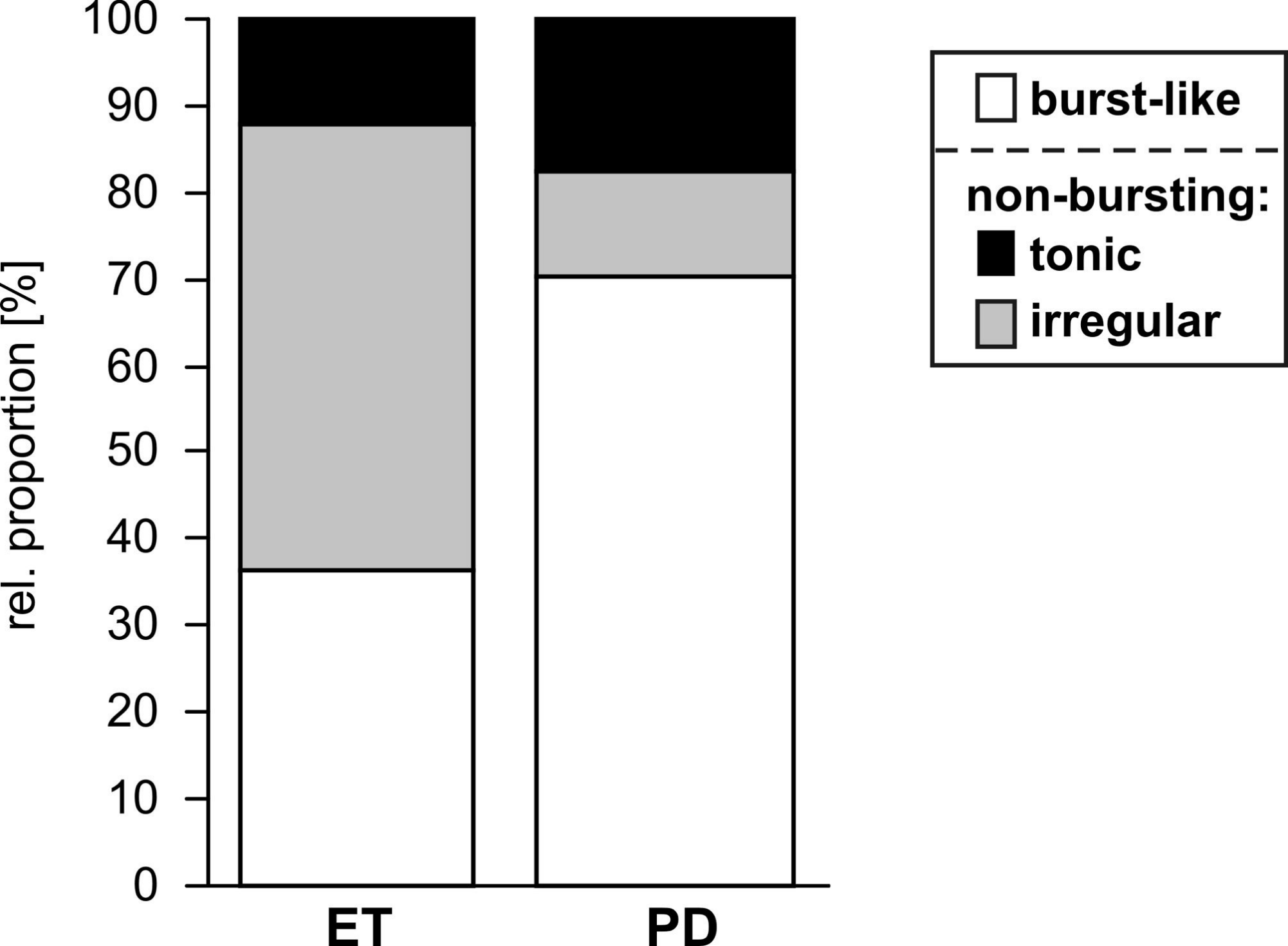
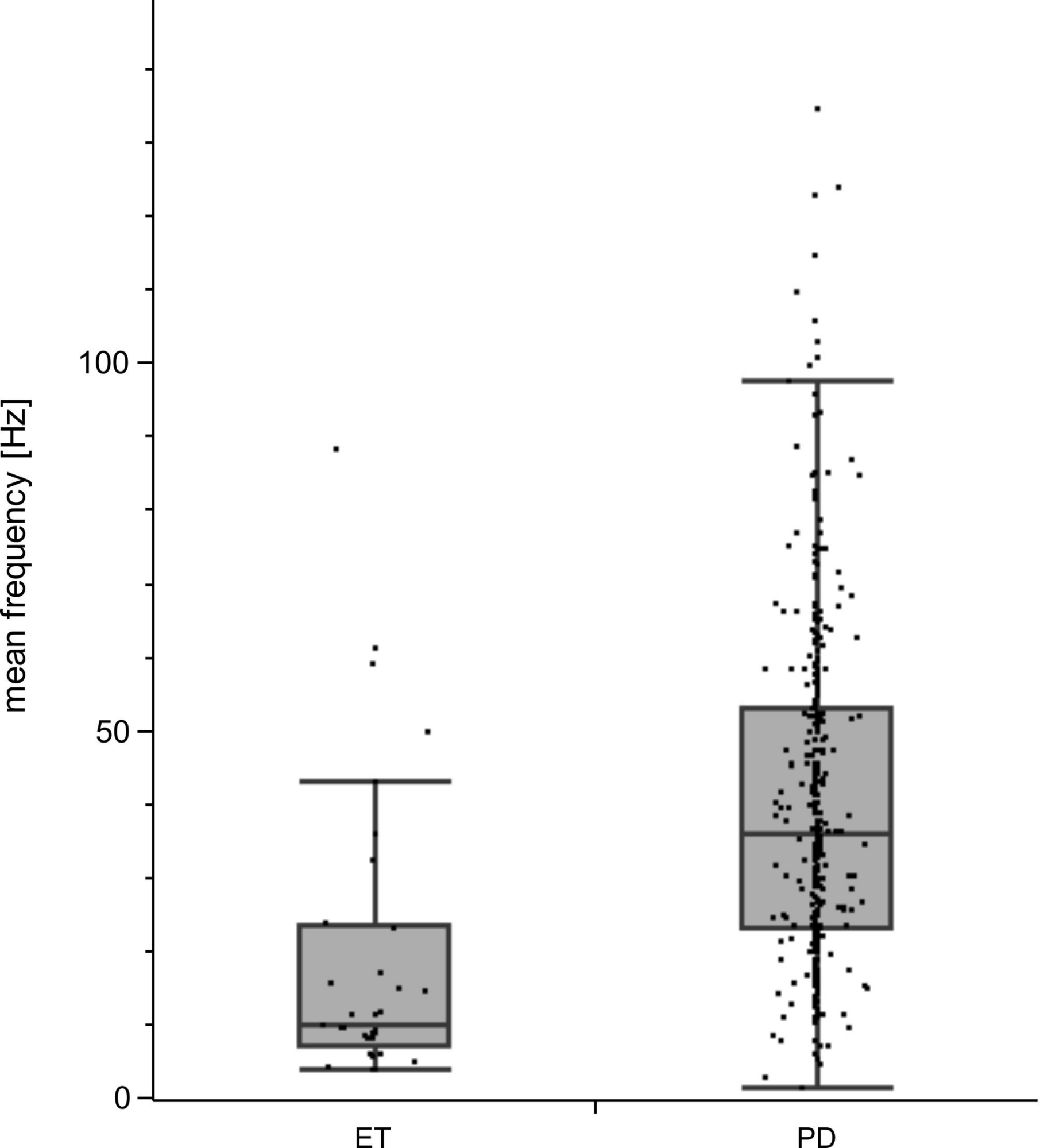


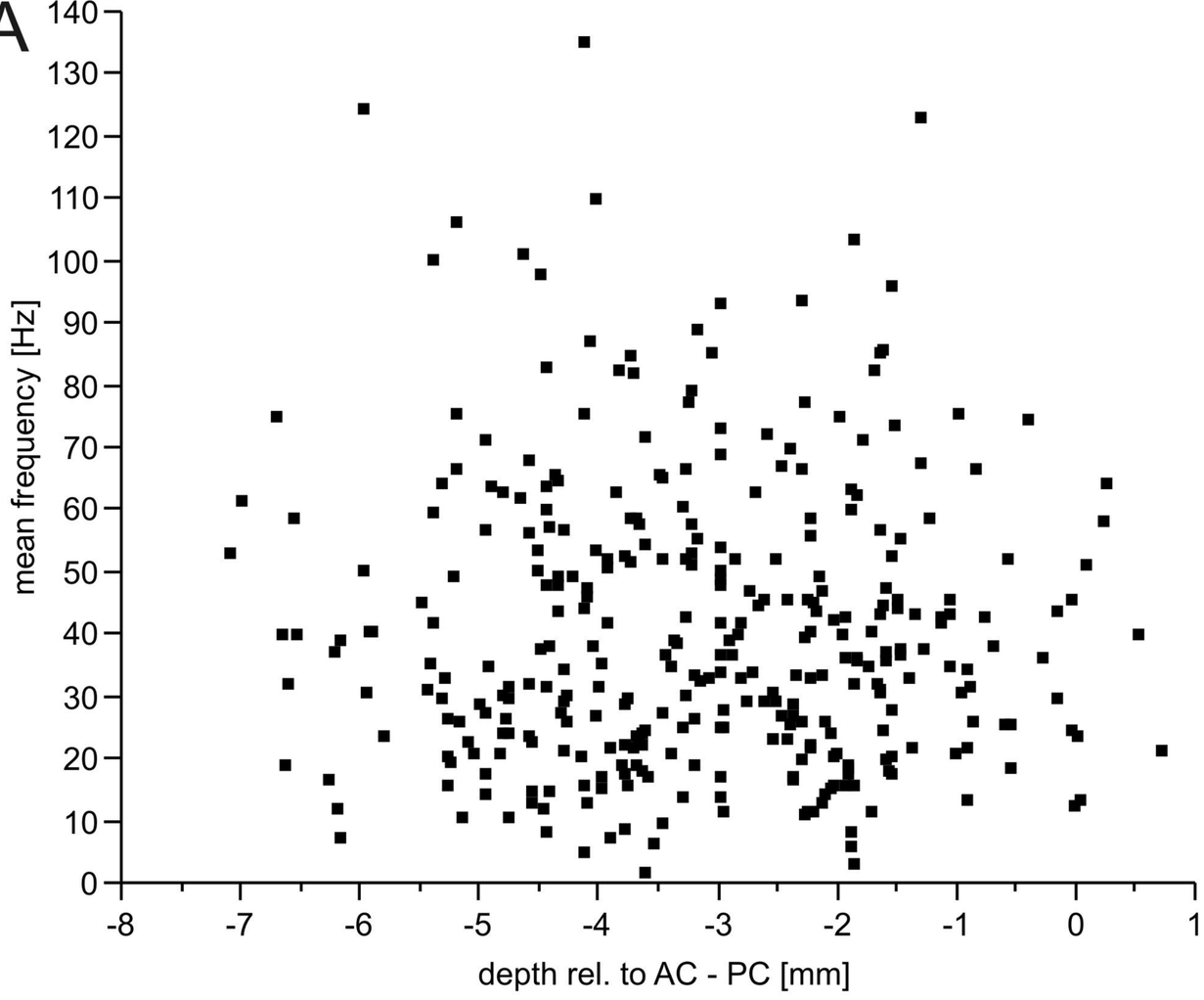





100
90
80
70
60
50
40
30
20
10
0
rel. proportion [%]
ET
PD
burst-like
non-bursting:
tonic
irregular


mean frequency [Hz]
100
50
0
ET
PD

A



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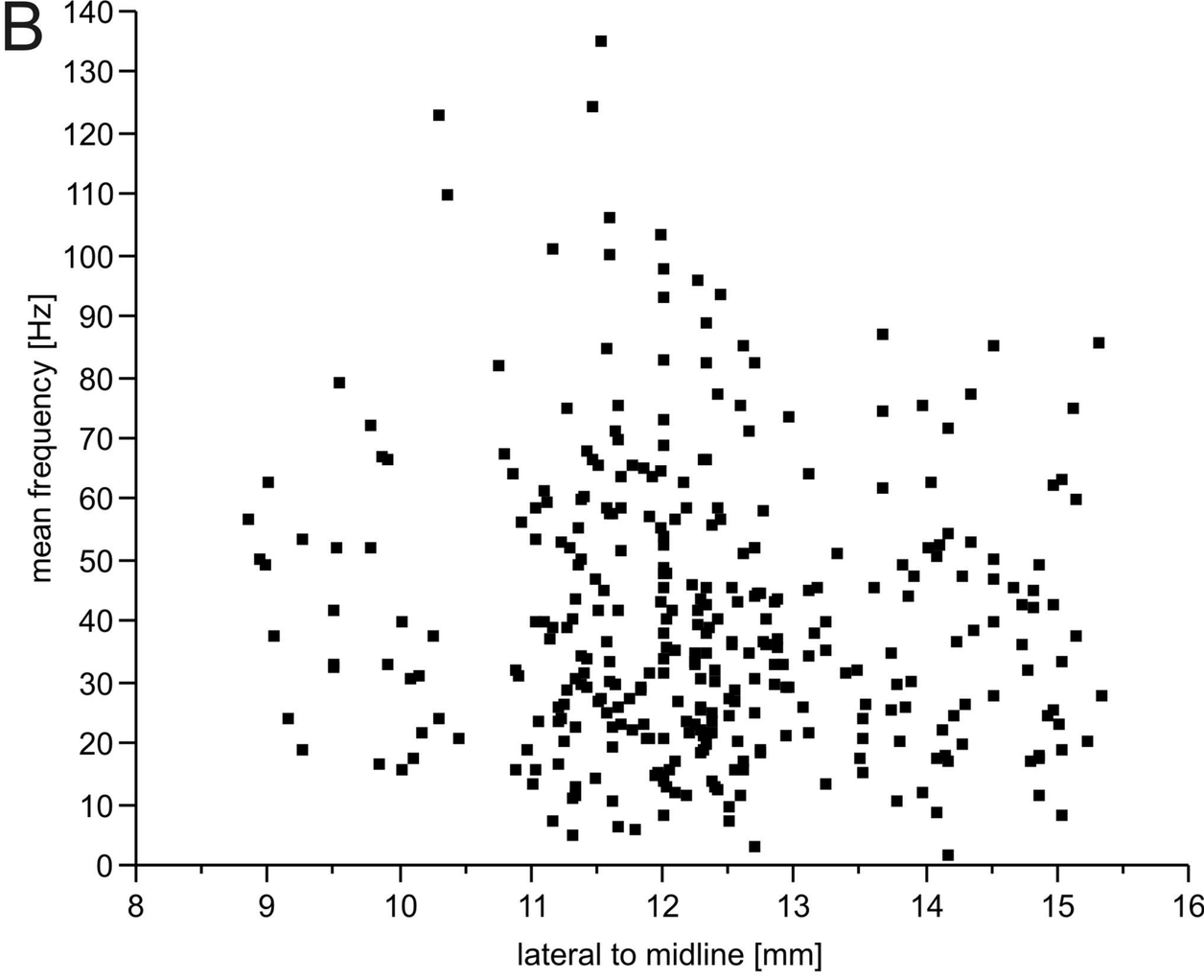

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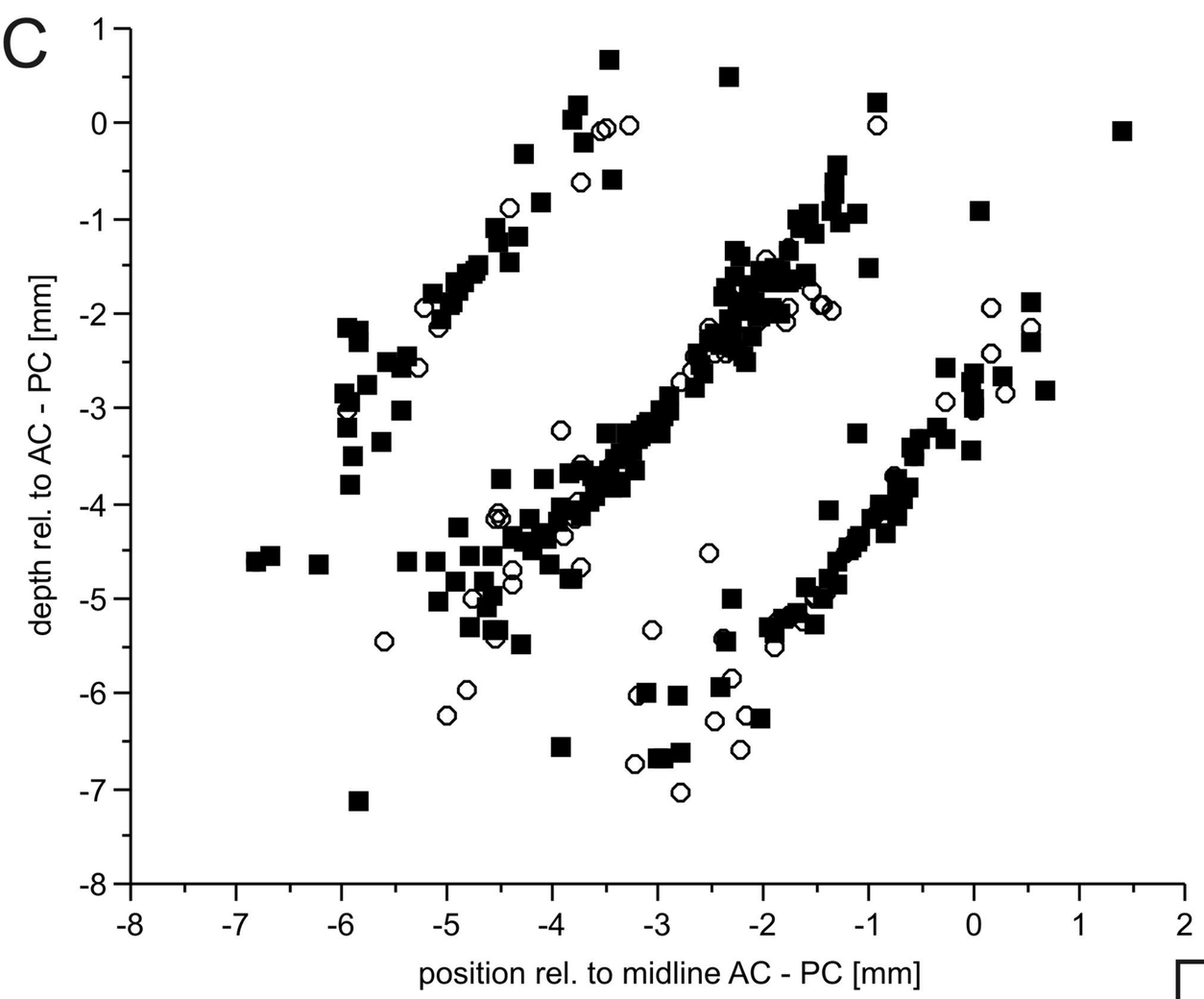

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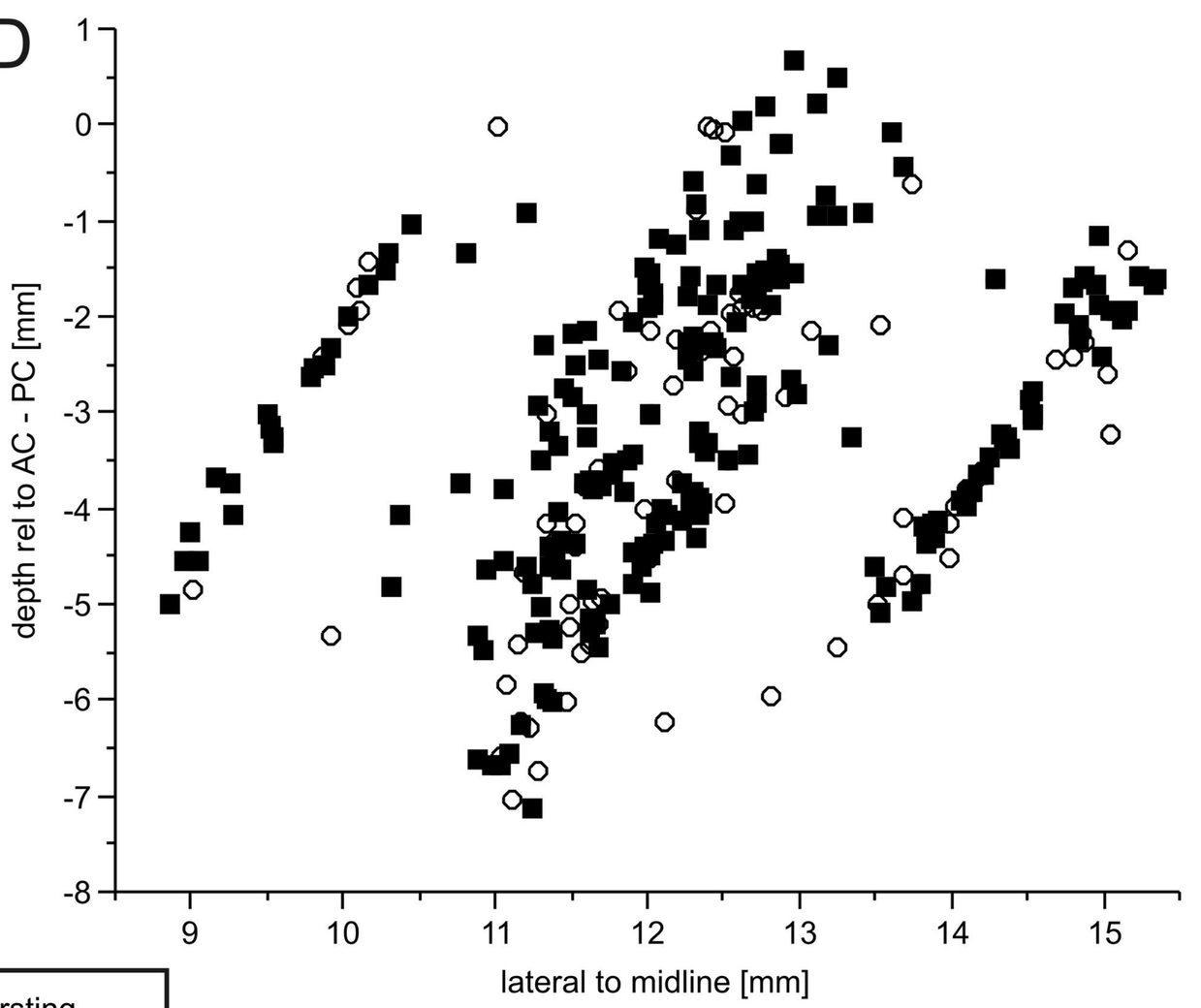

bursting

non-bursting