Distribution of striatopallidal projection neurons in the hemi-parkinsonian rat

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ABSTRACT

Introduction
The pathophysiology of Parkinson’s disease (PD) and the animal models of the disease, of which the most widely used is the 6-hydroxy dopamine-lesion (6-OHDA), include changes in firing pattern and oscillatory frequency that is pathologically synchronized throughout several nuclei of the basal ganglia, thalamus, and parts of the cortex. Additionally “functional coupling” has been detected, by analyzing invasive multielectrode data, to suggest that there is a connectivity change involving the striatum-globus pallidus cell projection (part of the indirect pathway of the basal ganglia). The pathognomonic dopamine neuron loss of the disease is probably not the only culprit in these electrophysiological changes.

Methods
To investigate a potential anatomical correlate to the increased functional coupling in the striatopallidal projection of the 6-OHDA-hemilesioned rat (one hemisphere is lesioned, the other is the control), retrograde tracers were injected into the GP of two rats and their striata studied through confocal microscopy for the purpose of mapping changes in number and distribution of tracer marked cell bodies.

Results
The results did not reach significance but pointed towards a decrease in number of cell bodies in the lesioned hemisphere and a shift from more dorsally located cell bodies to more ventrally located cell bodies in the lesioned hemisphere.

Conclusion
This study describes the distribution of the striatopallidal cell bodies in the rat striatum and indicates that structural changes in the form of neuronal death could follow basal ganglia dopamine-lesion and explain part of the aberrant oscillatory activity measured in 6-OHDA-hemilesioned rats and PD patients. A larger study is needed to attain conclusive evidence and to ultimately prove a correlation between morphology and function.
INTRODUCTION

Parkinson’s disease (PD) can generally be described as a dysfunction of dopaminergic pathways in the basal ganglia-thalamocortical loop. Most notably there is loss of dopaminergic nerve fibers in Substantia Nigra pars compacta (SNpc), the primary source of dopaminergic neurons in the basal ganglia. Symptoms of PD include bradykinesia, rigidity, postural instability, and resting tremor as well as non-motor symptoms like somnolence, gastric slowing, cognitive dysfunction, and mood-changes [1]

The most widely used rodent model of PD, the 6-hydroxydopamine-lesion (6-OHDA) was pioneered by Ungerstedt 1968 [2] and has since played a large part in advancing Parkinson research. 6-OHDA is transported into dopamine and norepinephrine neurons through their transporters, DAT and NET, respectively. [3] When injected into the medial forebrain bundle (MFB), 6-OHDA creates an 80-95 % depletion of dopaminergic neurons in the SNpc and a corresponding loss of terminals in the striatum. [4] The 6-OHDA model also provide consistent parkinsonian motor deficits. [5]

The etiology of PD remains unknown, but knowledge of the mechanisms behind the parkinsonian symptoms has developed significantly in recent years. The classic model describing the function of the basal ganglia, called “the rate model”, states that the indirect (“movement-inhibiting”) and direct (“movement promoting”) pathways jointly decide if a cortical input should be carried through or inhibited, through the sheer rate of the firing in the respective pathway’s neurons. This model has been revised in the light of recent studies showing that activation pattern, including burstiness (rapid action potential spiking followed by quiescent periods) and activity-synchronization between neurons, are equally important in function and dysfunction of the basal ganglia [6,7]

In the last 20-25 years, electrophysiological studies with intracranial electrodes, electroencephalograms (EEG) as well as electrocorticograms (ECoGs) have found and characterized the aberrant neuronal activation pattern in animal models of Parkinson’s disease and in human Parkinson patients. These studies have shown an increased coherency of neuronal oscillations throughout the basal ganglia-thalamocortical loop, in and between the direct and indirect pathways. Early research with animal PD-models [8,9] observed an aberrant oscillatory pattern and now we can characterize a distinct parkinsonian phenomenon in the “coherent beta-
band oscillation” that involves most structures of the basal ganglia as well as cortical structures in a low frequency oscillatory activation pattern. The phenomenon is also established in human PD-patients [10,11] and correlate (though not entirely consistently) with motor symptoms in PD. [12,13] It is also established that deep brain [14,15] and Levodopa can restore much of the aberrant oscillatory pattern; even though the latter comes with its own aberrant oscillation pattern [16] when L-Dopa induced dyskinesia start to develop in the treated animal.

The classic description of the pathophysiology of PD, essentially postulating a reduced direct pathway activation and enhanced indirect pathway firing, has thus been modified to include global activation pattern changes. In particular, increased oscillatory synchronization in the basal-ganglia-thalamocortical loop is in the revised model thought to hinder the propagation of normal nerve signals, thereby arresting the execution of cortical input. Overall the view of basal ganglia pathophysiology in PD has expanded beyond the old rate model, to a model emphasizing the importance of neural activation patterns.

For that reason, further studies have mapped out functional connectivity in the basal-ganglia-thalamo-cortical loop by analyzing what neuronal populations oscillate in a more or less correlated way. These data have been obtained, for example by measuring local field potentials (LFP:s) in several different structures simultaneously (using an in vivo intracranial multielectrode array) in 6-OHDA-lesioned rats and monkeys. The results have shown that several structures in the basal-ganglia-thalamocortical loop have a strong coherence, only in the parkinsonian low-frequency range (8–35 Hz = alpha to high beta-range which in normal animals are associated with less active or sleep states). [16-18]

The “parkinsonian oscillations” in the parkinsonian rat are not seen until about a couple of weeks after 6-OHDA-injection and most of the motor symptoms start developing at that time as well. [4,19] This fact, as well as the coherency of oscillations, suggests that a plastic change in several nuclei in the basal-ganglia-thalamocortical loop or changes in their functional connectivity may underlie the aberrant signaling and subsequently the motor dysfunction in PD. One hypothesis has been that increased coherency (between structures in dopamine depleted hemispheres) is caused by the sprouting of dendrites in dopamine associated neurons, as a sort of compensatory measure, leading to synchronized release in larger striatal volumes. Anatomical connectivity
could however potentially be altered between several different structures in the loop promoting synchronized oscillations. Interestingly, if coherent oscillations begin through some of these potential mechanisms, these oscillations could in turn possibly generate structural changes afterwards. This hypothesis has a foundation in the Hebbian-related theory of “spike-timing-dependent plasticity”; which is a basic theoretical concept in neuroscience that roughly assert that “cells that fire together, wire together” given that the temporal relation between spikes in connected cells occur with a relatively distinct time relation in critical interval.

6-OHDA into the MFB has been shown to increase the functional connectivity between striatal fast-spiking interneurons and striatopallidal neurons in mice. Advanced computer models have then showed that this in turn has the capacity to upturn the synchrony between the striatopallidal cells [20]

So far findings from plasticity-studies point to a loss of dendritic spines in the indirect pathway in parkinsonian animals and also reduction in dendritic length, branching and number of glutamatergic synapses. [21,22]Of special interest in the current study is the striatum and the globus pallidus. Striatum is the large input nucleus of the basal ganglia consisting of the, in rodents, structurally inseparable, caudate and putamen. Striatum functionally has two known groups of projection neurons – the striatonigral- (to Substantia Nigra pars reticulata) and the striatopallidal- (to globus pallidus (GP) in rats – corresponding to globus pallidus externa in primates) medium spiny neurons (MSN:s) and they are the major targets of dopaminergic axons from the Substantia nigra pars compacta with a dominance of D1-type dopamine receptors and D2-type receptors, respectively. They have GABA-ergic terminals and signify the start of the direct and the indirect pathways of the basal-ganglia-thalamocortical loop, respectively.

No studies, to my knowledge, have looked at changes in distribution of striatopallidal cellbodies in 6-OHDA-lesioned rats with neural retrograde tracers. Retrograde tracers mark cell bodies of neurons that project to a certain location in the brain through axonal retrograde transport, which can give a good estimation of potential change in number of cells sending projections to a specific target structure. If tracer-marked striatopallidal cell bodies decrease after a 6-OHDA-lesion this might mean neuronal cell death of striatal projection neurons. If they increase it could indicate “compensatory” neurogenesis of striatopallidal neurons or, more likely, sprouting of direct pathway neurons into the GP which could serve as a structural correlate to the increased
functional coupling seen in PD. If no change is found, physiological changes rather than direct increased structural connectivity, must be responsible for the synchronized beta-band oscillations.

**Aim**
The aims for this thesis is to map out the striatopallidal connections in 6-OHDA-lesioned rats and see if any changes in cell count or cell location can be observed when comparing the intact hemisphere to the lesioned, (based on the hypothesis put forward by the electrophysiological studies mentioned). This study seeks to contribute to the growing knowledge of the plastic changes that seem to occur in Parkinson’s disease, making up a potential anatomical correlate to the aberrant coherent oscillations in PD.

**METHODS**

**Ethical considerations**
The study was approved by the Malmö/Lunds djurförsöksetiska nämnd (The regional ethical review board of animal testing Lund) Permit: No. M23-14. The animal testing was supervised by trained staff and the rats were frequently inspected for any signs of weight-loss, chronic pain or distress in their well regulated housing during the trial period. No 6-OHDA-lesioning was done in this study; the rats had been lesioned before and been used in other trials.

**Animals**
Two female Wistar rats (350g and 420g) that were 6-OHDA-lesioned in the right hemisphere in November 2016, in a parallel trial with Tamté et. al. [4] was used in the current study. The lesion was evaluated in separate, identically treated rats by immunohistochemistry with anti-Tyroxin hydroxylase antibodies and DAB staining showing an 84% loss of dopaminergic afferents in striatum in the lesioned (right) hemisphere compared to the intact hemisphere (by comparison of optical density, in the image program ImageJ). This was considered a good lesion. The intact (left) hemisphere is considered the control hemisphere.
**Tracer injections**

Adapted from Apps and Ruigrok et al. [23] Red Fluorescent retrograde microbeads (Lumafluor Retrobeads™) were chosen as neuronal tracers for their accurate and detailed marking of cell bodies [24] and their good visualization in the microscope (compared with another retrograde tracer: Choleratoxine subunit B conjugated with Alexa fluor), as learned from previous trials in this lab.

**Materials:** 2 µm syringe (Hamilton 3000-series™) and a glass capillary with approximately 25 µm diameter tip and 1 mm diameter base connected to a Dual RN Coupler (Hamilton™ p/n 55752-01) and a Luer to RN Adaptor (Hamilton™ p/n 55753-01). Essential for the correct injection of the tracer was to have an airtight system, so the whole system was primed with paraffin oil. Stereotaxic frame was used to fasten the rats and measure the right xyz-coordinates for drilling and injecting tracer.

**Anesthesia:** The Rats were anesthetized with intra-peritoneal (I.P.) fentanyl/medetomidine (0.3/0.3 mg/kg) and Xylocain mixed with saline (1:9) was injected subcutaneously above the cranium to reduce nociceptive input. The rats were tested for pain-reactions to confirm they were deeply anesthetized and fixed to the stereotaxic frame. They there received bilateral injections (0.1-0.2µl) of the red flourescent microbeads-tracer into the Globus pallidus (GP) at following coordinates from Bregma (which is stereotaxic coordinate 0.0.0 in the midline at the cortical surface right below where the coronal suture intersects with the sagittal suture in the skull) [25] Antertposterior (AP): -1.0; mediolateral (ML): 3.0 and -3.0, dorsoventral (DV): 6.6. Both rats also received bilateral injections with green fluorescent microbeads-tracer into the Substantia nigra pars reticulate (SNr) for the purpose of mapping the overlap (double labelling) of striatopallidal and striatongral neurons. These injections were given at AP: -5.4; ML 2.4 and -2.4; DV: 8.4, but they missed their target (they did not hit any structures that could compromise the results, evident from looking at the sliced brain in confocal microscope) and were not further part of the study.

The surgery took approx. 4 hours and postoperatively the rats received glucose + saline (1:1, 5 ml I.P.), Buprenorfine + saline (1:9 1.7 ml/kg subcutaneously) and Atipamezol + saline (1:9 1 ml/kg) and were put back into their cages.
Time between injection and perfusion was chosen to be 4 and 6 days respectively, since previous trials showed no difference in tracer cell-marking between 4 and 7 days.

**Preparation of tissue**

Transcardial perfusion and dissection was carried out according to this protocol: “Gage et al.: Whole animal perfusion fixation for rodents”, with the addition of intraperitoneal Heparin (0.05 ml (5000 IE)) to avoid blood clotting.

The brains were put in Paraformaldehyde (PFA) (4 %) for three hours and then transferred to sucrose, for cryoprotection, (20% sucrose in PBS 0.1M) until saturated (ca 24 hours). They were then quickly frozen in dry ice and stored in a freezer (-18°C) for approximately 8 hours. Then the brains were sliced up in a cryostat microtome (Microm HM550, Thermo scientific™) into 50 µm thin parasagittal slices which were put on slides and stored in slide boxes in the freezer. The slides were later mounted with a glucose- and phosphate-buffered saline-medium (PBS, 9:1) and a glass coverslip and stored in a fridge until microscopy.

**Confocal microscopy**

Settings were made to mainly let the red tracer fluorescence (rhodopsin red) pass to the sensor, with some minimal background light for localization. Pictures (2-7 on each slice) of the whole mediolateral length of the striatum were taken at 10x magnification on every 9th slice (ca 50 µm between the slices) and stitched together using “Image Composite Editor”. The resolution of the images was 1024 x 1024 pixels, corresponding to 2380 µm x 2380 µm in length. The approximate coordinates for each slice was determined through comparison to the atlas, i.e. recognition of characteristic shapes of structures such as cerebellum, frontal cortex, hippocampus, thalamus and lateral ventricle (at least four out of five structures has been recognized to determine a slice’s coordinate with certainty).

**Injection documentation**

The injections left tracks of tracer fluid through the injection path and through diffusion into the tissue (diffusion estimated to be maximum 1 mm from injection with a 0.1-0.3 µm-injection. The injections did not spread to other areas with afferents to striatum, than GP. To be able to compare the marked cell bodies in striatum, the injection volume and location needed to be comparable between the hemispheres. The track and dense core of the injection were clearly visible in the microscope and were measured in all dimensions. The injections were all made into the dorsal
part of GP and the range of their spreads were 0.5-1.0 mm dorsoventrally (DV) through GP (counting from the dorsal edge of GP), 0.3-0.6 mm in mediolateral (ML) and 0.8-1.0 mm in anteroposterior (AP) axis (Fig. 1).

**Cell counting**

Cell bodies with tracer uptake were then counted using freeware image-program “ImageJ” with the cell body/nucleus counting plugin “ITCN”. The images were converted to 8-bit greyscale and inverted and settings were decided in ITCN for the width of the cell body, the minimum distance between cell bodies and the (intensity) threshold. These settings were continuously adjusted to manual counting, but was in average set to width: 7.0; Min. distance: 3.5; Threshold: 6.0. Striatum was marked by hand and cell count and marked area were measured in on all slices.

The pictures were cleaned and enhanced and fitted over the atlas slides that had the approximate corresponding coordinates, with “Paint Shop Pro”. To avoid miscalculation of cell bodies in slices with pollution of tracer injection, an anteroposterior cutoff through the striatum at Bregma was made. Some slices came out lacking a control slice (matching slice in other hemisphere) and were excluded from the diagrams (for better visualization) but included in the summed up comparison (Fig 2). The atlas slides for the overlaying of cell bodies were included if there were brain-slices that matched their coordinate. A pair of slices (same coordinate in corresponding hemispheres) fitted at the same atlas-slide as another pair and that meant that the pairs were added together for a correct measure of both total cell count and cell density in this specific mediolateral position. The outmost slices (lateral to 4.20 mm lateral to Bregma) were excluded due to uncertainty in discriminating striatum from neighboring areas. The Nucleus accumbens (Nacc) is included in the common definition of (ventral) striatum, and not possible to discern from the rest of the striatum structurally. Even so, Nacc could not be included in the pictures or the cell counting, because of uncertainty in discriminating it. But since the injection was made in the dorsal part of GP, the major part of cell bodies are expected to be in the dorsal striatum anyway (Källa: “Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study”).

A horizontal division was made at 5 mm ventral to Bregma for the purpose of classifying the distribution of striatopallidal cells in the dorsoventral axis. The 5 mm division mark was chosen for visually being the midpoint of cell-density – to give a sense of the potential distribution
change of cell bodies in different planes along the dorsoventral axis between lesioned and intact hemispheres. The parts were named “dorsal striatum” and “ventral striatum” (not to be confused with existing functional or anatomical divisions of striatum in other literature). ML-coordinates 1.33 mm was not included in this division, being considered too small to divide in a useful way.

**Fig 1:**

**RESULTS**

Pooled data of the two left (intact) hemispheres were compared to pooled data of the two right (lesioned) hemispheres, providing the following result:

Total striatopallidal cell body density in the left (intact) striatum was 56 % greater than in the right (lesioned) striatum (Left: 115.7 cell bodies/mm² (SD ± 52.2) Right: 74.3 cell bodies/mm² (SD ± 35.1) (Fig. 2A). Total cell count was 12 % greater in the left striatum than in the right one (Left: 1929 cell bodies (SD ± 122.3), Right: 1721 cell bodies (SD ± 79.4)) (Fig. 2B). Note that less total striatal area was sampled from the left hemisphere (average between the two left hemispheres: 56.2 mm² and the two right hemispheres: 67.5 mm²). In Fig. 3 is summarized the cell counts on several parasagittal slices along the mediolateral axis per area in lesioned and intact hemispheres. There were no obvious difference between hemispheres in this mediolateral distribution and with this small sample size it seemed unnecessary to make a statistical analysis of it.

*Dorsal/ventral distribution*

A 2-way ANOVA was performed on the striatopallidal cell counts of the ventral and dorsal divisions of striatum (at 5 mm ventral to Bregma) to detect differences in dorsal/ventral distribution between the two pairs of intact (left) and lesioned (right) hemispheres (Fig. 4). The results showed that the intact hemisphere had 58.6 % cells located in the dorsal striatum (1130 dorsal cell bodies and 799 ventral) and that the lesioned hemisphere had the opposite relation; 60.0 % of striatopallidal cells were here located in the ventral striatum (687 dorsal cell bodies and 1034 ventral) but the findings were non-significant (p = 0.07, n = 15, from 2 animals). Fig 5 (cell
numbers are approximate, but interrelations are valid) is showing the dorsoventral distribution along the mediolateral (ML) axis after the horizontal division at 5 mm from. Averaging the striatum in all four hemispheres the dorsal striatum contained 908 (SD ± 33.6) cells and the ventral striatum 916.5 (SD ± 30.6) , and thus this division was considered good for measuring differences in between the two hemispheres and mediolateral sections. Worth mentioning is perhaps one slice that stood out: 1.9 mm ML from Bregma, in the intact striatum the dorsal striatal cells made up 70% of the cells in that section (approximately 225 (SD ± 127 dorsal compared to 98 (SD ± 12.5) cell bodies in the ventral striatum, N=2). In the same ML-coordinate, in the lesioned hemisphere there were 53 (47 %, SD ± 24) dorsal and 61 (53 %, SD ± 31) ventral cell bodies. But the variation is too big for significance.

Lastly, Fig. 6 shows all cell distribution at their approximate three-dimensional location in the entire length of the Striatum (with exceptions mentioned in the methods).

DISCUSSION

The results from looking at the striatopallidal cell bodies of these two retrograde tracer-injected rats did not reach significance, but they suggest that there may be plasticity present in the 6-OHDA-hemilesioned rat, which could partly explain the increased synchronization of neural oscillations measured in this model of Parkinson’s disease. The data hint towards a decrease in number of tracer-marked striatopallidal cell bodies in the lesioned hemisphere. Furthermore there is an almost significant difference in striatopallidal cell distribution with more dorsal cell bodies in the intact striatum and more ventral in the lesioned striatum, something that has not been described before. Taken together, these findings could be interpreted as death of mainly dorsally located striatopallidal MSN:s after 6-OHDA lesions.

Other studies have shown a loss of dendritic spines and glutamatergic synapses on the striatopallidal neurons in animal models of PD and in PD-patients, but to my knowledge none of them have looked at total distribution of striatopallidal cell bodies. [21,26,27]

From the aspect of the classic “rate model” of the pathophysiology of PD it is a bit counterintuitive that a loss of striatopallidal neurons should be found in a 6-OHDA-lesioned hemisphere, since the indirect pathway should be the dominating pathway, leading to inhibition
of action. But we know now that changes in a broader activation pattern is a main mechanism as well, and that any plasticity fits well in with that picture. It is still also possible that some of the changes seen is associated with inflammation or other lesion-related mechanisms such as serotonergic [28] or noradrenergic denervation [29] Trials with Haloperidol (Dopamine-2-receptor antagonist) have shown dendritic dystrophy in these neurons, speaking for Dopamine loss as one major culprits in this mechanism. [30]

No significant numbers in comparison between hemispheres or striatal divisions were attained. This can be explained by the small sample size, but also by non-identical tracer injections possibly reaching more axons in one hemisphere than the other. This is a main limitation of the study.

A somewhat larger study, with a more standardized injection method and perhaps a blinded design, is needed to get significance in results. More focus can be laid on the different functional and anatomical subdivisions of the striatum and how they change with the 6-OHDA-hemilesion. A comparison of the striatopallidal projection with the striatonigral projection of the direct pathway in a double tracer study would also be valuable, to test the hypothesis of sprouting in the striatal projections in PD and broaden the search for anatomical connectivity. If plasticity in MSN:s were to be proven in a larger study, more research would center around the cellular mechanisms of plasticity and perhaps there would lie the answer to the aberrant oscillatory activity and an important part of the symptoms in PD.

ACKNOWLEDGEMENTS

Thanks to supervisor Per Peterson and co-supervisor Ivani Brys for their support through method learning and constant feedback. Thanks to Joel Sjöbom for support with image conversion among other things.
Fig 1 | Images depicting the main injection sites of the four tracer injected hemispheres. **Rat 1:**
left hemisphere GP-tracer-injection (A) spread: ML: 0.6 mm, AP: 1.0 mm DV: 1.0 mm, and right (B) hemisphere injection spread: ML: 0.4 mm, AP: 1.0 mm DV: 1.0 mm. **Rat 2:** left (C) injection
spread: ML: 0.3 mm, AP: 0.8 mm DV: 0.5 mm, and right (D) ML: 0.6 mm, AP: 0.7 mm DV: 0.6 mm.

Fig 2 | Tracer marked striatopallidal cell bodies counted throughout the striatum at every 9th slice (slices were 50 µm thick) of the rat brain. Because of differences in sample area, here is also cells per mm² presented (B). N = number of striatal slices. Non-significant numbers but an indication of a reduced number of striatopallidal cells in the lesioned striatum.
**Fig 3** | Visualization of striatopallidal cell counts in different mediolateral slices of the striatum, hemispheres (L and R) side by side on several parasagittal slices along the mediolateral axis per area in lesioned and intact hemispheres. No significant differences were seen in mediolateral distribution. N = 2 rats.

**Fig 4** | Not quite significant differences were seen in the dorso/ventral (horizontal division of the striatum) comparison between the two hemispheres with more ventral striatopallidal cell distribution in the 6-OHDA-lesioned hemisphere and more dorsal cells in the intact hemisphere (2-way ANOVA, p = 0.07, N = 15, from 2 animals).
Fig 5 | Dorsal/ventral cell distribution per mediolateral brain slice. Some differences noted between hemispheres and between coordinates, but none significant (standard deviations not presented). Note that the large quantity of cell bodies at left hemisphere 1.9 mm (225, SD ± 127, N = 2) has a large SD.
Fig 6 | Map of cell bodies in rat 1 and rat 2 in the near entire striatum. Cut off at Bregma for avoiding tracer-track pollution (other exclusions discussed in methods). Pictures of fluorescent
tracer markings taken through confocal microscope and counted in imaging program “ImageJ” with manual counting as ground truth.

REFERENCES


Distinct roles of cortical and pallidal beta and gamma frequencies in hemiparkinsonian and 

target-specific remodeling of fast-spiking inhibitory circuits after loss of dopamine. Neuron 
2011 09/08;71(5):858-868.

glutamatergic synapses on striatopallidal neurons in Parkinson disease models. Nat Neurosci 

22. BOLAM JP, HANLEY JJ, BOOTH PAC, BEVAN MD. Synaptic organisation of the basal 

23. Apps R, Ruigrok TJ. A fluorescence-based double retrograde tracer strategy for charting 

24. Schofield BR, Schofield RM, Sorensen KA, Motts SD. ON THE USE OF RETROGRADE 
TRACERS FOR IDENTIFICATION OF AXON COLLATERALS WITH MULTIPLE 
FLUORESCENT RETROGRADE TRACERS. Neuroscience 2007 03/26;146(2):773-783.


terminals following unilateral lesion of the dopaminergic nigrostriatal pathway: a 

specific plasticity of striatal projection neurons in parkinsonism and L-DOPA-induced 

U, et al. Adaptive down-regulation of the serotonin transporter in the 6-hydroxydopamine-
induced rat model of preclinical stages of Parkinson's disease and after chronic pramipexole 

Ethical considerations

There are of course many regulations and ethical considerations regarding animal testing and I will here only discuss a few.

In the trials leading up to these experiments neurotoxic lesions (6-OHDA) were performed on the rats to create the most widely used animal model of Parkinson’s disease (PD). Rats are affected in similar ways to PD patients, with symptoms like: bradykinesia, gastric slowing, somnolence, and impairment of executive function. The rats live and are experimented on for months up to a year after the lesion and are frequently inspected to make sure they do not lose more than 15% of their pre-surgical body weight. Any animal showing signs of excessive weight-loss, chronic pain or distress will be taken out of the study. In this study we used animals lesioned 6 months ago and injected “microbead tracers” (non-toxic substances) into their brains during anesthesia. Animals were then sacrificed through transcardial perfusion during deep (barbiturate) anesthesia 4-6 days after tracer injection. Same as before, the animals were inspected regularly in the meantime.

To be allowed to handle the animals alone, the researcher needs to go through a three weeks course with written and practical exams in the end. Other study participants get a short course and need to have a supervisor present when handling the animals.

It is important to evaluate continuously the need to include more animals, weighing the chance to get significant results against minimizing suffering. In a master’s thesis though, the margin is small to make changes in number of experiments and it is easier to argue for a small, well conducted trial than to try and fit in more animals, risking poorer methodology and in worst case, compromising the safety of the animals. Because coming researchers can use the data if it is good enough and expand it with more animals to reach the significant results aimed for.

There are dangerous chemicals also for the researcher carrying out the experiments. The preservative solution paraformaldehyde (PFA) is an irritant and a corrosive agent and should be handled with caution in a well ventilated area and with protective gloves and eyewear. In the lab (“Neuronano research center” in Lund) there are fume hoods and all necessary protection in place.

Importantly, the aims for this study would not have been possible to accomplish without the use of animal PD models. The data and knowledge gathered by studying these rats will hopefully help to further understanding of the pathophysiology of PD in an area that could potentially lead to new treatments to be considered for PD patients.
Letter of intent
May 25th, 2016

Editor in chief, Journal of Neuroscience

Dear Editor,

Attached to this letter I would like to submit the study: “Distribution of striatopallidal projection neurons in the hemi-parkinsonian rat” to be considered for publication in the Journal of Neuroscience.

In this experimental pilot study we use retrograde tracers to offer a near complete map of the indirect pathway’s striatopallidal (iMSN:s) cell body distribution in the striatum and show how plastic changes seem to shift this distribution in two 6-OHDA-hemislesioned rats.

The hypothesis builds on the electrophysiological findings of increased functional connectivity in the striatopallidal network and we can now show indications of a structural correlate to these findings. These results, if repeated and proven significant in a larger study, can hopefully spur more research into what mechanisms causes these changes and if they are correlated to the aberrant neural signaling.

This study is original and is not being published elsewhere.

Sincerely,

Gustav Lindmark
Pressmeddelande

Nya rön i Parkinsons sjukdom

Över 17 000 svenskar lever med Parkinsons sjukdom idag. Bland de vanligaste yttringarna av sjukdomen finns rörelsestörningar såsom svårighet att sätta igång rörelser, kuttryggig förlångsammad gång, ofrivilliga handskakningar, mm. Men även psykiska besvär såsom depression är en del av symtombilden.


I en ny studie har man genom att titta på hjärnan i mikroskop sett antydningar till att det sker vävnadsförändringar, sk. plasticitet, i andra celler än dopamin-nervcellerna också. Dessa fynd skulle kunna förklara de uppmätta kopplingarna i de parkinsonistiska råttorna. Med en större studie som bekräftar dessa fynd skulle forskningen kunna ledas in på nya spår i jakten på orsakerna till symtomen vid Parkinsons sjukdom, vilket skulle kunna resultera i nya behandlingsmetoder.