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RELATIONSHIP BETWEEN ENTROPY OF SPIKE TIMING AND FIRING RATE IN ENTOPEDUNCULAR NUCLEUS NEURONS IN ANESTHETIZED RATS: FUNCTION OF THE NIGRO-STRIATAL PATHWAY

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Abstract

The function of the nigro-striatal pathway on neuronal entropy in the basal ganglia (BG) output nucleus (entopeduncular nucleus, EPN) was investigated in the unilaterally 6-hydroxydopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD). In both control subjects and subjects with 6-OHDA lesion of the nigro-striatal pathway, a histological hallmark for parkinsonism, neuronal

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entropy in EPN was maximal in neurons with firing rates ranging between 15Hz and 25 Hz. In 6-OHDA lesioned rats, neuronal entropy in the EPN was specifically higher in neurons with firing rates above 25Hz. Our data establishes that nigro-striatal pathway controls neuronal entropy in motor circuitry and that the parkinsonian condition is associated with abnormal relationship between firing rate and neuronal entropy in BG output nuclei. The neuronal firing rates and entropy relationship provide putative relevant electrophysiological information to investigate the sensory-motor processing in normal condition and conditions with movement disorders.

Keywords

Entropy; basal ganglia; rat; globus pallidus internal; dopamine; 6-OHDA

Introduction

Executions of motor activities are envisaged in terms of selections and inhibitions of motor programs leading to the most appropriate motor sequences¹. Electrophysiological activities in sensory-motor circuitries are considered as the neuronal substratum for the control of motor activities. Conditions with motor disorders result from alterations in sensory-motor circuitry and consecutive neurotransmitter imbalances and pathological electrophysiological activities. Parkinson disease (PD)² and related conditions³ exhibit various histopathological hallmarks⁴ including the loss of catecholaminergic neurons (resulting in dopamine (DA) and noradrenaline (NA) depletions) which are key players in the dysfunctions of the sensory motor circuitry^{2, 5}. Identification of pathological neuronal activities in this circuitry may condition the efficiency of future neuro-modulation therapeutics to reduce the burden of parkinsonism⁶.

Briefly, cortical and sub-cortical areas also called basal ganglia (BG) contribute to sensory-motor processing. The BG, (Fig. 1) are a part of the cortico-cortical loops (via the thalamus)². The striatum is the most prominent input nucleus to the BG and receives dense excitatory (glutamatergic) projections from the cortex and dopaminergic (DA-ergic) inputs from the substantia nigra compacta (SNpc). This cortical excitatory drive exerts an excitatory control on the striatal efferent neurons (medium spiny neurons, MSN)⁷ which project to the BG output nuclei via two pathways. The 'direct' pathway is a GABA-ergic monosynaptic projection, while the indirect 'pathway' is a GABA-ergic multisynaptic projection. These two pathways are mostly segregated in two populations of medium spiny neuron (MSN) which express different types of DA-ergic receptors⁸. The MSN of the direct pathway express the D1 family receptors which have excitatory effects on these neurons. In contrast, the MSN of the indirect pathway express the D2 family receptors which inhibit their activities. In human and non-human primates, the output nuclei of the BG consist in the globus pallidus internal (GPi) and the substantia nigra reticulata (SNr); the GPi receives sensory-motor inputs from the dorsal striatum and it is a target for Deep Brain Stimulation therapy (an efficient treatment to alleviate PD symptoms)⁹. In rodents, the entopeduncular nucleus (EPN) also receives input from the dorsal striatum¹⁰ and its stimulation can alleviate dysfunctions induced by depletion in catecholamines¹¹. In both rodents and primates, the output nuclei of the BG are also under excitatory control arising from the cortex via a relay

in the subthalamic nucleus (STN, hyperdirect pathway)¹². This anatomo-functional model stresses the importance of the balance of activities between the direct, indirect and hyperdirect pathways on sensory-motor processing². In conditions with lesion of the DA-ergic nigro-striatal pathway (i.e. parkinsonism), the model predicts that loss of DA results in an imbalance between the direct and indirect pathway activities in favor of the indirect pathway, leading to decreased inhibition and increased neuronal firing activity in the output BG^{2, 3} and, secondary, to an inhibition of the thalamus¹³. Inhibition and excitation of the thalamus are considered promoting the selection and inhibition of movement respectively.

The concept of summation of disinhibitory/inhibitory drives onto the output nuclei of BG has given ground to consider that coding of information relies (solely) on the firing rate of neurons^{2, 14} and forged the “rate hypothesis”. Under this hypothesis, the BG are seen in a steady state; increased firing rate in BG output nuclei leads to hypokinesia while decreased firing rate leads to hyperkinesia. However, critics to the model^{15–17} have pointed out the fact that the parkinsonian condition may be seen as a breakdown of motor control with a wide range of motor symptoms including tremor, rigidity and impairment in performing voluntary movements; therefore, alterations in the spike timing of output nuclei of BG may not solely be characterized by changes in central tendency (aka, firing rate) but may also include a breakdown of its temporal organization^{9, 17–21}. We hypothesized that catecholaminergic regulation not only controls the ‘tone’ of the direct and the indirect pathways, but also the dynamic, or synergy, of their modulations on the output BG neurons². This hypothesis underlies that both rate and irregularity (as defined by entropy)^{22, 23} of the spike timing could be jointly altered following depletion in catecholamine. Specifically, we have investigated the entropy/rate properties of output EPN neurons in rats with dopamine depletion induced by the neurotoxin 6-OHDA directly administrated in the medial forebrain bundle to lesion the nigrostriatal pathway. Recordings of single neurons firing activity were performed in the anesthetized animals to limit movement-related sensory-motor inputs and environmental perturbations. Approximate entropy (ApEn) and firing rate were used to investigate changes in spike timing in EPN neurons following catecholamine depletion and in idle (aka, anesthetized) condition. In light to the changes in entropy/rate property observed after 6-OHDA lesion, we discuss the role of the nigrostriatal pathway on neuronal firing activity in output BG neurons and sensory-motor coding.

Material and Methods

2.1 Animals

Experiments were carried out in male Sprague-Dawley rats (N = 14; weighing 220–230 g; Charles River Laboratories, Germany). Rats were housed in groups of four in standard Macrolon Type IV cages (Techniplast, Hohenpeissenberg, Germany) under a 12-h light–dark cycle (on at 07:00 h) at a room temperature of 22 ± 2 °C, with food and water available at all times. All animal procedures were approved in accordance with the European Council Directive of November 24, 1986 (86/609/EEC) and approved by the local animal ethic committee. All efforts were made to minimize the number of animals used and their discomfort.

2.2 6-OHDA lesion

One group (N=8, 6-OHDA treated group) received 6-OHDA injection and a non-lesioned group (N=6, control) served as a naïve control. For surgery, rats were anaesthetized with 3.6 % chloral hydrate (1ml/100g body weight, i.p., Sigma, Germany) and placed in a stereotaxic frame (Stoelting, Wood Dale, Illinois, USA). The incision area was shaved and local anaesthetic (Xylocain, s.c.) was injected along the intended incision line. Ophthalmic ointment (Bepanthen, Bayer, Germany) was applied to the eyes to avoid corneal dehydration. The skull was exposed by a midline sagittal incision. Two holes were drilled over the targets above the right medial forebrain bundle and the dura was exposed. 6-OHDA was dissolved in 0.02% ascorbate saline at a concentration of 3.6 µg/µl and was injected (1 µl/min) in two deposits (2.5 µl and 3µl, respectively) at the following coordinates in mm relative to bregma and to the surface of the dura mater: anterior (A) = -4.0; lateral (L) = ±0.8; ventral (V) = -8.0; mouth bar at +3.4 and A = -4.4; L = ±1.2; V = -7.8; mouth bar at -2.4, respectively. The injection rate was 1µl/min. Following the infusion, the cannula remained at the target site for an additional 5 minutes to allow diffusion of the neurotoxin. We did not use desipramine to prevent putative effects of 6-OHDA on non-dopaminergic catecholaminergic or indolaminergic fibers.

Body temperature was maintained at 37–37.5 °C throughout surgery by a heating pad. After infusion, the incision was closed by stitches and the animals were returned to their home cages for recovery¹¹.

The efficacy of the 6-OHDA-induced lesion was assessed 3 weeks after surgery by challenge with apomorphine (0.05 mg/kg, s.c.; Sigma) as described by Schwarting and Huston (1996)²⁴. Apomorphine-induced rotation was used to functionally validate the lesion in 6-OHDA treated rats. A lesion was considered successful for subjects performing more than 80 net contraversive rotations in 20 min following apomorphine administration.

2.3 Single neuronal activity recording

Electrophysiological recordings were taken in the EPN of control and hemi-lesioned rats at least 3 weeks after vehicle or 6-OHDA injection. The rats were anesthetized with urethane (1.4 g/kg, i.p.; ethyl carbamate, Sigma, St. Louis, MO) and placed in a stereotaxic frame. The body temperature was maintained at 37 ± 0.5° C by a heating device (FHC, Bowdoinham, ME).

Small craniotomies were made over the target coordinate for the EPN to the ipsi-hemisphere to lesion side. A single microelectrode for extracellular recordings (quartz coated pulled with a ground platinum-tungsten alloy core (95%–5%), diameter 80 µm, impedance 1–2 MΩ) was connected to the Mini Matrix 2 channel version drives head-stage (Thomas Recording, Germany) and stereotaxically guided through the skull burr holes to the target coordinates in the EPN (A: -2.5 to -2.8 mm; L: +/- 2.5 to +/- 2.8 mm; V: -7.8 to -8.2 mm) under continuous recording of extracellular neuronal signals using a microdrive (Thomas Recording GmbH, Giessen, Germany). Single unit (SU) spike activities were recorded between 500 and 5000 Hz and amplified × 19,000 and sampled at 25 kHz. All signals were digitized with a CED 1401 (Cambridge Electronic Design, UK) and recorded

for 10 to 12 min after signal stabilization with Spike2 analysis software (Cambridge Electronic Design, Cambridge, UK). 300 s of the recording were analyzed and sorted on the base of a 3:1 signal to noise ratio. After termination of the experiment, electrical lesions were made at the proximal and the distal site of the trajectory to allow histological verification of the recording site (10 μ A for 10 seconds; both negative and positive polarities) as previously described²⁵. An illustration of histological-based reconstruction from the rat brain atlas²⁶ and of the localization of recording electrode in thionine-stained coronal section of the EPN is shown (Fig. 2a–b).

2.4 Spike sorting

Action potentials arising from a single neuron were discriminated by the template-matching function of the spike-sorting software (Spike2; Cambridge Electronic Design, Cambridge, UK). Only well isolated SUs were included in the analysis, which was determined by the homogeneity of spike waveforms, the separation of the projections of spike waveforms onto principal components during spike sorting, and clear refractory periods in ISIs histograms. All analyses were performed using custom-written Matlab routines (Mathworks, Natick, MA). Firing rate was calculated by taking the reciprocal value of the mean ISI for the whole 1000 spikes of recording.

2.5 Entropy and firing rate analyses

Entropy (as measured by Approximate Entropy, ApEn) is, by definition, a measurement for irregularity in a time series²⁷. ApEn was defined by Pincus^{23, 28} as follow:

$$\text{ApEn}(N, m, r) = (N - m + 1)^{-1} \sum_{i=1}^{N-(m-1)} \ln C_i^m(r) - (N - m)^{-1} \sum_{i=1}^{N-m} \ln C_i^{m+1}(r)$$

With

$$C_i^m(r) = (\text{number of } j \text{ such that } d[x(i), x(j)] \leq r) / (N - m + 1)$$

where

$$d[x(i), x(j)] = \max(|u(i+k-1) - u(j+k-1)|)$$

for $k=1,2,\dots,m$ and $i=j=1,2,\dots,N$.

$C_i^m(r)$ measures the regularity between patterns of windows length m and within a tolerance r . The value of ApEn increases when an observed pattern is not followed by additional similar patterns in the time series. A time series that is rendered highly predictable by repetitive patterns has a relatively small ApEn; a less predictable process (or more random-looking progress) has a higher ApEn.

In comparison to analyses in time, frequency and non-linear domains, the order of the data in the time series is the crucial factor. Briefly, the standard deviation (STD), mean or

coefficient variation (CV) measure the central tendency and dispersion of the magnitude of a series and these measurement are independent of the order of the data. In contrast, ApEn (and other entropy measurements) depends on the temporal organization of the data.

For example, let's consider the following sequences: A=[2 2 2 1 2 1 1 1 2] and B= [1 2 1 2 1 2 1 2 1 2]. Then, $STD(A)=STD(B)=0.53$; $mean(A)=mean(B)=1.50$; and therefore $CV(A)=CV(B)=0.35$. Using the previous data samples and for the m and r fixed at 2 and 0.1 respectively: $ApEn(A)=0.51$ and $ApEn(B)=0.01$. In the first case (A), the signal is irregular (aka, the next value is highly unpredictable) therefore the entropy of this signal is high; in the second case (B), the signal is very regular (aka, the next value is highly predictable), therefore entropy is very low.

Because ApEn is dependent on the record length N , contiguous segments of strictly 1000 ISIs were used. As previously discussed by others, the embedding dimension (m) was fixed at $2^{22, 28, 29}$. In the current study, the vector comparison length (tolerance, r) was set to an absolute value. This is a different approach from previous studies²² which used a r value relative to the standard deviation (STD) and this technical consideration is further developed in the discussion.

2.6 Statistics

Central tendencies are expressed in median and dispersion in percentiles (range 25th–75th percentiles). Differences between groups were tested using the Wilcoxon rank sum test. We used the Friedman test to determine the effects of 6-OHDA on the relation between entropy and firing rate.

Results

Comparison of the effects of treatments between control and 6-OHDA treated subjects

In this study, all 6-OHDA treated subjects responded to apomorphine challenge by developing over 80 net contralateral rotations in 20 min (110 turns/20min, range: 104–122). The level of anesthesia was assessed by testing reflexes to a hind-paw or tail pinch every 15 minutes and electrocardiographic activity. Control and 6-OHDA treated subjects did not exhibit flexor reflex to pinch during the recording sessions; heart beat rate was similar between groups with 354.87 b/min (312.87–370.4) for control and 358.85b/min (309.54–368.66) in 6-OHDA treated rats (Fig. 2c).

Comparison of the spike timing from EPN neurons between control and 6-OHDA treated subjects

One hundred eight neurons from 6-OHDA treated rats (10 neurons/rat; range: 6.5–20) and 80 neurons from naive control rats (13 neurons/rat; range: 13.0–15.0) were included in this study. An example of spontaneous single-unit activity in the EPN over 3 seconds in the control and 6-OHDA lesioned rats is shown (Fig. 2d). In control subjects, the mean firing rate of EPN neurons was 21.96 Hz (range: 15.21–28.23). In 6-OHDA treated rats, the mean firing rate was significantly increased ($RS=5466$, $z=-5.68$, $P<0.001$) to 33.36 Hz (range

23.82–40.88) (Fig. 3). On the over-all population, entropy was slightly increased from 2.54 (range: 2.32–2.69) to 2.62 (range: 2.39–2.71) ($P < 0.09$, $Z = -1.39$, $RS = 7048$) (Fig. 3).

When expressing the level of entropy as function of firing rate, our data shows that entropy increased from low frequency (below 15Hz) to reach maximal value for a plateau between 15Hz and 25Hz. In control subjects, entropy drastically decreased above 25Hz but not in 6-OHDA treated subjects. For low-frequency firing neurons (firing rate below 25 Hz), entropy levels were slightly different between control (median: 2.65; range: 2.50–2.71) and 6-OHDA treated subjects (median: 2.71; range: 2.53–2.76) ($P < 0.052$, $z = -1.63$, $RS = 2120$). However, entropy in high-frequency firing neurons (above 25Hz) was found to be significantly higher in 6-OHDA treated rats (median: 2.58; range: 2.67–2.33) comparatively to control rats (median: 2.31; range: 2.48–2.23) ($P < 0.001$, $Z = -3.74$, $RS = 866$).

Therefore, entropy difference between control and 6-OHDA treated subjects was mostly explained by neurons with firing above 25Hz (Friedman test, sigma: 0.71, $p < 0.009$ Fig. 4a) which were also more frequently found in 6-OHDA lesioned subjects (Fig. 4b).

Discussion

The main finding of this investigation is that entropy and firing rate are related in EPN neurons and that this relation is affected by dopamine depletion. In normal and 6-OHDA lesioned subjects, EPN neurons with firing rates ranging from 15Hz to 25 Hz exhibited high entropy. In 6-OHDA lesioned subjects, EPN neurons showed higher entropy and firing rate. Interestingly, the difference in entropy between intact and lesioned subjects was dependent on the neuronal firing rate. In comparison to control subjects, EPN neurons from 6-OHDA lesioned animals exhibited higher ISIs entropy in neurons with firing rate exceeding 25Hz.

It is worth first discussing a few technical aspects that characterize the current study from previous ones on BG entropy.

At the computational level, most of the previous studies using ApEn to measure irregularity in spike timing of BG neurons^{18, 22, 29} set the tolerance value of “ r ” to a percent of the standard deviation in order to filter the noise in the signal believed to be proportional to the magnitude of the signal. This strategy is adequate for bio-signals that have their magnitude dependent on technical methodologies²³. For example, the magnitude of the local field potentials can vary as functions of the impedance of the electrode and the distance between electrodes. However, in the case of single unit recording, noise (or error) on interval interspike series cannot exceed the minimal interval tolerated for differentiating two spikes which is defined by the refractory period (1ms or 0.001s). In addition, the relative r value as the mathematical result to de-correlate entropy value from the magnitude of the signal is used. Again, this is a valuable feature when the variability in the signal amplitude results from technical methodologies. However, sources of irregularities in the interval interspikes are not all scaled with mean frequency rate. For example the base line firing rate of DA-ergic cells is poorly predictive of the burst-firing frequency³⁰. We therefore used an r value fixed at the refractory period (0.001 s) for all the population of neurons analyzed under the

assumption that the systemic error on identifying the spike is not dependent on neuronal activity.

Also, and in contrast to previous investigation on non-linear dynamic of spike timing in basal ganglia neurons, the current study was performed under anesthesia. During recording sessions, the depth of anesthesia was carefully controlled by monitoring autonomic and nociceptive functions. Under urethane anesthesia, the heart rate and reflex suppression to pinch were similar between control and 6-OHDA. In agreement with previous studies¹¹, these observations support the view that depth of anesthesia was identical between groups and during the recording sessions. As previously reported under anesthesia³¹ or in awake state³², our data shows that loss of DA-ergic neurons in 6-OHDA lesioned rats increases neuronal firing rate in EPN. Therefore, and in addition to previous studies reporting that urethane anesthesia does not reverse the differences in neurochemistry between control and 6-OHDA treated subjects³³, our investigation confirms that this paradigm preserves the main electrophysiological hallmarks for parkinsonism (aka, higher firing rate in EPN)^{31, 34}. Because control and 6-OHDA subjects were recorded under anesthesia, the differences in linear and non-linear measurement of spike timing are unlikely dependant of movement, perception and arousal³⁵ but more likely related to the lesions induced by the neurotoxin. In agreement with our previous clinical investigation reporting that DA receptor agonist apomorphine decreases entropy and firing rate of spike timing in parkinsonian patients¹⁸, our current study reports without surprise that lesions induced by 6-OHDA increase entropy. Though 6-OHDA per se can affect both DA-ergic and noradrenergic cells, the noradrenergic component of intranigral 6-OHDA may be epiphenomenal³⁶ or elusive^{37–39} on BG circuitry activity. Data raised above supports the view that: (1) urethane did not mask the effects of 6-OHDA lesion on circuitry activity, (2) lesion of 6-OHDA effects on entropy corroborate those previously reported dependant on DA-ergic activity in awake PD patients.

Previous studies have described non-linear features in spontaneous discharge of BG neurons of animals or patients in awake state. Such spontaneous activity does not represent true 'idling' of the BG thalamocortical circuitry because the subjects were certainly engaged in cognition, small-amplitude movements or sensorial stimulation during recording^{22, 35}. In one hand, the use of experimental paradigm under anesthesia allowed to isolate the effects of 6-OHDA from such confounding factors²². On the other hand, under anesthesia, the loss of motor manifestation associated to the condition resulting from both peripheral and central effects of urethane limits the interpretation of the entropy/rate property in term of its relationship to specific movement disorders which is out of the scope of the current investigation.

Our data raised above supports the view that DA depletion increases entropy in the output nuclei of the BG, firing rate and alters the relationship between firing rate and entropy in EPN neurons. In the DA depleted subjects, increased firing rate in EPN-neurons is predicted by an overall imbalanced activity in favor of indirect pathways^{40–43} and an increased glutamatergic drive from the hyperdirect pathway⁴⁴. In agreement with the 'irregularity hypothesis'^{18, 45–47}, depletion in DA resulted in overall higher entropy in the spike timing of EPN neurons. The entropy/rate property observed in the population of EPN neurons and its alteration following nigro-striatal lesion provides new insights on the coding properties of

EPN neurons. In contrast to the ‘rate coding’ hypothesis, which underlies a static balance between the excitatory and inhibitory drives², the ‘entropy hypothesis’ underlies that irregularity in the spike timing is a reflection of a dynamic balance exerted by fluctuant excitatory and inhibitory drives.

In intact subjects, entropy was found maximal in EPN neuronal activity with firing rates ranging from 15Hz to 25Hz therefore high neuronal entropy *per se* may not be an abnormal feature of BG neuronal activity^{17, 45}. Our data indicate also that EPN is composed of heterogeneous subpopulation of neurons with distinct entropy/rate properties. Comparison of entropy/rate properties between groups shows that coexistence of high entropy and high firing rate in the spike timing of EPN neurons is a functional hallmark of EPN neurons associated to nigro-striatal lesion.

In dopamine depleted pathological condition, high entropy in EPN neurons was observed in high frequency neurons. These data can be interpreted as a consequence from EPN disinhibition which lower the threshold of neuronal excitability^{48, 49} and increase the efficiency of coincident excitatory postsynaptic potential (EPSPs) to induce neuronal firing¹³. Following lesion of nigrostriatal pathway, increased neuronal entropy in EPN may be envisaged as infiltration of neuronal coding likely caused by hyperexcitability to the coincident inputs⁵⁰. This hypothesis is also supported by computational approaches demonstrating that complex systems with high cohesion can exhibit low entropy, while systems with reduced cohesion can exhibit high irregularities causal to independent behaviors of their constitutive entities⁵¹. An alternative, but not exclusive, hypothesis is that increase in EPN neuronal entropy following DA-ergic depletion reflects¹⁸ an increase in uncoincidental events between the hyperdirect, direct and indirect pathways engendering irregularities in EPN neurons⁵². Since this investigation was performed under anesthetic condition, the relationship between entropy/rate property and movement disorders could not be characterized. In anaesthetized state, it cannot be ruled out that some of the effects of 6-OHDA might have been accentuated by urethane^{47, 53}. The robustness of the entropy/rate property to arousal and its relevance on motor symptoms in parkinsonism are warranted to be investigated. Since both irregularity and oscillatory activities are known to co-exist and interact in other circuitries (i.e. autonomic circuitry, cortex)^{54, 55}, future investigations in behaving subjects are warranted to investigate the relationship between oscillatory activity and entropy⁵⁵ in the BG of behaving subjects.

Conclusion

Irregularity in EPN neurons was found maximal in neurons with firing rates ranging between 15Hz and 25 Hz. DA depletion increased entropy in the EPN neurons and this increase in entropy was more pronounced in neurons with firing rates higher than 25Hz. Our data establish that lesion of the nigro-striatal pathway, a hallmark for parkinsonism, has synergic effects on firing rate and entropy in EPN neurons. The rate-irregularity relationship may be a relevant feature to identify, localize and quantify pathological electrophysiological activities of the BG in the context of movement disorders. Finally, integration of the entropy-rate feature into computational model^{56, 57} and/or brain interface system⁵⁸ may help to detect misprocessing episodes of sensory motor information.

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Figure 1. Schematization of the basal ganglia circuitry

See introduction for discussion.

The input nuclei of the BG consist of the STN and the striatum. The output nuclei of the BG are the GPi and the SNr. The BG input nuclei receive projection from the cortical area. The STN projects to the output nuclei through the glutamatergic hyperdirect pathway. The striatum projects to the output nuclei via two pathways: the direct pathway and the indirect pathway (through the GPe).

White arrow: the inhibitory GABAergic; black arrow: excitatory glutamatergic projection; gray arrow: dopaminergic projection projections. DA: Dopamine, D1: receptors dopamine D1-like, D2: receptors dopamine D2-like, SNc: substantia nigra compacta, GPe: globus pallidus external (globus pallidus in rodent, GP), GPi: globus pallidus internal (entopeduncular nucleus in rodent, EPN), STN: subthalamic nucleus, Th: Thalamus.

Figure 2.

(a) An illustration from the rat brain atlas of the EPN region at -2.5 mm posterior to bregma, 2.5 mm lateral to the midline and 7.8 to 8.0 mm to the skull surface, localization of recording neurons for control (blue dots) and 6-OHDA lesioned rats (red dots), image modified from Paxinos and Watson (1986). (b) Histological verification showing (black arrows) the localization of a recording electrode by electric coagulation in thionine-stained coronal sections of the entopeduncular nucleus (EPN). (c) A representation of heart rate and (d) single neuronal activity for total duration of 3 s in the EPN of control (blue color) and 6-OHDA lesioned rats (red color) during microelectrode recordings.

Figure 3.

The box plots illustrate the summary of changes in the firing rate (a), the ApEn values for all the neuronal activity between groups (b), the ApEn values for the neuronal activity less than <25Hz (c) and the ApEn values greater than >25 Hz (d) in the EPN of naïve control (N = 80) and 6-OHDA treated rats (N = 108). The central line of the box plots represents the median, the 25%–75% (interquartile) range, and the edge of the whiskers show the 5%-95% range.

Figure 4.

(a): The curves illustrate the quantitative relation of ApEn values on the function of firing rate across the naïve control rats ($N = 80$; blue dots) and 6-OHDA treated rats ($N = 108$; red dots). Solid curves express the trend (Legendre polynomials, 4th order) of ApEn as function of firing rate for control rats (blue curve) and 6-OHDA treated rats (red curve). Vertical lines indicate the range (25th–75th percentiles) of ApEn at different bins of frequency rates (bin size: 10Hz). Stars at the top indicate significant differences between controls and 6-OHDA subjects ($P < 0.05$). **(b)** This figure indicates the distribution of cells as function of the firing rate in the control and 6-OHDA treated animals. Y axis expresses the percent over the cell population of each group.