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Sex-differences in reported adverse side-effects caused by Deep Brain Stimulation therapy in the subthalamic nucleus

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Innehåll

Abstract

Parkinson's disease is a common neurological disease which will progressively damage dopaminergic neurons in the brain. Later stages of the disease will result in death of the neurons. The diagnosis is often made with respect to the motor symptoms, which include tremors, bradykinesia, and rigidity. In addition to motor symptoms, non-motor symptoms appear in many patients, such as cognitive changes and mood disorders. One method used to treat Parkinson's disease is deep brain stimulation, where electric pulses are emitted to a specific brain area. A common target is the subthalamic nucleus, which is part of the basal ganglia. By using deep brain stimulation, the dose of other medications for Parkinson's disease can be lowered. However, the mechanisms of deep brain stimulation are not yet entirely known, and there have been many reports of adverse side-effects caused by this method, including depression and other types of mood changes. Even so, information of a possible sex distribution of these side-effects is still limited. Here, a qualitative essay was made where 16 articles describing reported side-effects in men and women were compared. In addition, unpublished data from optogenetic studies on male and female mice were analysed in order to examine putative sex-differences upon experimental brain stimulation strategies. The results from the optogenetics results did not show any statistically significant sex-differences. In contrast, by comparing the selected articles in which results of deep brain stimulation treatment in patients were reported, some differences were found. First, it seems that women report more depressive-like symptoms than men. Second, while men also report depressions, they also report more aggressive behaviour upon the treatment. A preliminary conclusion of this essay is therefore that certain sex-differences can be observed among the adverse side-effects reported upon deep brain stimulation in Parkinson´s disease. However, since the studied material was limited, more research is required to make firmer conclusions.

Abbreviations

DBS	Deep brain stimulation
EP	Entopeduncular nucleus
GPi	Globus pallidus *interna*
GPi-DBS	Deep brain stimulation in the globus pallidus *interna*
PD	Parkinson's disease
SNr	Substantia nigra *pars reticulata*
STN	Subthalamic nucleus
STN-DBS	Deep brain stimulation in the subthalamic nucleus
UPDRS	Unified Parkinson's disease rating scale

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, and around 20 000 people are suffering from it in Sweden (Parkinsonguiden 2014). PD is a type of progressive neurological disease, which targets neurons in the brain. The earlier stages of the disease are recognised by neuronal dysfunction, however, the illness will ultimately lead to total degeneration and death of the neurons in later stages. Neurodegenerative disorders can affect any of the neurons involved in cognitive, motor, or sensory functions. In PD, the greatest damage is caused to the midbrain dopamine neurons, which will have a major effect on the body's motor function, the ability to control movement (Parkinsonguiden 2014, Sternudd 2020).

PD is one of many diseases included by the collective name parkinsonism. A common feature for parkinsonian diseases is that they show similar symptoms, such as rigidity and movement disorders. Since the term involves both PD and other similar diseases, several classifications of PD exist (Parkinson's UK 2019). The most common form of PD is idiopathic PD, which does not have a known cause. Most of the patients are affected by this type of the disease. A commonly used method to diagnose a patient with suspected PD is the Unified Parkinson's Disease Rating Scale (UPDRS). The UPDRS is a formula with extensive question concerning motor and non-motor symptoms of PD (Parkinson's UK 2020).

The motoric symptoms of PD are the most noticeable and usually the symptoms most well-known to the general public. In many cases, however, patients suffering from PD often experience a various number of both motor and non-motor symptoms. Common motoric symptoms among PD patients are muscle tremors, bradykinesia and rigidity (Li *et al.* 2020), while non-motor symptoms include anxiety, depression, fatigue and apathy. It is also common that the motoric symptoms in an early stage appear on one side of the body, while the other half is not affected. Although the diagnosis is usually made with regard to the motor symptoms, non-motor symptoms of PD, for example sleep disorders, mood disorders, olfactory dysfunction, and cognitive changes, may also appear before the expression of motoric symptoms (Widner & Marktorp 2015, Guillaumin 2020).

A majority of these non-motoric symptoms can be linked to the so called hypodopaminergic syndrome. The hypodopaminergic syndrome involves the deficit of dopamine, which primarily has an impact on the mesolimbic system in PD patients. Other non-motoric symptoms, such as difficulties with impulse control, depends on an overdose of dopamine, also known as the hyperdopaminergic syndrome. Symptoms depending on hyperdopaminergic syndrome are usually derived from an excessive dose of dopaminergic pharmaceuticals (Castrioto *et al.* 2014).

Deep brain stimulation

Deep brain stimulation (DBS), or high frequency stimulation, is a technique used as treatment for many neurological diseases, such as PD, obsessive compulsive disorder (OCD) and Tourette's syndrome (Mottaghi *et al.* 2020).

The first indication of effective treatment with DBS in patients with PD was obtained in the early 1960's. Researchers found that by stimulating the ventro-intermediate thalamic nucleus

with electrical pulses, motor tremors could be treated. In the 1990's, experimental treatments with DBS were executed in non-human primates. After that, the DBS treatment started to be performed in human parkinsonian patients in order to relieve the motoric symptom of the disease. Before the discovery of DBS, the most commonly used procedure to treat patients with motor tremors was surgical lesioning within the brain, in which part of the brain is removed. This often led to severe complications, such as hemichorea (Castrioto *et al.* 2014, Guillaumin 2020).

DBS is a type of neurosurgical treatment that do not require any pharmaceutical substances to induce an effective impact on the symptoms of Parkinson's disease (Widner & Marktorp 2015). The surgical procedure involves implantation of two electrodes bilaterally into the brain of the patient. The electrodes are cylindrical rods and consists of noble metals, such as Platinum and Iridium. After implantation, electrical signals are emitted from the electrodes with the function to stimulate the brain domain of interest. The electrical signals are initiated from a pacemaker, implanted in the patient. The electrical pulses are short, around 60-100 µs, and the optimal frequency of the stimulating electrical pulses is usually 130-180 Hz (Moreau *et al.* 2008, Mottaghi *et al.* 2020).

After implantation of the electrodes into the brain, the parameters of the electrical stimulation can be adjusted to accommodate the patient's individual needs. Mottaghi *et al*. (2020) have examined how different frequencies and the amplitudes of the electrical pulses affect the outcoming result of DBS. The results observed showed that rats treated with electrical pulses with higher amplitudes were more situated to travel a longer distance during DBS-treatment. This might suggest that higher amplitudes of the signals can have positive effects on the relieving of symptoms in PD.

The electrical parameters, such as voltage and frequency, can be changed independently in the two different electrodes. The duration of the stimulations and time between these can also be set differently, depending on if it is the right- or left side of the brain that needs to be the most stimulated. The electrodes consist of several separated contacts, emitting electrical signals in different part of the brain depending on where they are located. The correct position of the electrodes can be ensured by making a microelectrode recording of the electric activity in the brain domain. By doing this, the area of tissue affected by the pulse, together with the power of the signal, can be enrolled. Magnetic resonance imaging (MRI) analysis can also be performed in order to visually secure the correct position of the electrodes in the brain (Tommasi *et al.* 2008, Li *et al.* 2020).

DBS is today frequently used to palliate severe motor symptoms in patients suffering from an advanced staged of PD. The procedure is often favoured over traditional lesioning since it is believed to be a more safe surgical operation, especially in terms of a smaller risk of serious complications (Saint-Cyr *et al.* 2000). The decision to start the treatment is usually taken when the dopaminergic neurons have almost completely degenerated (Guillaumin 2020). In this advanced stage of the disease, the neurological damage is so advanced that treatment with dopamine-substituting pharmaceuticals have little effect. DBS can be performed in most patients in which the dopamine neurons have degenerated, no age or medical restrictions limit its usefulness. Neurosurgery can also be performed to relieve side effects of pharmaceuticals that substitutes dopamine (Widner & Marktorp 2015).

Side effects of deep brain stimulation

DBS has been shown to eliminate some of the adverse effects induced by dopamine-substituting pharmaceuticals, thus relieving some of the side-effects which the dopamine agonist medicines can give rise to. Another advantage of performing DBS is that it is a surgical method. Some of the adverse effects that may develop from different types of pharmaceuticals are therefore not present in DBS, since the treatment does not rely on any drugs. This reduces the risk of dopaminergic deficits and overdose (Castrioto *et al.* 2014, Widner & Marktorp 2015).

In addition, the surgical procedure usually does not lead to any complications. The risk of getting an infection from a DBS-operation is low, under 3 % (Widner & Marktorp 2015). DBS has been shown to effectively relieve many of the motoric symptoms in PD (Castelli *et al.* 2004). Side effects from the surgical procedure *per se* are unusual. However, many patients suffering from PD experience adverse side-effects upon DBS treatment, primarily reported as cognitive symptoms (Guillaumin, 2020). The underlying mechanisms for these side-effects are not yet fully understood. One strong theory in the field suggests that the electrical DBS-impulses are not selective for a specific area of the brain, but instead reach beyond the intended brain area. Thus, electrical pulses with higher voltages than 3 V may be able to diffuse from the electrodes and affect other, adjacent areas of the brain.

The subthalamic nucleus and its implementation as target area for DBS

The subthalamic nucleus (STN) is a small, lens-shaped nucleus in the brain located close to the thalamus and hypothalamus. The dimensions of an average human STN are about 3x5x12 mm and was discovered the first time in 1865. It was named corpus Luysii after J B Luys, who first described this nucleus (Castrioto *et al.* 2014). The STN is located between the entopeduncular nucleus and the midbrain, and the zona incerta and the cerebral peduncle (Guillaumin 2020).

The STN is a part of the basal ganglia, a brain system known for its regulatory function of motor, cognitive and emotional behaviour. The basal ganglia receive information mainly from the cerebral cortex, and in turn projects to the thalamus. The basal ganglia consist of three different loops: the motor loop, the limbic loop, and the cognitive loop. These parallel loops are responsible for different functions mediated by the basal ganglia. The motor loop is the most investigated loop, and it is highly relevant in PD. The motor loop is constructed of three different pathways, which regulate the output projection to the thalamus. These pathways are known as the direct, indirect and hyperdirect pathways. The dynamic regulation of inhibition and excitation between the structures of these pathways determines the function of the basal ganglia (Guillaumin 2020, (Rodriguez-Sabate *et al.* 2019).

The role of the STN has been investigated for a long time. In 1927, researchers examined the effects after lesioning the STN. The results showed severe cases of involuntary movements, such as seen in Huntington's disease, but also symptoms of emotional and behavioural changes. This physiological state was named as "the syndrome of the body of Luys". With these interesting findings, investigations into the role of the STN and the impact of its lesioning were initiated by scientists. In this early stage, only the adverse motor effects of STN-lesioning were investigated, whereas the cognitive and affective side effects were largely disregarded for a long time (Saint-Cyr *et al.* 2000, Castrioto *et al.* 2014).

DBS-treatment in the STN (STN-DBS) is an efficient method in relieving motor symptoms associated with the later stages of the PD, such as tremors and bradykinesia (Castelli *et al.* 2004). This is because STN-DBS has been shown to inhibit STN neurons, which in turn induces a negative effect on the inhibition of the brain cortex. This will ultimately lead to the relief of motor symptoms commonly seen in PD (Li *et al.* 2020). Today, deep brain stimulation of the subthalamic nucleus (STN-DBS) is a common method to treat patients with PD. Since the first successful STN-DBS treatments in PD patients in the 90's, STN-DBS is now considered as a standard procedure for patients with advanced PD (Benabid *et al.* 2009).

Mechanisms of deep brain stimulation

The mechanisms behind DBS, and how it changes brain function, are very complex and not fully understood. However, three major hypotheses exist (Guillaumin 2020). One states that the consequences of DBS correspond to those of a brain lesion, that is, removal of function. This hypothesis is based on the theory of inhibition of STN neurons upon applying the STN-DBS procedure. Another theory instead suggests that the mechanism is based on the excitation of STN neurons (and other neurons) as studies have reported an increase in firing rate upon DBS (Chiken & Nambu 2013)

The electric signals from the DBS-rods may also affect other areas, for example the pedunculopontine nucleus, or PPN, which is a brain domain situated close to the STN. A vast number of nerve fibres between the PPN and the STN connects the two domains with each other. Electric signals diffused from the STN could therefore have an impact on the PPN. The PPN is a part of the constitution of the mesencephalic locomotor region (MLR) domain, which is strongly connected to the sense of balance. Electric stimulation of the PPN could therefore have an influence over the balance function in humans (Li *et al.* 2020).

Additional DBS target areas, and comparison with the subthalamic nucleus

Several studies have shown that STN-DBS is the most effective to decrease parkinsonian motor symptoms (Guillaumin 2020). Some researchers, however, have proposed an alternative option for DBS-treatment. In this case, the target of interest is not the STN, but instead the globus pallidus *interna* (GPi) (Odekerken *et al.* 2016)

The GPi is one of the output structures to the basal ganglia motor loop. The GPi is involved in the direct pathway of the motor loop. In the direct pathway, the GPi gets signals from the striatum, another brain area included in the motor loop. The GPi then transmits this signal to the substantia nigra *pars reticulata* (SNr) and the entopeduncular nucleus (EP), resulting in inhibition of these structures. This inhibition of the SNr/EP ultimately leads to the promotion of movement from the ventro-lateral thalamus (Guillaumin 2020).

The main reason for performing DBS in the GPi (GPi-DBS) is that some researchers associate STN-DBS with a higher risk of cognitive deficits and behavioural changes in patients. Some studies have found that the alleviation of motor symptoms associated to PD had a similar effectivity when comparing STN-DBS and GPi-DBS. At the same time, GPi-DBS treatment resulted in fewer seriously adverse side effects. Other studies, however, indicated no such

relation in adverse effects, whilst reporting a higher rate of alleviation of motor symptoms when DBS-treatment was performed in the STN (Odekerken *et al.* 2016).

Observations of side effects after DBS-treatment of the STN and GPi, that were made three years after the surgical procedure, also showed different results. While some studies state that it is more safe to perform a GPi-DBS procedure in order to minimise the risk of long term adverse cognitive effects, other showed that there was no significant difference in cognitive decline between DBS treatment of the STN versus the GPi (Boel *et al.* 2016). Further, GPi-DBS has also shown to be a safe surgical method for the relief of motor symptoms including tremors, rigidity, and bradykinesia in patients with PD, just as STN-DBS. However, the STN is often preferred due to practical causes (Anderson *et al.* 2005).

Advantages of deep brain stimulation

One common method for the treatment against PD is medication with levodopa. Levodopa has been used in many years and have been shown to be very effective in alleviating the motor symptoms of Parkinson´s disease. While levodopa treatment is effective in the first period of the disease, after a few years many parkinsonian patients experience major disabilities in motor functions, such as dyskinesia and loss of therapeutic efficacy. As discussed above, DBS has been shown to improve motor symptoms, and is therefore a better treatment for many patients in later stage of PD (Faggiani & Benazzouz 2017).

One of the principal advantages of DBS compared to lesioning is that it is a reversible method. After a performed brain lesioning, a significant part of the brain has been removed which in worst-case could lead to serious side-effects. Since the brain has been significantly damaged, these adverse effects are irreversible. The putative adverse side-effects of DBS can be reversed by removing the DBS-stimulation rods, or simply but turning the power off. (Anderson *et al.* 2005).

As discussed above, studies have found that STN-DBS is effective in relieving motor symptoms in PD (Tommasi *et al.* 2008). Motor disability in patients have shown to decrease immediately after bilateral DBS surgery, resulting in improved balance performance and ultimately to fewer accidents connected to falling. This significantly improves the quality of life for many parkinsonian patients (Li *et al.* 2020).

Disadvantages of deep brain stimulation

While the STN-DBS procedure is considered a safe method by many researchers and clinicians, some studies have reported adverse side-effects in the form of decreased cognitive function. Cognitive dysfunction was observed especially among patients suffering from the disease at a high age (Witt *et al.* 2008). Other studies have stated that while electrical pulses are efficient in reducing some of the motor symptoms, for example tremors and rigidity, they can have an impact on the occurrence of symptoms such as impaired gait and periods of freezing (Moreau *et al.* 2008).

Studies performed by other researchers have also found that some patients undergoing STN-DBS have shown cognitive deficits and psychiatric symptoms in forms of depressions (Tommasi *et al.* 2008). There have also been reports of depression and decreased sexual

satisfaction in both sexes, but especially in female patients undergoing STN-DBS (Castelli *et al.* 2004).

Aim

Many studies have been performed in order to examine both the beneficial and adverse effects of DBS in patients with PD. The firm conclusion has been that DBS is a procedure that effectively reduces the motor symptoms associated with this disease. However, different types of adverse side-effects have also raised concerns about DBS as therapeutic method. Consequently, clinical follow-up procedures have been designed, so that both short- and long-term effects of the DBS-procedure can be revealed. While adverse side-effects have been reported, potential sex-differences of their distribution have not yet been extensively investigated.

The aim of this essay is to analyse the putative occurrence of sex-differences in terms of reported adverse side-effects upon DBS treatment in advanced stage PD.

Methods

For this thesis, 16 research and review papers were studied and analysed. The papers were found by using the Web of Science Core Collection database. Frequently used search terms included: STN-DBS, subthalamic nucleus, adverse effect, and sex differences. The articles were then selected for analysis primarily based on whether there was a clear description of the adverse effects reported by the participating patients. The sex distribution between the patients was also an important factor that was considered for the selection. However, some papers not stating the sex distribution was also included in the analysis.

Statistically analysis of optogenetic data was performed in Microsoft Excel. For analysis, a two-way ANOVA with replication was used for each of the examined behaviours. Moreover, if the ANOVA showed a significance difference, a two-sample t-test assuming unequal variances was performed for further examination of the significance.

Due to ongoing pandemic, this degree project was conducted by comparative analysis of current literature paralleled by statistical analysis on data obtained by Serra/Mackenzie in a previous experimental study (Guillaumin *et al.* 2021).

Results

Studied material

The number of participating patients differed greatly between the different studies. Some of the articles included multiple patients, whereas some of them only included one or a few participants. The sex distribution of the patients involved were also not noted in all the papers (table 1). In some reports, the sex distribution of the participants was mentioned, but the

resulting side effects reported in the study was not. The studies using this method are listed with a "No" in the sex division field in table 1.

In many studies, the authors have also not reported if the adverse effects were reported by men or women. All the articles that reported sex distributed side effects are included in table 2. However, even among those articles, some did not report a detailed list of distributed effects. One article was just reporting the adverse effects experienced by three of the men and three of the women, yet the article included 24 participants in total (Berney *et al.* 2002). Another article showed the results as adverse effects obtained by the group of men and women in total, and not as individual numbers (Montaurier *et al.* 2007). This does not show the frequency of each reported side effect, and they are therefore only reported one time in table 2. The number of participants in the different reports could also have an impact on the outcoming result of this report since the articles focusing on a small number of contestants generally could report more, detailed adverse outcomes.

The distribution of sex reported in the studies differed greatly between the articles. There was, however, more men than women among the patients in almost all studies reporting more than one participant. The only exception to this was in the article written by Romito LMA *et al.* (Romito LMA *et al.* 2002). In this study, an equal number of men and women have been reported. Further, some of the articles with only a few (five or less) contributing patients have only examined men in their study (table 2). Some studies have also examined a large number of patients, but only mentioned the sex distribution between a few of them (table 1). In those cases, only the sex distributed patients will be collated in table 2.

Table 1. The studies covered in this thesis and which information they report. The participants are reported as the total number of patients covered. Some studies only distributed a small group of patients in terms of sex. The number of sex distributed patients in those articles are listed in parenthesis.

Study	***No. of participants***	***Sex distribution***	***Domain of DBS***	***Dopamine medication***
(Montaurier et al. *2007)*	24	Yes	STN	Yes
(Moretti et al. *2002)*	2	Yes	STN	
(Li et al. *2020)*	36	No	STN	Yes/No
(Berney et al. *2002)*	24	Yes	STN	Yes
(Sensi et al. *2004)*	1	Yes	STN	Yes
(Houeto et al. *2002)*	24 (5)	Yes	STN	Yes/No*
(Bejjani et al. *2000)*	12	Yes	STN/ZI	Yes/No*
(Moreau et al. *2008)*	13	No	STN/GPi	Yes

(Doshi et al. *2002)*	3	Yes	STN	Yes
(Diederich et al. *2000)*	1	Yes	STN/ZI	No
(Romito LM et al. *2002)*	2	Yes	STN	Yes/No*
(Funkiewiez et al. *2004)*	70 (4)	Yes	STN	Yes/No*
(Kulisevsky et al. *2002)*	3	Yes	STN	Yes
(Romito LMA et al. *2002)*	22	Yes	STN	Yes/No*
(Stefurak et al. *2003)*	1	Yes	STN	Yes
(Tommasi et al. *2008)*	1	Yes	STN	

* = All medication against Parkinson's disease were withdrawn for some patients.

Definitions

Physiological side effects

Physiological side effects in this essay included both motor effects and other physiological effects. Motor side effects included dyskinesia and impaired gait, while other physiological effects for example included autonomic effects such as sweating.

Affective side effects

Affective side effects included both clinical diseases, such as depression, and emotional effects. The emotional effects include decreased state of mood, anxiety, aggression, and euphoria. The affective side effects also included behavioural symptoms, such as addictive behaviour and higher propensity to take risks.

Cognitive side effects

Cognitive side effects include difficulties concerning speech, such as decreased verbal fluency and hypophonia, but also other effects, *e.g.*, visuospatial dysfunction.

Reported adverse side-effects and their distribution among men and women

The adverse side effects in the tables below were categorised in three groups: affective, physiological, and cognitive side effects. This was done for both women (figure 1) and men (figure 2), along with the table over studies not reporting any sex distribution (figure 3). If a side effect is reported more than once in an article in tables 2 & 3, the number of times will be

represented in parenthesis behind the specific side effect. The only exception is in the article by Moreau *et al.* and Montaurier *et al.* where the adverse effects was observed in all patients (table 3).

The largest number of side effects reported in the articles were of physiological type. This was the case for both women, men, and the undistributed group (figures 1, 2 & 3). The most frequently reported side-effect by both men and women was increase of weight. Weight gain was in total reported 26 times in men and 17 times in women. Among women, however, depression was the most common side effect in terms of number of reports by different articles. This was also true regarding the articles about side effects in men. Depression was reported in three of the seven articles with participating women, and in five of the 13 articles with men (table 2). Depression was also reported three times in the articles not containing a sex distribution (table 3).

Some articles did not specify the distribution of the sexes in the study. The side-effects reported in these studies are shown in table 3. One article did not report any negative side effects, but focused on the positive outcome (Li *et al.* 2020). The results stated that STN-DBS led to improved physiological ability, such as higher stability, improved sense of balance and improved motor functions (table 3). Another common result reported by all articles was that the dose of levodopa and other dopamine substituting pharmaceuticals could be lowered in all cases after the DBS surgery.

Physiological side effects

The motor symptoms of PD were reported as relieved in most of the studies. Some individual participants, however, experienced withstanding or worsened motor symptoms as a side effect of DBS. These types of side effects were reported by both men and women (table 2) in the studies by (Bejjani *et al.* 2000), and by the undistributed group (table 3) in the study by Romito LMA *et al.* (2002). Another motor effect was the emergence of tremors. The tremors occurred in the blepharon- and the buccinator muscles (Romito LMA *et al.* 2002). In table 3, those effects have been summarised as “facial spasms”. Other physiological, but not motor, adverse effect reported by both sexes was weight gain and loss of weight. Weight-related effects of DBS were overall shown to be similar between men and women Noticeably, increased weight was reported more frequently than loss of weight (table 2). An increase of fat mass was also reported by both men and women, while men, but not women, also experienced an increment of muscle mass after STN-DBS. Another side effect of STN-DBS was that the energy expenditure decreased in both sexes.

<u>Men</u>

The reported motor side effects included difficulties considering gait and the sense of balance in men, and dyskinesia in both women and men (table 2). Another side effect reported in men, but not in women, was sexual hyperactivity. Sexual hyperactivity was also reported in the articles without sex distribution. There were five reported cases of sexual hyperactivity in men, and four cases among the PD patients in the undistributed group (tables 2 &3). Sexual hypoactivity was also reported in men. However, it was not as common as sexual hyperactivity, as it was only reported once.

Women

The most common motor side effect in women was dyskinesia. Dyskinesia was reported twice by female patients. The other motor side effect reported in women were impaired ipsilateral movement (table 2). The study made by Stefurak *et al.* (2003), showed that the woman patient experienced an improvement of parkinsonian motor symptoms, such as bradykinesia and tremors, when treaded with stimulations from the left deep brain stimulation (LDBS) rod only. During treatment with the right deep brain stimulation (RDBS) rod only, the patient showed signs of a depression-like state: dysphoric mood, apathy, and emptiness.

Affective side effects

Affective side effects were, as stated above, the type of effects reported by most articles. Most of the reported affective dysfunctions observed were different behavioural changes, primarily among men.

Men

While some men became more enraged and irritated, other reported emotions of euphoria and joy of life. One man was reported to show a more violent behaviour and made both aggressive verbal and physical outburst against people in his surroundings. Furthermore, he developed a kleptomaniac behaviour. At the same time, he could switch between enraged and vivid expressions (table 2).

Mania was also reported 5 times among the men, with symptoms including a large increased self-esteem, elation, and higher involvement in different activities. In addition, several cases of side effects similar to manic behaviour, such as increased self-esteem, were reported. Increased self-esteem was reported 2 times in men. Furthermore, one case of addictive behaviour was observed, along with another case concerning a man who developed a more risk-taking behaviour (table 2).

Other men developed depression, or a decreased state of mood. Depression was reported nine times in men. Additionally, side effects indicating a decreased state of mood, were reported 9 times. This included side effects such as loss of interest, energy, and initiative. Anxiety was also reported once. Other men reported a more emotional behaviour, but not necessarily a decrease in state of mood. This type of "emotional hyperactivity" was reported twice. Apathy was also reported once in men (table 2).

Women

Depression and decreased state of mood was also reported in women. However, these effects were the only affective side effects reported from STN-DBS in women. Depression was observed twice, and a decreased state of mood was reported three times. In women, the symptoms of a decreased state of mood included anxiety, hysterical crying, and apathy.

Additionally, affective symptoms were also reported in those studies not containing a sex distribution. Depression was reported three times, and other side effects concerning a decreased state of mood, apathy and psychic akinesia, was reported 3 times as well. However, there was also two observations of participants developing side effects of manic psychosis and one case of hypomania (table 3).

Cognitive side effects

Cognitive side effects were observed in some men, and in the group not considering sex divided participants (figures 2 & 3). Hypophonia was the only cognitive dysfunction reported in the group with no sex distribution (table 3). In other reports, cognitive dysfunctions were not specified by the authors. They mentioned, however, an increase of scores in the UPDRS-scale, which states the cognitive skills among persons suffering from PD.

Men

In men, cognitive side effects regarding speech were also the most common. Speech-related side effects were reported in five cases. There were also two reports of attention difficulties, two reports of a reduction of semantic fluency, one report of impaired visuospatial function and one report of psychomotor retardation (table 2).

Women

Cognitive dysfunction was not discovered in women (figure 1).

Table 2. The number of men and women participating, and the side effects reported by both sexes. Numbers in parenthesis represents the number of times the side effects were reported in the study, if more than once.

Study	***Male***	***Female***	***Reported side effects (men)***	***Reported side effects (women)***
Montaurier et al.	17	7	Increased weight, increased muscle mass, slightly increased fat mass, decreased testosterone levels, decreased energy expenditure, reduced energy expenditure after eating. [+]	Increased weight, increased fat mass, unchanged muscle mass, decreased energy expenditure. [+]
Moretti et al.	2	0	Attention difficulties (2), (2), reduction of: phonological fluency (2), semantic fluency (2) and syllabic task (2)	ND
Berney et al.	15	9	Depression (3)	Depression (3)
Sensi et al.	1	0	Verbal aggressive outburst, violent behaviour, kleptomania, alternation between vivid and enraged expressions, alternation between euphoric and aggressive feelings.	ND

Houeto et al.	5	0	Lack of energy, mood switches, sexual hyperactivity (2), exhibitionism (2), addictive behaviour, sexual dysfunction, depression, loss of appetite, insomnia, emotional hyperactivity, decreased verbal fluency, fatigue, sexual hypoactivity, loss of initiative.	ND
Bejjani et al.	8	4	Gait (3), akinesia (3), motor fluctuations (3), depression, suicidal tendencies, loss of energy, loss of interest	Dyskinesia (2)
Doshi et al.	3	0	Walking difficulties/freezing (2), depression (3), insomnia, anorexia, suicidal tendencies	ND
Diederich et al.	1	0	Hallucinations, sleepiness, psychomotor retardations, visuospatial dysfunction.	ND
Romito LM et al.	2	0	Mania (2) increased self-esteem (2), decreased need for sleep, risk behaviour, sexual hyperactivity (2), prodigality, reckless behaviour, loss of appetite, increased empathy and emotions, lability,	ND
Funkiewiez et al.	3	1	Anxiety, fatigue, apathy, loss of initiative, unmotivated, depression, suicidal tendencies, energetic behaviour, hyperactivity, decreased need for sleep.	Depression, hysterical crying, reoccurring of parkinsonian symptoms.
Kulisevsky et al.	3	0	Mania (3), euphoria, press speech, sexual hyperactivity, delusions, foot dyskinesia	ND
Romito LMA et al.	11	11	Increased weight (9), decreased weight (2), insomnia,	Increased weight (10), decreased weight,
Castelli et al.	21	10	Decreased sexual dissatisfaction	ND
Stefurak et al.	0	1	ND	LDBS: decreased bradykinesia & contralateral tremors

				RDBS: dysphoric mood, apathy, anhedonia, emptiness.
Tommasi et al.	0	1	ND	Acute depression, anxiety, sweating, impaired ipsilateral movement.
Total	102	47		

ND = No Data
[+] = Side effects reported by all the participants.

Table 3. Number of patients with corresponding side effects in the studies with no reported distribution between the sexes. Numbers in parenthesis represents the number of times the side effects were reported in the study, if more than once.

Study	***No. of participants***	***Reported side effects***
Romito LMA et al.	22	Sexual hyperactivity (4), seizure, hypophonia (4), depression (2), psychic akinesia (2), manic psychosis (2), facial spasm (3), paraesthesia (7), limb dystonia, diplopia.
Li et al.	16	Improved sense of balance, improved motor functions, higher stability.
Moreau et al.	13	130 Hz, high voltage: freezing periods, worsening of gait. 60 Hz, high voltage: Improvement of gait, tremors, akinesia, and rigidity. [+]
Montaurier et al.	24	Dysarthria (7), apathy, hypomania, depression.
Total	75	

[+] = Side effects reported by all the participants.

To summarise, the results show that physiological side effects were most common for all three groups. Affective effects were the next most frequently reported type of side effect among women and men (figure 1 & 2). Among the undistributed group, cognitive side effects were slightly more common than affective effects (table 3). Both motor effects, such as dyskinesia and seizures, and other physiological effects were included by the group "Affective effects". Examples on other physiological effects were sexual hyperactivity and fatigue.

In addition to analysis of reported side-effects from human patients, statistical data analysis was performed on a group of mice that had been analysed for different types of behaviour upon experimental optogenetic stimulations in the STN. The examined behaviours included

velocity, distance moved, and grooming. Data from optogenetic studies on mice were also obtained in order to see if there was a difference between the sexes. After analyses, however, it showed that there was no significant difference. These results are presented in the appendix.

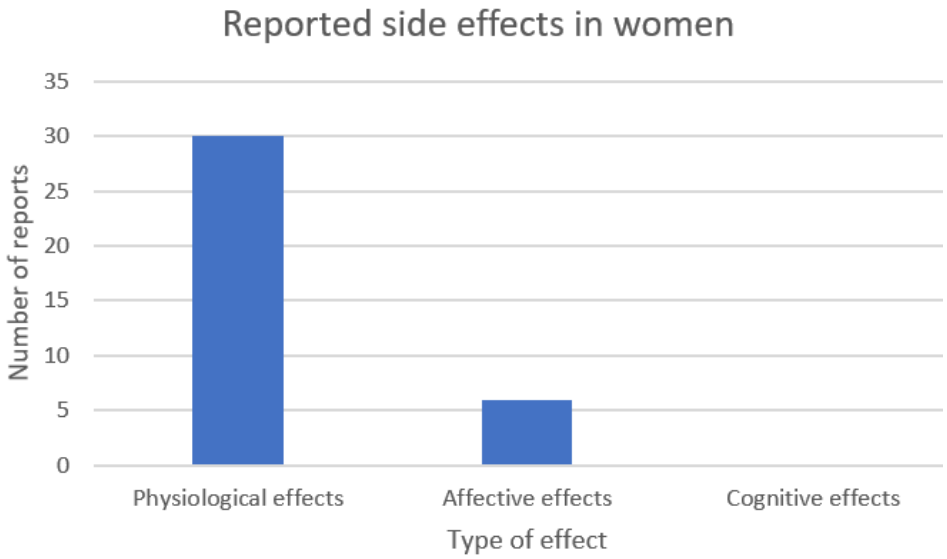

Figure 1: Bar plot over the number of adverse physiological, affective, and cognitive side effects reported by women.

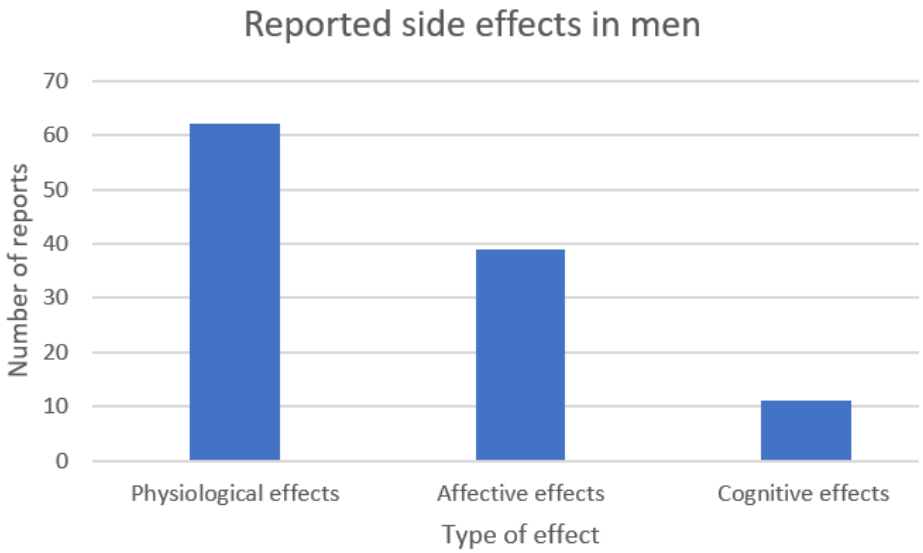

Figure 2. Bar plot over the number of adverse physiological, affective, and cognitive side effects reported by men.

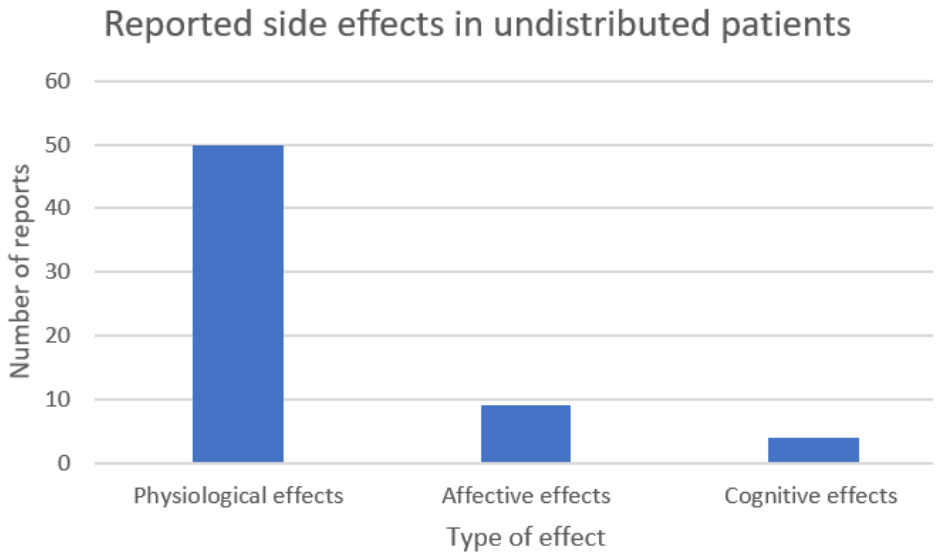

Figure 3. Bar plot over the number of adverse physiological, affective, and cognitive side effects reported by patients in the articles not presenting a sex distribution.

Discussion

The results in this essay are based on presented results in different studies made by other authors. These authors have been focusing on different aspects of the outcome of DBS-treatment in PD patients. Whilst some researchers have been concentrated on the cognitive side effects of DBS-treatment, others have focused more on the physiological outcomes. The difference in perspectives might also mean that some adverse effects could have been overlooked as non-important, and therefore not noted in that particular study. The time frame between the surgical procedure and the follow up reporting visits of side effects have also differed between the reports. Some of the follow up visits were made some months after the operation, whilst other visits were made after several years. Some articles also reported continuously repeated follow up visits. The differences in time between the various reports may have contributed to a wider spectrum of side effects presented, since some effects might develop at different times. However, the side effects reported a long time after the operation relies to a great extent in the parkinsonian patient and his or her family's ability to remember those effects. This could have a negative impact on the outcoming results if the patient and its relatives forget to report some effects.

The obtained results showed that both men and women experienced physiological side effects the most. This was a bit surprising since most literature states that DBS treatment is efficient in reducing the motor symptoms of PD. This could however be due to the consolidation of both motor effects and other physiological effects into one group. Still, data from table 2 & 3 shows some cases with side effects quite similar to these also present in PD. A possible explanation could be different settings of the implanted electronical rods. Parameters such as frequency and amplitude might be able to worsen some physiological parkinsonian symptoms (Mottaghi *et al.* 2020).

One difference in the adverse effects of DBS treatment between the sexes indicates that male patients have a higher tendency to elaborate sexual hyperactivity and altered sexual behaviour than female patients, since none of the participating women reported an alternation in sexual

activity. One cause could be that STN-DBS improves the urogenital dysfunction sometimes experienced by PD patients (Castelli *et al.* 2004). However, this does not explain why none of the women reported sexual side effects. The most common sexual adverse effect was sexual hyperactivity. Even if not directly concluded in the articles above, it would be interesting to see if the sexual hyperactivity is a symptom of maniac behaviour. Symptoms of mania included for example increased self-esteem and gambling. Sexual hyperactivity, on the other hand, was reported as a separate side effect. Furthermore, all men that had mania as a reported side effect of the DBS treatment, also showed sexual hyperactivity. Nevertheless, male patients have also showed sexual hyperactivity without a reported manic behaviour.

Affective side effects were more common among men than among women. Not only in terms of number, which can be explained by the higher number of male participants, but also in terms of percentage. Another interesting result was that while women only experienced depression-like symptoms, men would also develop other types of affective changes, such as aggressive behaviour or euphoria. Since the range of men and women included in this essay is quite limited, this could be one explanatory factor. Nonetheless, it would be interesting to investigate if men develop more types of behaviours than women in larger studies.

Both men and women in the articles replied that they have been experienced symptoms such as depression, anxiety, and apathy during STN-DBS. However, the adverse effects experienced by men were usually more detailed than those of women. This could depend on the fact that men were examined in a greater extent than women, so that more effects were reported. Except apathy and anxiety, side effects such as lack of motivation, anorexia and suicidal tendencies were also reported by some men. Other men however, developed feelings of euphoria and happiness. This kind of effects were not reported by any of the women. It was interesting to see that from the results obtain from these articles, men could experience both depressive and euphoric feelings along with an increased aggressive behaviour. Family members have witnessed of, for example, verbal outbursts towards hospital personnel and a violent behaviour. That person also had several fluctuations between aggressive and vivid expressions (Sensi *et al.* 2004). Women on the other hand, do not tend to be euphoric or aggressive. It would be interesting to see if there exist a sex difference between the affective side effects of STN-DBS, and in that case, what the difference depends on.

Cognitive side effects were not that common in either men or women. Women reported zero cases of cognitive dysfunction. This indicates that STN-DBS is an effective method in relieving these kind of PD symptoms. However, men did report some cognitive dysfunction. The most reported type of cognitive effect was speech impairments. Conclusions about why cognitive dysfunctions are seen in men but not in women cannot be drawn in this essay. It may be due to the limited number of participants, or there is a physiological difference in the brain structures between men and women, which makes men more receptive to cognitive dysfunctions.

A study made by Jahanshahi *et al.* (2000) also investigated the effects of deep brain stimulation in the GPi domain. The ability to compare the two brain domains is an interesting aspect in the study. The result showed that both GPi-DBS and STN-DBS was effective in reducing PD symptoms. However, patients who underwent a STN-DBS operation had a higher improvement of the performed TMT B test, when stimulated. During a TMT B test, the person is required to alter its mental state. The difference in score between the TMT B and a similar TMT A test is often used as a measurement of cognitive speed. Patients with STN-DBS had both greater scores in the TMT B test, and in the difference between the two tests

than persons with GPi-DBS, post operatively. This may be an indication on that STN-DBS is a more effective option for reducing adverse side effects with cognitive nature in PD patients. However, due to the limited range of selected patients, more research is needed in order to make conclusions.

Another interesting feature found in one article was that in 75 % of the participating women, the right side of the brain induced the worse parkinsonian symptoms. Among the participating men, the results were the opposite- most of them (78 %) had the worse symptoms induced by the left side of the brain (Jahanshahi *et al.* 2000). This could maybe be depending on if there were physiological differences in the pathway of Parkinson between men and women. As mentioned in the introduction, the signalling pathways in PD is not entirely known yet. It would be interesting to get further information on this result, since it shows a difference between the sexes.

The methods used to obtain and analyse the results differed between the studies. Most articles asked the participants and their relatives what kind of adverse effects the patients experienced. The patients also had to implement several tasks before and after surgery, which measured the effective impact on motor- and cognitive symptoms of the DBS-treatment. However, one article examined the participants in a calorimetric chamber in order to analyse the individual energy expenditure. They also took blood samples in order to examine the levels of testosterone in the blood (Montaurier *et al.* 2007). These types of methods elucidate other physiological side effects which other articles have not investigated. Testosterone was the only hormone tested in this article. It would be of interest to investigate if the level of other hormones also altered. That would maybe give an explanation in why some PD patients reported gain of weight, and some reported weight loss. Eleven of the participants were over-weighted or obese before the surgery. It would be interesting to see if this had any impact on the sex differences of weight gain in the study. Unfortunately, the article did not list the outcoming adverse effect of every individual so, in this essay, it was not possible to see if there existed a difference between the sexes.

Even though the doses of dopamine pharmaceuticals were lowered in all cases, many of the patients participating in the studies were still on some medication after the DBS surgery (table 1). This raises the question whether the medication has a major impact on the reported adverse effects. Would there be a greater difference in the reported the side effects with a total withdrawal of dopamine substituting pharmaceuticals? While some of the articles only mentioned minor side effects caused by DBS treatment in patients without any medication (Romito LM *et al.* 2002), other reported major disabilities such as loss of motivation (Houeto *et al.* 2002). The impact of DBS and dopamine pharmaceuticals combined, should be further investigated to collect more information about how the medication of dopamine substitutes affects men and women.

One article have reported that the post-operative improvement of bradykinesia among men are higher than the improvement among the participating women (Accolla *et al.* 2007). There is no known explanation on how or why bradykinesia is more resistant to the DBS-treatment in women than in men. Since it was only statistically assured in one article, this could also be a coincidence and only apply to that article. Nonetheless, it would be interesting to see if there is a difference in the improvement of bradykinesia between men and women.

Limitation of the study

The range of this study is not enough to include all the reported symptoms, or a fully distribution of these, between the sexes. Neither is it capable of explaining why adverse effects might arise from DBS, or the mechanisms behind the procedure. The primarily intention with this written report is merely to picture the problematics of PD, and if they are experienced differently between the sexes. Further, it may touch the question if there are reports of a difference in sex divided symptoms as a consequence of DBS between different domain of the brain.

Conclusion

In conclusion, DBS is a relatively safe method in order to treat PD. However, there are many reports of adverse side effects. Both women and men seem to experience similar side effects after STN-DBS. However, men tend to have a higher tendency to develop cognitive and affective changes, and more types of affective changes, than women. However, since the number of articles investigating the side effects of DBS was limited in this essay, no conclusion about the sex distribution could be made. Hopefully, with future research it will be possible to draw significant solutions.

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Supplementary data analysis

Background

The STN is a brain domain highly involved in the regulation of motor movement. Although its importance is known in neurological disorders such as Parkinson's disease, the impact of STN as a regulatory factor has still not been extensively understood. There has however been shown that dysfunctions in the STN frequently results in impaired function of voluntary movements. Deep brain stimulation (DBS) of the subthalamic nucleus (STN-DBS) is today a common method to treat and to examine the excitatory function of the STN in advanced Parkinson's disease. However, since the mechanism of DBS is not yet fully known, and that other factors may have an impact on the disease, this method has limitations. Another method used to examine the STN is optogenetics. The research of STN-DBS based on the optogenetic method is mainly focused on Parkinson model mice. In optogenetics, excitatory and inhibitory opsins are used to control the activity of neurons. Results from optogenetic experiments have given contradictory results, as there are both reports of performed optogenetics in the STN not leading to improvement of the disease, and that inhibition of the STN using optogenetics led to motor movement recovery in mice (Guillaumin *et al.* 2021).

Aim

The aim of the experiment was to further examine the role of the STN in motor movements, such as locomotion and the coordination of movements.

Aim of data analysis

The aim of the data analysis was to analyse data from the results of the study, to draw conclusions whether there was a difference in behaviour, based on the sex of the mice.

Method

The optogenetic experiment were performed by Guillaumin *et al.* (2021). Via injection of a viral vector in the STN, carried by an AAV2 virus, mice were injected with either a control vector only encoding for the reporter eYFP, or a vector encoding eYFP and a Cre-dependent channelrhodopsin (ChR2). Mice injected with ChR2 and eYFP is referred to as Pitx2/ChR2 mice. After the injection, optical cannulas needed for the experiment were attached bilaterally above the STN.

After a recovery period of at least 4 weeks, the mice were placed in an open field arena, consisted of a plastic chamber. The chamber measured 50 x 50 cm, and had two fields: one central zone, which accounted for about 25 % of the area, and a peripheral zone. Before each experiment, the mice were placed in the arena for 5 minutes to explore it. After that, the open field test was performed. The test was constituted of 4 periods of 5 minutes each where the stimulation was either on (ON) or off (OFF), the total length was 20 minutes. Each test followed the pattern OFF-ON-OFF-ON. Tested behaviour included rearing, grooming, escape behaviour, total distance moved, speed, body elongation, and time spent and frequency in the crossing centre. The results from the experiments covering total distanced moved, speed, body elongation and time and frequency in the crossing centre was automatically recorded. The

results from the experiment covering rearing, grooming, and escape behaviour were manually recorded.

The mice were exposed for two types of stimulations: 0.5 Hz and 20 Hz light stimulations. In the 0.5 Hz light stimulation, the mice were exposed to light stimulation pulses of 0.5 Hz and 5–8 mW, during a total time of 100 s. The length of the pulses was 5 ms long. The results from the light stimulation were then analysed to see if the neurons were excited, inhibited, or not responding to the stimulation. Thereafter, the mice were exposed to the 20 Hz light stimulation. This stimulation was performed in a similar way to the 0.5 Hz light stimulation, but the light stimulation pulses were 50 Hz. To measure the activity of the STN during STN-photostimulation, an optrode were attached to a recorder glass micropipette. The optrode also had a laser-connected optic fiber. The recorder pipette contained 2 % pontamine blue sky, mixed with 0.5 µM sodium acetate.

I got the data results from the behaviour experiments covering velocity, total distance moved and grooming in order to do a data analysis. A two-way ANOVA with replication was used to analyse the data. If the ANOVA showed a significant result, a two-sample t-Test assuming unequal variances was performed to further examine the significance. The analysis of data was made in Microsoft Excel.

Results

The result from the data analysis stated no significant difference in either the Pitx2/ChR2 mice or the mice in the control group, regarding the sex of the mice.

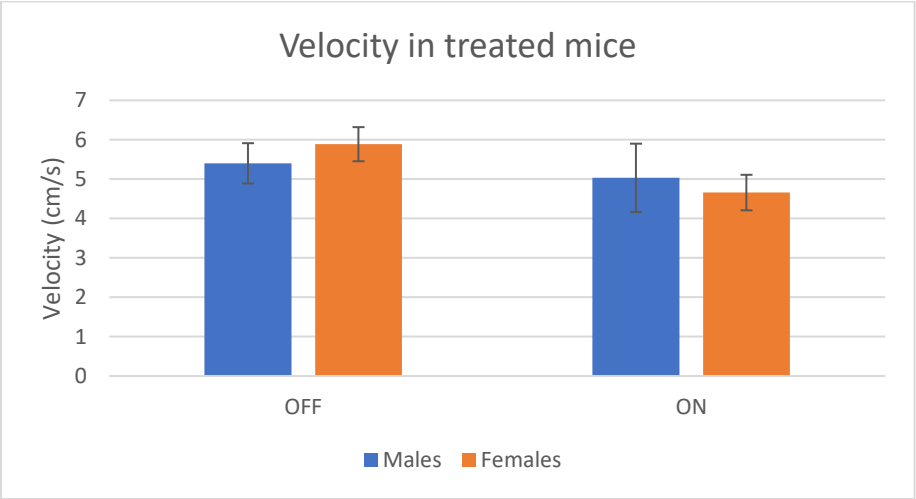
<u>Velocity in treated mice</u>

The ANOVA showed no significant difference between males and females regarding the velocity among treated animals.

	OFF	ON
Males	7.353535	7.198905
	5.482135	4.5789
	2.828665	2.46917
	4.93416	2.650075
	4.59615	3.72764
	5.3066	3.65884
	5.51234	9.409385
	7.202555	6.562745
Females	6.963195	6.01718
	7.11159	5.99517
	3.796255	2.75214
	6.82748	4.585765
	5.842705	4.98593
	5.712385	3.159905
	6.45374	3.97044
	4.3846	5.801715

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.024664	1	0.024664	0.008771	0.92605	4.195972
Columns	5.107356	1	5.107356	1.816352	0.188552	4.195972
Interaction	1.471996	1	1.471996	0.523493	0.475357	4.195972
Within	78.7325	28	2.811875			
Total	85.33651	31				



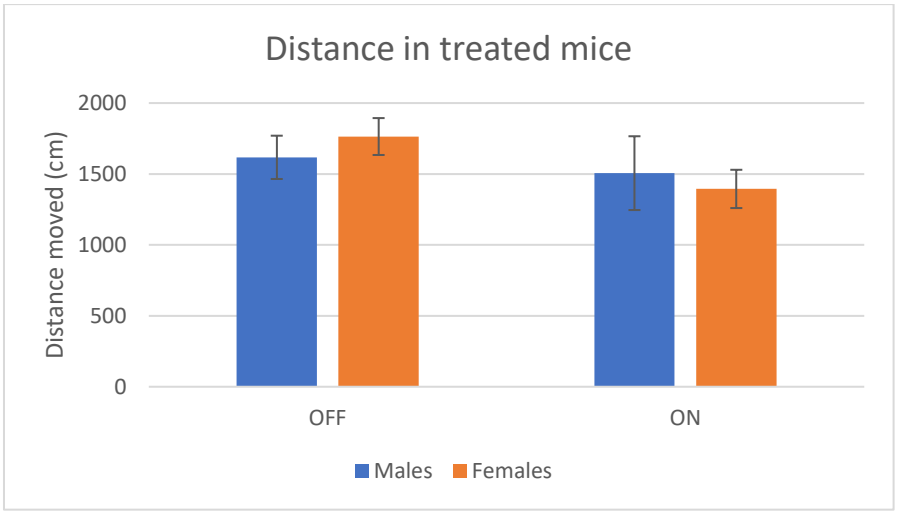
Distance moved in treated mice

The ANOVA showed no significant difference between males and females regarding the distance moved among treated animals.

	OFF	ON
Males	2202.505	2155.105
	1637.045	1361.221
	847.1005	738.719
	1476.98	794.037
	1377.65	1117.495
	1591.025	1096.405
	1651.97	2817.205
	2154.75	1967.19
Females	2086.305	1803.295
	2131.88	1796.73
	1137.675	825.0955
	2045.72	1374.77
	1750.995	1483.5
	1712.67	947.3265
	1934.955	1189.495
	1312.069	1737.345

ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Sampel	2510.278	1	2510.278	0.009955	0.921234	4.195972
Kolumner	462327.5	1	462327.5	1.833437	0.186551	4.195972
Interaktion	133007.2	1	133007.2	0.527462	0.473704	4.195972
Inom	7060602	28	252164.4			
Totalt	7658447	31				



Grooming in treated mice

The ANOVA showed a significant difference between males and females regarding grooming among treated animals.
However. the performed t-tests showed no significant results.

	OFF	ON
Males	21.08	89.1
	37.58	120.5
	43.14	92.54
	16.02	180.44
	22.26	117.14
	22.28	104.52
	18.26	42.68
	27.34	63.76
Females	9.02	69.66
	1.66	89.58
	10.52	154.16
	3.94	82.22
	59.12	153.7
	18.58	104
	4.56	104
	41.36	90.96

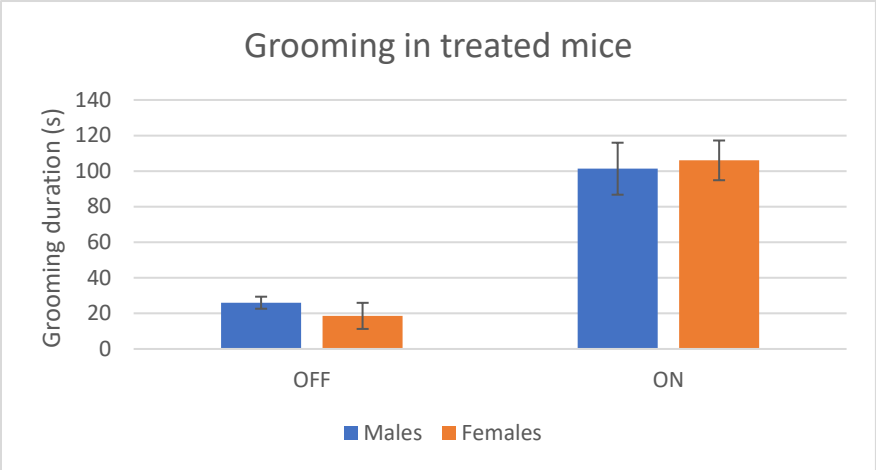
ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Sampel	14.58	1	14.58	0.018059	0.89406	4.195972
Kolumner	52994.66	1	52994.66	65.63994	8.04E-09	4.195972
Interaktion	292.82	1	292.82	0.362691	0.551862	4.195972
Inom	22605.91	28	807.3539			
Totalt	75907.97	31				

t-test: Två sampel antar olika varianser

	Variabel 1	*Variabel 2*
Medelvärde	101.335	106.035
Varians	1709.168	997.7954
Observationer	8	8
Antagen medelvärdesskillnad	0	
fg	13	
t-kvot	-0.25551	
P(T<=t) ensidig	0.401165	
t-kritisk ensidig	1.770933	
P(T<=t) tvåsidig	0.80233	
t-kritisk tvåsidig	2.160369	

t-test for ON-values



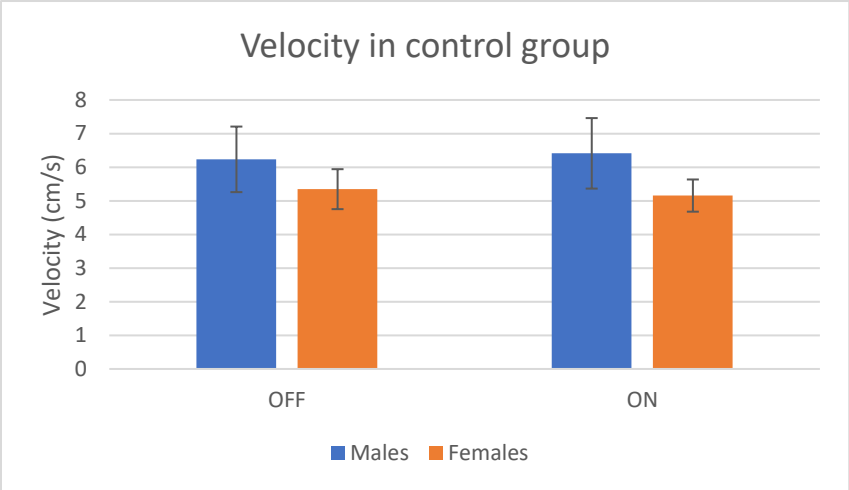
Velocity in the control group mice

The ANOVA showed that there was no significant difference between males and females regarding Velocity among mice in the control group.

	OFF	ON
Males	10.39315	10.56748
	4.853575	3.86619
	4.317225	4.75493
	4.055985	4.350495
	6.79757	7.363495
	6.984855	7.569345
Females	4.880375	4.66765
	5.262975	4.733365
	4.11082	5.07555
	3.979805	3.49061
	5.92604	6.75705
	7.922895	6.21387

ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Sampel	6.883622	1	6.883622	1.743459	0.201613	4.351244
Kolumner	0.000236	1	0.000236	5.98E-05	0.993909	4.351244
Interaktion	0.204313	1	0.204313	0.051748	0.822359	4.351244
Inom	78.9651	20	3.948255			
Totalt	86.05327	23				



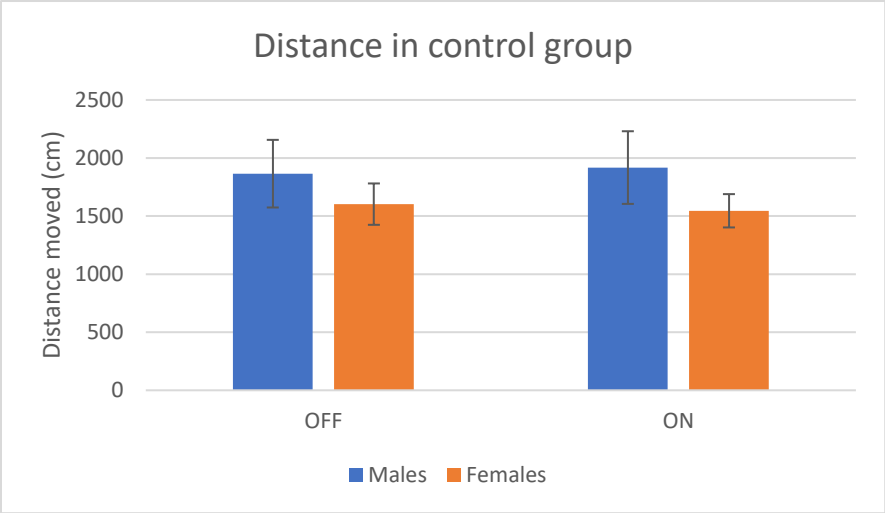
Distance moved in the control group mice

The ANOVA showed that there was no significant difference between males and females regarding the distance moved among mice in the control group.

	OFF	ON
Males	3108.235	3156.845
	1449.675	1157.888
	1294.14	1423.755
	1216.055	1304.265
	2035.34	2202.755
	2087.72	2261.46
Females	1462.16	1398.54
	1577.945	1419.14
	1232.492	1521.85
	1193.21	1046.624
	1775.92	2024.795
	2375.43	1862.83

ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Sampel	603948	1	603948	1.714323	0.205269	4.351244
Kolumner	31.68138	1	31.68138	8.99E-05	0.992528	4.351244
Interaktion	18104.9	1	18104.9	0.051391	0.822961	4.351244
Inom	7045906	20	352295.3			
Totalt	7667990	23				



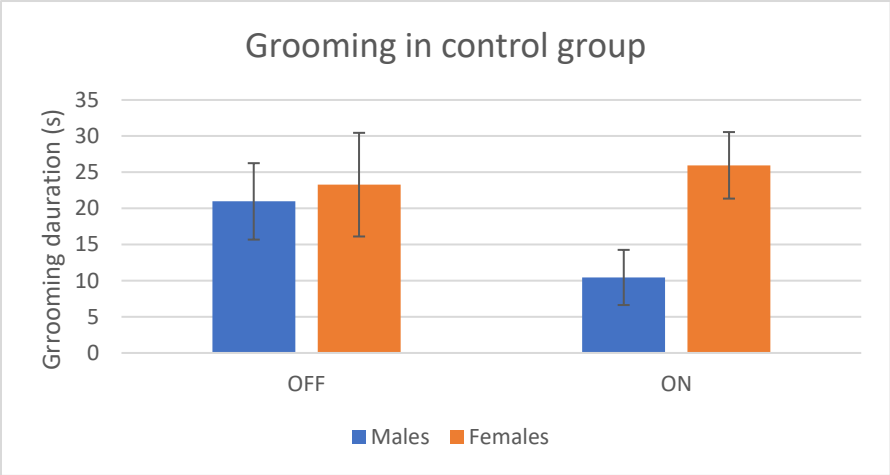
Grooming in the control group mice

The ANOVA showed that there was no significant difference between males and females regarding grooming among mice in the control group.

	OFF	ON
Males	10.94	0.82
	43.26	8.46
	21.38	10.12
	27.32	26.82
	8.36	2.66
	14.54	13.78
Females	36.56	33
	20.14	21.16
	45.12	8.06
	31.54	38.44
	2.7	21.14
	3.62	33.92

ANOVA

Variationsursprung	*KvS*	*fg*	*MKv*	*F*	*p-värde*	*F-krit*
Sampel	476.5068	1	476.5068	2.761008	0.112183	4.351244
Kolumner	92.43375	1	92.43375	0.535586	0.472757	4.351244
Interaktion	261.228	1	261.228	1.513625	0.232862	4.351244
Inom	3451.687	20	172.5844			
Totalt	4281.856	23				


Grooming in control group
Grrooming dauration (s)
35
30
25
20
15
10
5
0
OFF
ON
Males
Females